17 Bacterial Biosorption of Trace Elements

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CONTENTS

17.1 INTRODUCTION

Metals are ubiquitous in environments and are essential to organisms, e.g., K, Na, Mg, Ca, Mn, Fe, Co, Ni, Cu, Zn, and Mo; other metals have no known essential biological functions, e.g., Al, Ag, Cd, Sn, Au, Sr, Hg, and Pb [1]. All these elements can interact with microorganisms and be accumulated by physicochemical mechanisms and transport systems associated with cell growth and metabolism [2,3]. Heavy metals are not biodegradable and tend to be readily accumulated in living organisms, causing various diseases [4–6]. Virtually all metals can exhibit toxicity to above certain threshold concentrations whether essential or nonessential [1,7].

Heavy metal contamination has been increased in aqueous environments near many industrial facilities, such as metal plating facilities, mining operations, and tanneries. The soils in the vicinity

of many military bases are also reported to be contaminated and pose a risk of groundwater and surface water contamination with heavy metals [8].

Physicochemical methods such as chemical precipitation, membrane filtration, ion exchange, and activated carbon adsorption have been developed for the removal of heavy metals from wastewater [9,10]. However, the practical application of such processes is sometimes restricted due to technical or economical constraints. The biological removal of metals through biosorption has distinct advantages over conventional methods: the process rarely produces undesirable or deleterious chemical byproducts and it is highly selective, efficient, easy to operate, and cost effective in the treatment of large volumes of wastewater containing toxic heavy metals [11–13]. Algae, fungi, yeast, and bacteria can remove heavy metals from aqueous solutions by binding the cationic metals onto negatively charged functional groups distributed on their cell walls, such as carboxyl and phosphoryl groups [7,14,15].

According to Beveridge [16], bacteria can be used as an excellent biosorbent for metal sorption because it has high surface-to-volume ratios related with the active sorption site in bacterial cell walls. Particularly, the pure microbial strains have shown extremely high capacities for the selective uptake of metals from dilute metal-bearing solutions [17,18]. The advantages of bacteria as a sorbent for metal removal from wastewater have been well documented and reviewed [19–21]. Although bacterial biosorption can be applied as a useful strategy for metal removal in soil and preventing contamination of groundwater, the funding is insufficient to study the microbial remediation of heavy metals in pilot scale applications comparing with the bioremediation of organic contaminants.

This chapter investigates the characteristics and mechanisms of bacterial biosorption and provides the potential applicability of bacterial biosorption as advanced technology in contaminated environment sites.

17.2 BACTERIAL BIOSORPTION

17.2.1 Bacteria as a Sorbent

The bacterial biomass has been successful for the selective removal of heavy metals and radionuclides as sorbents [22–25]. The use of bacteria offers a potential feasibility with cost effectiveness and high removal efficiency because microbial biomass is made up of abundant natural materials and can be grown extremely fast.

17.2.1.1 Structure of Bacterial Cells

Bacteria generally can be divided into two kinds of bacteria: Gram positive and Gram negative by Gram straining. The walls of Gram-positive bacteria consist of three primary components: cytoplasm mixed with a peptidoglycan, to which teichoic acids are covalently bound [26,27]. Each of these polymers is an amphoteric group, but with a net negative charge [16,28]. The envelope of Gram-negative bacteria is more complex than that of Gram-negative bacteria. It consists of two membrane bilayers (the outer and plasma membrane) that are chemically and functionally distinct from one another and sandwich a thin peptidoglycan layer between them [27].

The cell surfaces of Gram-positive and Gram-negative bacteria, whether living or nonliving, possess abundant functional groups that bind metal ions in EPS. These also include phosphate, carboxyl, hydroxyl, and amino functional groups, among others [29]. In Gram-negative cell, the EPS is composed of polysaccharides and protein, which are less firmly bound to the cell surface. External polysaccharides of Gram-negative bacteria offer many functional groups such as carboxylate, hydroxyl, sulphate, phosphate, and amino that can coordinately interact with heavy metals ions. In Gram-positive cells, teichoic acids as well as polysaccharides and proteins that are not anchored in the cell wall contribute to the EPS [7,28]. The Gram-positive bacteria, therefore, can accumulate more heavy metal ions than Gram-negative bacteria.

