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Review Article

Bioremediation of Heavy Metals through Fresh Water Microalgae: A Review

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Abstract: Industrial development has created a serious problem by emitting waste water containing many different kinds of pollutants including heavy metals. There are several chemical and physical processes for heavy metals removal but bioremediation technology has also good prospect. As the waste water from different industries is deposited in the rivers nearby so aquatic flora are needed to be studied more to be used to remove all these heavy metals. Fresh- water microalgae are one of the potential organisms which can be safely used without any hazard to the environment. Instead of that the algal flora will help to reduce the carbon component in the waste water, as well as to remove the nitrate and phosphate component, whereas the oxygen cotent will increase in the water. There are various reports about the heavy metal uptake capacity of microalgae by different means, by adsorption or by intercellular uptake which are ultimately detoxified by metallothioneins or phytochelatins or are sequestered into the vacuoles or polyphosphate bodies. There are various Marine Algal flora which can really remove various heavy metals from aquatic environment, but we are more concerned about the heavy pollutants in rivers and waste water bodies where the fresh water microalgae can be used successfully. High Rate Algal Ponds or Algal Turf Systems are being used successfully for removal of pollutants in some places. But more studies should be done for using other combined technologies like immobilized algal cultures along with other to improve the different pollutants removal capacity by freshwater microalgae. Transgenic algae production must be attempted to increase their potentiality in these aspects by manipulating the detoxifying mechanisms. In this review article a brief account has been presented about the present status in this area of research and some suggestions what may be attempted in future to implement this technique to make our environment pollution free.
Keywords: Fresh water Microalgae, Bioremediation, Heavy metals, Uptake, Detoxification.

INTRODUCTION

The term Heavy metal is collectively applied to a group of metals or metal like elements which are directly related with environmental pollution and biological toxicity problems. These kind of metals tend to bio accumulate and ultimately get transferred to higher level of food chain. Industrial development has created the increased emission of waste waters containing various kinds of pollutants such as different organic pollutants as well as other toxic substances and heavy metals also. Heavy metals can easily enter the food chain if heavy metal contaminated soils are used for food crops cultivation. These also may contaminate the water bodies like different rivers and affect the aquatic ecosystem. Productivity may get decreased due to this heavy metal effects as bioaccumulation occurs when a portion of metal is retained by an organism which may have some effects on plant metabolism. In human being accumulation of some toxic metals like mercury, nickel, copper, cadmium, chromium and zinc has several bad consequences such as growth and developmental abnormalities, carcinogenesis, neuromuscular control defects, mental retardation, renal malfunction and a wide range of other kinds of illnesses [1]. Chemistry of heavy metals in solutions is an important aspect which must be taken into account when bio sorption of heavy metals from industrial wastewater is studied. The main factors which influence metals speciation in solution are pH, heavy metal concentration in solution, presence of light metals and anions in solution. It is known that almost all metals tend to precipitate at basic pH value, as hydroxide or carbonate forms. Precipitation of heavy metals is a disadvantageous method for heavy metals removal because can generate toxic chemical sludge. From this point of view bio sorption is recommended as an appropriate method for acidic effluent treatments which contain high concentrations of ionic species in solution (mining effluents, electroplating industry effluents, metals finishing industry batteries etc.) [2].

Removal of metals from waste water is achieved principally by the application of several processes such as adsorption, sedimentation, electrochemical processes, ion exchange, cementation, coagulationflocculation, filtration and membrane processes, chemical precipitation etc. Phytoremediation has emerged as the most desirable technology which uses plants for removal of environmental pollutants or detoxification to make them harmless. There are several plants used in the phytoremediation has a considerable capacity of metal absorption, its accumulation and reducing the time of decontamination of an ecosystem. As the industrial waste waters are mostly discharged into the water bodies so we are more concerned about the effect of those heavy metals on the aquatic flora. There are various experiments and reports on the basis of those we can think of freshwater microalgae to be used as agent of phytoremediation [3].

The algae have many features that make them ideal for the selective removal and reducing the concentration of heavy metals, which include high bio sorption capacity, high tolerance to heavy metals, ability to grow both autotrophically and heterotrophically , large surface area/ volume ratios, phototaxy, phytochelatin production and its potential for genetic manipulation [4].

