Phycoremediation: Future Perspective of Green Technology

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1 Introduction

The unrestricted developmental activities such as rapid industrialization and urbanization carried out during the past few decades have given rise to serious problems of environmental contamination. The load of pollutants including toxic metals is ever rising in the environment as a result of enhanced industrial activities. These heavy metals enter the environment through a variety of human activities, such as mining, refining, electroplating industries, etc. (Micheletti et al. 2007). Thus, the deposition of toxic metals in the environment and their speciation between abiotic and biotic components of ecosystem is posing toxicity in the latter group as it is impossible to degrade these pollutants by any means; the only way to overcome the effects of toxic heavy metals is their physical removal from the contaminated sites (Volesky 1997). The accumulation of toxic heavy metals in the aquatic environment has become a significant problem worldwide, and, therefore, it is a matter of great concern (Khoshmanesh et al. 1996; Dönmez et al. 1999; Gupta et al. 2006). At present, heavy metals are one of the most widespread pollutants, and their continuous accumulation in water bodies, soil and water sediments constitutes a serious hazard to both the environment and human health (Wase and Forster 1997). To avoid the adverse effects of metal-contaminated wastewater, it is necessary to treat them prior to their discharge into the environment.

The conventional physicochemical techniques for the removal of inorganic pollutants from wastewater involve lime precipitation, chemical oxidation or reduction, ion exchange, electrochemical treatment, filtration, reverse osmosis, membrane technologies and evaporative recovery (Barakat 2011). However, these techniques have significant shortcomings, for instance, low efficiency at lower concentrations of individual metal pollutants, high capital investment and operational costs and production of toxic sludge (Khoshmanesh et al. 1996). Therefore, it is imperative to have new technologies for reduced contamination of environmental components, which are not only environment friendly but also cost-effective.

The removal of toxic inorganic environmental pollutants has received an ever-increasing attention in recent years, and various biomaterials such as bacteria, fungi, algae and plants have been employed as bioremedial agents to decontaminate the metal-polluted environment (Kotrba and Ruml 2000; Kiran et al. 2008). This chapter mainly emphasizes the removal of certain deadly inorganic heavy metals by using algal strains and their phycoremediation potential.

2 Pollution in the Aquatic Environment

There are several ways in which we can classify contaminants of a water body. Broadly there are two classes of pollutants: organic pollutants and inorganic pollutants (Fig. 2.1).

2.1 Organic Pollutants

These are the compounds which consist of mainly carbon and hydrogen. The toxicity of organic pollutants depends upon the functional groups present in them. There are several subgroups of organic pollutants, as follows.

2.1.1 Hydrocarbons

They can be divided into two classes: aliphatic hydrocarbons (alkanes, alkenes and alkynes) and aromatic hydrocarbons which contain carbon ring. Aromatic hydrocarbons such as poly aromatic hydrocarbons (PAHs) are much more reactive than any other class of aliphatic hydrocarbons.

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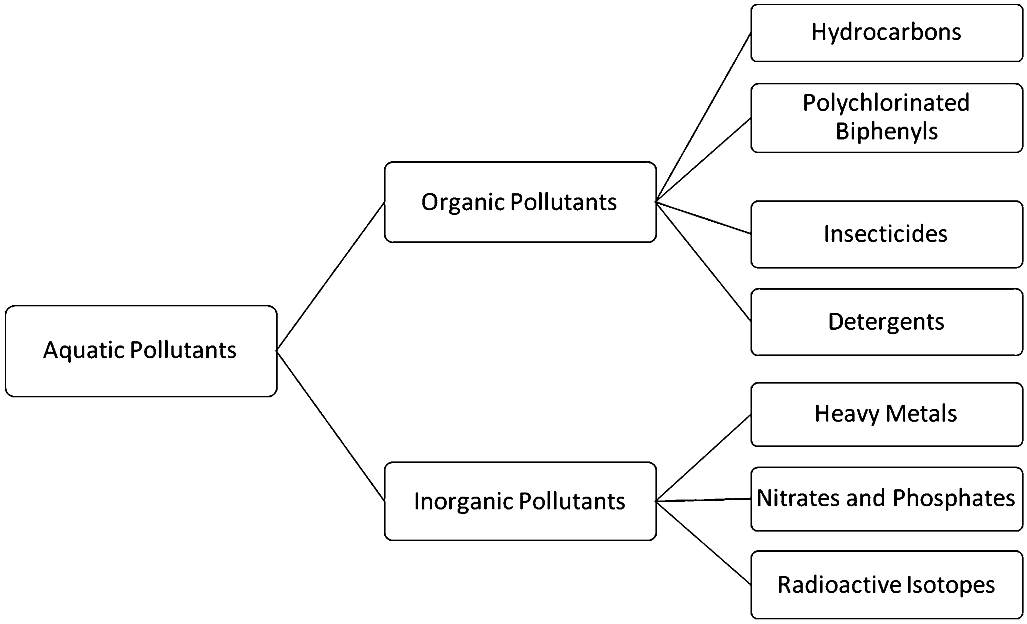

Fig. 2.1 Classification of the water pollutants

2.1.2 Polychlorinated Biphenyls (PCBs)

These are stable and unreactive fluids, relatively insoluble in water. They are mostly used as hydraulic fluids, coolants/insulation fluids and plasticizers in paints.

