CHAPTER: 5

PERSPECTIVES IN THE USE OF BIOPOLYMER COMPOSITES IN MICROBIAL-MEDIATED BIOREMEDIATION OF HEAVY METALS

Dr. Viveeyan Saikia[1], Dr. Moon Mandal[2] & Dr. Archana Deka[3*]

[1]Department of Botany, Dibru College, Dibrugarh-786003, Assam, India

[2]Department of Chemistry, Pandit Deendayal Upadhyaya Adarsha Mahavidyalaya, Dalgaon, Darrang-784116, Assam, India

[3] ICMR-Regional Medical Research Centre, N.E. Region, Dibrugarh-786001, Assam, India

***Corresponding Author** Email: [1]viveeyan2008@gmail.com, [2]mnmandal8@gmail.com, [3*]archanadeka001@gmail.com

Abstract

In recent times, heavy metal pollution has become a major problem worldwide and is reported to have both direct and indirect impacts on the human health. Industrial activity, along with modernization, has caused massive environmental pollution with toxic heavy metals affecting different levels of ecosystems. Conventional approaches available for heavy metal remediation are expensive, non-ecofriendly, and do not meet the criteria of specificity to treat target metals against a background of competing ions and for large scale surface contamination. In recent years, there has been growing research interests on the use of resistant microorganisms that have adapted themselves to adverse environmental conditions in the field of heavy metal bioremediation. Immobilization of metal resistant microorganisms to support carriers such as biopolymers offers several advantages in bioremediation process. This review provides an overview on heavy metal pollution and its serious implications on human health. Further, the review provides insights into the microbe mediated bioremediation process and explores potential application of biopolymer composites for immobilization of microorganisms for the purpose of heavy metal bioremediation.

Keywords

Bioremediation, heavy metals, biopolymers, immobilization, polymer composites

INTRODUCTION

1. Heavy metal pollution

The term ‘Heavy metal’ has been basically used to represent metals with an atomic number of 23 or more and with a density above 5 gm cm-3 [1-2]. Among the 90 natural elements known, 53 are heavy metals and are broadly categorized into – essential and non-essential heavy metals [3]. Essential heavy metals are crucial for maintaining several biological processes in living organisms. For instance, heavy metals such as copper (Cu), Iron (Fe), Manganese (Mn), Cobalt (Co), Zince (Zn) and Nickel (Ni) are important cofactors of enzymes and vital for functionality of other proteins. However, the non-essential heavy metals such as Cadmium (Cd), Chromium (Cr), Mercury (Hg), and Lead (Pb) have no documented physiological functions in living organisms and are not required even in trace amounts. Heavy metals are non-toxic in nature but when the concentration exceeds certain level in organisms, it becomes highly toxic. The bioaccumulation of heavy metals occurs in the body due to absence of a definite route of excretion in organisms, which further magnifies its toxic effects [4]. Their distribution in environment is result of natural geological processes and human anthropogenic activities including mining, release of industrial effluents over-exploitation of fossil fuels, etc [5].