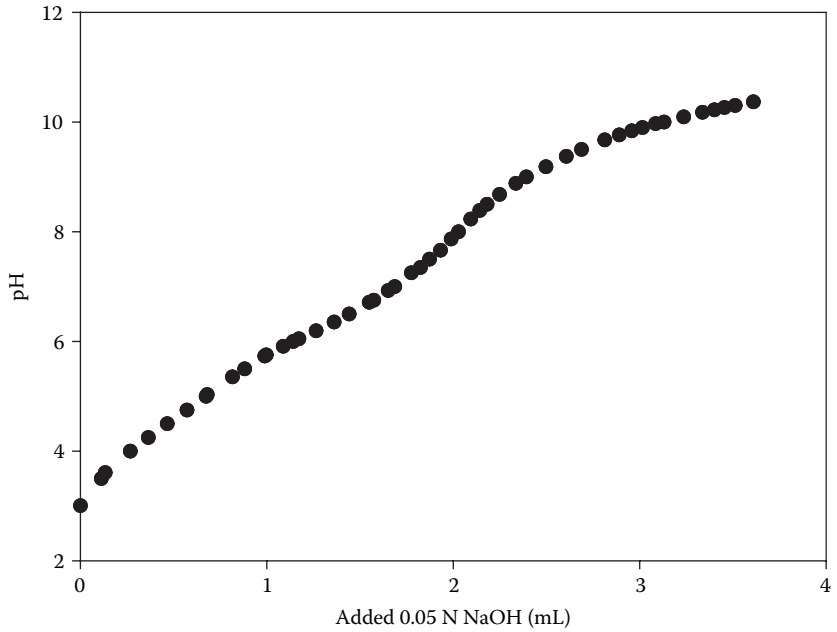

FIGURE 17.1 Biomass potentiomatric titration: 1 g of *P. aeruginosa* was washed by distilled ionized water and then potentiometrically titrated with 0.05 *N* NaOH.

17.2.1.2 Case Study: Identification of Functional Groups

Several investigations have shown that *Pseudomonas aeruginosa*, which is a Gram-negative bacterium commonly distributed in environmental sources such as soil, water, and plant surfaces, has high efficiency for metal uptake [30]. For instance, Chang and Hong [31] have found that the amount of mercury adsorbed on *P. aeruginosa* biomass was higher than that bound to a cation-exchanger resin (AG 50W-X8 resin) with 180 mg Hg/g dry cells and 100 mg Hg/g dry resin, respectively. Hu et al. [32] have identified that *P. aeruginosa* strain CSU showed the highest affinity and maximal capacity for uranium (100 mg U/g dry weight) and was also competitive with commercial cation-exchange resins.

In this part, the study was performed to obtain more information of functional groups of *P. aeruginosa* by potentiometric titration of an aqueous cellular suspension and IR analysis of the lyophilized biomass in solid phase. The washed protonated biomass was potentiometrically titrated with 0.05 *N* NaOH. The biomass titration data are shown in Figure 17.1. It would seem that the *P. aeruginosa* cell wall has two main functions. To distinguish the different groups of binding sites, IR analysis of the lyophilized biomass was used.

In Figure 17.2, the pattern for biosorbent revealed a complex and additive impact of chemical texture. The amines group presents relative transmittance at 3500 to 3300 cm^{-1} (N–H stretching) and at 1140 to 1080 cm^{-1} (C–N stretching). The N–H stretching peak lies in a spectrum region occupied by a broad and strong band (3600 to 3300 cm^{-1}). This may be due to hydroxyl groups that are hydrogen bonded to various degrees. The presence of intense OH peaks in the spectrum could be due to the great water content of lyophilized biomass and to the real presence of hydroxyl groups in the biomass.

The C–N stretching peak is also covered by another strong band (1125 to 1090 cm^{-1}) that can be attributed to an alcoholic C–O stretching. The amide group presents relative transmittance at 1490 to 1440 cm^{-1} (N–H stretching) and at 1661 cm^{-1} (C–O stretching) at 1550 cm^{-1} (C–N–H stretching). Again, the C–N stretching peak is covered, while a peak at 1414 cm^{-1} can be assigned

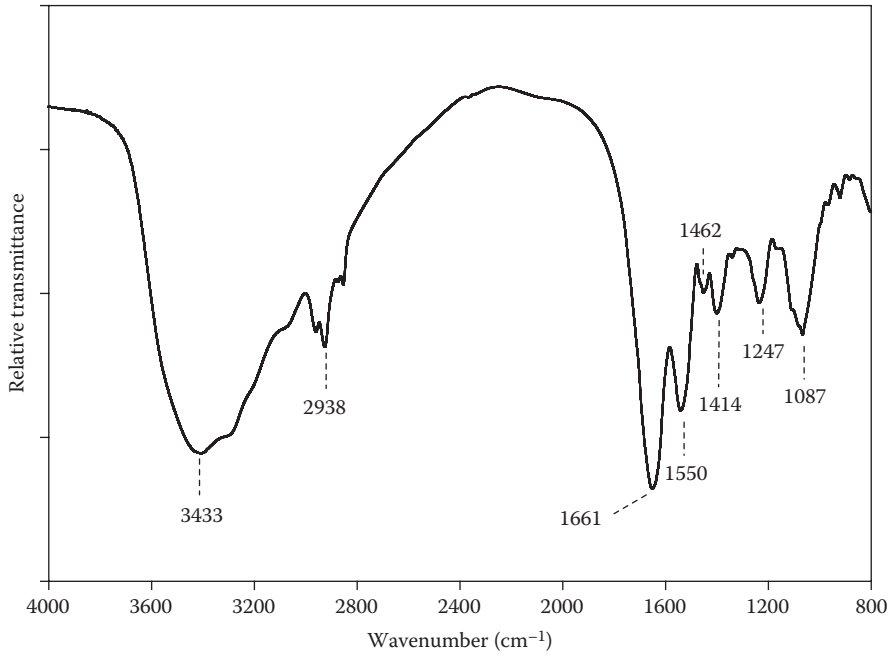

FIGURE 17.2 IR spectrum in solid phase of the lyophilized *P. aeruginosa* in KBr disk.

to O–H stretching of the acidic group. The carboxyl group presents some relative transmittance peaks (O–H stretching at 3100 to 2900cm^{-1} and at 1414 cm^{-1}; C–O stretching at 1320 to 1211 cm^{-1}).