2.1.3 Insecticides

Some of the insecticides are found to be highly dangerous for living tissues as they accumulate in fat tissues and enter in the food chain. Examples are DDT, lindane, carbamate, azadirachtin, etc.

2.1.4 Detergents

These are classified as phosphate detergents and surfactants. Phosphate detergents are used to soften the hard water and they have caustic property. Surfactants are used to enhance the foaming and emulsifying properties of detergents and are very toxic.

2.2 Inorganic Pollutants

Mostly inorganic pollutants include highly toxic metals (lead, cadmium, zinc, mercury, etc.), while some of these are non-metallic inorganic substances such as nitrates and phosphates but still dangerous for the quality deterioration of aquatic system and partly toxic for the living system. An excessive input of nitrates and phosphates in aquatic bodies received through inorganic fertilizers is the major cause of algal blooms in surface waters, leading to eutrophication of water bodies.

2.2.1 Heavy Metals

The heavy metals, commonly defined as metals having a specific density of more than 5 g/cm^{-3} (Hawkes 1997), include Fe, Mn, Cu, Mo, Zn and Co, which are required in traces as nutrients by the living organisms, but they become toxic at higher concentrations. The other group of metals like Cd, Hg and Pb exert their potential toxic effects even at extremely lower concentrations. These metals are found in surface water bodies in their stable ionic forms. They mostly interfere with electron transfer reactions, and their interaction with oxygen often leads to the formation of toxic oxyradicals. Metalloids can bind with organic compounds, leading to the formation of lipophilic substances which are highly toxic, and can be stored in the fat tissues of animals including humans. Since the heavy metals cannot be broken down into less harmful components as they are non-biodegradable, they can only be remediated to reduce their toxic effects.

2.2.2 Radioactive Isotopes

Radioactive isotopes are either present in nature or created by the humans in the nuclear industry. The decay of radioactive isotopes and their half-life determines the potential danger of these elements to humans. Different kinds of radiations can cause damage to the living tissues, depending upon the type of radiation and its energy level.

3 Bioremediaton

Bioremediation is a biological process used to clean up the hazardous chemicals present in the environment (Gianfreda and Rao 2004). It has several obvious advantages over physicochemical remediation methods in terms of cost-effectiveness, convenience, complete removal of organic pollutants and lack of collateral destruction of the site materials or its impact on indigenous flora and fauna (Timmis and Pieper 1999). With the advances in the field of biotechnology, bioremediation has become one of the major developing fields applied for environmental restoration. The bioremediation technique involves the use of microorganisms to reduce the concentration and toxicity of various chemical pollutants such as heavy metals, dyes, pesticides, etc. A considerable effort is devoted for developing a low-cost environmental friendly bioremediation technology that can effectively immobilize the dissolved toxic metals; a variety of living biomass has been tested (Say et al. 2001; Adhiya et al. 2002; Sheng et al. 2004) for the removal and/or recovery of metals for their probable reuse potential. Because of high metal-adsorbing capacity, low cost and widespread abundance, the algal biomass has attracted the attention of scientists all over the world (Davis et al. 2003). Various types of either living or dead microalgal biomass have been employed to absorb the dissolved toxic metals.

3.1 Phycoremediation

Phycoremediation is a part of bioremediation where macroalgae or microalgae are being used for the removal or biotransformation of pollutants, including nutrients, xenobiotics and CO_2. It simply offers cleanup technology, which is cost-effective, nonintrusive and safe.

3.1.1 Algae

Algae represent a large group of aquatic, most primitive photoautotrophic organisms that include around 30,000 species, ranging from unicellular (microalgae) to more complex multicellular organisms (macroalgae). Cyanobacteria (blue-green algae) were also included under the microalgae by some authors (Priyadarshani et al. 2011). Algae possess chlorophyll and are able to transform light energy into chemical energy in a similar way to higher plants but lack true roots, stems and leaves. They grow comparatively faster, which results in fixation of CO_2 being 10–50 times faster than in plants (Subashchandrabose et al. 2013). When compared to plants, microalgae have a simple cell structure, and they are also often surrounded by fluid allowing easier uptake of water and nutrients (Chacoón-Lee and González-Mariño 2010). Algae are taxonomically divided based on their pigments, storage compounds and the main compounds present in their cell wall. The major classes are Chlorophyta (green algae), Rhodophyta (red algae), Phaeophyta (brown algae), Euglenophyta, Pyrrophyta, Chrysophyta and Cyanophyta (blue-green algae).

Advantages of Using Algae

- The blue-green alga (cyanobacteria) uses light energy source and CO_2 for its growth and survival. This way it helps in carbon sequestration and mitigation of global warming.
- They are economically more viable and an eco-friendly tool.
- They are capable of not only photosynthesis but also fix up atmospheric nitrogen, and they can survive better under the nutrient-limited conditions.
- Microalgae cultures can be cultivated in open ponds or in large-scale water reservoirs. At the same time, the algal growth under laboratory conditions provides reliable and consistent supply of biomass.
- They have regenerative and metal recovery potentiality.
- They generate lesser volume of chemical and/or biological sludge to be disposed off.
- They have high efficiency in dilute effluents and have large surface area to volume ratio.
- They have the potential to treat sites polluted with more than one type of pollutant.