REPORTS ON THE ROLES OF DIFFERENT MICROALGAE IN HEAVY METAL UPTAKE

The most common microalgae of aquatic bodies *Euglena gracilis* has been reported to have the uptake capacity of zinc at the rate of 5mg /g of dry weight of algal mass [5]. Another report states that a diatom *Phaeodactylum tricornutum* is highly tolerant to cadmium (22.3 mg /L) and has also proved to have high removal capacity [6].

The blue green algae, *Phormidium sp*. can successfully hyper-accumulate heavy metals like Cadmium, Zinc, lead, Nickel and Copper [7]. It has been reported that *Scenedesmus sp*. could tolerate the metals like copper, nickel, cadmium and zinc which ranged from 0.5 mg/L to 2 mg/L, whereas in case of lead it was 30mg/L [8]. Biosorption of chromium, cadmium and copper ions by another blue green algae *Spirullina sp* was reported by [9].

Chlorella sp. could efficiently reduce 76% - 96% of cadmium and 78% to 94% of nickel from the medium within 7-28 days when cultured under laboratory condition [10]. *Dunaliella salina* has high tendency for zinc accumulation followed by copper and cobalt, but lowest tendency towards cadmium. It also showed that uptake of copper, cobalt and zinc increased with the increasing concentration of metal ions in the medium whereas cadmium concentration had no effect [11].

Scenedesmus bijuga and *Oscillatoria quadripunctulata* showed heavy metal removal capacity 37-50 % for copper, 20-33% for cobalt, 35-100% for lead and 32-100% in case of zinc from the sewage and petrochemical industry effluent [12].

Cladophora glomerata and *Oedogonium rivulare* have been tested to remove copper, lead, cadmium and cobalt within a short time period,[13] *Spirogyra halliensis* has the capacity for bio sorption of heavy metals like nickel, chromium, iron and manganese. There are several reports that different species of several fresh water microalgae like *Chlorella sp., Anabaena sp., Westiellopsis sp., Stigeoclonium sp., Synecococcus sp.* etc. have high tolerant capacity for various heavy metals [14]. The uptake or heavy metal removal capacity of different microalgae as reported earlier by different authors have been summarised in Table 1.

HEAVY METAL TOLERANCE, ACCUMULATION AND DETOXIFICATION MECHANISMS IN ALGAE

The principal mechanism of metallic cation sequestration involves the formation of complexes between a metal ion and functional groups on the surface or inside the porous structure of the biological material. Metals are taken up by algae through adsorption over the cell surface very quickly which is called as physical adsorption and then these ions transported slowly into the cytoplasm through the process called chemisorption. When living *Spirullina* cells were used to remove lead from the aqueous medium containing 50mg/L lead, it showed that initially the adsorption rate was rapid i.e., 74% of metal was biologically adsorbed [15]. The adsorption, phytosorption and affinity of algae for these metal cations because of its high negatively charged cell wall components.

Ionic strength of the media also plays an important role on metal ion uptake, decrease in ionic strength helps increase in removal efficiency of metal ions from the media. It was reported that when ionic strength decreased the removal efficiency is increased which may be due to the competition for the functional groups between the metal ions and other ions which played an important role [9]. One of the most influencing factor in adsorption is the pH of the media, with increasing pH more negative sites became available for the adsorption of copper ions so that the removal of copper increased at high pH. It was also reported that with decreasing pH the number of binding sites reduced and during the metal ion uptake the media pH increased. The effect suggests that metals can form complexes with acetate and EDTA (strong chelating agents) and does bind at pH 5 [16].

Schiewer and Volesky [17] proposed a model for binding of heavy metal ions and protons as a function of metal concentration and equilibrium pH. They used the modified Langmuir adsorption model based on two types of binding sites i.e., strongly acidic and weakly acidic sites along with multi-sites and multi-ion system behavior.

Polyphosphate bodies within the algal cell enable the fresh water algae to store other kind of nutrients. Several researchers have established that heavy metals are sequestered in polyphosphate bodies in green algae. These bodies perform two different functions in algae, provide a storage pool for the heavy metals and act as a detoxification mechanism [14]. Different organic acids i.e., carboxylic, malic, oxalic, amino acids like histidine, nicotinamine, and some phosphate derivatives (phytate) are the potential ligands for heavy metals and are found to play a role in tolerance and detoxification [4].