17.2.2 Mechanisms of Biosorption

According to the location where metal biosorption occurs, the mechanisms of biosorption are classified as the following processes:

- Intracellular interaction
- Cell surface interaction
- Extracellular interaction

Figure 17.3 schematically summarizes alternative process pathways to remove heavy metals in trace elemental environments. The biosorption mechanisms of heavy metals show that the heavy metals are adsorbed by physicochemical interactions between metal ions and the bacterial surface. Biosorption is mainly the passive interaction of metals independent of metabolisms. Nutrients are not supported for continuing the bacterial activity and dead cells as well as living cells are effectively used in the removal of heavy metals.

17.2.2.1 Intracellular Interaction

Active transport of the metal across the cell membrane yields intracellular accumulation, which depends on the bacterial metabolism [33]. Essential metals are actively taken up by specialized uptake systems because they are needed, but other, nonessential metals may also be taken up because they are mistaken for an essential metal [34]. In high concentration of toxic metals, microorganisms actively take up the metal ions to detoxify the surrounding environment. Actually, a variety of bacteria is capable of converting metal and metalloid ions to organometallic and organometalloid compounds by intracellular ligands such as metallothioneins [35]. The pathway

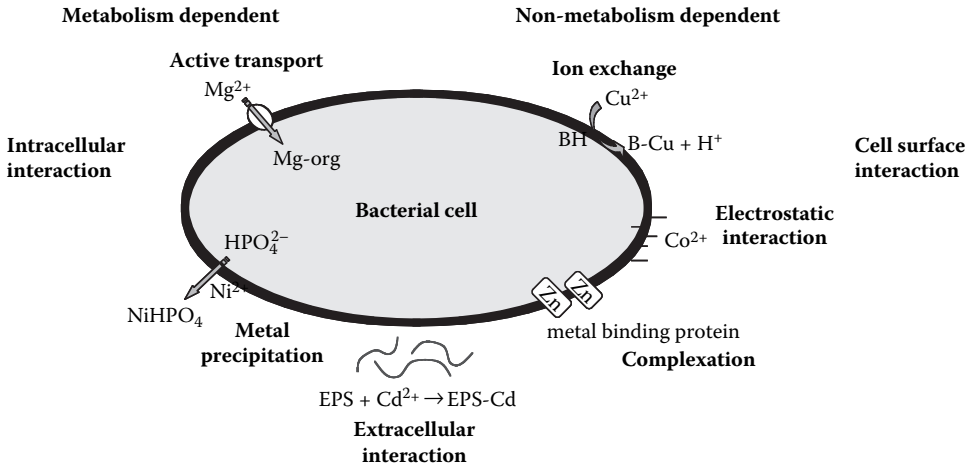

FIGURE 17.3 Schematic diagram showing the mechanisms of bacterial biosorption.

of the formation of organometallic and organometalloid compounds has been comprehensively reviewed [36,37].

The microorganism also has a metabolically sponsored process such as bioprecipitation to enhance the metal uptake [38,39]. The insoluble metal compound precipitates as metal ions combined with anoic species produced by cell metabolism [37]. For instance, Basnakova and Macaskie [40] reported that *Citrobacter* sp. could accumulate high levels of uranium, nickel, and zirconium through the formation of metal phosphate precipitates.

17.2.2.2 Cell Surface Interaction

In the case of physicochemical interaction based on physical adsorption, ion exchange, and complexation between the metal and functional groups of the cell surface, metal uptake does not depend on the metabolism [1,41]. The mechanisms by which metal binds onto the cell surface most likely include electrostatic interactions, van der Waals forces, covalent bonding, or some combination of these processes [27,28]. These passive parts showed a rapid initial uptake and surface-mediated mechanism.

Electrostatic interactions have been demonstrated to be responsible for cobalt biosorption by main algae by Kuyucak and Volesky [42]. The negatively charged groups, such as carboxyl, hydroxyl, and phosphoryl groups of the bacterial cell wall, adsorb metal cations by electrostatic forces. The ion exchange is related to cellular metal accumulation because cell walls of microorganisms contain polysaccharides as basic building [33]. Cell-based on their displacement by ion exchange, the following ascending order of light metal affinity toward biomass was observed: $Na^+ < K^+ < Mg^{2+} < Ca^{2+}$ [43].

The metal sorption may also take place through complex formation on the cell surface by interaction between metals and metal-binding proteins of the organism [19]. Uranium and thorium biosorption onto *Rhzopus arrhizus* has a mechanism based not only on physical adsorption but also on complexation on the cell wall network [44].

17.2.2.3 Extracellular Interaction

As has already been pointed out, some bacteria can produce large quantities of extracellular polymeric substances (EPS), including negatively functional groups [22,45]. The EPS can bind and accumulate cation heavy metals such as magnesium of cadmium. Recent studies from Loaëc et al. [46] showed that the polymer from *Alteromonas macleodii* possessed affinity for lead, cadmium,

and zinc. Lead was preferentially absorbed, but between zinc and cadmium competed for the same binding site. In a study using extracted EPS from activated sludge, Liu et al. [47] demonstrated that the metal capacity had 1.48 mg of Zn^{2+}; 1.12 mg of Cu^{2+}; 0.83 mg of Cr^{3+}; 0.90 mg of Cd^{2+}; 1.10 mg of Co^{2+}; and 0.25 mg each of $Ni^{2+}$and CrO_4^{2-} by polymers.