4 Removal of Heavy Metals by Algae

Microalgae are sensitive indicators of environmental changes, and their ubiquitous presence serves as the basis of most freshwater and marine ecosystems, widely being used in the assessment of risk and development of environmental regulations for metals (Levy et al. 2007). Algae are known to accumulate heavy metals and bind with metal ions in uncomplicated aquatic environment in a short period of time by biosorption without any problem of toxicity (Afkar et al. 2010). Algae have many features that make them an ideal tool for the selective removal and concentration of heavy metals, which include high tolerance to heavy metals, ability to grow both autotrophically and heterotrophically, large surface area/volume ratios, phototaxy, phytochelatin expression and potential for genetic manipulation (Cai et al. 1995). An important biochemical function of algae is their involvement in the shaping of proper ecological relationships and interaction between organisms in the aquatic environment (Wilde and Benemann 1993; Sandau et al. 1996; Bajguz 2000) by way of accumulating high concentration of heavy metals depending on their concentration in the external environment. The threshold level of heavy metals varies greatly for different algal species, but it increases as the metal concentration in the water decreases (Kelly 1988; Sharma and Azeez 1988). However, little attention has been paid to metal removal and detoxification by algae in the natural environment.

The studies on biosorption of metals by marine algae revealed an interesting adsorption potential of some algal species such as *Ascophyllum nodosum*, *Sargassum baccularia* (Volesky 1994; Chong and Volesky 1995; Holan et al. 1998), *Scenedesmus abundans* (Terry and Stone 2002), *Ecklonia radiata* (Matheickal and Yu 1996) and *Sargassum fluitans* (Fourest and Volesky 1996). Marine alga *Dunaliella tertiolecta* has been shown to have high phytochelatin (PC) content attributed to its capability to hyperaccumulate Zn and Cd (Tsuji et al. 2002, 2003). Similarly, a periphytic green alga *Stigeoclonium tenue* is also known to produce high amounts of PC-related peptides when adapted to high Zn concentrations (Pawlik-Skowronska 2003). Ettajani et al. (2001) reported the hyperaccumulation of Cd in microalgae *Skeletonema costatum* and *Tetraselmis suecica*. The use of both marine and freshwater algae for adsorption and elution of gold, silver and cobalt has been reported (Fujita et al. 1992; Hamdy 2000).

The growth of large amounts of algae due to eutrophication of water body is commonly seen in most of the water bodies. Such eutrophic algae may help to eliminate the toxicity of heavy metals and exert a major influence on the behaviour and fate of trace metals entering freshwaters. Metal accumulation capacity of algal biomass is either comparable or sometimes higher than chemical sorbents (Mehta and Gaur 2005). It has also been reported that metal uptake capacities of certain algae are much higher than the activated carbon, natural zeolite and synthetic ion-exchange resin (Volesky 1992). It provides a cost-effective solution for industrial wastewater management. Algae have been used for pharmaceutical reasons for detoxification of heavy metals in the human body due to a very efficient adsorption of the toxic ions (David and Volesky 1998). Moreover, algae possess high metal-binding capacities, because polysaccharides, proteins, or lipids on the surface of their cell walls contain some functional groups such as amino, hydroxyl, carboxyl and sulphate, which can act as binding sites for metals (Holan and Volesky 1994) (Table 2.1).

4.1 Factors Affecting the Removal of Heavy Metals

Biosorption of metals by algae may be affected by several factors, including concentration of metals and biomass, pH, temperature, the presence of competing ions, etc.

4.1.1 Effect of pH

Most of the studies have shown that sorption of metal ions is a function of pH of the solution. Earlier studies have indicated that ambient pH condition is an important parameter affecting the biosorption of heavy metal ions (Matheickal et al. 1991; Fourest et al. 1994; Matheickal and Yu 1996). pH affects the chemistry of the metal, the activity of the metal-binding functional groups in the biomass and the competition of metallic ions (Selatina et al. 2004). pH strongly influences the speciation and biosorption ability of the metal ions (Esposito et al. 2001). Since a majority of metal-binding groups of algal cell are acidic (e.g. carboxyl), their availability is pH dependent. These groups generate negatively charged surface groups at acidic pH, and electrostatic interactions

