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Use of Alga *Anabaena ambigua* for Bioremoval of Some Metals from Aqueous Solution

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ABSTRACT

In recent years, the biosorption processes have been studied extensively using microbial biomass as biosorbents for heavy metal ions removal. In these studies, metal ions removal abilities of various species of bacteria, algae, fungi and yeasts were investigated. The discharge of heavy metals into aquatic ecosystems has become a matter of concern over the last few decades. The biosorption of six metal ions from artificial wastewaters containing single metal ions was investigated in batch experiments. Microalgae Anabaena ambigua was obtained from National Chemical Laboratory (NCL), Pune. The selected microalgae was tested for Chromium (Cr), Copper (Cu), Iron (Fe), Manganese (Mn), Selenium (Se) and Zinc (Zn) removal from aqueous solution. The concentration of all the six metal ion species in the absorption medium was varied between 5 and 25 mg/L. The residual concentration of metals from the absorption medium was determined by using UV spectrophotometer. After seven days incubation period at 5 mg/L metal concentration, the percent bioremoval for Chromium, Copper, Iron, Manganese, Selenium and Zinc was 98.46%, 82.93%, 98.13%, 97.73%, 99.33% and 57.53% respectively.

Key words: Algal biomass, percent bioremoval, heavy metal ions, aqueous solution, UV spectrophotometer.

Introduction

Several living organisms including algae, according to their nutrient need, are used to evaluate most of the environmental characters. Algae were considered good bioindicators to several qualitative and quantitative characters of aquatic environment (Al-bergoni et al. 1980, Favero et al., 1996, Markert and Ohelmann, 1998). Their widely distribution enhanced their use in monitoring program through simple models (Rachlin et al., 1983, Piccinni and Gutierrez, 1995). Heavy metals are one of the main water pollutants. They are more danger due to their bioaccumulation. Some conformer organisms can tolerate high concentration of these metals, while others can regulate their internal concentrations (Albergoni et al., 1980, Favero et al., 1996). Algae are able to accumulate these metals mainly by two ways, firstly by chemical-physical way which is fast due to their adsorption on the external algal surfaces, and secondly by regulated cellular uptake which is slow and energy depended. Several authors have been searching for alternative and better performing remediation strategies pertaining to toxic heavy metals, because conventional physico-chemical methods (e.g. precipitation and ion exchange) are not fully effective; in addition, they are rather expensive (Bayramoglu and Arica 2008; Doshi et al., 2007b), especially when the metal levels are of the ppm order of magnitude (Gupta and Rastogi 2008a; Yu and Kaewsarn, 1999). A more feasible approach relies on the metal binding and uptake capacities of living materials, which include microalgae in particular (Doshi et al. 2007b; Fraile et al., 2005; Leborans and Novillo, 1996; Rollemberg et al., 1999; Solisio et al., 2008). Application of microbial biomass to remove toxic heavy metals has become relatively popular, owing to its high adsorbing capacity and low cost (Bayramoglu and Arica 2008; Doshi et al., 2007b). Additionally, metals removed by

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adsorption onto the cell surface, may be successfully recovered, after desorption brought about by chemical agents: (Costa and Franca, 1998) reported that a 10.0 g/l EDTA solution could totally recover the Cd previously removed by adsorption onto the cell walls of the microalga Tetraselmis chuii, whereas (Gupta and Rastogi, 2008b) obtained 85 and 80% recoveries of Cd ions from Oedogonium sp. biomass, when using HCl or EDTA as desorbing agents, respectively.

Many investigations have been carried out for biosorption of heavy metals by the other important divisions of algae Green algae (Chlorophyta) and Red algae (Rhodophyta) (Handy, 2000; Lee, et.al, 2000). Ulva reticulate was the focus of the study of Kuppusamy Vijayaraghavan et al. The influence of several factors such as pH, initial metal concentration and contact time were analyzed (Vijauaraghavan, 2004). Green algae Cladophora fascicularis was to be an effective and economical biosorbent material for the removal of heavy metal ions (Dend et al., 2006). In the present study, the biosorption of heavy metal ions such as Cr, Cu, Fe, Mn, Se and Zn by fresh water algae Anabaena ambigua was investigated. The effect of initial metal concentration was studied.

