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BIOSORPTION OF CD^{2+}, CU^{2+}, NI^{2+}, ZN^{2+} AND CR^{6+} CATIONS BY GREEN ALGA *SCENDESMUS OBLIQUUS*

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Abstract

Experiments were conducted comparing the individual removals of cadmium, copper, nickel, zinc and chromium from water via biosorption using *Scendesmus obliquus*, a common green alga. The goal was to characterize the biological treatment of water contaminated with heavy metals using life aquatic species and the effect of different concentrations of these metals on chlorophyll a content. In addition, experiments were performed to measure cell viability as a function of metal concentration and also to compare removal using living cells to nonliving ones. The data indicate that both living and nonliving cells of *Scendesmus obliquus* removed nearly all heavy metals from water, living alga cells significantly outperformed nonliving one in case of Cd metal removal. The alga exhibited high uptake capacities for five metal ions. Maximum Cr (VI) removal reached around 97%, Cu (II) removal was at its maximum value which reached 98.5 % of its original initial concentration, as for Cd (II), Ni (II) and Zn (II), similar removal ratios were obtained ranging between 88% and 99.4% .

Keywords: Biosorption, *Scendesmus obliquus*, heavy metals, toxicity.

Introduction

The disturbance of aquatic ecosystems provoked by heavy metals pollution from industrial and domestic sources has as consequence the loss of biological diversity, as well as increased bioaccumulation and magnification of toxicants in the food chain (He *et al*., 1998). Toxic heavy metal contamination of industrial water is a significant universal problem. They accumulate in living tissues throughout the food chain, which has humans at its top. These toxic metals can cause accumulative poisoning, cancer and brain damage when found above the tolerance levels. The use of biological processes for the treatment of metal enriched wastewaters can overcome some of the limitations of physical and chemical treatments and provide a mean for cost- effective removal of metals. A great deal of interest has recently been generated using different kinds of inexpensive biomass for adsorbing and removing heavy metals from wastewater (Volesky and Holan, 1995). In this context, accumulation of metals by microorganisms, including algae, has been known for a few decades, but has received increased attention only in recent years because of its potential for application in environmental protection or recovery of precious or strategic metals (Tsezos, 1985, 1986; Volesky, 1987; Malik, 2004).

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Metal accumulation capacity of algal biomass is either comparable or sometimes higher than chemical sorbents. Biosorption, the process of passive cation binding by dead or living biomass, represents a potentially cost-effective way of removing toxic metals from industrial waste waters (Volesky, 1990). Biosorption could be employed most effectively in a concentration range below 100mgl^{-1}, where other techniques are ineffective or costly (Schiewer and Volesky, 1995).

Efforts made to use the algae and aquatics plants, moss and ferns for heavy metals include the use of silica –immobilized algae *Chlorella pyrenoidosa* at low pH for Cr (VI) (Greene *et al*.,1987) ; *Chlamydomonas rheinhardii* for Cu (II) and Cd (II) (Xue *et al*.,1988) pretreated biomass of nine common species of marine macro algae for lead(II),copper(II)and cadmium(II) (Yu and Kaewsarn, 1999).

Although dead algae have been utilized successfully in heavy metal adsorption experiments (Leusch *et al*., 1995; Holan *et al*., 1998), living algae may be more advantageous due to metabolic uptake and continuous growth.

The response of algae cells to metal exposure is essential to determining the viability of biological treatment. Responses of algae cells to metal exposure are typically measured in terms of cell density, biomass, growth rate, chlorophyll content or absorbance. Carr *et al*. (1998) studied the inhibitory growth effect of cadmium on the unicellular algae *Chlorella vulgaris* and found that the 48h 50% cell growth inhibition was 15.7 mg/L cadmium. Photosystems of algae can be damaged by excessive amounts of cadmium resulting in a reducing of photosynthetic pigments, such chlorophyll levels. In addition, high cadmium concentrations reduce cell size and cause a decrease in growth (Leborans and Novillo, 1996). Prasad *et al*. (1998) has shown that 11mg/L cadmium linearly reduces chlorophyll a in *Chlamydomonas reinhardtii* in some cases by 76%. Nassiri *et al*.(1997) found no growth inhibition at cadmium concentrations <1mg/L, but at 2mg/L *Tetrselmis suecica* had 10% growth inhibition after 4 days and 30% and 50% growth inhibition in solutions containing 5 and 10 mg/L cadmium, respectively.