17.3 METAL REMOVAL BY BACTERIAL BIOSORPTION

17.3.1 Biosorption Isotherms

Sorption phenomena can be quantified and evaluated by fitting experimental data to one of several sorption isotherms. The adsorption isotherms describe the relation between the activity or equilibrium concentration of the adsorptive and the quantity of adsorbate on the surface of adsorbent at constant temperature. The most widely used sorption models are the Langmuir isotherm and the Freundlich isotherm. Although they are rather simplistic when applied to biological systems, mathematical sorption isotherms can be used to understand the surface behavior of biosorbent, mechanisms of biosorption, and distribution of metal ions between the liquid and solid phases [12,14].

The Langmuir adsorption isotherm equation is shown in the following equation [48]:

$$\Gamma = \Gamma_{\max} \frac{K_{ads} C_e}{1 + K_{ads} C_e} \tag{17.1}$$

where

Γ is the amount of adsorbed metal ion per wet mass of resin (μmol/g)
Γ_{max} is the maximum adsorption capacity of metal ion (μmol/g)
C_e is the equilibrium concentration of adsorbate in solution (μmol/L)
K_{ads} is the equilibrium adsorption constant (L/μmol)

The parameter represents the uptake capacity when the surface is completely covered with metal ions and is an indication of the biosorbent maximum uptake capacity, Γ_{max}. Constant K_{ads} is related to adsorption energy, reflecting quantitatively the affinity between the biosorbent and the metal ion [49].

The Freundlich isotherm suggests a concentration-dependent increase of metal sorption onto the adsorbent. It is based on a heterogeneous distribution of active sites as well as on the interaction between sorbed metals.[50]. One of the major disadvantages of the Freundlich equation is that it does not predict an adsorption maximum.

The Freundlich isotherm can be explained by the following equation [48]:

$$\Gamma = K_d C_e^{1/n} \tag{17.2}$$

where

K_d is the distribution constant
n is the Freundlich exponent known as adsorption intensity

To evaluate the sorption capacity and to understand the pattern of metal biosorption on *P. aeruginosa*, the experimental metal biosorption isotherms were obtained (Figure 17.4). Metal sorption studies carried out at varying initial metal concentrations revealed that specific metal uptake increased with increase in initial metal concentration. Equilibrium sorption isotherm studies showed

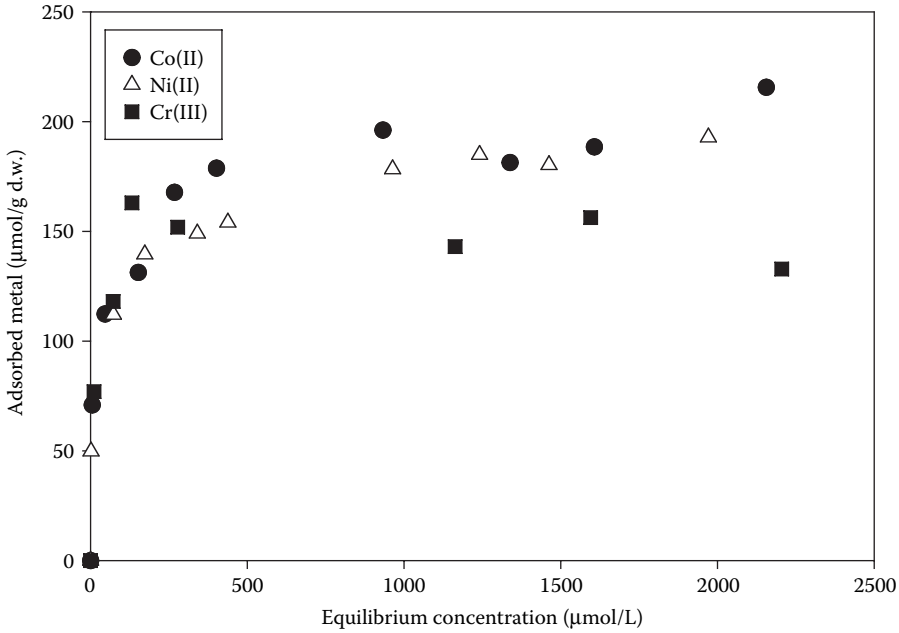

FIGURE 17.4 Biosorption isotherms of Co^{2+}, Ni^{2+}, and Cr^{3+} onto *P. aeruginosa*. The biomass was contacted with metal solution for 10 h at 25°C and 180 rpm in shaking incubator.

that metal uptake by *P. aeruginosa* was a chemically equilibrated and saturated mechanism. When experimental data were applied to adsorption models such as Langmuir and Freundlich, the data were found to fit in the Langmuir isotherm model reasonably. From the result, the maximum amounts of metals taken up by *P. aeruginosa* were 188.7 µmol Co^{2+}/g dry weight; 166.7 µmol Ni^{2+}/g dry weight; and 149.3 µmol Cr^{3+}/g dry weight.

17.3.2 Comparing Metal Capacity

Uptake of toxic metal ions may contribute to the detoxification of polluted environments. Therefore, the investigation of the metal capacity of bacteria is fundamental for the field application of biosorption because it gives information about the removal efficiency of metal ions in the process [33]. As a necessary factor for the design of equipment, the metal capacity of bacteria is usually used by the parameter Γ_{max}. Table 17.1 shows the maximum metal capacities reported in literature by bacteria.

