POTENTIAL OF *Nostoc muscurom* AND *Anabaena subcylindrica* FOR THE 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. Algae have been proven efficient biological vectors for heavy metal uptake. In order to further study their biosorption potential, two strains *Nostoc muscurom and Anabaena subcylindrica* have been studied under different initial metal concentrations. In recent years, the biosorption processes have been studied extensively using microbial biomass as biosorbent 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. Both the strains of algae collected from National Chemical Laboratory (NCL) 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. From the tested algae after seven days of incubation period, the highest percent bioremoval by *Nostoc muscurom* for Cr (98.70%), Cu (74.13%), Fe (98.73%), Mn (98.26%), Se (99.12%) and Zn (73.20%) respectively. The same by *Anabaena subcylindrica* for Cr (97.00%), Cu (75.66%), Fe (98.00%), Mn (93.53%), Se (98.93%) and Zn (51.53%) respectively at 5 mg/*l* initial metal concentration.

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 bioindicator to several qualitative and quantitative characters of aquatic environment[1,2,3]. Their widely distribution enhanced their use in monitoring program through simple models[4,5].

Heavy metals are more danger due to their bioaccumulation. Some conformer organisms can tolerate high concentration of these metals, while others can regulate their internal concentrations[6,2]. 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.

Heavy metals are one of the most widespread causes of pollution, both in water and in the soil and increasing levels of heavy metals in the environment are causing mounting concern in public opinion. Because of their chemical characteristics, heavy metals cannot be

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biodegraded by micro-organisms into non-toxic eventually assimilable or volatile compounds as is frequently the case with organic pollutants, but they remain in the environment, changing from one chemical state to another and eventually accumulating in the food chain. For this reason, chemical and physicochemical methods have traditionally been utilized to remove heavy metals from polluted water bodies but such methods have a number of disadvantages: they are not very efficient, in particular at low metal concentrations, and they are expensive. Recently, great interest has been aroused by a new technique, biosorption, which exploits the cell envelopes of microorganisms to remove metals from water solutions[7]. Biosorption is a passive process of adsorption by either living or dead microbial biomass and it offers some interesting advantages such as (i) low operating cost, (ii) high efficiency in removing metals even from very dilute solutions, (iii) the possibility of recovering the valuable metals sorbed by the biosorbent, and (iv) a lower amount of metal containing biological sludges that have to be disposed of after treatment[8]. Microbial cells can be viewed as natural ion-exchange material because they have many anionic groups on their cell surface[8] and this enables them to fix metal ions, mainly by means of an ion-exchange mechanism[9,10].

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[10-12], especially when the metal levels are of the ppm order of magnitude[11,12].

A more feasible approach relies on the metal binding and uptake capacities of living materials, which include microalgae in particular [15-18]. Application of microbial biomass to remove toxic heavy metals has become relatively popular, owing to its high adsorbing capacity and low cost[11,12]. Additionally, metals removed by adsorption onto the cell surface, may be successfully recovered, after desorption brought about by chemical agents[19] 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[20] obtained 85 and 80% recoveries of Cd ions from *Oedogonium sp.* biomass when using HCl or EDTA as desorbing agents, respectively.

In the present study, the biosorption of heavy metal ions such as Cr, Cu, Fe, Mn, Se and Zn by fresh water algae *Nostoc muscurom* and *Anabaena subcylindrica* was investigated. The effect of initial metal concentration was studied.

MATERIAL AND METHODS

Anabina ambigua cultivation

The starting culture was obtained from the National Chemical Laboratory (NCL), Pune. *Nostoc muscurom* and *Anabaena subcylindrica* in log phase used in the experiment was inoculated in the Fog's medium at pH 7.5. The medium was sterilized by autoclaving at 121^{0}C for 15 minutes. Medium was stored at 4^{0}C until inoculated. Culture was grown in liquid media in 2*l* glass Erlenmeyer flasks and incubated at 25^{0}C 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 strains of *Nostoc muscurom and Anabaena subcylindrica* 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.