Table 2.1 Some work on algae as heavy metal removing agent

Algae	Metal removed	Description of metal-rich surrounding	References
Anacystis nidulans	Cu	Metal solution	Singh and Yadava (1986)
Tolypothrix tenuis	Cd	Aqueous solution	Inthorn et al. (1996)
Chlorella vulgaris, *Scenedesmus obliquus* and *Synechocystis* sp.	Cu, Ni, Cr (VI)	Aqueous solution	Dönmez et al. (1999)
Nostoc muscorum	Cu and Cd	Multimetal solution	Prasad and Pandey (2000)
Nostoc linckia and *N. rivularis*	Zn and Cd	Sewage water	El-Enany and Issa (2000)
Chlorella vulgaris	Cu and Ni	Single and binary metal solution	Mallick (2003)
Spirulina sp.	Trace element	Copper smelter and refinery effluent	Chojnacka et al. (2005)
Aulosira fertilissima	Cr and Ni	Free-cell condition	Banerjee et al. (2004)
Nostoc muscorum and *Anabaena subcylindrica*	Cu, Pb, Co and Mn	Sewage and industrial wastewater	El-Sheekh et al. (2005)
Cladophora fascicularis	Cu and Pb	Aqueous solution	Liping et al. (2006)
Spirulina platensis (*Spi*SORB)	Cu	Column reactor system	Vannela and Verma (2006)
Nostoc calcicola and *Chroococcus* sp.	Cr	Metal-contaminated soil	Anjana et al. (2007)
Lyngbya and *Gloeocapsa*	Cr	Contaminated sites	Kiran et al. (2008)
Gloeocapsa sp.	Pb	Residue of cyanobacterial EPS	Raungsomboon et al. (2008)
Spirulina platensis and *Aphanothece flocculosa*	Hg	Wet biomass	Cain et al. (2008)
Gloeothece sp. strain PCC 6909	Cu	Wastewater	Micheletti et al. (2008)
Hapalosiphon welwitschii Nägel	Cd	Metal solution	Guzman and Cao (2010)
Oscillatoria sp. NTMS01 and *Phormidium* sp. NTMS02	Cr (VI)	Aqueous solution	Kamaraj et al. (2011)
Sargassum wightii and *Caulerpa racemosa*	Cr (III and VI), Pb, Cd	Aqueous solution	Tamilselvan et al. 2012
Nostoc muscorum	Pb and Cd	Metal solution	Dixit and Singh (2014)

between cationic species and the cell surface are responsible for metal biosorption.

There are numerous studies showing an increase in metal sorption with increasing pH of the solution (Gupta et al. 2006; Solisio et al. 2008; Liping et al. 2008). A decreased metal sorption by algae has been frequently observed at extremely acidic pH (<2) (Özer et al. 1994; Mehta and Gaur 2001a). The marine algae *Sargassum* sp., *Padina* sp., *Ulva* sp. and *Gracilaria* sp. were investigated for their biosorption performance in the removal of lead, copper, cadmium, zinc and nickel from dilute aqueous solutions. Maximum biosorption was found to be at pH 5.0 for lead and copper and at pH 5.5 for cadmium, zinc and nickel (Sheng et al. 2004).

There is also a great variability in optimum pH for sorption of a particular metal ion by different algal species. For example, the optimum pH for sorption of Cu by cyanobacteria (*Microcystis aeruginosa* and *Spirulina platensis*) is far greater than that for Cu sorption by green algae (*Cladophora prolifera*, *Chlorella vulgaris*, *C. kessleri*). This variability may be related to the differences in chemical composition of cell surface of various algal species. Chojnacka et al. (2005) found a distinct relationship between pH of aqueous metal solution and involvement of functional group in binding of Pb onto *Spirulina maxima*; for pH ranging between 2–5, 5–9 and 9–12, respectively, the functional groups involved in the binding of Pb were carboxyl, carboxyl and phosphate and carboxyl, phosphate and hydroxyl.

4.1.2 Effect of Contact Time

The contact time is of great importance in adsorption for the assessment of the suitability of microbes to serve as biosorbents in a continuous flow system. The biosorption consists of two phases: a primary rapid phase that accounts for the major part in the total metal biosorption and a second slow phase that contributes to a relatively small part. Metal uptake increases with an increase in contact time but remains constant after equilibrium time period (Murugesan et al. 2006). Equilibrium time varied with metals due to the difference in initial metal concentration and affinity of the biosorbent for a particular metal ion.

The metal removal rates were rapid, with 90 % of the total adsorption taking place within 60 min (Sheng et al. 2004). Some authors observed that at the initial stage (0–12 min), the adsorption rate of Pb was so rapid that 74 % of the metal was biologically adsorbed by *Spirulina* (Chen and Pan 2005). Heavy metal ion (Cr^{3+}, Cu^{2+} and Cd^{2+}) removal from solutions by *Spirulina* species showed that the equilibrium reached after 10 min (Chojnacka et al. 2005). An increase in the biosorption of Cu by *Spirogyra* species was observed with increase in contact time from 0 to 120 min and after that becomes almost constant up to 180 min (Gupta et al. 2006).

