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USE OF MICROALGAL BIOMASS FOR THE REMOVAL OF HEAVY METALS FROM AQUEOUS SYSTEMS

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Abstract

A number of microalgal species isolated from untreated industrial wastewater containing bodies were screened for their ability to remove heavy metals from contaminated water. A blue green alga *Phormidium* sp. was noted to have excellent metal removing ability. The alga removed 98.4, 64.1, 96.6, 86.4, 84.3 and 88.2% of Pb^{2+}, Ni^{2+}, Cd^{2+}, Cr^{3+}, Cu^{2+} and Zn^{2+}, respectively, from aqueous metal solutions. The metal uptake process was extremely rapid with, >95% of the sorption completed within 5 minutes. Reuse of algal biomass as a biosorbent was possible as the bound metals, except chromium, desorbed with 0.1 M HCl. These observations indicate the potential of an endemic strain of *Phormidium* sp. for application as an efficient biosorbent for the removal of toxic metals in aqueous wastes at low concentrations.

Introduction

Pollution of the environment by toxic metals is a result of many man-made activities, largely industrial, although sources such as agriculture and sewage disposal also contribute. These pollutants are discharged or transported into the atmosphere, aquatic and terrestrial environments and may reach high concentrations, especially near sites of entry. Effects of metals on ecosystem function vary considerably and are of economic and public-health significance. Consequently, environmental awareness is growing among consumers and industrialists, and legal constraints on emission are becoming increasingly strict, leading to a need for cost-effective emission control.

Many algae, bacteria, fungi or higher plants that remove and/or accumulate large amounts of heavy metals have been reported (Sandau *et al.*, 1996; Kapoor and Viraraghavan, 1997; Lu and Wilkins, 1996; Chang *et al.*, 1997). Most studies on the use of microorganisms for the removal of metal ions from their external environment have been done on easily available biomass from fermentation industrial waste or marine algae. These studies showed that whereas these biomass have possibilities of application as effective biosorbents for toxic metals, their abilities were restricted owing to limitations of species available.

To develop an efficient and low-cost method for the elimination of metal pollutants, it is important to search for microorganisms that remove and accumulate toxic metals efficiently. To this end, a screening program for the selection of most efficient strain of microalgae with highest metal removing capacity was conducted. The objective of the present study was to isolate and screen a number of metals sequestering microalgal species and select a more suitable strain capable of detoxification, biosorption and recovery of heavy metals. As a result of these investigations, a very efficient strain of blue green microalga *Phormidium* sp.,

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capable of removing most of the toxic metals, has been isolated from wastewater containing bodies. The results of these investigations are reported here.

Materials and Methods

Algal Strains and Cultivation: Wastewater samples were collected in sterile glass bottles from different sites of untreated industrial effluent containing bodies of Lahore-Kasur and Lahore-Sheikhupura regions. Isolation of microalgae from wastewater samples was carried out using the method of Hoshaw and Rosowski, 1979. Out of the initial 24 isolates, 10 isolates, namely, PTCC-WA-02, 05, 06, 07, 11, 13, 16, 17, 18 and 20 with higher metal binding capacity, were selected and compared for their metal adsorption ability. Isolate PTCC-WA-05 showed the best metal sequestering ability and was identified as *Phormidium* sp. This species was cultivated in batch culture using 100 ml inorganic DSN medium (Pohl *et al.*, 1987) contained in 250 ml Erlenmeyer flasks at 30±2 ^{o}C and maintained under continuous illumination with fluorescent cool-white light at 25μmol photon m^{-2} s^{-1}. For detailed biosorption studies the alga was cultivated in 10 l aspirator jars containing 8 l DSN medium under continuous illumination at 75 μmol photon m^{-2} s^{-1}. The cultures were continuously aerated with air and maintained at 30±2 ^{o}C. After four weeks of growth, cultures were harvested by centrifugation, washed twice with deionized water and freeze dried.

Biosorption Experiments: Heavy metal solutions of different required concentrations were prepared from standard metal solutions (Merck) containing 1000 mg/l of Cd^{2+}, Cr^{3+}, Cu^{2+}, Pd^{2+}, Ni^{2+}, and Zn^{2+} each. The pH of the solutions was adjusted using either 0.1 M NaOH or 0.1 M HCl.