Industries such as metallurgical, electroplating, metal finishing, tanneries, chemical manufacturing, mine drainage and battery manufacturing are accountable for discharge of heavy metals in the environment. Removing metallic contaminants from the environment is a real challenge because, unlike organic contaminants, they cannot be destroyed; they can only be transformed or contained [6]. Several toxic heavy metals have the ability to act as "molecular mimics" of nutritionally essential trace elements, competing with vital metallic cofactors for entry into cells and incorporation into biological enzymes. Heavy metals possess ability to interact with the cellular components and cause oxidative dysfunction of biological macromolecules (DNA, protein). They also have the capacity to generate free radicals resulting in depletion of enzyme activities, damages lipid bilayers and DNA. The detrimental effects of heavy metal in the cells is mainly attributed to the presence incompletely filled *d* orbitals which can form complex unspecific compounds in the cells [7]. Prolong exposure to high levels of heavy metals may lead to several long-term diseases such as respiratory complications, renal failure, bone disorders, immune suppression and cancers in human beings [8] (Figure 1). Heavy metal cadmium is recognized as category #1 human carcinogen by the International Agency for Research on Cancer (IARC), USA. It also has been associated with skeletal damage and the first reported case was from Japan in 1950, when cadmium-contaminated irrigation water caused '*itai-itai*' (ouch-ouch) condition (a combination of osteomalacia and osteoporosis) [9]. The chronic exposure to Chromium and Nickel are reported to cause obstructive airway diseases and various types of cancers [10-11]. Studies on acute exposure of human populations to arsenic have demonstrated the occurrence of cancers, increased risk of cardiovascular diseases and long-term neurological effects [8]. Non-essential heavy metal Lead is considered as possible human carcinogen, which is likely to cause lung cancer, stomach cancer and gliomas [12-14]. High levels of mercury may cause deleterious effects on lung, kidney and other vital organs. The infamous Minamata disease or methyl mercury poisoning in Japan was the first reported case of human toxicity from organic mercury. Methyl mercury intoxication is characterized by neurological symptoms including ataxia, dysarthria, tremors, vision impairment, dementia, constriction of the visual field, and hearing difficulties [8].

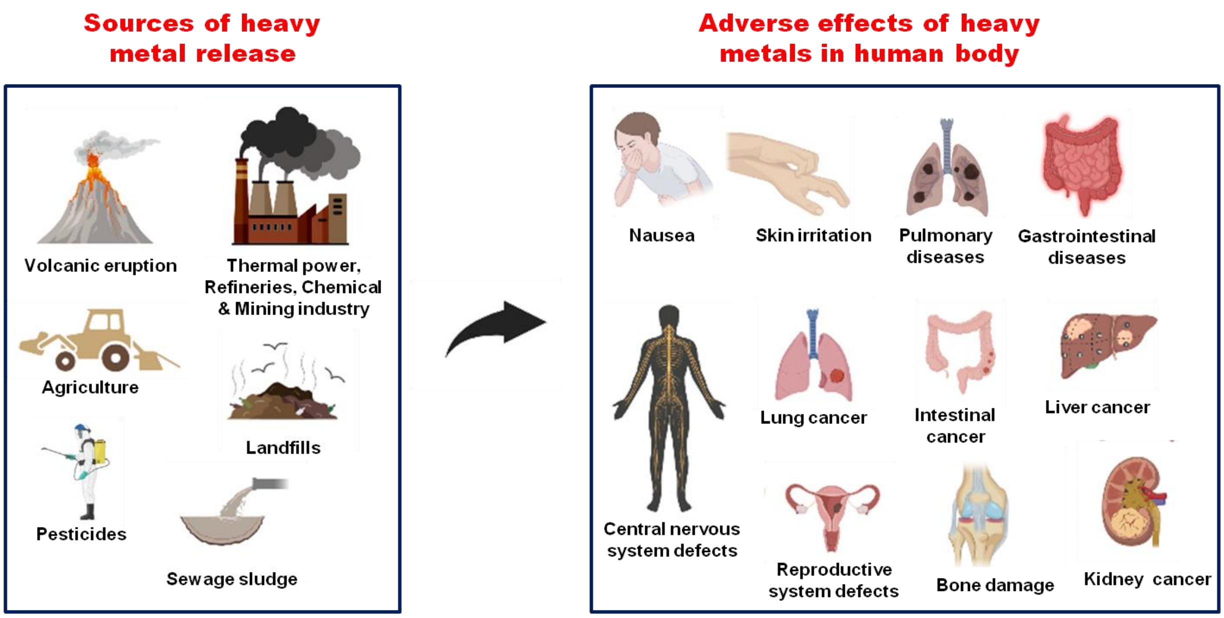

Figure 1: Sources of heavy metal pollution and its adverse effects in human body.

