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Bioremediation of Heavy Metal by Algae: Current and Future Perspective

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Abstract: Instead of using mainly bacteria, it is also possible to use mainly algae to clean wastewater because many of the pollutant sources in wastewater are also food sources for algae. Nitrates and phosphates are common components of plant fertilizers for plants. Like plants, algae need large quantities of nitrates and phosphates to support their fast cell cycles. Certain heavy metals are also important for the normal functioning of algae. These include iron (for photosynthesis), and chromium (for metabolism). Because marine environments are normally scarce in these metals, some marine algae especially have developed efficient mechanisms to gather these heavy metals from the environment and take them up. These natural processes can also be used to remove certain heavy metals from the environment. The use of algae has several advantages over normal bacteria-based bioremediation processes. One major advantage of the removal of pollutants is that this is a process that under light conditions does not need oxygen. Instead, as pollutants are taken up and digested, oxygen is added while carbon dioxide is removed. Hence, phytoremediation could potentially be coupled with carbon sequestration. Additionally, because phytoremediation does not rely on fouling processes, odors are much less a problem. Microalgae, in particular, have been recognized as suitable vectors for detoxification and have emerged as a potential low-cost alternative to physicochemical treatments. Uptake of metals by living microalgae occurs in two steps: one takes place rapidly and is essentially independent of cell metabolism – "adsorption" onto the cell surface. The other one is lengthy and relies on cell metabolism – "absorption" or "intracellular uptake." Nonviable cells have also been successfully used in metal removal from contaminated sites. Some of the technologies in heavy metal removals, such as High Rate Algal Ponds and Algal Turf Scrubber, have been justified for some practical application in China and abroad and limitations of these methods in large-scale still exist. As an innovative clean-up technology, it mainly depends on the biosorption and bioaccumulation abilities of algae, and the former is dominated in the whole process of bioremediation. Studies suggest that the constituents of algae cell wall such as alginate and fucoidan which have key functional groups are chiefly responsible for biosorption of heavy metal ions.

Keywords: Heavy metals, Metal pollutants, Microalgae, Wastewater.

1. Mechanism of Bioremediation

Thus bioremediation can be defined as the process of using specific microorganisms to transform hazardous contaminants in soil/water to nonhazardous waste products. However, some definitions that give a broader outlook define bioremediation as biological treatment systems to destroy or reduce the concentration of hazardous waste from a contaminated site. Thus some definitions restrict the use of microbes only while others seem to incorporate all the biological entities such as plants (phytoremediation). Whatever, barriers we define, in fact in nature the process of biological remediation involves both plants and microbes and rather the plant-microbe interaction in root zone has a very important role.

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Table 1. Advantages and disadvantages of different types of bioremediation strategies.

Techniques	Examples	Advantages	Limitations
In situ	*In situ* bioremediation Bioventing Biostimulation Bioaugmentation	Most cost efficient Noninvasive Relatively passive Natural attenuation Treats soil and water	Environmental constraints Extended treatment times Monitoring difficulties
Ex situ	Land farming Composting Biopiles/Biocells	Cost-efficient Can be done on site	Extended treatment time Need to control abiotic loss Mass transfer problem Bioavailability limitation
Bioreactors	Slurry/aqueous reactors	Rapid degradation kinetics Optimized environmental parameters Enhances mass transfer Effective use of inoculants and surfactants	Requires excavation Relatively high capital cost & operating cost

(Source: M. Vidali (2001), Bioremediation. An overview Pure Appl. Chem., 73, 1163–1172)

2. Influence of pH

pH is the major factor influencing the adsorption. Surface charge studies showed that the availability of free sites depended on pH. With increasing pH, the surface charged sites of calcium alginate became more negative, so the uptake of metal ions increased with increasing pH. Crist *et al*., (1994) reported that with decreasing pH, the number of binding sites reduced and that pH increased during the metal ion uptake. Chen *et al*., (1993) reported that with increasing pH, more negative sites became available for the sorption of copper ions, so that the removal of copper increased at high pH; and that pH increases about 0.1 - 1 unit from the initial pH during the adsorption. But no correlation between the increase in pH and the uptake of copper ions was observed. They assumed that the pH change might be due to the mass balance resulted from the copper ion sorption. Schiewer and Volesky (1995) studied *Sargassum fluitans* in their work by raising pH from an initial value of 3.5 to a final value of 6.0 after the addition of *S. fluitans*. They also found that at higher pH values, more sites were available for metal ion sorption and that as a result, the removal efficiency increased with pH.