In various experiments it has been found that the algal cells respond to heavy metal stress using different defense systems such as exclusion, compartmentalization, making complexes and the synthesis of binding proteins such as metallothioneins (Mts) and phytochelatins (PCs) and translocate them into vacuoles [18]. Lee *et al*. [19] reported the metallothionein production in *Thalassiosira weissflogii* under heavy metal stress, whereas in *Phaeodactylum tricornutum* the degradation of Mt III has been reported complexed with metal. It was observed that many diatoms and Dinoflagellates capable of producing Metallothioneins (Mt III) under heavy metal stress, and they also produce exo-cellular polysaccharides which are effective bio-absorber [20].

Ahner, B.A. *et al*. [21] reported that *Thalassiosira sp*. produces phytochelatins in great amounts due to higher activity of phytochelating synthase which has a greater affinity for metal ions. But Rejstenbil *et al*. [22] reported that under copper, zinc and cadmium stress there is an increased activity of reactive oxygen species scavenger -SH molecules in *Thalassiosira pseudonana.* Wu *et al*. [23] reported the accumulation of proline in response to copper stress observed in *Chlorella.* Mehta and Gaur [24, 46] suggested that proline accumulation in *Chlorella* was highest when cells are treated with copper and chromium, which showed proline may act as protective agent on metal toxicity through inhibition of lipid peroxidation. Siripornadulsil *et al*. [25] reported that free proline acts as antioxidant in cadmium stressed cells of *Chlamydomonas reinhardtii*.

Howe and Merchant [26] reported that in the green algae *Chlamydomonas reinhardtii* mercury was not chelated by MtIII but by Glutathione which indicates that glutathione acts not only as Glu-Cys donor of MtIII but also plays as detoxifying molecule itself. Whereas in case of *Tetraselmis tetrathele* showed that under mercury stress it did not produce MtIII through Glutathione pool depletion but used a novel peptide Arg-Arg-Glu with mercury scavenging capacity [27]. Pawlik-Skowronska [28] reported a different kind of metallothionein which has an additional –SH group observed in *Stigeoclonium tenue* when exposed to high zinc and lead containing medium.

Table 1: Tolerance and uptake capacity of different heavy metals by fresh water algal genera as reported by different authors

Name of algal genera	Name of heavy metals		References
	Tolerance capacity	Uptake capacity	
Phormidium sp. *P. bohner* *P.ambigunum* *P. corium*	Cd, Zn, Pb, Ni, Cu Chromium Hg(<20 mg/l), Cd, Pb (20-100mg/l) Cd, Ni, Zn,		Wang *et al*. [7] Dwivedi S *et al*. [14] Shanab S *et al*. [35] Rana L *et al*. [36]
Oscillatoria quadripunctulata *Oscillatoria tenius*	Cd, Ni, Zn	Cu (37-50%), Co (20-33%), Pb (35-0%), Zn (32-100%)	Ajavan K.V *et al*. [12] Azizi S N *et al*. [37] Rana L. *et al*. [36]
Scenedesmus acutus *Scenedesmus quadricauda*	Cd, Zn	Cr (31% from 20mg/l conc.) Hg 97%, Cd 86%, Pb 70%,	Travieso *et al*. [34] Cannizares-Villanueva *et al*. [38] Shanab S *et al*. [35]
Euglena gracilis		Zinc 5 mg/g	Fukami *et al*. [5]
Chlorella vulgaris *Chlorella sorokiniana* *Chlorella sp*		Cd 65%, Cr 48%, Cd 43ug, Zn 42ug, Cu 46ug /mg dry wt. Cr 78-94% Cd 76-96%	Matsunaga *et al*. [39] Travieso *et al*. [34] Yoshida N *et al*. [40] Rehman & Shakoori [10, 43]
Spirogyra hyaline *Spirogyra halliensis* *Spirogyra sp.*		Cd, Hg, As (40mg/l) Pb , Co (80mg/l) Ni, Cr, Fe, Mn, Cr 98%, Cu 89%, Fe99%,Mn 99%, Zn81%	Kumar & Oommen [41] Mane & Bhosle 2012[42]
Spirullina sp.		Cr 98%,Cu81%,Fe98%, Mn99%, Zn79%	Mane & Bhosle [42]
Cladophora glomerata		Zinc higher removal & Cu,	Vymazal J. [13]