17.3.3 Influence of Environmental Conditions

In the biosorption process, many environmental factors can influence the metal capacity of bacteria due to the change of bacterial surface properties and the characteristics of metal-bearing streams such as a variable pH and competing ions.

17.3.3.1 Bacterial Growth Phase

The physiological changes between the exponential and stationary phases are often reported to be significant so that cells in the stationary phase have distinct characteristics. When *Arthrobacter*, for example, reached the stationary phase, t its factor changed from rod to coccoid [26]. Thus, the cell growth phase can be an important factor that affects metal sorption.

TABLE 17.1
Comparison of Maximum Metal Capacities (Γ_{max}) by Bacterial Biosorption[a]

		Metal biosorption		
Metal	**Bacteria**	Γ_{max}	**pH**	**Ref.**
Ag	*Streptomyces noursei*	38.6	6	Mattuschaka and Straube, 1993
Al	*Chryseomonas luteola*	55.3	5	Ozdemir and Baysal, 2004
Cu	*Arthrobacter* sp.	148	6	Veglió et al., 1997
	Arthrobacter sp.	6.6	4.5	Pagnanelli et al., 2000
	Brevibacterium sp.	34.3	6.3	Vecchio et al., 1998
	Pseudomonas aeruginosa	79.5	5.5	Chang et al., 1997
	Pseudomonas putida	6.6	6	Pardo et al., 2003
	Thiobacillus ferrooxidans	23.12	5	Ruiz-Manriquez et al., 1998
	Zoogloea ramigera	270	5.5	Norberg and Persson, 1984
	Zoogloea ramigera	29	4	Aksu et al., 1992
	Zoogloea ramigera	34.05	4	Sag and Kutsal, 1995
	Streptomyces noursei	9	5.5	Mattuschaka and Straube, 1993
Cd	*Arthrobacter* sp.	13.4	6	Pagnanelli et al., 2000
	Bacillus laterosporus	159.5	7	Zouboulis et al., 2004
	Bacillus simplex	1.8	6	Valentine et al., 1996
	Brevibacterium sp.	15.7	6.3	Vecchio et al., 1998
	Pseudomonas aeruginosa	42.4	6	Chang et al., 1997
	Pseudomonas cepacia	130	7.4	Savvaidis et al., 1992
	Pseudomonas putida	8	6	Pardo et al., 2003
	Streptomyces noursei	3.4	6	Mattuschaka and Straube, 1993
Cr(III)	*Pseudomonas aeruginosa*	7.7	4	In this study
	Streptomyces noursei	10.6	5.5	Mattuschaka and Straube, 1993
Cr(VI)	*Bacillus laterosporus*	72.6	7	Zouboulis et al., 2004
	Bacillus licheniformis	62	7	Zouboulis et al., 2004
	Bacillus simplex	0.4	6	Valentine et al., 1996
	Chryseomonas luteola	3	5	Ozdemir and Baysal, 2004
Co	*Pseudomonas aeruginosa*	11.1	4	In this study
Hg	*Pseudomonas aeruginosa*	180	7.4	Chang and Hong, 1994
Mg	*Arthrobacter* sp.	406	5.5	Veglió et al., 1997
Ni	*Arthrobacter* sp.	12.7	6	Veglió et al., 1997
	Bacillus simplex	0.8	6	Valentine et al., 1996
	Pseudomonas aeruginosa	9.8	4	In this study
	Zoogloea ramigera	57.43	4.5	Sa and Kutsal, 1995
Pb	*Arthrobacter* sp.	130	5	Veglió et al., 1997
	Streptomyces noursei	36.5	6.1	Mattuschka and Straube, 1993
	Zoogloea ramigera	81.23	4.5	Sa and Kutsal, 1995
	Brevibacterium sp.	74.6	6.3	Vecchio et al., 1998
	Pseudomonas putida	56.2	6.5	Pardo et al., 2003
	Pseudomonas aeruginosa	79.5	5.5	Chang et al., 1997
Zn	*Pseudomonas cepacia*	200	7.4	Savvaidis et al., 1992
	Streptomyces noursei	1.6	5.8	Mattuschaka and Straube, 1993
	Thiobacillus ferrooxidans	9.7	4.5	Celaya et al., 2000
	Pseudomonas putida	6.9	7	Pardo et al., 2003

[a]Milligrams per gram of dry weight sorbent.

In the case of *P. aeruginosa*, the amount of cobalt did not change substantially with the cell growth phase. The quantities of cobalt taken up by the cells were 180.3 µmol/g dry cells at midexponential phase and 178.9 µmol/g dry cells at stationary phase, respectively (no data shown). From the result, the sorption of cobalt by *P. aeruginosa* appeared to be independent of the cell growth phase. Chang et al. [51] reported an increase in sorption of lead and cadmium by *P. aeruginosa* with increasing culture age; however, copper uptake was also found to be independent of growth phase.

17.3.3.2 pH

Numerous studies show that the biosorption of heavy metal from aqueous solution depends strongly on pH. Rao et al. [52] studied Cu^{2+} biosorption by *G. lucidum* and *A. niger* at initial copper concentration of 0.5 m*M* and found that the metal binding had an increasing trend from pH 2 to 6, with the maximum occurring between pH 5 and 6.