2. Heavy metals removal

Numerous methods have been adopted for the removal of the heavy metals from the contaminated environment. The commonly used approaches include chemical precipitation, chemical oxidation and reduction, ion exchange, filtration, electrochemical treatment, reverse osmosis, evaporative recovery and solvent extraction, soil vapor extraction, soil flushing, excavation, solidification/stabilization, land farming etc. Although these approaches have shown immense potential in removal of heavy metals but possess several disadvantages. Several of these approaches are expensive, non-eco-friendly, sometimes ineffective, require high energy consumption for maintaining temperature and extreme pressure, high operational costs, generates large toxic sludge and produces mutagenic derivatives [15-18].

Considering the short-comings of the conventional methods, microbe-mediated bioremediation has shown promising potential as an alternative and economically attractive strategy for toxic metals removal from contaminated sites. Bioremediation is the process of reclaiming polluted sites using biological agents such as microorganisms. The advantages of remediation process involving microorganisms includes environment friendly process, technically easy, low cost of operation, small quantities of disposable sludge, highly efficient in detoxification of dilute effluents and potential usage for *in situ* remediation of contaminated groundwater and/or sediments [19-20]. The first reported use of bioremediation began in 1891 with opening of biological sewage treatment plant in Sussex, UK [21]. Following this, extensive research has been focused on the biological approaches for removal of highly toxic metals with operational flexibility and its applicability both *in situ* and *ex situ*. Various strategies are implemented for *in situ* and *ex situ* bioremediation including biosorption, bioaccumulation, biostimulation, biotransformation, and bioaugmentation. For instance, biostimulation requires addition of nutrients to the contaminated sites to increase natural microbiota while bioaugmentation involves incorporation of microorganisms into contaminant site for biodegradation process.

Heavy metal removal by microorganisms involves complex process which depends on several factors including cell wall composition, its physiology and physicochemical factors (pH, temperature, contact time, ionic strength), the chemistry of metal ions and concentration of metal concentration [22]. Microorganisms, in particular bacteria, have adapted to numerous heavy metals and developed the tolerance which is often plasmid-borne with capability to be transferred throughout the bacterial community by the lateral gene transfer. Heavy metal resistant microorganisms act as indicators of potential toxicity to other forms of life. It is of immense importance to characterize the microbial communities that has the ability to acclimatize to harsh conditions and to understand the resistance mechanisms regulating microbial interactions with heavy metals [23-24]. The mechanistic knowledge at biochemical and molecular levels on the metal detoxification is indispensable for exploitation for the remediation and better management of heavy metal-based wastes. Recent advances in technological developments in the field of proteomics and genomics have aided in better interpretation of role of microorganisms in the mineral cycling and implications of such mechanisms in the bioremediation of heavy metals. Using genomic approach, complete genome sequences of several environmentally relevant microorganisms have been determined which have proved beneficial in understanding the mechanisms of metal-microbe interactions. Further, proteomics approaches have been useful in identification of protein markers expressed during stress conditions and comprehend the metal detoxification mechanisms in microbial systems.

The significant role of microorganisms in the biogeochemical cycle of toxic metals has been well illustrated previously and their applications in composting, sewage treatment, and certain types of fermentation have been known to mankind since the beginning of recorded history [25]. Several mechanisms are adopted by microorganisms particularly bacteria to tolerate the uptake of heavy metals (Figure 2). These mechanisms include efflux of metal ions outside the cell, precipitation and complex formation of the metal ions as insoluble salts, reduction and oxidation of the heavy metal ions to a less toxic state [1], generation of chelating agents, partitioning [26-27], alteration in membrane permeability, biochemical modifications of metal ions [28]. Studies reported presence of active efflux mechanism involving plasmid genes cadA and cadC in *Bacillus subtilus* [29], *Staphylococcus aureus* [30] and *Lactobacillus plantarum* [31]. Similar membrane transports efflux systems have been reported in Chromium metal resistant bacteria. For instance, ChrA protein reported in *Pseudomonas aeruginosa* function as chemiosmotic pump that aid in removal of chromium from the cytoplasm. NikABCD import pump or high affinity nickel/cobalt permeases involved in nickel uptake have also been reported from several gram positive and gram negative bacteria [32]. Heavy metals are known to induce oxidative stress in the bacterial system, which in turn results in activation of antioxidant enzymes that play pivotal role in combating metal-induced oxidative stress [33]. Antioxidant enzymes are involved in scavenging reactive oxygen species (ROS) or reduction of oxidized glutathione to reduced form to prevent oxidative stress [34]. In addition to bacteria, algae and fungi are considered appropriate for removal of heavy metals from contaminated sites. Algal species such as *Chlorella*, *Scenedesmus* and *Spirulina* are widely used for heavy metals removal due to their high biomass production which benefits high adsorption and accumulation of toxic metals [35]. Fungi belonging to the genera *Rhizopus* and *Penicillium* have shown potential to be used as biomass for effective removal of heavy metals from aqueous solutions [36-37]. Few fungal species such as *Trichoderma*, *Candida*, *Zygorhynchus*, *Mucor*, *Saccharomyces* and *Fusarium* have also displayed ability to biosorb different metal ions from aqueous environments [38-40].

