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Heavy Metal Binding and Removal by Phormidium

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Bioremediation is growing in importance for the clean up of contaminated soil and ground water. The technology of bioremediation is not only useful and economic, but also practicable. Among the different applications of bioremediation, this paper deals specifically with bioremediation of trace metals using *Phormidium sp.*, an attached filamentous microalgae. The study was done mainly to identify metal adsorption capacity of this algae (for five different metals) under different pH conditions. Its potential was identified by the ability of the plant to reach high specific adsorption ranging between 13,600 mg/Kg for Pb and 10,100 mg/Kg for Cu. The algae could also reduce metal concentrations to very low residual levels (0.01 mg/L) within 60 minutes.

Aquatic plants and algae are known to accumulate metals and other toxic elements from contaminated water (Wang et al. 1995; Muramoto and Ohi 1983; Green and Bedell 1990; Wilde and Benemann 1993). Bioremoval process typically exhibits two stages: an initial fast, reversible metal binding process "biosorption," followed by slow irreversible, ion sequestration "bioaccumulation." Biosorption can occur via complexation, coordination, chelation, ion exchange, and other processes of greater or lesser specificity. Bioaccumulation processes are sometimes due to active (metabolism dependent) metal accumulation by living cells. In other cases, bioaccumulation is a strictly aggressive process in which metal ions are sequestered by metal binding sites in the interior of the cells. In the removal of toxic elements from contaminated water, bioremoval process has potential advantages over the conventional treatment process (ion exchange, precipitation, membranes, etc.) (Kuyack and Volesky 1989, 1990). Our initial screening experiments identified *Phormidium sp.*, an attached, filamentous microalgae, as one potential biomass for bioremoval process (Wang et al. 1995). The goal of this study was to determine the range of metal adsorptive capabilities by *Phormidium* under different pH conditions. A slate of five metals, Cd, Zn, Ni, Pb and Cu were used. Both the maximum specific adsorption capacity (mg metal adsorbed/kg of dried biomass) and the lowest residual metal concentration attainable were measured during this study.

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MATERIALS AND METHODS

Phormidium sp. was cultured in the lab in glass-sided tanks with inclined planes made of roughened translucent plastic rising out of a shallow (3-5 cm) pool of media. The media was pumped over the inclined planes, starting at the top and forming a stream of flowing water. The loose tangles became tightly bound to each other forming a flattened mat. The cultures were grown using modified Allen media. *Phormidium sp.* was collected from the culture tank, weighed and blended at low speed for 12 sec. The blended liquid was centrifuged at 1500 rpm for 15 min. The supernatant was discarded and remaining biomass used for the contacting experiments. The blending was necessary to disrupt the impenetrable algal mat. Weighted amounts of biomass were added to metal solutions yielding 50 mL total volume in 125 mL Erlenmeyer flasks equipped with screw caps and incubated in a shaker for 60 minutes. All experiments were conducted in triplicate. The pH and temperature of the contact solution both before and after the experiment was measured. AT the end of the experiment, the contents of the flasks were centrifuged (microalgae) to separate the biomass from the supernatant. The supernatant was immediately analyzed with an atomic absorption spectrophotometer to measure metal concentration left in the solution. The biomass was stored for digestion with nitric acid in a microwave digester (MDS 2100). Both dry weight and ash free dried weight analyses were performed as per AWWA Standards Methods, pH of the solutions was measured using a Cole-Palmer portable pH meter and temperature was recorded with a thermometer.

All samples were analyzed using an Atomic Absorption Spectrophotometer (AAS) Perkin Elmer Model 3100 or an Inductively Coupled Argon Plasma (ICAP) Trace Analyzer Thermo Jarrell Ash Model 61E. The latter was used for determining low metal concentrations (<0.1 ppm). Before running the samples, the instruments were optimized, and a calibration blank was run. A five point standard curve was then established. Prior to sample analysis, one of the median standards was rerun. At the end of 20 samples, the 20th sample was rerun as a duplicate. A matrix spike was also performed before starting the next run of samples. The recovery of the standards, duplicates and the matrix spikes was within 85-115%. The mean values from triplicate runs of both initial and final metal concentration were used to calculate the following parameters: (1) Percent metal remaining in solution, (2) Percent metal removed by biomass, (3) Specific adsorption capability (mg of metal adsorbed/kg of ash free dried biomass AFDW biomass). Specific adsorption was computed in two ways. The amount of metal adsorbed was calculated from the difference in metal concentration before and after contacting. It was also determined from the metal content measured in the separated biomass. A linearized form of the Langmuir adsorption isotherm equation was plotted: $C/Y = C/Y,_m + 1/kY_m$, where Y_m= the maximum specific adsorption; k = equilibrium constant, related to the affinity of the binding site; Y = specific adsorption at residual metal concentration C, C = residual metal concentration. From a plot of C/Y vs. C, the slope ($S = 1/Y_m$), give Y_m, the maximum adsorption capability, and the intercept ($I = 1/kY_m$) gives k, the affinity constant of metal on the biomass.