The aim of this work was to assess that the efficiency of the green alga; *Scendesmus obliquus* to remove chromium, nickel, copper, zinc and cadmium from the water. Cell viability is measured as chlorophyll a level and cell counting before and after exposure. Heavy metals removal is measured by the quantity remaining in solution after 72 h exposure.

Materials and Methods

Scendesmus obliquus in all experiments was obtained from the author's collection, Women's College, Ain shams university, Egypt and grown in the laboratory in modified Bold's basal medium (Bischaffi and Bold, 1963). Prior to characterizing metal removal, experiments were preformed to determine the effect of metal concentration on cell viability of chlorophyll a. Pre- exposure and post-exposure chlorophyll a was measured following 72 h metal exposure. Chlorophyll a was determined according to APHA (1995). Copper, in the form of copper sulphate was added to 100 ml flasks at concentrations 0, 1, 3, 5, 7, 10, 15 and 20mg/L, respectively. Chromium in the form of potassium dichromate, cadmium in the form of cadmium chloride, zinc in the form of zinc sulphate and nickel in the form of nickel chloride were added to separate sets of flasks for each and in the same series of concentrations. Exposure tests for each of these metal concentrations were performed with *S. obliquus* with initial cell number 54 x 10^6cells/L. Algae controls were run with no metal to compare the natural chlorophyll a decline to that resulting from tested heavy metals.

After 72 hours of the addition of tested heavy metals, the growth was determined by counting the organisms on Rafter cell under a binoclear microscope and Chlorophyll a content. The tests were carried out in triplicate and in axenic conditions with a 16 –h light and 8- h dark photoperiod.

Metal removal experiments were performed in batch tests. For each experiment 100 ml glass flasks were filled with 50 ml media containing 54 x 10^6 cells/L of *Scendesmus obliquus* in triplicate. To compare the quantity of each tested heavy metal removed by lived cells to that removed by nonliving cells. Cultures of the tested alga in the two cases were subjected to similar metal concentrations. Nonliving alga samples were obtained by heating live alga samples to a fatal temperature. All tested heavy metals were added to specific flasks at concentrations of 0, 1, 3, 5, 7 and 10 mg/L. Residual tested heavy metals in water were filtered and analyzed using plasma optical emission mass spectrometer (POEM III) using Merk (multi element 1000ppm) as standard solution.

Results and Discussion

Cell viability

Table (1) compare the initial Chlorophyll a levels and cell number of *S. obliquus* those following 72h exposure to 0, 1, 3, 5, 7,10, 15and 20 mg/L of copper, cadmium, zinc, chromium and nickel. The results show clearly the inhibitory and/or stimulatory effects of each of the heavy metals used depend on concentration. Higher doses of these metals affected strongly and adversely all growth parameter tested. Lowest values of chlorophyll a content and cell number were recorded at 20 mg/L of cadmium or nickel. The results also showed that inhibitory effect of cadmium on algal growth and chlorophyll a content is more pronounced than that of the other tested heavy metals.

Table 1: Effect of different concentrations of Cd^{2+},Cu^{2+} , Zn^{2+} ,N^{2+} and Cr^{6+} on chlorophyll-a content and cell number (No.x10^6 / L^{-1}) of *Scendesmus obliquus*