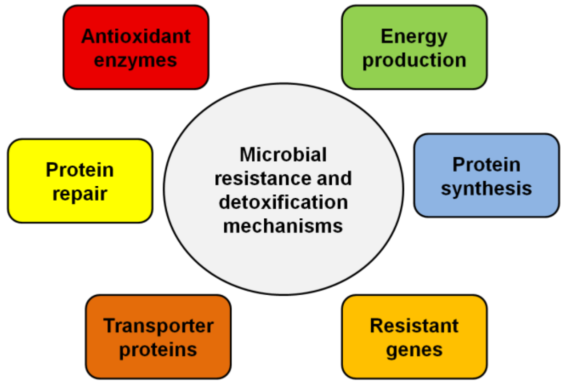

Figure 2: Heavy metals detoxification mechanisms in microorganisms

3. Immobilization of microorganisms for bioremediation

Maintaining high biomass of microbial populations is crucial for effective implementation of bioremediation process. In order to improve the survival and retention of microorganisms involved in bioremediation in contaminated sites, immobilization of cells is vital [41-42]. Whole cell microbes used as enzymatic systems are immobilized on substrates to ensure durability and efficacy. Immobilization of microorganisms onto appropriate substrates allows easy recovery and reutilization of microorganisms for bioremediation process [43]. The operational stability of microbial cells and metabolic activity is enhanced when immobilization approach is implemented. Further, it protects the cells from high temperature, toxic components, harsh conditions, and reduces the risk of contamination from other microbes [44-46]. Further, immobilized microbial cells used for bioremediation process increases its efficiency. The technique is better, faster and can be used for a longer period.

Different methods of microbial immobilization on substrates include adsorption, covalent binding, entrapment and encapsulation. Adsorption is reversible immobilization method involving physical interaction between microorganisms and the surface of matrix. This method is quick, simple, cost-effective, do not require chemical additives and is easy to perform with the option of reloading of the support [47]. Covalent method is irreversible process based on covalent bond between activated inorganic substrate and cell in the presence of a cross linking agent. This method, however, is not often implemented for immobilization of whole cells due to high toxicity of coupling agents involved in the process [48]. Entrapment method is based on capturing of cells inside a support matrix which prevents cell leakage and creates a protective barrier. Both natural (e.g. chitosan, alginate, agar) and synthetic polymers (e.g. polyvinyl alcohol, polyvinylchloride, polyacrylamide, polyacrylnitrite, polypropylene) are employed for production of cost-effective and non-toxic substrates for effective entrapment of microorganisms [49]. Another irreversible method called encapsulation is obtained by enveloping the cells within various forms of spherical semi permeable membranes with a selective controlled permeability [50]. These methods extensively used for immobilization of microbial cells have their own set of advantages and limitations. For instance, adsorption method of immobilization causes high rate of leaching of bacteria from matrix due to weak and unstable interactions [51].