3. Influence of Ionic Strength

Many previous studies showed that Ionic strength plays an important role in the metal ion uptake. It is generally considered that with decreasing Ionic strength, the removal efficiency increased. Chen *et al*., (1997) studied the influence of Ionic strength on the metal ion adsorption. They used sodium perchlorate to adjust the Ionic strength at 0.005, 0.05, 0.5mol/L, respectively and found that when the Ionic strength decreased from 0.5mol/L to 0.005mol/L, the removal efficiency increased from 80% to 95%. Chang and Hong (1994) obtained the same result in their study. For this reason, Chen *et al*., (1997) believed that during the adsorption, the competition for the functional groups between metal ions and other ions played an important role. At a fixed pH, the number of functional groups is fixed, so the sites available for metal ion uptake decreased with increasing Ionic strength. As a result, the ion removal was less at higher Ionic strength.

4. Models of Adsorption

Many adsorption experiments showed that the Freundlich equation fitted very well the adsorption isotherms (Lin, 1998; Chen *et al*., 1997). But the Langmuir and Freundlich isotherms failed to predict the effect of several important factors, such as pH and ionic strength. Some models were recently developed to overcome these problems. First was the multi-component Langmuir model (Crist *et al*., 1994) which considered the effect of competing ions. Its assumption of a constant number of free sites did not hold for a system with changing pH. Then Schiewer and Volesky (1995 & 1996) modeled the binding of heavy metal ions and protons as a function of metal concentration and equilibrium pH. They used a modified multi-component Langmuir sorption model based on two types of binding sites, i.e., strongly acidic and weakly acidic sites and described multi-site and multi-ion system behavior.

As noticed above, if favorable nutritional and environmental conditions occur, the bacteria are able to readily incorporate the simple organic substances into their cells and oxidize them. However, degradation of complex organic compounds with longer molecular structures is slower. Some compounds are so complex that they cannot be degraded at all, which are termed as recalcitrant or refractory compounds. Still, another may be toxic and thus inhibit the growth of microorganisms and their metabolic activity. Such compounds need special techniques or integration of physicochemical and biological techniques for effective remediation (Fig. 1).

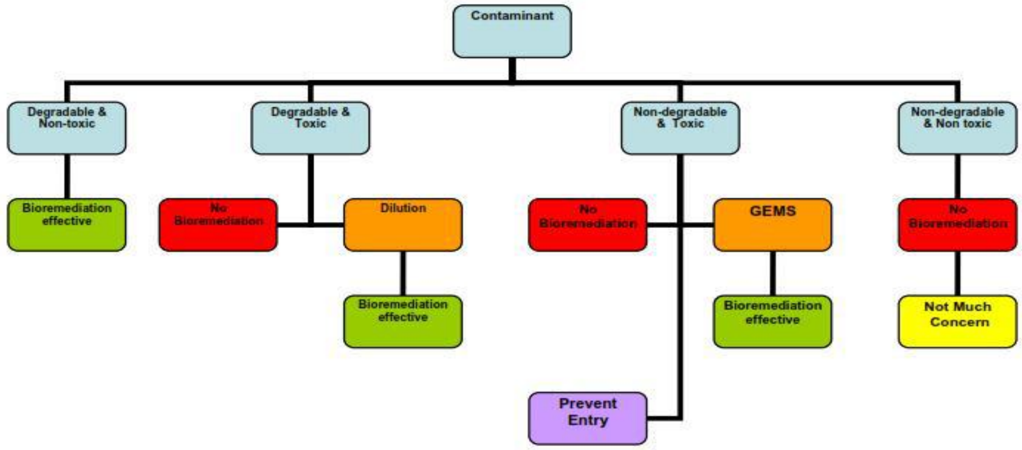

Fig. 1. Bioremediation: Influence of contaminant type.

Table 2. Mechanisms of energy generation by the microbes.

