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Studies on Zinc(II)-Biosorption Capability of a Filamentous Green Algal Species (*Mougeotia viridis*) Isolated from Electroplating Wastewater

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Abstract. The biosorption capacity of zinc(II) by a filamentous green alga *Mougeotia viridis*, isolated from the wastewater discharged by electroplating industry was tested under laboratory conditions as a function of contact time, pH, and initial metal ion and biomass concentrations. Optimum pH value for zinc(II) biosorption was determined as 5.0. At 10 mg l^{-1} zinc(II), the biosorption equilibrium with 1 g l^{-1} algal biomass was reached in 10 min showing a relative efficiency of 59.24% metal removal. When the biosorbent biomass was increased to 2 g l^{-1}, the metal removal capacity was enhanced to 81.4%. At optimum conditions, zinc(II) uptake increased with the increase in initial metal ion concentration from 5-300 mg l^{-1}. Langmuir isotherm model was found to be suitable for describing the biosorption of zinc(II) by *Mougeotia viridis*. The q_{max} obtained experimentally and theoretically calculated from the Langmuir isotherm model at the regression coefficient value of 0.9975, respectively, were 27.7 mg and 26.71 mg zinc(II) g^{-1} algal biomass, at the initial metal concentration of 200 mg l^{-1}. In five repeated biosorption-desorption cycles, the zinc(II) desorption from the metal-loaded algal biomass remained around 99%. The observations reported in the present study indicate that the alga can be used as an efficient biosorbent for the removal of zinc(II) from aqueous solution.

Keywords: filamentous microalga, metal biosorption, zinc(II) adsorption, heavy metals, wastewater treatment, *Mougeotia viridis*

Introduction

Heavy metal contamination is a serious threat to global ecosystems (Gong *et al*., 2005). The main sources of heavy metal contamination include mining, milling, electroplating, tanneries, polymers and plastics, agrochemicals, and surface finishing industries, discharging a variety of toxic metals such as cadmium, copper, nickel, cobalt, zinc and lead into the environment (Malik, 2004). These metals, even at low concentrations, are toxic to living organisms including human beings (Sheng *et al*., 2004). Various treatment procedures are used to remove such heavy metal contaminants from effluents and industrial wastewaters before discharging into natural water bodies (Xiangliang *et al*., 2005). The most often used treatment technologies include chemical precipitation and coagulation, ion-exchange, reduction, osmosis and reverse osmosis, membrane processes, and electrolytic technologies (Chong *et al*., 2000; Aksu *et al*., 1998; Yetis *et al*., 1998; Kapoor and Viraraghavan, 1995; Wilde and Benemann, 1993). However, conventional treatment technologies, like chemical precipitation and coagulation, become less cost-effective when high effluent volumes and low metal concentrations are encountered (Kratochvil and Volesky, 1998; Kapoor and Viraraghavan, 1995). Precipitation process, furthermore, has other disadvantages, such as the generation of toxic sludge (Krishnan *et al*., 1992). The application of membrane technology and activated carbon are also prohibitive due to high operational cost (Xiangliang *et al*., 2005; Kapoor and Viraraghavan, 1995). As a result of these shortcomings, the foregoing concerns have led to an interest in the development of safe technologies that can reduce heavy metal concentrations to environmentally acceptable levels at affordable cost (Aksu, 1998; Atkinson *et al*., 1998). Biosorption, based on the metal binding capacities of various biological materials, has gained attention during recent years due to its high efficiency and cost-effectiveness, particularly at low metal concentrations (Andrade *et al*., 2005; Pagnanelli *et al*., 2003; Sag *et al*., 2001). Included in the range of biological materials used for this purpose are marine macroalgae (seaweeds) and fresh water algae (Davis *et al*., 2000; Aksu and Acikel, 1999; Yu *et al*., 1999), fungi (Bai and Abraham, 2002), bacteria (Karana *et al*., 1999), and bioindustrial and agrowastes (Saeed *et al*., 2005; Saeed *et al*., 2002). These materials have proven to be environmentally safe and extremely cheap for removing heavy metals from contaminated waters (Xiangliang *et al*., 2005).

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Various biological materials such as microalgae possess high metal-binding ability due to their large surface areas in addition to high metal binding affinity of their cell walls, which are composed of fibre-like and amorphous embedding matrix of various polysaccharides where both electrostatic attraction and complexation can play a role (Sheng *et al*., 2004; Schiewer and Volesky, 2000; Kuyucak and Volesky, 1989). Several functional moieties such as carboxyl, hydroxyl, amines, phosphates, sulfates are present in the cell wall, which act as binding sites for metals (Al-Qunaibit *et al*., 2005).