4.1.3 Effect of Temperature

Owing to the dependence on metabolism, metal uptake by live cells is considerably affected by variation in temperature. There are reports showing altered metal uptake by live organisms with change in temperature regime (Skowron'ski 1986; Mehta and Gaur 2001a; Mehta et al. 2002a), with maximum uptake occurring at specific temperature optima. Higher temperature usually enhances sorption due to the increased surface activity and kinetic energy of the solute (Sag and Kutsal 2000; Vijayaraghavan and Yun 2007). An increase in metal sorption with increasing temperature (Tsezos and Volesky 1981; Kuyucak and Volesky 1989; Aksu and Kutsal 1991; Aksu 2002) suggests that metal biosorption by algae is an endothermic process. On the contrary, some studies indicate exothermic nature of metal sorption by algae (Cruz et al. 2004; Aksu 2001; Benquell and Benaissa 2002). Due to the exothermic nature of some adsorption processes, an increase in temperature has been found to reduce the biosorption capacity of the biomass (Mameri et al. 1999; Suhasini et al. 1999). A temperature change affects the number of factors which are important in heavy metal biosorption. Some of the factors include (i) the stability of the metal ion species initially placed in solution, (ii) microorganism-metal complex depending on the biosorption sites, (iii) the effect of temperature on the cell wall of microorganisms and (iv) the ionization of the chemical moieties on the cell wall (Sag and Kutsal 2000). Increased biosorption of heavy metals with increasing temperature has been ascribed to bond rupture, which perhaps enhances the number of active sites involved in metal sorption or higher affinity of sites for metals. There are also some reports which show no effect of temperature on metal sorption (Norris and Kelly 1979; Zhao et al. 1994). It is always desirable to conduct biosorption at room temperature, as this condition is easy to replicate.

4.1.4 Effect of Biomass Concentration

The dosage of a biosorbent strongly influences the extent of biosorption, and also the amount of metal ion recovered from a solution is affected by biomass concentration. Biomass concentration in solution seems to influence the specific uptake: for lower value of biomass concentrations, there is an increase in the specific uptake (Gadd et al. 1988; Fourest and Roux 1992). An increase in biomass concentration leads to interference between the binding sites (Gadd et al. 1988). Hence, this factor needs to be taken into consideration in any application of microbial biomass as biosorbent. Conversely the quantity of biosorbed solute per unit weight of biosorbent decreases with increasing biosorbent dosage, which may be due to the complex interaction of several factors.

An increase in the biomass concentration generally increases the amount of solute biosorbed due to the increase in the surface area of biosorbent, which in turn increases the availability of metal-binding sites (Esposito et al. 2001; Mehta and Gaur 2001c). However, there is no straightforward relationship between biomass concentration and metal removal as some workers have noticed a decrease in sorption of heavy metals by different algae with increasing biomass concentration (Hamdy 2000; Nuhoglu et al. 2002; Gong et al. 2005). This may be due to the limited availability of metal, increased electrostatic interactions, interference between binding sites and poor mixing at higher biomass concentrations (Meikle et al. 1990; Fourest et al. 1994).

4.1.5 Effect of Initial Metal Ion Concentration

Sorption and removal of heavy metals largely depend on the initial metal ion concentration in the solution. Several workers have reported that metal sorption initially increases with an increase in the metal ion concentration in solution and then becomes saturated after a certain concentration of metal (Da Costa and Leite 1991; Aloysius et al. 1999; Mehta and Gaur 2001a, b, c; Mehta et al. 2002a, b). This is because at lower initial metal concentrations, the ratio of the initial moles of metal to the available surface area is low; subsequently, the fractional sorption becomes independent of the initial concentration. However, at higher concentrations, the sites available for sorption become fewer compared to the moles of metal present, and hence, the binding of the metal is strongly dependent upon the initial metal concentration. It is always necessary to identify the maximum saturation potential of a biosorbent, for which experiments should be conducted at the highest possible initial metal concentration. Algal cell surface has several kinds of functional groups with varying affinity for an ionic species. Low- and high-affinity functional groups are involved in sorption of metal ions at high and low concentration of metal ions, respectively.

4.1.6 Effect of the Presence of Anions and Cations

Actual industrial wastewaters contain different kinds of impurities, which may significantly affect metal biosorption (Ho and McKay 2000). Some studies indicated that cations and anions in addition to the ion of interest have a generally detrimental impact on metal accumulation (Suh and Kim 2000). Among such impurities, cations such as Na^+, K^+, Mg^+ and Ca^+ and anions like sodium salts of chloride, nitrate, acetate and EDTA exist in most of the industrial effluents, and they greatly interfere with the metal sorption potential of biosorbents (Chen and Yiacoumi 1997; Lee and Volesky 1997; Low et al. 2000). Earlier low level of accumulation of Co and Cu by algae in the presence of carbonate, orthophosphate, sulphate, nitrate, EDTA and chloride ions has been observed (Rai et al. 1981). However, the accumulation of ionic species like nitrate and ammonium also increased in the presence of Cu and Fe in *Anabaena doliolum* (Rai and Mallick 1992). The presence of other cations including metal ions significantly affects metal sorption by algae (Mehta and Gaur 2001a, b; Mehta et al. 2002a, b). Reduced heavy metal uptake in the presence of light metals is attributed to competition for cellular-binding sites or precipitation or complexation by carbonates, bicarbonates, or hydroxides of Ca and Mg (Rai et al. 1981). High concentrations of salts like NaCl in solution can also decrease the rate of metal sorption by algae (Cho et al. 1994). The inhibitory effect of Na is more pronounced with weakly bound metals such as Zn or Ni. It is important to note that Na^+ and K^+, being monovalent cations, do not compete directly with covalent binding of heavy metals by the biosorbent. Other compounds that could be considered as impurities in metal removal process are surfactants and some chelating agents. The nature of impurities differs depending on the type of effluent to be treated. Whereas most of the studies reported the inhibitory effect of light metal ions on sorption of heavy metals by biosorbent, a few of them showed no effect (Pawlik and Skowronski 1994; Adhiya et al. 2002; Axtell et al. 2003; Jalali-Rad et al. 2004).