Several factors are involved for effective immobilization of microbial cells on to substrates [52]. The surface properties of supports used, pore size, nature of the material, physiological state of cells and environmental conditions highly influence the retention immobilized microorganisms on the substrates [53]. Different organic (word or bark chips) and inorganic substrates (metal oxides, zeolites, cement particles) are used for microbial immobilization. The organic substrates have advantage of being chemically stable while inorganic substrates are affordable and resistant to biological degradation [54]. Among wide variety of synthetic polymers, polyacrylamide (PAM), polyvinyl alcohol (PVA), polyethyleneglycol (PEG) and polycarbamoyl sulphonate (PCS) have been extensively explored as supports for entrapment of microorganisms. The organic natural polymers (such as chitosan, alginate) are also widely used as substrates for immobilization of microbial cells [55-56]. Although the natural polymers are more susceptible to biological degradation than the synthetic polymers, the major advantages is that biopolymers are easy to handle, non-toxic, highly abundant, cost-effective, and possesses reactive functional groups for effective cell-substrate interaction.

4. Biopolymer composites for microbial-mediated bioremediation

New environmental regulations and societal concerns have triggered the urgency to develop novel bio-based products and other innovative technologies that can unhook widespread dependence on fossil fuels [57]. Bio-based materials are crucial for sustainable development as they hold the potential to reduce greenhouse emissions, raw material costs, and create opportunities for growth and employment in agriculture. Most of the conventional polymer composites are produced using synthetic polymers and reinforcing agents derived from synthetic fibers [58]. Polymer composites made from synthetic reinforcements are losing popularity due to various environmental problems and cost issues. They are non-renewable, require high energy consumption in the manufacturing process, pose a health risk when inhaled, and are non-biopolymer. One major initiative has been the synthesis and application of a wide range of bio-based materials from sustainable and environment-friendly starting materials, such as animals, algae, plants and bacteria [59]. Biopolymers are categorized into three groups based on the production methods – (i) extracted from biomass in the form of polysaccharides and proteins; (ii) chemically synthesized from bioderived monomers; (iii) produced by microorganisms. Figure 3 represents schematic overview of classification of biopolymers with examples. These materials offer the advantages of low cost, being readily available from renewable natural resources, and often possessing comparable or better properties than those of widely used industrial polymers. Also, the incorporation of bio-resources in composite materials can reduce further dependency on petroleum feedstock [60].

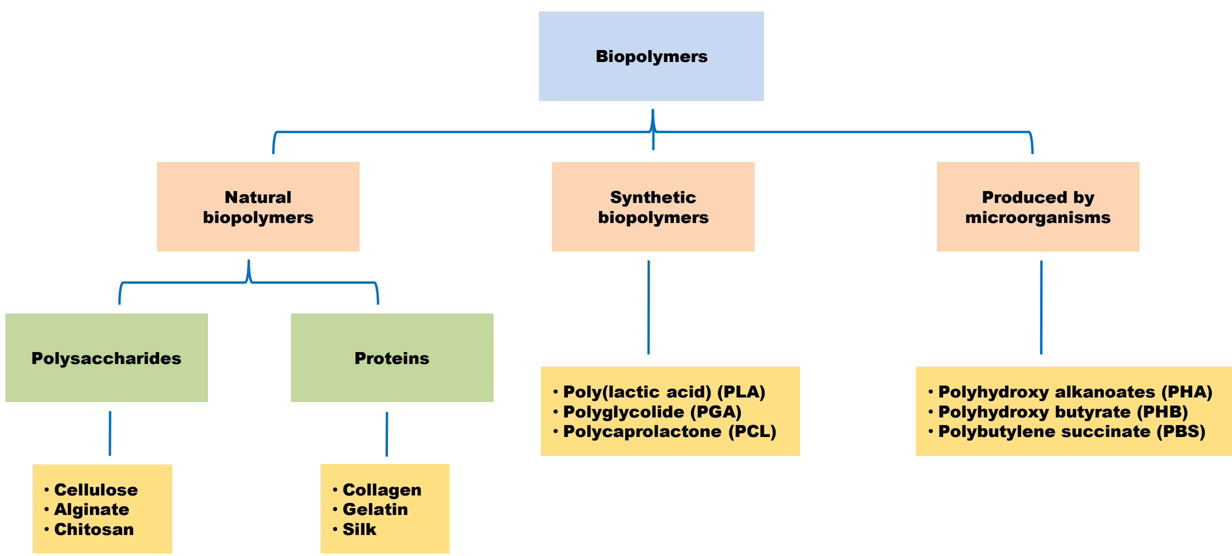

Figure 3: Pictorial representation of biopolymers classification based on their source