Materials and Methods

Anabaena ambigua cultivation

The starting culture was obtained from the National Chemical Laboratory (NCL), Pune. Anabaena ambigua in log phase used in the experiment was inoculated in the Fog's medium at p^H 7.5. The medium was sterilized by autoclaving at 121^oC for 15 minutes. Medium was stored at 4^oC until inoculated. Culture was grown in liquid media in 2 L glass Erlenmeyer flasks and incubated at 25^oC in a growth chamber with a light and dark cycle of 8 h and 16 h and 3000 – 3500 lux, light intensity provided by cool white day light fluorescent tube lamps.

Metal bioremoval experiment

Algal metal bioremoval was assayed by exposing the strain of Anabaena ambigua to various metals concentrations, in triplicate. Defined aliquots of each metal stock solution were added to 90 mL of algal culture in a 250 mL conical flask separately, in order to obtain the desired final concentrations i.e. 5, 10, 15, 20 and 25 mg/L. The conical flasks were stoppered with a cotton wool bung and incubated for seven days. The total concentration of all the metals removed by micro algal cells was calculated as the difference between the initial and the remaining metal concentration in the supernatant. Replicated blank controls, containing culture medium plus metal at each concentration tested, were considered; all the metal concentration remained stable in those flasks for the time frame of each experiment, so no redox reaction or adsorption onto the vessel walls took place to any measurable extent.

The bioremoval efficiency (R) of the algae was calculated by the following formula (Zhang et al, 1998).

$$R\ (\%) = \frac{(Ci - Ce)}{Ci} 100$$

Where, R = Bioremoval efficiency (%); C_i = initial concentration of metals in aqueous solution (mg/L); C_e = equilibrium concentration of metals in aqueous solution (mg/L).

Analytical methods

After completion of incubation period, 10 mL sample was drawn from the flask, centrifuged at 5000 rpm for 15 minutes and then the supernatant was filtered through filter paper. The filtrate was analyzed for residual metal in the solution by UV Spectrophotometer. Chromium was analyzed by s-Diphenylcarbazide method, copper by Neocuproine, Iron by Thiocyanate, Manganese by Persulphate, selenium by 3-3$'$ Diamino Benzidine and zinc by Dithiozone methods.

Results and Discussion

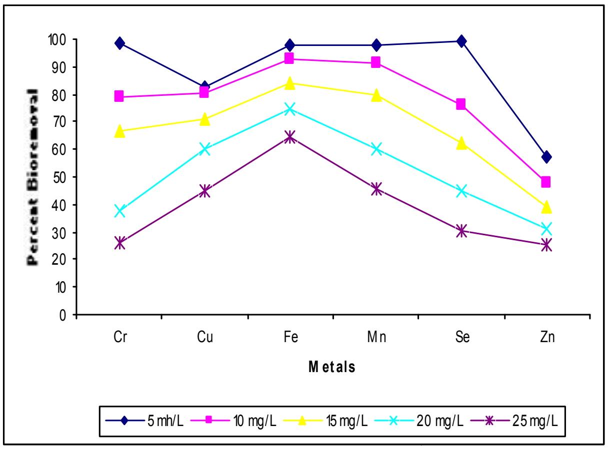
In the present study the bioremoval characteristics of the algal strain Anabaena ambigua was examined with regard to as Cr, Cu, Fe, Mn, Se and Zn ions. The bioremoval capacity of Anabaena ambigua was high for all the tested metals except Zn. Within all the tested metals the bioremoval capacity for Cr, Cu, Fe, Mn, Se and Zn was highest [98.46%, 82.93%, 98.13%, 97.73%, 99.33% and 57.53% resp.] at initial concentration 5 mg/L after seven days of incubation period as shown in Fig. 1. The biosorption of metals onto the cell surface allows for cross-binding of anionic ligands on different cells by multivalent cations. The residual concentrations of metals in the medium after seven days of incubation period are shown in Table 1. This bioremoval study of metals reveled that, the removal decreased with increasing initial metal concentration. Living microalga biomass has been used for bioremediation processes of heavy metal-contaminated wastewaters, owing to its ability to remove such contaminants, either by adsorption onto the cell surface or by incorporation into the cells themselves.