Mechanism	Electron donor	Electron acceptor	Product
Aerobic respiration	Organic compound	Oxygen	CO_2, H_2O
Anaerobic respiration	Organic compound	NO_3, SO_4, Fe^{3+}, Mn^{4+}, CO_2	N_2, H_2S, CH_4, Reduced metals
Fermentation	Organic compound	Organic compound	Organic acids, alcohols, H_2 & CO_2

5. Bioremediation by Algae

Industrialization has led to increased emission of pollutants into ecosystems. Metal pollutants can easily enter the food chain if heavy metal-contaminated soils are used for production of food crops. Farm productivity has decreased in toxic metal polluted areas (Gosavi *et al*., 2004). Accumulation of toxic metals e.g. Hg, Cu, Cd, Cr and Zn in humans has several consequences such as growth and developmental abnormalities, carcinogenesis, neuromuscular control defects, mental retardation, renal malfunction and wide range of other illnesses. Elevated levels of such metal ions are generally toxic and cause major damage to cell (Inouhe *et al*., 1996). Conventional technologies, such as ion exchange or lime precipitation, are often ineffective and/or expensive, particularly for the removal of heavy metal ions at low concentrations (below 50mg/L). Furthermore, Most of these techniques are based on physical displacement or chemical replacement, generating yet another problem in the form of toxic sludge, the disposal of which adds a further burden on the techno-economic feasibility of the treatment process. In View of this, the development of new techniques is necessary to meet the environmental standards at affordable costs. They that can provide solutions to the twin challenges of energy security and environmental pollution. They have great potential for the removal of excess nitrogen and phosphorus from wastewater including the farm runoff. They can capture carbon dioxide in the flue gas from coal-fired power plants thereby reducing greenhouse gas and also producing algal biomass, which can be converted into biofuel *Chlorella*, *Scenedesmus* and *Spirulina* are the most widely used algae for nutrient removal.

Metals are taken up by algae through adsorption. At first, the metal ions are adsorbed over the cell surface very quickly just in a few seconds or minutes; this process is called physical adsorption. Then, these ions are transported slowly into the cytoplasm in a process called chemisorption.

Polyphosphate bodies of algae enable freshwater unicellular algae to store other nutrients. Several researchers have established that metals such as Ti, Pb, Mg, Zn, Cd, Sr, Co, Hg, Ni and Cu are sequestered in polyphosphate bodies in green algae. These bodies perform two different functions in algae; provide a “storage pool” for metals and act as a “detoxification mechanism”. The alga *Scenedesmus obliquus* was also found to accumulate some metals on increasing the amount of phosphorus in the media. It was able to accumulate increased Cd and Zn with higher phosphorus concentrations, whereas Selenium (Se) accumulation was found to be inhibited.

Shehata *et al*., cultured *Scenedesmus* in different concentrations of copper, cadmium, nickel, zinc and lead to evaluate their effects on the growth of algae. The concentration of metal that reduced *Scenedesmus* growth was 0.5mg/L for Cu, 0.5mg/L for Ni and 2mg/L for Cd, 2mg/L for Zn. The nickel solution was less toxic than copper for *Scenedesmus* growth. The alga tolerated high lead concentrations up to 30mg/L. Cd^{2+} contamination in surface water comes mainly from phosphatic fertilizers used in agricultural operations, which is reflected in municipal water supplies drawing water from river sources. The major route of exposure of Cd^{2+} to humans is via the consumption of vegetables homegrown on Cd-contaminated soil. It is well known that soil pH is one of the main soil properties controlling bioavailability of Cd in plants (Millis *et al*., 2004). Cd^{2+} is carcinogenic, embryotoxic, teratogenic and mutagenic and may cause hyperglycemia, reduced immunopotency and anemia, due to its interference with iron metabolism (Sanders, 1986). The toxicity of Cd has also been well documented in other eukaryotes (Rainbow, 1995; Unger and Roesijadi, 1996).

Nickel is a problematic heavy metal (Joho *et al*., 1995). Higher concentrations of nickel are toxic. Nickel contamination may come from desorption of the metal to natural waters from the earth's crust after the global climatic change or from growing electroplating/steel industries. It can cause contact dermatitis, particularly in young women using nickel-containing earrings. Acute inhalation exposure to nickel can cause metal fume fever and acute exposure to nickel carbonyl can cause pneumonitis. Nickel compounds are found to be nephrotoxic, hepatotoxic, immunotoxic and teratogenic (Ross, 1995).