There exist different types of environmental pollutants that harm the human health and ecosystem. Heavy metals, dyes, pesticides and surfactants are some common pollutants that cause effects like genetic mutation, gastrointestinal dysfunction, birth defect, neural disorders, conjunctivitis and allergic reactions. Biopolymers have gained a lot of interest in the scientific community because of their distinctive characteristics. These biopolymers are long chain like molecules containing repeating units of monomers which are environmentally degradable. The majority of biopolymers are derived from biological sources including micro-organisms, agrowastes and plant biomass. Biopolymer comprises proteins, nucleotides, fats and polysaccharides. Recent competing interest with biopolymers has increased with global consideration for environmental pollution remediation. Biopolymers and biopolymer-based industrial products are therefore considered as major candidates to replace synthetic polymers [61]. The key aspect of biopolymers is their synthesis from renewable resources, a feature that guarantees successful, long-term production processes. A large number of novel applications have been explored in which biopolymers and biopolymer-based products can offer a complete alternative to the use of existing non-degradable synthetic polymers [62]. Among the numerous applications of biopolymers, the most important are the ones in the medical and environmental fields. In medicine, these materials have been used as drug carriers, in tissue-regenerating materials, and in the development of artificial organs, among other applications [63]. Furthermore, these biomaterials have a direct role in the resolution of various environmental hazards. Their most important environmental advantages are their easy disposal and quick degradation, which results in their natural recycling, reducing their environmental implications.

Composite materials are solid multiphase materials formed through the combination of materials with different structural, physical and chemical properties. It not only retains the key feature of the original component materials but also gets the performances that are not depicted by the original components through the combined effects. Biopolymer composites were intended to address some of the shortcomings of polymeric properties, such as poor mechanical performance, low resistance, limited processing capability, and long-term ability. They are created by reinforcing biopolymers with appropriate fillers such as metal, metal oxides, and natural fibres [64]. Usually, biopolymers are synthesized from monomers through polymerization, extraction using solvent and using fermentation while, the preparation methods for composites differ from conventional techniques. Fillers can be reinforced into polymers by methods such as molding, extrusion, solvent casting, electro spinning and intercalation. The choice of composite fabrication method plays a critical role in mechanical and physical properties of polymer biocomposites. Composites of biopolymers have dominant properties such as improved durability, processing capability, high functionality, and a large surface area, which increases the removal of contaminants or pollutants from the environment *via* adsorption [65]. Recently, biopolymers were being used as membrane modifiers in waste remediation processes to alleviate membrane fouling issues. The inherent properties of biopolymers with additional functional moieties, as well as their environmentally sustainable nature, allow for their use in membrane-based treatment processes. Chitosan, chitin, alginate, and carrageenan are some of the most common polymers used in membrane modification.

The properties of biopolymers and their composites have prompted a focus on waste water treatment and gas purification. Many inorganic nanoparticles including Fe(III), Mn(IV), and $M(HPO_4)_2$ (M = Ti, Zr, Sn) have been applied as adsorbents for target pollutants [66-67]. These nanoparticles have been incorporated into biopolymers including alginate, porous resins, and cellulose to avoid the transition loss caused by the nano size of these particles [68]. Cellulose−$Fe(OH)_3$nanocomposites were applied for the adsorption ofAs(III) and As(V) [69]. Alginate−Fe_3O_4nanocomposites were also applied for the removal of methylene blue and methyl orange. Biopolymers also offer a wide range of chemical and mechanical properties for the fabrication of sensor devices. A chemical sensor comprised of chitosan− Au nanocomposites has been utilized for the detection of Zn^{2+} and Cu^{2+}.Chitosan−Au-composite-based biosensors have been applied for the detection of glucose. Cellulose derivatives including carboxymethyl cellulose (CMC), sodium carboxymethylcellulose (SCMC), and cellulose acetate (CA) have been utilized as the matrix for the fabrication of nanocomposites for environmental applications. CMC− Fe^0 composites have been utilized for Cr(VI) degradation, and its removal efficiency was 94% after 60 min of treatment [70]. CMC−Fe^0 composites were also utilized for the degradation of trichloroethylene [71]. SCMC−Fe/Pd composites were utilized for the degradation of para-nitrochlorobenzene (p-NCB).