4.2 Mechanism of Heavy Metal Removal

The mechanism of metal biosorption is a complicated process. The status of biomass (living or nonliving), types of biomaterials, properties of metal solution chemistry and environmental conditions such as pH influence the mechanism of metal biosorption. The uptake of heavy metal ions by algae was found to occur in two principal ways: passive uptake due to surface adsorption (metabolism independent) followed by cellular uptake (metabolism dependent) via intracellular transport and chelation (absorption) (Khummongkol et al. 1982; Cho et al. 1994; Yee et al. 2004). Some metals such as Pb and Sr may be passively adsorbed by charged polysaccharides in cell wall and intracellular matrix (El-Sheekh et al. 2003; Osman et al. 2004; Fathi et al. 2000; 2005); other metals (e.g. Zn, Cd) are taken up actively against large intracellular concentration gradients. As passive biosorption mainly depends on the binding to functional surface ligands, the cell wall structure is the most important for rapid metal ion uptake.

The probable sites of an algal cell for the binding of metal ions are shown in Fig. 2.2. Adsorption occurs directly onto the cell wall in some algae, but the presence of various amounts of mucilage or extracellular polymeric substances (EPS) (Leppard 1995; Lee 1997) in others (e.g. Cyanophyta) may play a key role in metal binding (Weckesser et al. 1988). The algal cell wall contains many functional groups, such as hydroxyl (–OH), phosphoryl, amino ($-NH_2$), carboxyl (–COOH), sulphydryl (–SH), etc., which confer a negative charge on the cell surface. Since metal ions in water are generally in the cationic forms, they are adsorbed onto the cell surface. The functional group involved in the metal sorption

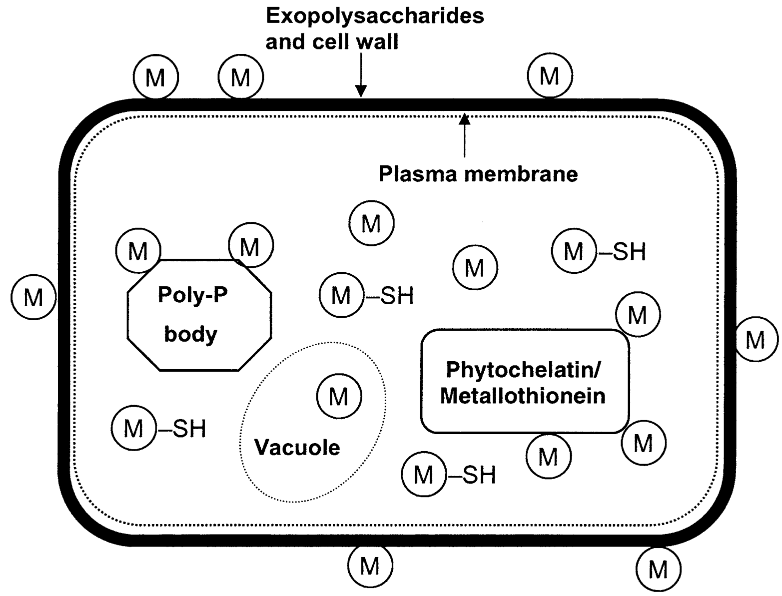

Fig. 2.2 Metal-binding sites of a typical algal cell. The alphabet M represents the metal species (independent of its oxidation state) (Source: Mehta and Gaur 2005)

by algae have been identified by FTIR spectroscopy, pH titration, potentiometric and conductimetric titration techniques and also after blocking of functional groups by certain chemicals. Each functional group has a specific dissociation constant (pKa), and it dissociates into corresponding anion and proton at a specific pH (Niu and Volesky 2000). The cell wall functional groups are found linked with various cell wall components, e.g. peptidoglycan, teichoic acid, polysaccharides and proteins. Among different cell wall constituents, polysaccharides and proteins have most of the metal-binding sites (Kuyucak and Volesky 1989). Since the distribution and abundance of the cell wall components vary among different algal groups, the number and kind of functional group also vary among them.

Hamdy (2000) reported that metal uptake is dependent on the type of biosorbent, with different accumulation affinities towards the tested elements, and the amount of metal uptake increased steeply by increasing the weight of the biomass. Fathi et al. (2005) reported that the uptake of an element from the surrounding medium is seldom exactly proportional to the amount present in the water. Verma and Singh (1990) reported Cu uptake in a diazotrophic cyanobacterium *Nostoc calcicola* to be biphasic. During the first 10 min, there was a rapid binding of cations to the cell wall, followed by subsequent metabolism-dependent intracellular uptake for at least 1 h.

5 Potential Applications of Algae in Biotechnology

Algae are one of the potential organisms, which are useful to mankind in several ways. Algal cells constitute a vast potential resource in various applications as follows.