The ability of algae to absorb metals has been recognized for many years (Megharaja *et al*., 2003). In natural environments, algae play a major role in controlling metal concentration in lakes and oceans (Sigg, 1985 & 1987). Algae possess the ability to take up toxic heavy metals from the environment, resulting in higher concentrations than those in the surrounding water (Megharaja *et al*., 2003; Shamsuddoha *et al*., 2006). Bioaccumulation studies reveal the accumulation of the contaminant in the organism via uptake of food or water containing the contaminant

Algae the species of *Chlorella*, *Anabaena inaequalis*, *Westiellopsis prolifica*, *Stigeoclonium tenue*, *Synechococcus* sp. tolerate heavy metals. However, several species of *Chlorella*, *Anabaena*, marine algae have been used for the removal of heavy metals. But the operating condition limits the practical application of these organisms. Rai *et al*., (1998) studied biosorption i.e. both adsorption and absorption of Cd^{++} by a capsulated nuisance cyanobacterium, *Microcystis* both from field and laboratory. The naturally occurring cells showed higher efficiency for Cd^{++} and Ni^{++} as compared to laboratory cells. Microalgae capable to synthesize peptides metallothioneins, mainly the post-transcriptionally synthesized class III metallothioneins or phytochelatins those effectively bind to heavy metal.

Chen Hong *et al*., examine the possibility of using live spirulina to biologically remove aqueous lead of low concentration (below 50mg/L) from wastewater. Afterward, the lead adsorption by live spirulina cells was conducted. It was observed that at the initial stage (0–12 min.) the adsorption rate was so rapid that 74% of the metal was biologically adsorbed. The maximum biosorption capacity of live spirulina was estimated to be 0.62mg lead per 105 alga cells.

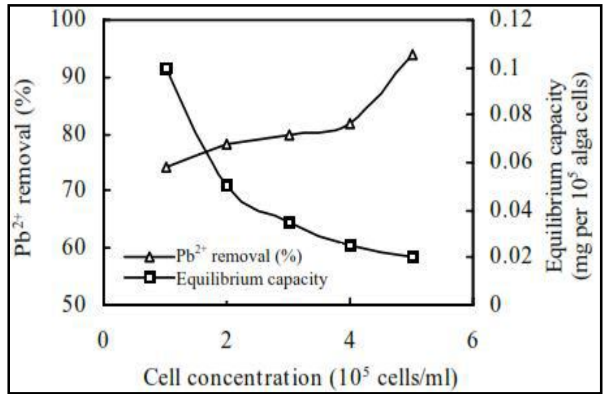

Fig. 2. The effect of spirulina concentration on Pb^{2+} Removal and maximum adsorption capacity (Pb^{2+}: 30mg/L, temperature: 25°C, agitation speed: 166 rpm) (Adopted from Chen Hang *et al*.,).

Various studies have been carried out to show the role of algae in the bioremediation of heavy metals. Some metals such as Cu, Pb, Cd, Co are removed by *Cladophora glomerata* and by *Oedogonium rivulare* as short-term and others such as Ni, Cr, Fe, Mn as continuous uptake biosorption of heavy metals from aqueous solution by freshwater filamentous algae *Spirogyra hatillensis*. Removal of Cadmium, Mercury and Lead from aqueous solution using marine macroalgae as low cost adsorbents.

Travieso *et al*., treated distillery wastewater from an anaerobic fixed-bed reactor in a microalgae pond and obtained 90.2%, 84.1%, and 85.5% organic nitrogen, ammonia and total phosphorus removal, respectively. Kim *et al*., reported 95.3% and 96% removal of nitrogen and phosphorus, respectively, by *Chlorella vulgaris* in 25% secondarily treated swine wastewater after four days of incubation. Hodaifa *et al*., used industrial wastewater from olive oil extraction to remove potassium salts and other minerals with *Scenedesmus obliquus*.

The other mechanism of wastewater treatment is the immobilization of algal cells. It eliminates the harvesting step which is most difficult in the treatment process. A gel matrix prevents cells from freely moving in its environment. Immobilized cells have increased reaction rates because of higher cell density. Further, they show no cell wash out. As a result, they are preferable to their free-living counterparts. Travieso *et al*., reported higher nutrient removal from raw sewage treatment through internal immobilization of *Chlorella vulgaris* in sodium alginate beads.

6. Prospects

With the advantages of low-cost raw material, big adsorbing capacity, and no secondary pollution etc., algae is promising for purification of wastewater containing heavy metals. Many years ago, the accumulation of heavy metals by algae had been studied extensively for biomonitoring or bioremediation purposes. For example, attempts to recover uranium from the sea and freshwater by using immobilized cells of algae, yielded satisfactory results (Nakajima, 1982). Recently, the immobilization of whole cells attracted much attention due to their potential in industrial applications. Further studies should focus on the mechanism of adsorption, such as the relationship between the binding capacity and alginate components, and on research on the biosorption properties of each alginate component. This work will lead to effective use of algae, and subsequently, accelerate the development of more effective biosorbents.

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