On the other hand, for some biosorbents, pH plays a different role in biosorption when the initial ion concentrations are different. Ke et al. [53] reported results for the biosorption of Ag^{+} by using *Datura* cells. The binding was pH independent when the initial concentration was 0.1 m*M*, but it became strongly pH dependent when the initial concentration increased to 1 m*M*. These investigations suggested that at least two binding sites are involved: one site is pH independent and displays a greater affinity and lower availability than the other site, which is pH dependent.

Figure 17.5 shows that the adsorption of metal ions by the *P. aeruginosa* depended highly on solution pH. The adsorption percentage of metals in the lower pH levels (e.g., pH 2 or 3) was significantly low due to competition with the H^{+} ions for binding sites on the surface of bacteria; the increase in pH favored metal sorption mainly because of the elevated levels of negatively charged groups on the cell surface.

The negatively charged groups existing on the surface of the microbial cell wall may undergo protonation at low pH, leading to an increase in the positive charge density on the cell surface. Such a process results in a competition between H^{+} and cationic ions for the same binding sites, as suggested by Hu et al. [32]. These coworkers noted that an increased H^{+} concentration resulted in suppressed uranium uptake by *P. aeruginosa* CSU biomass. The metal biosorption capacities declined substantially under lower pH condition. They concluded that the reduction in uranium-loading capacities with decreasing pH may be due to protonation of the cell wall, high solubility of uranyl ions, and cell structure damage at very acidic conditions.

17.3.3.3 Competing Cations

Most studies on biosorption using microorganisms have involved the removal of only one kind of metal ion from aqueous solutions. However, the presence of only one kind of heavy metal is a rare situation in nature or in wastewater.

To study the competitive biosorption behaviors between trivalent chromium and bivalent cobalt and nickel ions using *P. aeruginosa*, mixed metal solution was used in a batch sorption system (Figure 17.6). In a ternary system, cobalt (–93%) and nickel (–91%) uptake was strongly affected by the presence of chromium when compared with uptake in a single system. The decrease in uptake of chromium ion was negligible in the presence of bivalent ions.

At pH 4 of mixture solutions, cobalt, nickel, and chromium ions are present in the positively charged forms. In this state, they can compete with each other for negatively charged surfaces of the biomass. It is well documented that the ionic charge and ionic radii of cations affect the ion exchange as well as adsorption phenomena [42]. Because monovalent or bivalent ionic species are sorbed to a lesser extent than polyvalent ones, cobalt and nickel do not suppress the prevailing affinity of chromium to the binding sites of *P. aeruginosa*.

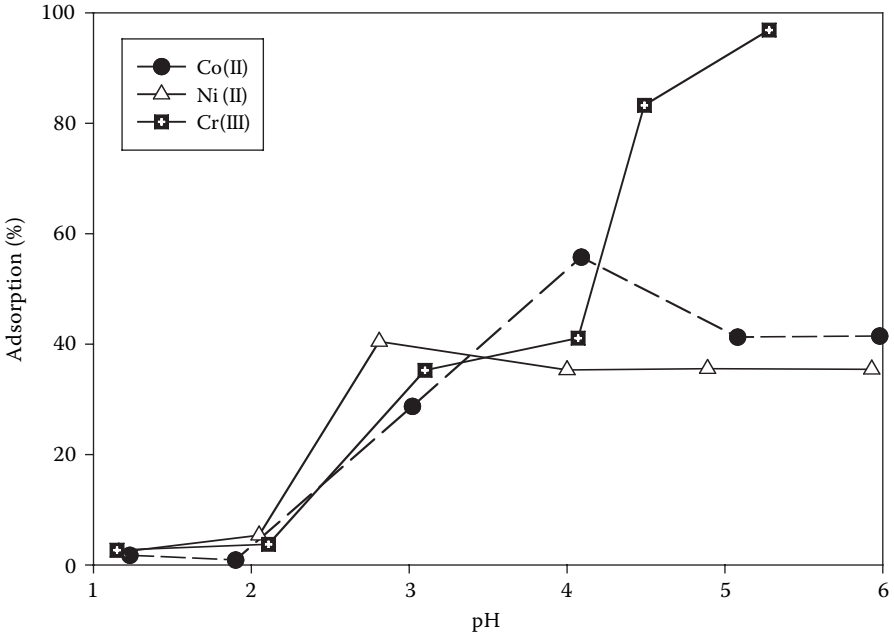

FIGURE 17.5 Effect of initial pH on biosorption of Co(II), Ni(II), and Cr(III) by *P. aeruginosa*. The effects of solution pH on heavy metal sorption were studied by adjusting the initial pH of the metal solution (50 mg/L) over the range of pH 1 to 6. Solution pH was adjusted with NaOH or HNO_3.

17.3.3.4 Ionic Strength and Organics

Ionic strength also plays an important role in the metal biosorption. Chang and Hong [31] reported that the mercury uptake by *P. aeruginosa* decreased with increasing ionic strength. Cho et al. [54] showed that no significant decrease in the binding of Cd^{2+} and Zn^{2+} occurred up to the ionic strength of 10^{-3} *M*.