Natural biopolymers and their composites have recently gained significant attention as support carriers for immobilization of enzymes/cells owing to their widespread abundance, availability, non-toxicity, biocompatibility, biodegradability, flexibility, renewability, presence of reactive functional sites [72]. Immobilization is a process by which enzymes/whole cells are confined to a phase distinct from the one in which the substrates and the products are present. It allows the ease of removal of the product, reuse of the enzymes, and enhanced effectiveness due to the reuse of enzyme. Various natural polymers such as chitosan, sodium alginate have been used to immobilize enzyme and microorganisms. Chitosan is the product obtained from deacetylation of chitin, which is the second most abundant biopolymer after cellulose. Chitosan has been used as a raw material for biomedical applications such as surgical sutures, artificial skin and immune-suppressant [73]. One of the most important applications of chitosan is as support for immobilization of enzymes or whole cells as it is non-toxic and user-friendly, and has protein affinity [74]. Enzymes/cells that have been immobilized on functionalized and non-functionalized chitosan based matrices include pepsin on chitosan beads [75], hydrolases onto chitosan micro particles, amylase, glucoamylaseon chitosan-clay composite beads [76], etc. Alginate is also one of the most extensively used polymers for various biomedical applications and in pharmaceutical industries as gelling agent [77]. Alginate has the capacity to extract metal ions from dilute aqueous solutions. Reported cell immobilizations on sodium alginate beads include, immobilization of *Acidithiobacillus ferrooxidans* on PVA and sodium alginate [78], and *Saccharomyces cerevisiae invertase* on chitin-sodium alginate beads [79]. Recently, reports on the immobilization of carbonic anhydrase enriched microorganism have been revealed. Various materials based on biopolymers have been used for effective immobilization of microbial cells and carbonic anhydrase for their applications in the conversion of CO_2 to mineral carbonates. The active site of the enzyme is responsible for the acceleration of CO_2 hydration as well as for the hydrolysis of esters [80].

The detailed description of various biopolymer composites, its methods of production and their application in immobilization of microorganisms have been well illustrated by Martines et al. [81]. The use of different matrices for immobilization of microorganisms with the capacity of bioremediation of toxic heavy metals from polluted sites has been extensively studied [82]. Sarin et al. employed alginate as support carrier for immobilization of *Pseudomonas fluorescens* G7 for bioremediation of heavy metals cadmium and zinc from contaminated soil [83]. Tariq et al. examined the alginate based immobilization of nitrogen-fixing bacteria displayed promising potential in detoxification of higher concentration of mercury as compared to free cell cultures [84]. Several studies reported advantages of using natural biopolymers for bioremediation process, in recovery of polluted sites, and removal of heavy metals and petroleum derivatives [85-87]. By combining Aspergillus oryzae and biodegradable polymer (poly(ε-caprolactone)) (PCL), bioremediation of formaldehyde has been successfully achieved [88]. The potential use of polyhydroxyalkanoate and poly(butylene succinate) as nutrient-releasing system for bioremediation and biodegradation of toxic chemical solvents and petroleum derivatives by *Pseudomonas aeruginosa* have been studied [89-90]. The production of biopolymer composites as support carriers having mechanical and chemical stability, porosity, elasticity, and biocompatibility suggests that these materials have potential to be used for immobilization of metal-resistant microorganisms for biosorption of heavy metals from contaminated sites.

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