Metal Conc. mg/L	Cd		Cu		Zn		Ni		Cr	
	Chlorophyll a content	Cell number No x10 / L^{-1}	Chlorophyll a content	Cell number No x10 / L^{-1}	Chlorophyll a content	Cell number No x10 / L^{-1}	Chlorophyll a content	Cell number No x10^6/ L^{-1}	Chlorophyll a content	Cell number No x10^6/ L^{-1}
Control	0.77	54.00	0.77	54.0	0.77	54.0	0.77	54.0	0.77	54.0
1	0.69	49.00	0.68	37.3	0.89	15.3	0.89	64.0	0.44	52.0
3	0.36	38.00	0.61	36.0	0.56	107.0	0.86	38.0	0.32	38.0
5	0.25	26.70	0.53	35.0	0.44	78.0	0.80	28.7	0.31	34.0
7	0.17	25.30	0.42	28.0	0.33	43.3	0.40	16.0	0.28	28.0
10	0.14	16.00	0.37	19.3	0.14	33.3	0.31	15.3	0.25	24.0
15	0.13	12.00	0.23	16.0	0.18	28.0	0.16	12.0	0.14	20.7
20	0.10	6.70	0.10	12.0	0.13	20.7	0.12	8.7	0.12	18.7
LSD at 0.05	**0.074**	**4.50**	**0.10**	**5.3**	**0.082**	**6.80**	**0.10**	**7.3**	**0.12**	**8.10**

The inhibitory effects of heavy metals on pigment accumulation, observed in this investigation particularly at higher doses, may be attributed to inhibition in reductive steps in their biosynthetic pathway (De Filippis *et al*., 1981). Heavy metals can induce oxidative stress in chloroplasts of the unicellular alga *Gonyaulax*, particularly under acute conditions in addition to oxidative damage to proteins and lipids occurred in cells. The observed concentration dependent reduction in chlorophyll and cell count is in a good agreement with the findings of (Hofner *et al*., 1987; Rai *et al*., 1991; Fathi *et al*., 2000 and Sponza, 2002).

Metal removal

Figure 1 from (a - e) show the percentage of heavy metals removal by living and nonliving cells of green algae *Scendesmus obliquus* at different metal concentrations. The experiments were performed with 54 x 10^6 cells/L of *S. obliquus*. The results from triplicate samples revealed that the average residual metal concentrations for the nonliving and living algae varying according to metal concentration and its type. At 1mg/L initial zinc concentration living *S. obliquus* reduced the metal to 0.11 mg/L, while the nonliving reduced the metal to 0.13 mg/L (Fig .1a). For copper, the residual was reduced from 1mg/L to 0.15 mg/L and 0.1 mg/L (Fig.1b). In case nickel metal, the 1mg/L initial concentration is reduced to 0.046 mg/L by living cells and 0.063 mg/L by nonliving cells of *Scendesmus obliquus* (Fig.1c). The concentration of chromium residual reduces from 1mg/L to 0.078 and 0.138 mg/L by living and nonliving cells respectively (Fig.1d). In contrast, nonliving cells removed 93.4% from cadmium metal (1mg/L concentration) while the living ones removed only 88% of metal only (Fig.1 a - e), this was in agreement with Mehta and Gaur (2005), they stated that dead cells of algae sorbs more metal than live cells

Conclusion

Experiments were performed to characterize the biosorption of zinc, copper, chromium, nickel and cadmium from water using *Scendesmus obliquus* common green algae. Chlorophyll a measurement and cell count were performed at varying metal concentration to determine the effect of each metal on cell viability. Most metal concentrations decrease chlorophyll a and cell number. Different metals biosorption experiments were performed on both living and nonliving *S. obliquus* for metal concentrations of 0, 1, 3, 5, 7 and 10 mg/L. It was found that the living cells of alga were more efficiently for removing metals from solution than the nonliving ones at low metal concentration except in case of cadmium metal the nonliving cells were more effective at this concentration and at all other concentrations.