In natural systems, the metal removal can be affected by the presence of other organics. The presence of organic and inorganic ligands that act as chelators is of particular concern [55]. Such ligands may compete with the microorganisms for heavy metals, and once metal–ligand complexes are formed, they may not be adsorbed by the cells. Metal uptake will be most affected if the binding constants for the ligands are greater than those for the bacteria. In studies using *Thiothrix* species strain to adsorb copper, nickel, and zinc, the bacteria did not bind copper chelated to ethylenediaminetetraacetic acid and nitrilotriacetic acid [56].

17.4 APPLICATION AND POTENTIAL BENEFITS IN METAL-CONTAMINATED ENVIRONMENTS

The main target of the biosorption process is to remove heavy metal, which can be quite toxic even at low concentrations. Biosorption is particularly suited as a polishing step in wastewater with a low to medium initial metal concentration from a few to about 100 ppm [57]. In high initial metal concentrations, biosorptive treatment of wastewaters may be economically applied by combining the pretreatment using other technologies such as precipitation, which is currently used for 90% of heavy metal removal or electrolyte recovery. As a result of the biosorptive process, it offers high effluent quality and avoids the generation of toxic sludge. Figure 17.7 shows several designs utilizing biosorption in the contaminated sites.

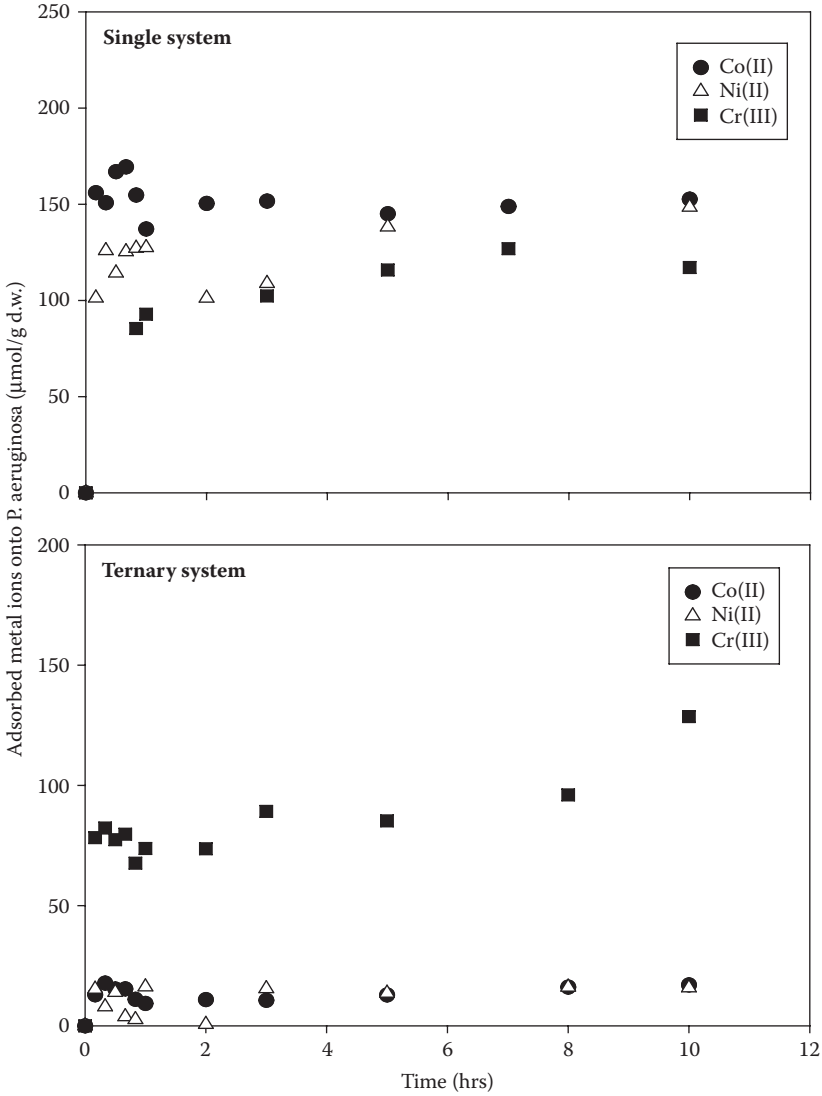

FIGURE 17.6 Adsorption equilibrium for each metal ion in synthesized wastewaters. The solution pH was adjusted to pH 4.

17.4.1 Wastewater Treatment

Recent research demonstrated that bacteria can enable recovery of valuable elements or further containment of highly toxic or radioactive species [15,20,58]. It is clear that some microbiological methods for the treatment of metal-containing wastes offer potentially efficient and cost-effective alternatives [59]. Actually, the high removal efficiency (almost 99%) of heavy metals could be obtained by using bacteria in batch system containing mixture metal solution (Figure 17.8). To increase the removal efficiency of heavy metal, the sequent bioreactor can be conducted in bacterial biosorption or adjunct to existing treatment technologies.