5.1 Food and Feed

Blue-green algal protein has received worldwide attention either as a supplement or as an alternative source of food. Some strains of *Spirulina* and *Nostoc* are consumed as human food in Chile, Mexico, Peru and the Philippines. *Spirulina* is used as a food supplement because of its excellent nutrient composition (60–70 % protein, 20 % carbohydrate, 5 % lipids, 7 % minerals and 6 % moisture) and digestibility. It is also a rich source of β-carotene, thiamine and riboflavin and is one of the richest sources of vitamin B12. *Nostoc commune* has high amount of fibre and moderate protein; therefore, this species is used as a new dietary fibre source in human diet (Jeraci and Vansoest 1986). Halotolerant marine algal species *Dunaliella* is also a rich source of β-carotenoid (Gudin and Chaumont 1991). Green alga *Chlorella vulgaris* is used as a food supplement in many countries including China, Japan, Europe and the USA (Yamaguchi 1997).

5.2 Fine Chemicals

A variety of fine chemicals such as pigments, vitamins and enzymes with various applications can be obtained commercially from different algal strains. Some marine algae are a potential source for commercial production of vitamins such as vitamin B complex and vitamin E (Borowitzka 1988). *Haematococcus pluvialis* accumulate the highest level of astaxanthin which is a potent radical scavenger and a singlet oxygen quencher that surpasses the antioxidant benefits of β-carotene, vitamin C and vitamin E (Lorenz and Cysewski 2000). A number of cyanobacteria are also rich in vitamins and many excrete them into the surrounding environment (Borowitzka 1988). The products obtained from cyanobacteria like carotenoids and phycobiliproteins are used as natural food colourants and also as food additives and have high commercial value (Emodi 1978). *Dunaliella salina*, a halotolerant microalga, is able to accumulate very large amount of β-carotene and also a valuable chemical, glycerol (Avron 1992; Oren 2005). Feed grade *Phormidium valderianum* is an excellent source of phycocyanin, a blue natural colourant useful as a phycofluor in diagnostics. Cyanobacteria being photoautotrophs have the ability to photosynthetically transform simple, labelled compounds such as $14CO_2$, $13CO_2$, $33H_2O$ and $15NO_3$ into complex organic compounds. In addition, cyanobacteria are a rich source of polysaccharides, lipids, amino acids, fatty acids, halogenated compounds, etc., which are used as flocculants, surfactants and others (Patterson 1996).

Enzymes that can be exploited commercially such as chitinase, L-asparaginase, L-glutaminase, amylase, protease, lipase, cellulase, urease and superoxide dismutase have been reported from several algal strains (Prabhakaran et al. 1994; Wikstrom et al. 1997). Several common and unique sequence-specific endonucleases are known from *Anabaena cylindrica* (Acy I), *A. flos-aquae* (Afl I and Afl III), *A. variabilis* (Ava I and Ava II), *A. variabilis UW* (Avr II), *Microcoleus* sp. *UFEX* 2220 (Mst II) and *Nostoc* sp. *PCC* 7524 (Nsp C I), which can be marketed at low cost since the relative biomass production of cyanobacteria is much less expensive than bacteria (Elhai and Wolk 1988). Marine microalgae *Isochrysis galbana* and *Diacronema vlkianum* produce long-chain fatty acids, mainly eicosapentaenoic acid (EPA, 20:53) and docosahexaenoic acid (DHA, 22:6ω3) (Liu and Lin 2001).

5.3 Pharmaceuticals

Algae are one of the richest sources of known and novel bioactive compounds with wide pharmaceutical applications (Raghavan et al. 2002). The reported biological activities comprise cytotoxic, antitumor, antibiotic, antimicrobial, antiviral (e.g. anti-HIV) activities as well as biomodulatory effects like immunosuppressive and anti-inflammatory properties (Burja et al. 2001). *Chlorella vulgaris* has been used as an alternative medicine in the Far East. It is considered an important curing agent for many kinds of health disorders such as gastric ulcers, wounds, constipation, anaemia, hypertension, diabetes, infant malnutrition and neurosis (Yamaguchi 1997). A preventive action of *Chlorella* against atherosclerosis and hypercholesterolemia is attributed to glycolipids and phospholipids and antitumor actions assigned to glycoproteins, peptides and nucleotides (Yamaguchi 1997). However, the most important substance in *Chlorella*, i.e. beta-1,3-glucan, is an active immunostimulator, a free radical scavenger and a reducer of blood lipids (Spolaore et al. 2006). The anti-HIV activity of marine cyanobacterial compounds from *Lyngbya lagerheimii* and *Phormidium tenue* was also reported (Gustafson et al. 1989). Halophilic marine algae *Dunaliella* has anticancerous, anti-atherosclerosis, anti-inflammatory, anti-allergic, antidiabetic, antibacterial and antiviral properties (Hennekens et al. 1996; Fujitani et al. 2001; Ayelet et al. 2008; Francisco et al. 2009; Nakazawa et al. 2009). Medically important gamma-linolenic acid (GLA) is relatively rich in cyanobacteria *Spirulina platensis* which is easily converted into arachidonic acid in the human body and then into prostaglandin E2, which has lowering action on blood pressure and contracting function of the smooth muscle, thus playing an important role in lipid metabolism (Thajuddin and Subramanian 2005).