Although several algal species have been identified, yet very few have been investigated for their ability to sequester heavy metal ions. Most of these studies, furthermore, have been done on strains that are easily available from academic and commercial culture collections. These studies have shown that the biomass of these algal species have the potential of application as effective toxic metal biosorbents. Nevertheless, the need to explore the metal removal ability of a wider diversity of available algal species, particularly those isolated from metal contaminated wastewaters, remains significant. The present study is the first report of metal biosorption by a local strain of a filamentous green alga isolated from the wastewaters discharged from an electroplating industry in Pakistan. The algal isolate was thus understood to have acclimatized to metal-loaded habitat. Investigations on this filamentous alga, *Mougeotia viridis*, were carried out to determine the optimum operational conditions for the sequestering of zinc from contaminated waters. The adsorption capacity was evaluated from equilibrium adsorption isotherms, and results indicate that the filamentous alga is an efficient material for the development of a high capacity biosorbent for heavy metal removal from aqueous media. The study is of significance as no previous study has been reported in literature on the capacity of *M. viridis* to biosorb zinc(II) and an addition to the present knowledge of only a few filamentous algae reported of having the affinity to sequester metal ions.

Materials and Methods

Organism and growth medium. A filamentous green algal strain, growing in metal-rich environment, was isolated from wastewater bodies contaminated with the effluents discharged from an electroplating industry in the vicinity of Lahore, Pakistan. The algal culture was maintained in test tube slants of Bold's basal agar medium (Nichols and Bold, 1965) at 25 ±2 ^{o}C under continuous illumination with cool-white fluorescent light at the intensity of 50 μE^{-2} s^{-1}. The isolate was identified as a filamentous species *Mougeotia viridis* (Chlorophyta: Zygnemataceae) by Dr. F. M. Sarim, Professor of Botany, University of Peshawar, Pakistan. Biomass for the inoculum and metal sorption was prepared by growing axenic algal culture to exponential phase of growth in 100 ml Bold's medium contained in 250 ml Erlenmeyer flasks shaken at 100 rpm and maintained at 25 ±2 ^{o}C under continuous illumination with cool-white light at the same intensity as for algal culture maintenance. The filamentous algal biomass was harvested, washed with deionized water, and freeze dried at -58 ±2 ^{o}C (Eyela Freeze Dryer FD-550) for metal sorption studies.

Biosorption investigations. Metal sorption studies were carried out on solutions made from standard stock solution of zinc(II) (nitrate salt in HNO_3 0.5 mol l^{-1}), concentration 1000 ±2 mg l^{-1} (Merck Ltd., UK), which was diluted in ion-free double distilled water to the desired metal concentrations. The pH of metal solution was adjusted to 5.0, unless otherwise stated, using 0.1 M NaOH. Fresh dilutions were made for each study. Biosorption capacity of the filamentous algal biomass was determined by contacting 100 ml of the metal solution of known concentrations (5-300 mg l^{-1}) in 250 ml flasks. The filamentous algal biomass, along with the metal solution, was incubated in tightly stoppered flasks on an orbital shaker at 100 rpm. The flasks were shaken for 1 h, unless otherwise stated, for determining the optimum metal-biomass contact time for biosorption. The algal biomass was separated from metal solution by centrifugation at 5,000 rpm for 5 min. Concentration of the metal remaining in solution was determined using atomic absorption spectrophotometer (UNICAM-969, UK). The effect of time period was noted for determining the sorption equilibrium time by contacting the biosorbent algal biomass with the metal solution for 5-120 min. The effect of pH on the sorption of metal ions by the filamentous algal biomass was determined at different pH values of 2, 3, 4, 5, 6 and 7. The quantity of biomass was varied from 0.25 g l^{-1} to 2.0 g l^{-1} to determine the effect of algal biomass on metal biosorption. This study was done to determine the limiting effect of the quantity of algal mass for the biosorption equilibrium. Metal-free solution and algal biomass-free metal solution were used as blanks and controls, respectively. Analyses were done on triplicate samples and the results reported are the mean values of three separate experiments. Statistical analyses were done to determine standard deviation in accordance with Steel and Torrie (1996).

Desorption studies. The metal-laden filamentous algal biomass was separated from metal solution by centrifugation, transferred into screw-capped test tubes containing 10 ml of 0.1 M HCl, and shaken on test tube rotator at 100 rpm for 30 min. The filamentous algal biomass was separated by centrifugation at 5,000 rpm for 5 min. The supernatant was analyzed for zinc(II) released in 0.1 M HCl for determining the efficiency of desorption by atomic absorption spectrophotometer. After desorption of zinc(II), the algal biomass was washed with double distilled water to regenerate it and transferred to fresh zinc(II) solution for the next biosorption cycle. Biosorption-desorption studies were done for five repeated cycles.