Contacting large volumes of metal-bearing aqueous solutions with microbial biomass in conventional unit processing operation is not typical because of the solid and liquid separation problems. Recent cell immobilization technology is therefore often studied for its potential to improve

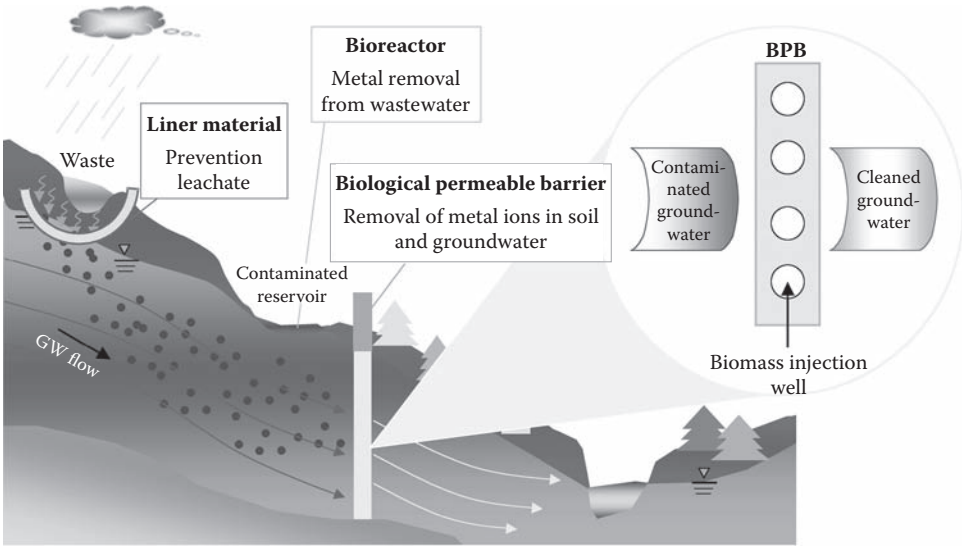

FIGURE 17.7 Bioremediation in contaminated environmental sites using biosorption.

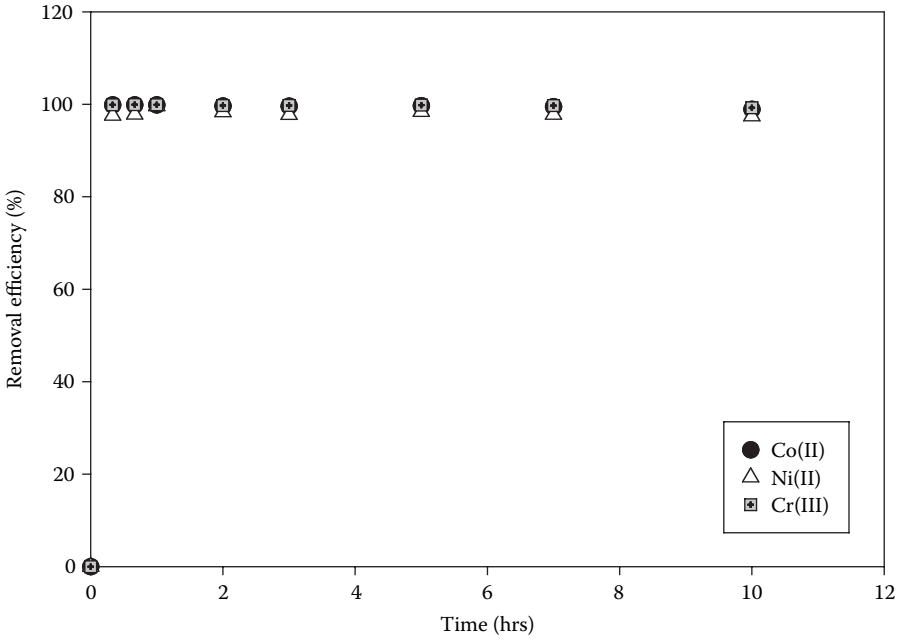

FIGURE 17.8 Removal efficiency of each metal ion in ternary system at pH 4. The initial concentrations of Co, Ni, and Cr were 1 mg/L.

fermentation productivity [21]. The bacterial immobilization can provide remarkable stability and prevent the loss of bacteria. A packed bed or fluidized bed reactor containing the immobilized biomass also can effectively remove contaminants using a much smaller reactor than biosorption processes containing free cells.

17.4.2 Groundwater Treatment

The biosorption process can be applied *in situ* without the expense of pumping out the contaminated groundwater or excavating the soil. This technique provides low-cost, easy operating, and safe treatment of contaminants in groundwater. The immobilized microbial stratum may be placed in an engineered trench across the flow path of a contaminated plume to create a BPB (biological permeable barrier). Contaminated groundwater enters the BPB, to which electron donor and nutrients may be supplied through the groundwater gradient, while the remediated groundwater exits the BPB. Selective removal has the potential and flexibility to treat a wide range of wastewater contaminants beyond nitrates. The most relevant work on true bacterial biosorption has been done by Brierley [60]. He found that the biosorption process was useful for cost-effective treatment of high-volume, low-concentration wastes such as mine runoff using artificial wetlands with undefined biota.

17.4.3 Protection from Pollutant Plumes

The migration of contaminants from a hazardous site is a concern for the protection of downstream resources. Biobarriers serve as an alternative technology for controlling the migration of contaminants from hazardous waste sites. The biobarrier can be applied in the field by injecting starved bacteria and then nutrients into a series of injection wells. The pore space is sealed by bacterial growth and EPS production and then a biobarrier is formed in soil [61]. The biobarrier has applicability as an alternative liner material in landfills to the contaminated sites. It is able to immobilize heavy metals *in situ*, thus protecting environments from the hazardous lechate.

ACKNOWLEDGMENT

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