Freeze dried algal biomass (10 mg) was suspended in 10 ml solution of appropriate metal (10 ppm, pH 5.0) contained in 12 ml polystyrene tubes and agitated on a tube rotator at room temperature. After 30 minutes of incubation sample tubes were removed from the rotator and cells were separated by centrifugation at 5000 rpm for 5 min. For sorption kinetics experiments the sample tubes were removed from rotator at an interval of 5, 15, 30, 45 and 60 minutes. The heavy metal ions concentration in the resulting supernatant was determined by Atomic adsorption spectrophotometer (UNICAM-969). 100 μl nitric acid (65%) was added in the supernatant before measurement. Control experiments were also performed using the metal ions in the absence of biomass. All experiments were performed in triplicates.

For desorption and regeneration experiments, metal-laden algal biomass was washed twice with deionized water to remove any residual metal solution. After washing the biomass was separated by centrifugation. The biomass was then mixed with 0.1 M HCl for 15 min and suspension was centrifuged at 5000 rpm for five minutes. The concentration of desorbed metal was determined in the supernatant. The regenerated biomass was washed twice with deionized water and again suspended in metal containing solutions for the next adsorption cycle.

Results and Discussion

The first step in this work was to identify the most suitable microalgal strain, from a wide variety of microalgal species isolated from untreated wastewater containing bodies. The initial investigations on screening for highly effective metal removing isolates resulted in the selection of ten most effective microalgal isolates. Out of these ten, six strains were from green algae while the other four were found as blue green (cyanobacteria) algae. Comparative metal uptake potential of these 10 strains namely PTCC-WA-02, 05, 06, 07, 11, 13, 16, 17, 18 and 20 is presented in Table 1. Although, all the ten strains showed quite high metal adsorption capability, isolate PTCC-WA-05 and PTCC-WA-13 exhibited the highest metal uptake potential for all the metals tested (Table 1). Isolate PTCC-WA-05 was identified as *Phormidium* sp. This particular isolate removed lead almost completely from the solution while cadmium, chromium, copper, zinc and nickel removal efficiencies were found 96.59, 86.4, 84.3, 88.25 and 64.1%, respectively. The adsorption of all the six metals by freeze-dried biomass of *Phormidium* sp. was extremely rapid. Approximately >95% of the metals were removed in the first 5 minutes. For all the metals a plateau was reached in about 10-15 minutes (Fig. 1). Because of the rapid sorption rates, 15 min was selected as a standard assay period during subsequent biosorption studies.

The biosorption of toxic metal ions from single metal solution containing various amounts of the metal ions by *Phormidium* sp. is shown in Table 2. The results show that the amount of the metal ions bound by the algal biomass depends on the metal ion type and the concentration of the metal ion solution. It can be seen from Table 2 that although the amount of metal ions removed increased with an increase in the initial concentration of metal ion solution, the sorption efficiency of metal ions removed from solution by algal biomass decreased with increase in the initial metal ion concentration.

Because of the high sorption potential of *Phormidium* sp. for all the metals tested, the reusability of the algal biomass was also investigated. For this purpose adsorbed metal ions were desorbed from the algal biomass by dilute hydrochloric acid (0.1 M). Almost 100 % recovery of cadmium, lead, zinc and nickel and 86% of copper was achieved (Table 3). In the case of chromium only 26.4 % of the adsorbed metal was recovered, indicating that HCl is not suitable for the recovery of chromium. Like adsorption and desorption, regeneration efficiency of Pb, Cd and Cu was also found very high (Table 3). The regeneration efficiency of nickel, chromium and zinc decreased by 24.4, 47.8 and 53.6%, respectively.

Conclusion

The endemic strain of microalga *Phormidium* sp. was found to remove all the six heavy metals, very efficiently from aqueous solution. The rapid and extremely higher adsorption capacity of *Phormidium* sp., excellent recovery and regeneration efficiency for Pb^{2+} and Cd^{2+} show that *Phormidium* sp. has the potential to be used as an adsorbent for the removal and recovery of these metals, particularly, when the metals are present at a low concentration. Further studies are required to be conducted on the operative parameters acting on metal ions elimination, with special attention on pH, temperature, physiological state and metal ion or biomass concentration.

Table 1. Screening of microalgae, isolated from industrial wastewater containing bodies of Lahore-Kasur and Lahore-Sheikhupura regions, for the removal of heavy metals from aqueous solution.