Results and Discussion

Appropriate conditions for the removal of zinc(II) from aqueous solutions by *Mougeotia viridis* were studied in detail. The parameters investigated were the effect of contact time, pH, and metal ion and biosorbent concentrations. Evaluation of the potential application of zinc(II) removal by *M. viridis* for wastewater treatment was investigated in several repeated sorption-desorption cycles.

Effect of contact time. The effect of time on the biosorption of zinc(II) by *M. viridis* biomass is shown in Fig. 1. For this purpose 10 mg l^{-1} zinc(II) solution was contacted with 1 g l^{-1} algal biomass. Biosorption equilibrium was achieved rapidly within the first 10 min, which was 5.92 mg zinc(II) g^{-1} algal biomass. The relative efficiency of zinc(II) removal, at the active phase of biosorption equilibrium, was 59.24%. This active phase of biosorption was followed by a very short passive sorption phase, which showed only insignificant incremental difference to the first phase. The completely stable equilibrium, comprising of both the active and passive phases of sorption, was achieved after 20 min of zinc-algal biomass contact which was 5.94 mg zinc(II) g^{-1} algal mass.

Effect of pH. The effect of different pH values on the biosorption of zinc(II) by *M. viridis* is shown in Fig. 2, from which it is evident that maximum zinc(II) biosorption occurred at pH 5. The higher rate of metal biosorption in the slightly acidic conditions may be attributed to the adjustment of the *M. viridis* strain to its natural growing habitat in the acidic contaminated water bodies from which it was isolated.

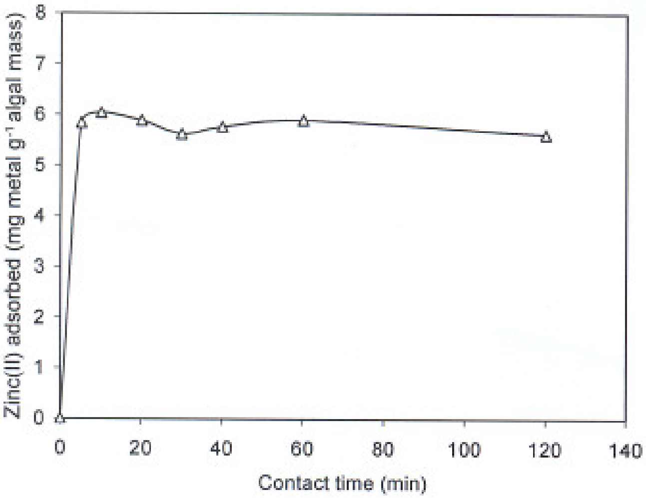

Fig. 1. The time course of the biosorption of zinc(II) from 10 mg l^{-1} metal solution, pH 5, by 1 g l^{-1} biomass of *Mougeotia viridis*.

Effect of biosorbent concentration. Increase in the algal biomass from 0.25 g l^{-1} to 2 g l^{-1} was associated with decrease in zinc(II) biosorption from 10 mg l^{-1} metal solution for the contact time of 30 min as shown in Fig. 3. This decrease in zinc(II) biosorption was quite steep when algal biomass was increased up to 1 g l^{-1}, which however tended to level off when the biomass was further increased up to 2 g l^{-1}. When these values

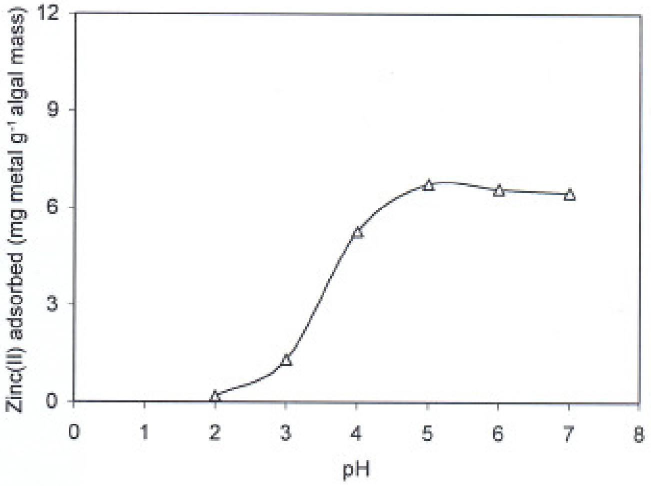

Fig. 2. The effect of pH on the biosorption of zinc(II) from 10 mg l^{-1} metal solution by 1 g l^{-1} biomass of *Mougeotia viridis* as the biosorbent in shake flasks at 100 rpm during the contact period of 30 min.