Oedogonium rivulare		Pb, Cd, Co slow removal	

DIFFERENT ALGAL SYSTEMS USED FOR BIOREMEDIATION

High Rate Algal Ponds (HRAP) and the Algal Turf Scrubber (ATS) employs the culture of suspended biomass of common green algae *(Chlorella, Scenedesmus, Cladophora*) or the Cyanobacteria (*Spirullina, Oscillatoria, Anabaena*). These kinds of systems have been tested for heavy metal removal. Toumi *et al*. [29] compared the heavy metal removal rate by HRAP and traditional system which showed that HRAP is about ten times more efficient than traditional Waste Stabilisation Pond (WSP) which had higher removal rate per unit volume per day. ATS system has also been tested for treating polluted underground waters which also revealed the efficiency of this system in heavy metal removal along with the removal of chlorine and other aromatic compounds. Rose *et al*. [30] reported a patented hybrid process which recombines the HRAP and sulphate reducing bacteria (SRB), where heavy metals are precipitated into HRAP with high pH values and that water is used as source for SRB. Phillips *et al*. [31] showed that consortia of algae and cyanobacteria could effectively reduce prohibitive manganese concentration to an environmentally safe level. In this case manganese was removed by means of biomass adsorption, high pH precipitation and immobilization.

Sibhi *et al*. [32] used a diatom, *Planothedium lanceolatum* as an economic biosorbant cheap material for the removal of the heavy metals like copper, cadmium and zinc. They reported that the cells can accumulate high amounts of these elements until they reach a toxic level and these algae showed more resistance than any other algae reported earlier like *Chlorella, Thalassiosira, Nitzchia* etc.

The other mechanism of waste water treatment is the immobilization of algal cells. It eliminates the harvesting step which is most difficult in the treatment process. Immobilised cells have increased reaction rates because of higher cell density [33]. *Scenedesmus acutus* and *Chlorella vulgaris* when immobilized on Polyurethene foam and Kappa-carrageenan gel are more tolerant to cadmium, chromium and zinc over normal concentration of these ions in industrial water [34, 47]. This fact implies the great possibility for heavy metal removal by immobilization, more experiments are needed on this aspect.

CONCLUSION

Bioaccumulation by adsorption or by uptake and accumulation of them into cellular compartments is an unique criteria of several microalgae. The above reports by various authors prove that microalgae provide more advantage over the other existing methods which are more costly and not environment friendly. The algae has the advantages of low cost raw materials, more adsorbing capacity, no secondary pollution, moreover waste water cleaning capacity removing nitrate and phosphate. There are several reports that immobilized cells of microalgae yielded satisfactory results in heavy metal uptake and those heavy metals can be recovered easily from algal cells and utilized.

Genetic engineering is another thrust area where the transgenic algal cells can be produced by over-expressing the genes for metallothionein and phytochelatin expression which will have more binding capacity with various heavy metals and translocate them into vacuoles. The introduction and over expression of metal binding proteins have been widely exploited in prokaryotic as well as eukaryotic system to increase the metal binding capacity, tolerance or accumulation of heavy metals into the cells. Modification of phytochelatin synthesis pathway has also been reported to enhance the metal accumulation in plant system and various metal binding peptide residues are important in prokaryotic system for enhancing the binding capacity in prokaryotes. All these mechanisms can be exploited both in cyanophycean and chlorophycean algae by using the recombinant DNA technology. Those transgenic algal species will have more capacity for tolerance and can uptake more amount heavy metals if used in HRAP or ATS system.

Further studies in this area should focus on the more detailed mechanism of physical adsorption, binding capacities and translocation or compartmentalization of those metals within the cells without affecting the metabolic activities and growth by regulating different parameters like pH and ionic strength. Thorough survey of different microalgal species naturally grown in different kinds of waste waters i.e., from different types of industries is needed. The algal species which can grow well in waste water naturally must have the more capacity to tolerate more pollutants. Considering all these aspects we are very sure that microalgae isolated from waste water bodies must have a promising future in bioremediation.

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