Isolates	Metal removed (%)					
	Ni	Cd	Cr	Pb	Cu	Zn
Green algae						
PTCC-WW-06	89.59	95.68	83.02	91.87	78.15	86.35
PTCC-WW-07	76.24	91.11	86.85	90.25	80.61	92.00
PTCC-WW-13	82.29	98.35	81.84	98.04	87.67	94.23
PTCC-WW-16	70.15	92.65	91.56	82.39	65.19	98.74
PTCC-WW-17	95.38	80.97	70.82	86.11	94.06	79.12
PTCC-WW-20	62.41	87.03	76.93	93.67	81.07	69.38
Cyanobacteria						
PTCC-WW-02	47.42	59.36	35.60	80.64	51.92	59.68
PTCC-WW-05	64.06	96.59	86.36	98.37	84.30	88.25
PTCC-WW-11	71.62	98.07	75.12	73.15	90.78	90.70
PTCC-WW-18	79.19	86.21	96.67	92.53	76.32	88.41

Table 2. Biosorption of heavy metal ions from aqueous solutions containing different amount of metal ions by freeze-sried biomass (1 mg/ml) of *Phormidium*.sp.

Metals	Sorption capacity (mg/g biomass dw)			Sorption efficiency (%)		
	q5	q10	q50	q5	q10	q50
Nickel	3.53± 0.14	6.62± 0.37	17.22± 0.43	70.6± 2.8	66.2± 3.7	34.4± 0.9
Lead	4.98± 0.28	9.74± 0.44	38.82± 0.42	99.6± 5.6	97.4± 4.4	77.6± 0.8
Copper	4.28± 0.37	8.43± 0.53	24.50± 0.56	85.6± 7.4	84.3± 5.3	49.0± 1.1
Chromium	4.36± 0.11	8.64± 0.21	32.07± 0.38	87.2± 2.2	86.4± 2.1	64.1± 0.8
Cadmium	4.89± 0.25	9.57± 0.37	32.64± 0.61	97.8± 5.0	95.7± 3.7	68.3± 1.2
Zinc	4.64± 0.20	8.89± 0.32	29.16± 0.37	92.8± 4.0	89.9± 3.2	58.3± 0.7

q5, q10 and q50 are the metal uptake at the residual concentrations of 5, 10 and 50 mg/l respectively. All values are mean of three, ± = standard deviation

Table 3. Sorption and desorption of heavy metal ions by freeze-dried biomass of *Phormidium* sp. Biomass concentration: 1g/l; metal concentration: 10 mg/l; pH 5.0

Metals	Metal adsorbed mg	Metal desorbed mg	Percentage of recovery	Regeneration efficiency (%)
Nickel	6.54±0.11	6.53±0.33	99.84	75.64
Lead	9.67±0.41	9.68±0.21	100.00	99.45
Copper	8.37±0.28	7.23±0.13	86.45	95.64
Chromium	8.58±0.26	2.26±0.18	26.40	52.16
Cadmium	9.61±0.19	9.58±0.15	99.68	96.64
Zinc	9.06±0.34	9.04±0.61	99.78	46.38

q5, q10 and q50 are the metal uptake at the residual concentrations of 5, 10 and 50 mg/l respectively. All values are mean of three, ± = standard deviation

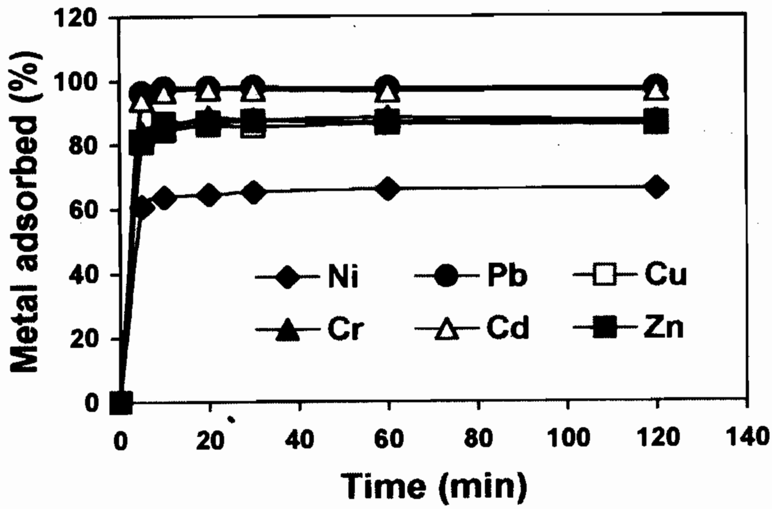

Figure 1. Biosorption of heavy metals by *Phormodium* biomass as a function of time.

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