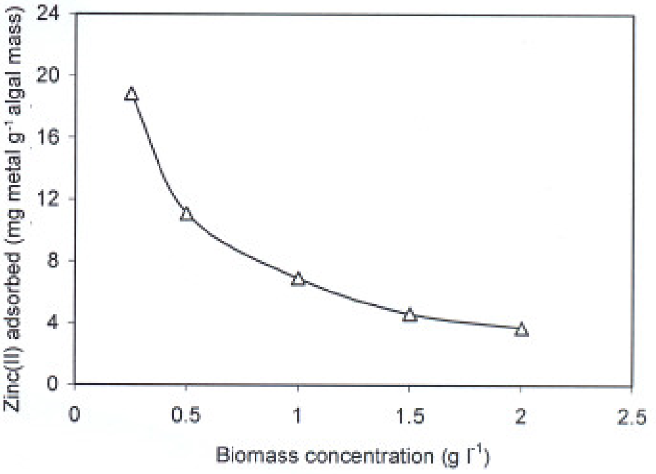

Fig. 3. The effect of biomass quantity of *Mougeotia viridis* on biosorption of zinc(II) from 10 mg l^{-1} solution, pH 5, in shake flasks at 100 rpm during the contact period of 30 min.

were converted to percentage zinc(II) sorption, 1 g l^{-1} seemed to be near optimum quantity of biomass for equilibrium stage with 83.2%. Further increase in algal biomass resulted in only minor increase in zinc(II) sorption as indicated by sorption ability at 2 g l^{-1} of 86.4%.

Maximum biosorption capacity. For determining the maximum biosorption capacity of zinc(II) by *M. viridis*, the initial metal concentration was varied between 5 mg l^{-1} to 300 mg l^{-1}. The sorption equilibrium for zinc(II) is shown in Fig. 4, which was achieved at 200 mg l^{-1} with the maximum sorption (q_{max}) of 27.7 mg zinc(II) g^{-1} algal mass. Conversion of the data at different metal concentrations to the Langmuir adsorption isotherms equation showed the q_{max} value of 26.71 mg g^{-1}. The calculated value was very close to the experimental value. The biosorption data were thus found to fit perfectly to the Langmuir adsorption isotherms model, as evident from the linear isotherm with the regression co-efficient (r^2) value of 0.9975 (Fig. 5).

Regeneration efficiency. Efficient desorption of the metal-loaded biosorbent for repeated use is an important attribute for the potential of the biosorbent for large scale or commercial applications. Regeneration efficiency of the metal-laden *M. viridis* was investigated in several repeated sorption-desorption cycles. Higher biosorption and desorption efficiency of zinc(II) was observed in the first cycle (Fig. 6). However, the net difference in elution and decline of zinc(II) at the end of each cycle was insignificant (Duncan's multiple range test, $p = 0.05$), when compared to net biosorption in the corresponding cycles. Therefore, the biosorption efficiency may appear to decrease from 100% to 78.11% during the operation of five cycles due to slightly lesser quantity of zinc(II) eluted after each time. Loss in weight was also determined during the adsorption-desorption cycles as shown in Table 1. From the tabulated results it can be argued that the decrease in biosorption capacity of the biosorbent may be attributed to the loss in biomass during repeated cycles. The losses in biomass during adsorption-desorption cycles are believed to be due to the repeated mixing by shaking and separation of cells by centrifugation. This loss of biomass can be reduced by immobilizing the filamentous alga in some suitable biomatrix as reported previously for other microorganisms (Iqbal and Edyvean, 2005; Akhtar *et al*., 2003).

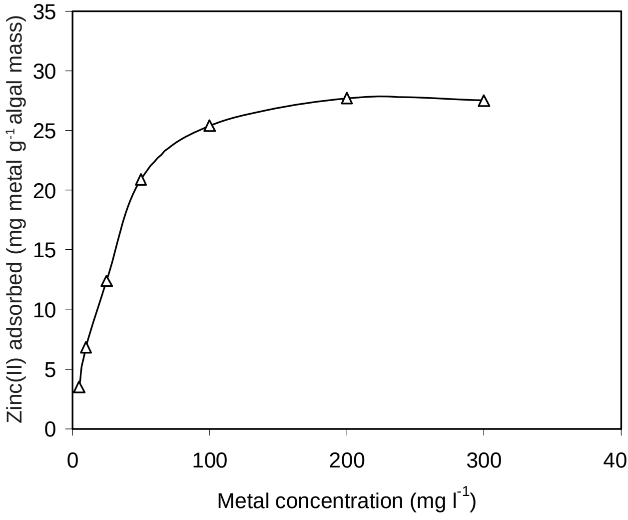

Fig. 4. The effect of initial concentration of zinc(II) solution, pH 5, by 1 g l^{-1} biomass of *Mougeotia viridis* during 30 min biosorbent-metal contact in shake flasks at 100 rpm.