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

The bioremoval efficiency (R) of the algae was calculated by the following formula[34].

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 strains, Nostoc muscurom and *Anabaena subcylindrica* were examined with regards to Cr, Cu, Fe, Mn, Se and Zn ions from aqueous solution. The residual concentrations of respective metals after seven days of incubation period with *Nostoc muscurom* and *Anabaena subcylindrica* is given in **Table 1** and **Table 2**

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

	Metal Concentrations [mg/*l*]				
Metals	**5**	**10**	**15**	**20**	**25**
Cr	0.065 ± 0.0055	2.220 ± 0.0624	4.520 ± 0.0500	8.210 ± 0.0400	14.17 ± 0.0750
Cu	1.293 ± 0.0208	3.203 ± 0.0321	5.620 ± 0.0300	8.966 ± 0.0251	15.93 ± 0.0264
Fe	0.063 ± 0.0057	0.630 ± 0.0100	1.836 ± 0.0351	4.236 ± 0.0057	8.350 ± 0.0264
Mn	0.086 ± 0.0057	0.840 ± 0.0360	2.886 ± 0.0208	7.683 ± 0.0152	13.05 ± 0.0208
Se	0.044 ± 0.0040	2.440 ± 0.0300	6.026 ± 0.0115	11.41 ± 0.0208	17.77 ± 0.0208
Zn	1.340 ± 0.0173	3.116 ± 0.0288	5.736 ± 0.0404	8.836 ± 0.0351	14.52 ± 0.0208

respectively.

It was observed that the metal removal is highly dependent on metal concentration system. Cr, Fe, Mn and Se removal capacity by *Nostoc muscurom* is high as compared to Cu and Zn at lower initial metal concentration (5 mg/L). The same in case of *Anabaena subcylindrica* revealed that with Cr, Fe, Mn and Se removal capacity is high as compared to Cu and Zn at lower initial metal concentration (5 mg/L). In case of Zn bioremoval *Nostoc muscurom* (73.20%) have higher potential than *Anabaena subcylindrica* (51.53%) at 5 mg/L of initial metal concentration.

Table 2. Residual concentration of metals in the medium after seven days of incubationperiod with *Anabaena subcylindrica* .

	Metal Concentrations [mg/*l*]				
Metals	**5**	**10**	**15**	**20**	**25**
Cr	0.150 ± 0.0264	2.213 ± 0.0450	4.596 ± 0.0416	8.383 ± 0.0503	14.66 ± 0.0750
Cu	1.216 ± 0.0404	3.113 ± 0.0251	4.993 ± 0.0351	8.416 ± 0.0152	14.88 ± 0.0173
Fe	0.100 ± 0.0100	0.750 ± 0.0173	2.130 ± 0.0173	5.066 ± 0.0305	8.353 ± 0.0321
Mn	0.323 ± 0.0251	0.873 ± 0.0152	3.123 ± 0.0251	8.293 ± 0.0115	14.92 ± 0.0264
Se	0.053 ± 0.0020	2.676 ± 0.0251	6.203 ± 0.0251	11.67 ± 0.0173	18.07 ± 0.0300
Zn	2.423 ± 0.0251	5.920 ± 0.0200	9.166 ± 0.1527	14.320 ± 0.0200	19.16 ± 0.0251

Within all the tested metals the bioremoval capacity for Cr, Cu, Fe, Mn, Se and Zn was highest [98.70%, 74.13%, 98.73%, 98.26%, 99.12% and 73.20%] respectively at initial concentration 5 mg/L after seven days of incubation period by Nostoc muscurom as shown in **Fig. 1.**

The same by *Anabaena subcylindrica* for Cr, Cu, Fe, Mn, Se and Zn was highest [97.00%, 75.66%, 98.00%, 93.53%, 98.93% and 51.53%] respectively at initial concentration 5 mg/L after seven days of incubation period as shown in **Fig. 2.**