5.4 Biofertilizer

Several cyanobacterial strains colonize paddy fields where heterocystous species are capable to fix atmospheric nitrogen (Mishra and Pabbi 2004). However, a variety of non-heterocystous cyanobacteria are also able to fix atmospheric nitrogen under microaerophilic conditions. The role of N2-fixing cyanobacteria in maintenance of the fertility of rice fields has been well substantiated and documented all over the world (Saadatnia and Riahi 2009). In India alone, the beneficial effects of cyanobacteria on yield of many rice varieties have been demonstrated in a number of field locations (Venkataraman 1981). Beneficial effects of cyanobacterial inoculation have also been reported on a number of other crops such as barley, oats, tomato, radish, cotton, sugarcane, maize, chilli and lettuce (Kaushik and Venkataraman 1979; Thajuddin and Subramanian 2005).

Red marine algae *Laurencia obtusa*, *Corallina elongata* and *Jania rubens* were also used as biofertilizers by some workers to enhance the growth of maize (*Zea mays* L.) plants, and it was reported that the mixture of these algae is more suitable for the growth of maize in the field (Safinaz and Ragaa 2013). Lozano et al. (1999) stated that the application of an extract from algae to soil or foliage increased ash, protein and carbohydrate content of potatoes.

5.5 Wastewater Treatment

Algal species have been used for many decades in wastewater treatment because of its high capacity to uptake inorganic nutrients (Talbot and De la Noue 1993; Bajguz 2000; Ettajani et al. 2001; Tsuji et al. 2002, 2003; Afkar et al. 2010). The importance of microalgae in wastewater treatment has increased in recent years due to the biotechnological potential for producing valuable substances for biofuel production and animal feed (Pulz and Gross 2004; Spolaore et al. 2006). Marine cyanobacteria *Oscillatoria* sp. BDU 10742 and

Table 2.2 Important microalgal species, their products and applications

Species	Product	Application areas	References
Spirulina sp.	Phycocyanin, biomass	Health food, cosmetics, wastewater treatment	Lee (2001) and Costa et al. (2003)
Chlorella vulgaris	Biomass	Health food, food supplement, feed surrogates, wastewater treatment	Lee (2001)
Dunaliella salina	Carotenoids, β-carotene	Health food, food supplement, feed	Jin and Melis (2003) and Del Campo et al. (2007)
Haematococcus pluvialis	Carotenoids, astaxanthin	Health food, feed additives, pharmaceuticals	Del Campo et al. (2007)
Odontella aurita	Fatty acids	Pharmaceuticals, cosmetics, baby food	Pulz and Gross (2004)
Porphyridium cruentum	Polysaccharides	Pharmaceuticals, cosmetics, nutrition	Fuentes et al. (1999)
Isochrysis galbana	Fatty acids	Animal nutrition	Molina Grima et al. (2003) and Pulz and Gross (2004)
Phaeodactylum tricornutum	Lipids, fatty acids	Nutrition, fuel production	Yongmanitchai and Ward (1991)
Lyngbya majuscula	Immune modulators	Pharmaceuticals, nutrition	Singh et al. (2005)
Muriellopsis sp.	Carotenoids, lutein	Health food, food supplement, feed	Blanco et al. (2007) and Del Campo et al. (2007)

Aphanocapsa sp. BDU 16 were able to treat a factory effluent rich in calcium and chloride and enabled 100 % survival of *Tilapia* fish with only cyanobacteria as the feed source (Uma and Subramanian 1990). Shashirekha et al. (1997) found that *Phormidium valderianum* BDU 30501 was able to treat phenol-containing effluents. Studies at the National Facility for Marine Cyanobacteria (NFMC) have identified suitable cyanobacteria for treating a number of noxious effluents containing organophosphorus pesticides, detergents, antibiotics and other molecules (Subramaninan and Uma 1996) and also for the degradation of solid wastes like coir pith by their lignolytic action (Malliga et al. 1996). There are several reports for the treatment of heavy metal-contaminated wastewater by using marine and freshwater algae and also sorption/desorption of heavy metal for the recovery of this valuable resource (Hamdy 2000; Ettajani et al. 2001; Terry and Stone 2002; Dixit and Singh 2013, 2014) (Table 2.2).

6 Conclusion

During the last few decades, the researchers have started viewing the algal cells as photobioreactor which can be exploited by many ways in different spheres of biotechnology at minimal cost. The algal cells require only water, minimum quantity of nutrients, sunlight and CO_2 for their growth and survival. Phycoremediation using these algal cells is finding favour for the treatment of variety of wastewaters due to their minimal need for space and easy-to-grow characteristics. The resulting biomass after bioremediation (phycoremediation) has the potential to be used as animal feed, production of biofuel and other industrially relevant bioproducts. Thus, phycoremediation technology of wastewater treatment is considered as a low-cost technology which promises a more sustainable and environment-friendly way of life.

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