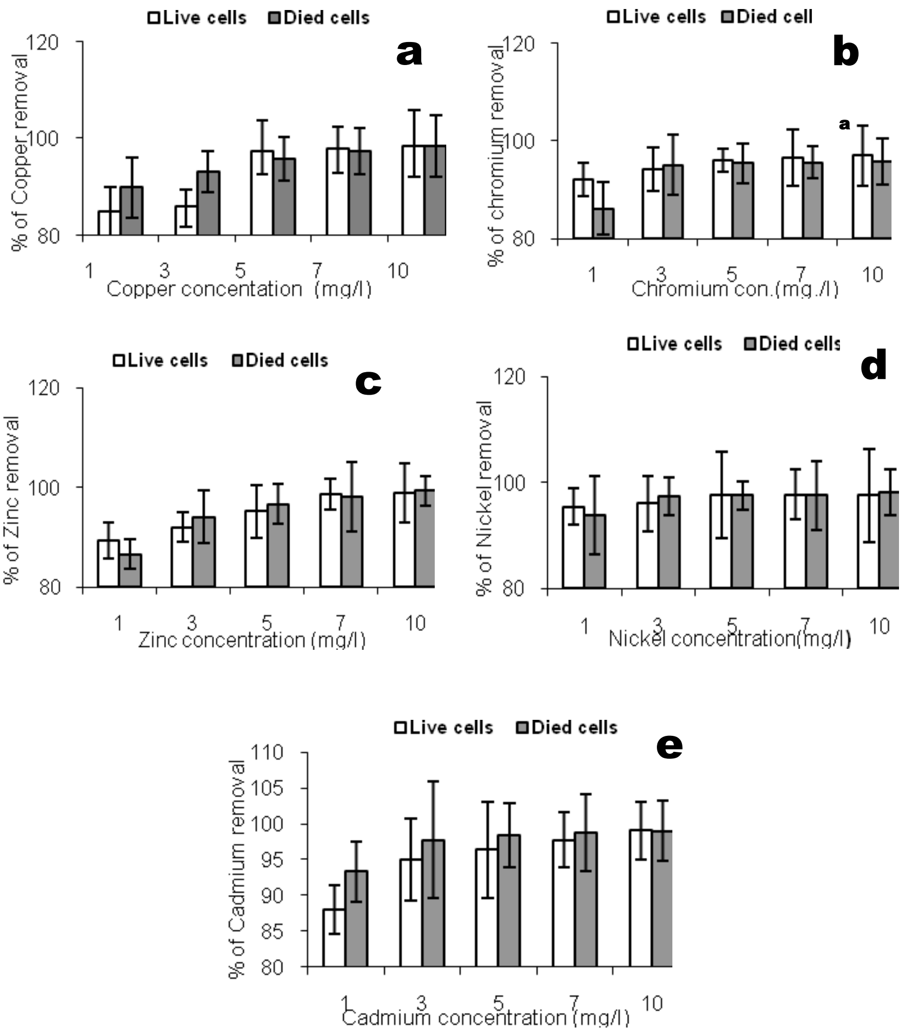

Figure 1(a – e): The percentage of heavy metals removal (Cu, Cr, Zn, Ni and Cd) by living and nonliving cells of green alga *Scendesmus obliquus*.

Hence, it is possible to remove zinc, copper, chromium, nickel and cadmium from water by *Scendesmus obliquus* using living cells and nonliving cells. Living cells were more effective for removing zinc, copper, chromium, nickel while in case of cadmium metal nonliving cells come first.

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الأمتصاص الحيوي لكاتيونات الكادميوم والنحاس والنيكل والزنك والكروم بواسطة الطحلب الأخضر سندزمس اوبليكس

عليه انور الشيمي
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لتوضيح ذلك اجريت عدة تجارب لمقارنة الأمتصاص الحيوي لكاتيونات الكادميوم والنحاس والنيكل والزنك والكروم بواسطة الطحلب الأخضر سندزمس اوبليكس اجري هذا البحث لدراسة كيفية المعالجة الحيوية للمياة الملوثة بالعناصر الثقيلة. ايضا تمت دراسة تأثيرالتركيزات المختلفة لهذه العناصرعلي محتوي الكلورفيل أ و حيوية الخلايا لهذا الطحلب. دلت النتائج علي:

1- ان الخلايا الحية كانت اكثر كفاءة للتخلص من عناصر النحاس والنيكل والزنك والكروم مقارنة بالخلايا الغيرحية.
2- ان الخلايا الغير حية كانت اكثر كفاءة للتخلص من عنصر الكادميوم مقارنة بالخلايا الحية.
3- كانت قدرة الطحلب علي امتصاص هذه العناصر عالية وقدرت بنسبة 97 % للكروم 98.5 % للنحاس وتراوحت هذه النسبة بين 88 % و 99.4 % لكل من الكادميوم والنيكل والزنك.