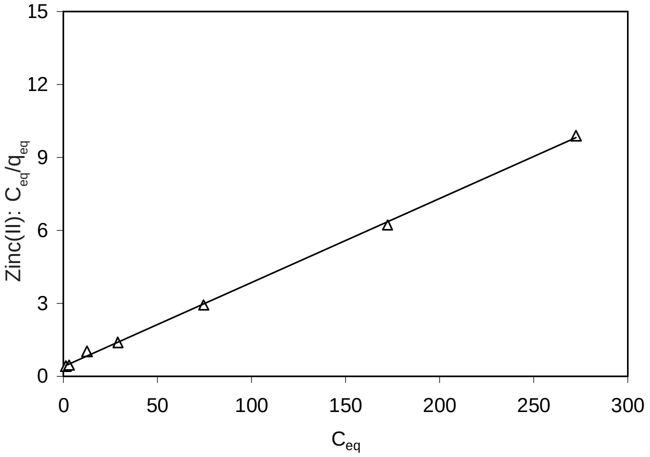

Fig. 5. The Langumir adsorption isotherms for zinc(II) biosorption by 1 g l^{-1} biomass of *Mougeotia viridis*; conditions: initial metal concentration range 5-300 mg l^{-1}, pH 5, flask shaking at 100 rpm during 30 min of contact, where q_{eq} is the metal biosoerbed g^{-1} algal mass and C_{eq} is the equilibrium metal concentration l^{-1}.

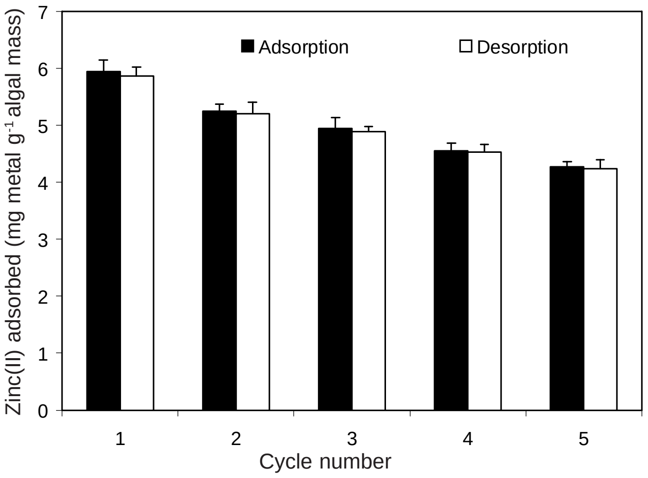

Fig. 6. Biosorption and desorption of zinc(II) by *Mougeotia viridis* in five repeated cycles.

Table 1. Biosorption and desorption of zinc(II) in batch cycles by *Mougeotia viridis*; conditions: algal biomass 1 g l^{-1}, 100 ml of 10 mg l^{-1} zinc(II) solution, contact time of 30 min at 100 rpm flask shaking, 10 ml of 0.1 M HCl desorbent, desorption time 30 min at 100 rpm flask shaking

Cycle no.	Metal biosorbed (mg zinc(II) g^{-1} algal biomass)	Metal desorbed (mg)	Desorption efficiency (%)	Biosorption decline (%)	Loss in biomass (%)
1	5.95	5.86	98.6	-	-
2	5.25	5.21	99.2	11.76	8.81
3	4.95	4.89	98.9	16.81	6.14
4	4.55	4.53	99.5	23.52	5.14
5	4.27	4.24	99.3	28.23	5.23

Conclusions

The maximum removal of zinc(II) by *Mougeotia viridis* was 26.71 mg g^{-1} algal biomass. This report adds to the recently known biosorption capacities of other filamentous algae of chromium(VI) by *Spirogyra* sp. of around 14.7 mg per g biomass (Gupta *et al*., 2001), and copper(II) by *Ulothrix zonata* of around 39.20 mg per g biomass (Nuhoglu *et al*., 2002). The results of the present study have indicated that the biomass of *M. viridis* is a suitable biosorbent for the development of a system for both the efficient removal (biosorption) and recovery (desorption) of heavy metals from wastewater.

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