Two distinct biochemical paths can thus be followed: biosorption or adsorption of metal ions onto the cell surface and bioaccumulation or absorption of metal into the cell (Rangsayatorn et al., 2002). The cell wall of microorganisms has been claimed to play a crucial role as a defence mechanism—in that it is the first barrier to the uptake of toxic metals (Ozer et al., 1999; Rangsayatorn et al., 2002).

Table 1 Residual concentration of metals in the medium after seven days of incubation period.

	Metal Concentrations [mg/L]				
Metals	5	10	15	20	25
Cr	0.07 ± 0.0070	2.11 ± 0.0400	4.99 ± 0.0800	12.48 ± 0.0450	18.48 ± 0.0750
Cu	0.85 ± 0.0404	1.96 ± 0.0251	4.40 ± 0.0360	07.92 ± 0.0251	13.69 ± 0.0152
Fe	0.09 ± 0.0057	0.73 ± 0.0200	2.41 ± 0.0152	05.11 ± 0.0200	08.94 ± 0.0251
Mn	0.11 ± 0.0230	0.85 ± 0.0115	3.08 ± 0.0173	07.94 ± 0.0251	13.66 ± 0.0264
Se	0.03 ± 0.0057	2.39 ± 0.0200	5.67 ± 0.0264	10.97 ± 0.0264	17.41 ± 0.0100
Zn	2.12 ± 0.0208	5.21 ± 0.0208	9.13 ± 0.1527	13.82 ± 0.0251	18.68 ± 0.0115

Figure 1Percent bioremoval of metals by Anabaena ambigua from aqueous solution



Our results indicated that the algal strain Anabaena ambigua had very high for Cr, Cu, Fe, Mn, Se and Zn. The green alga Chlorella vulgaris is often used to study adsorption of heavy metals (Hans JG and Urbach, 1983; Ting et al, 1991; Cho et al, 1994; Wong et al., 1979) but in this study, the alga Anabaena ambigua had a higher removal capacity. In general heavy metals are taken in by blue green algal cells by adsorption followed in sequence by metabolism-dependent intracellular cation intake as applicable to Zn, Cu, Cd and Zn, Cd, Al, Ni and Hg (Shehata and Whitton, 1982; Les, 1984; Singh and Yadava, 1985; Pettersson et al, 1986; Campbell and Smith, 1986; Johnson and Shubert, 1986). In other studies, blue green algae had lower capacities than those found herein. The blue green algae Aphanothece halophytica and Spirulina platensis could remove only 22% and 35% Pb, respectively, from battery factory wastewater (Kitjaharn and Incharoensakdi, 1992; and Incharoensakdi and Kitjaharn, 1998). In spite of this, we selected blue green algae to test for heavy metal adsorption because they have high growth rates and are easy to separate from solution by simple filtration. Literature reports that the accumulation of metal ions depends on external concentration of metal ions in the solution until their concentration leads to toxic effects and thus to decreased performance of bioaccumulation (Wong and Tan, 1998).

In the studied range of initial metal ions concentration this phenomenon was observed. Each of the metals showed different affinity toward algae. This could be explained with the difference in cell wall composition and the intra group differences which cause significant differences in the type and amount of metal ion binding to them. The cell wall consists of variety of polysaccharides and proteins which offers a number of active sites capable of binding metal ions. The brown algae Cystoseira barbata and C. crinita exhibited highest uptake with regard to most of the heavy metal ions. In brown algae biomass, alginate in the cell wall is the main component responsible for the metal sorption. It is present in a gel form which appears very porous and easily permeable (Fourest and Volesky, 1997). This could explain the highest uptake of metal ions.

Conclusion

In the present study it was found that, the micro alga Anabaena ambigua is well suited to remove Cr, Cu, Fe, Mn and Se from aqueous solution. In case of Zn there is less removal from the aqueous solution.

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