In order to simplify the ability of each alga to

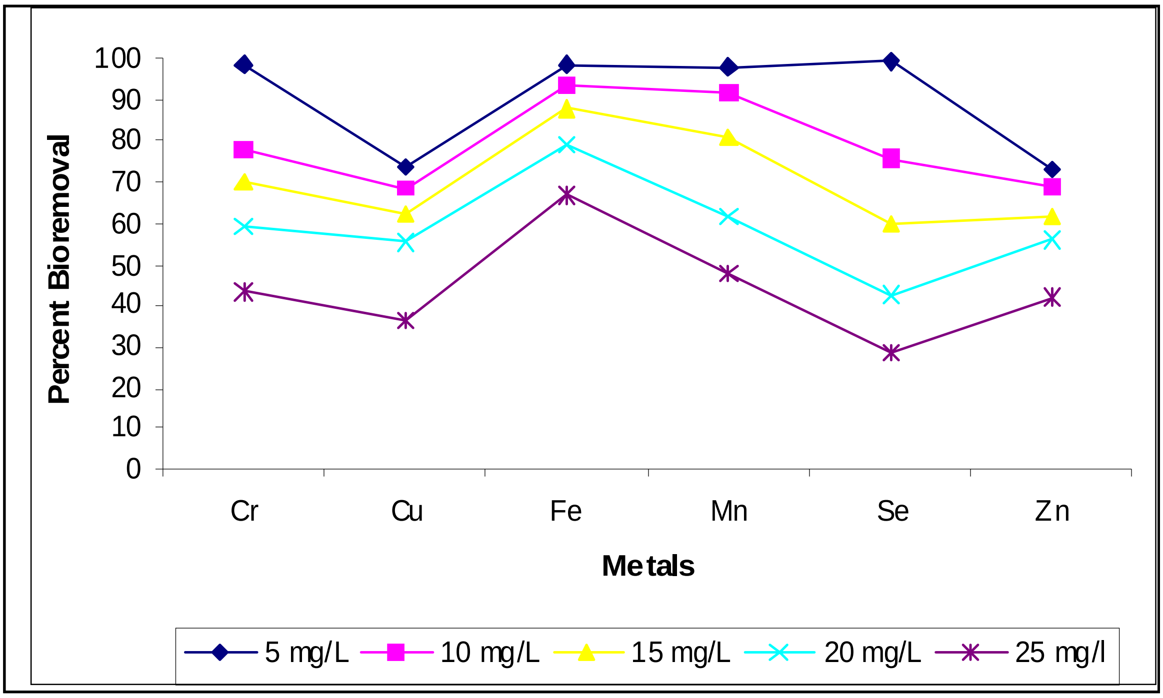

Fig. 1 : Percent bioremoval of metals by *Nostoc muscurom* from aqueous solution

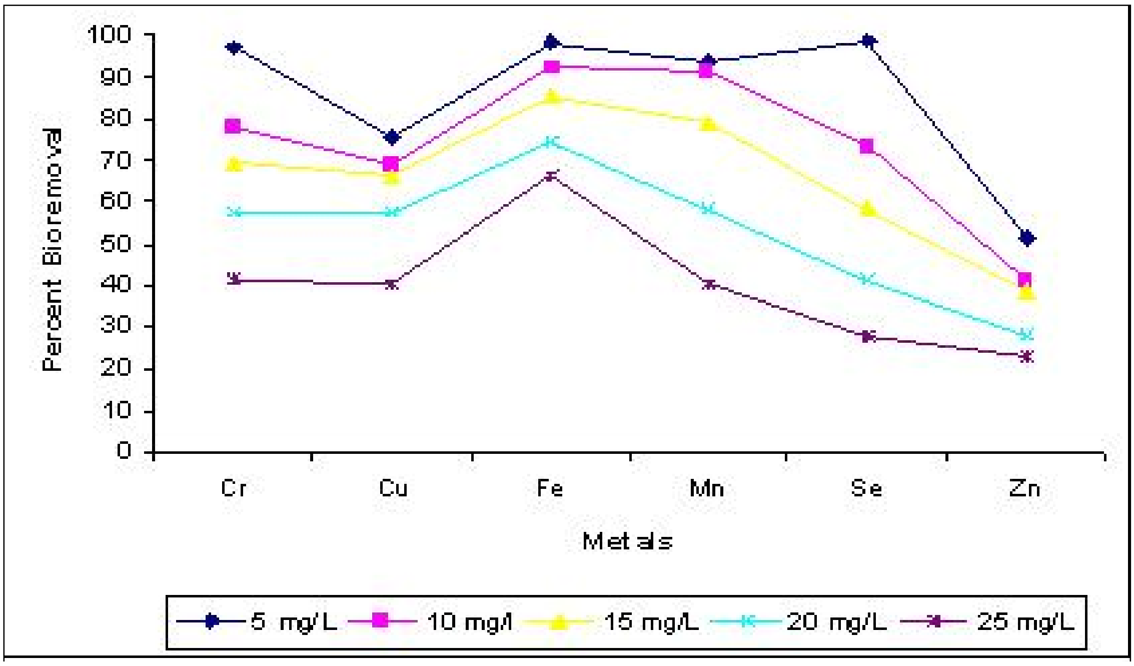

Fig. 2 : Percent bioremoval of metals by *Anabaena subcylindrica* from aqueous solution.

remove each heavy metals, we converted % removal into removal index by dividing % removal by the highest % removal and multiply the value obtained by 10. This would facilitate the comparison of each heavy metal removal ability by various microalgae. **Table 3** was very helpful as it clearly denotes the effectiveness of each alga in removing the each metals viz. Cr, Cu, Fe, Mn, Se and Zn from aqueous solution.

Table 3. Heavy metals removal (at 5 mg/L) in aqueous solution by *Nostoc muscurom* and *Anabaena subcylindrica*.

	Percent removal						Removal index					
Algae	Cr	Cu	Fe	Mn	Se	Zn	Cr	Cu	Fe	Mn	Se	Zn
Nostoc muscurom	98.70	74.13	98.73	98.26	99.12	73.20	10	9.79	10	10	10	10
Anabaena subcylindrica	97.00	75.66	98.00	93.53	98.93	51.53	9.82	10	9.92	9.51	9.98	7.03

In the present investigation both the algal strains showed highest metal removal index to all the tested metals except Cu and Zn. 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 [19,20-25]. In other studies, blue green algae had lower capacities than those found herein. For example, the blue green algae *Aphanothece halophytica and Spirulina platensis* could remove only 22% and 35% Pb, respectively, from battery factory wastewater[26,27]. 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[28]. 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.

Study indicates that both algae are able to remove the metals from aqueous solution at lower concentrations. At higher concentration there might be toxicity of metals which can reduce the biosorption capacity of the microorganisms. It has been described that microalgae can protect themselves against the toxicity caused by heavy metals using several approaches: exclusion mechanisms, adsorption to cell surface or intracellular (segregated) accumulation[1,29-30]. Because of the lack of published data regarding metal removal by *D. pleiomorphus*, results reported in others studies, with similar microalgae were chosen for the sake of comparison. Some researchers stated that small amounts of cellular Zn were observed in C*hlorella kesslerii* cells[30], whereas some described that Chlorella sp. accumulated intracellular the highest proportion of Zn removed from the medium in the range 0.1-2 mg/L[30] and some reported, in turn, that *Closterium lunula* cells did not undergo damage in the presence of 50 µg Cu/L, owing to their ability to exclude this metal[31-34].

CONCLUSION

Biomass of both the algal strains of Nostoc muscurom and Anabaena subcylindrica proves efficient towards removal of Cr, Cu, Fe, Mn, Se and Zn ions, to extents comparable to those reported for other algae, thus unfolding the potential of those strains for water and wastewater treatment processes.

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