RESULTS AND DISCUSSIONS

The assessment of the pH and buffer's effect on metal precipitation was performed using 1 mg/L of metal solution with different types of buffer. The results show that both Pb and Cu were precipitated when pH is ≥ 7. Cd, Zn and Ni had insignificant amounts of metal precipitated between pH3 and pH9. By knowing the type of buffer and pH on metal precipitations (metal loss), the effect of pH on metal adsorption by *Phormidium* in 1 mg/L metal solution was performed. The results demonstrate the higher specific adsorption was observed between pH5 and pH7, and lower adsorption generally occurred in lower acidic solutions (<pH5). Concentration curves (specific adsorption vs. residual metal concentration) for each metal were determined with metal concentrations varying from 1.0 to 16 mg/L at three different pH (3, 5, and 7) levels. There was no buffer used for pH3 experiments. pH5 solution was obtained by adjusting initial metal solution with NaOH and then maintained in 5 mM acetate solution. For pH7 metal solutions, phosphate buffer (20 to 40 mM) for Cd, Zn and Ni and acetate buffer (10-40 mM) for both Pb and Cu were employed. The increasing buffer concentration (10-40 mM) was required to maintain constant pH as initial metal concentration increased from 1 to 16 mg/L. Maximum specific adsorption by *Phormidium* obtained from Langmuir isotherms at different pH solutions are shown in Table 1. The maximum specific adsorption obtained at pH5 solution was 13,600±1,900 mg/kg for Pb; followed by 10,100±800 mg/kg for Cu; 9,600±1,200 mg/kg for Cd; 9,400±1,100 mg/kg for Zn; and 5,700±600 mg/kg for Ni. One of the major requirements of a practical bioremoval process is the capability of the biomass to reduce the residual metal concentration to meet regulatory levels. Experiments were conducted to test the ability of the biomass to lower the metal concentration below the EPA water quality criteria within 60 minutes. The results from the experiments indicate that the minimum residual concentration obtained for Cd, Zn, Ni and Cu were about 0.01 mg/L and Pb was below detection (≤0.004 mg/L). All metals except Cd were within EPA water quality criteria (1986) as shown in Table 2.

A comparison was also made to demonstrate the effect of different concentrations of acetate buffer used at the same pH solution. Concentration curves for both Pb and Cu at pH5 solution were established in two different ways to determine the maximum specific adsorption. Adjusting initial pH to 5 with NaOH and maintained in a 5 mM acetate solution was used to establish one set of concentration curves. The other curve was obtained with buffer concentration ranging from 10 to 40 mM as concentration increased from 1 to 16 mg/L. The results demonstrate that adsorption of Pb increased from 9,300±400 mg/kg performed with 10-40 mM buffer to 13,600±1,900 mg/kg obtained with 5 mM acetate solution. Similarly, the specific adsorption of Cu was increased from 6,800±300 mg/kg with 10-40 mM buffer to 10,100±800 mg/kg, at 5 mM acetate buffer. These results are shown in Table 3. The reproducibility of data was demonstrated in repeating entire experimental runs to establish concentration curves on separate days. Table 4 shows the reproducibility of maximum adsorptions for Pb and Cd at different pHs. Within each set of experiments, the Y_m obtained were nearly within a standard deviation of each other. Confidence in data is further demonstrated by comparing specific adsorption determined by direct measurement of metals in the biomass pellet and from Langmuir calculations shown in Table 5.

Requirements for developing a practical bioremoval process include low-cost production of plant biomass, ease of removing the biomass from suspension, high maximum specific adsorption, and the capability to reduce metal concentration to very low residual levels. The filamentous microalgae discussed here are also easy to harvest and potentially productive in mass culture. The experimental data presented here indicate that these types of plants may have the promising metal adsorbing characteristics.

Table 1. Maximum specific adsorption by *Phormidium* obtained from Langmuir isotherms at different pH solutions.

Metal	**Y_m**	**pH**	**Buffer**
Cd	3,100 ± 200	3	No buffer
	9,600 ± 1,200	5	Acetate (5 mM – NaOH)
	8,800 ± 1,300	7	Phosphate (10-40 mM)
Zn	5,000 ± 1,300	3	No buffer
	9,400 ± 1,100	5	Acetate (5 mM – NaOH)
	8, 700 ± 400	7	Phosphate (10-40 mM)
Pb	6,800 ± 800	3	No buffer
	13,600 ± 1,900	5	Acetate (5 mM – NaOH)
	9,300 ± 300	7	Acetate (10-40 mM)
Ni	3,200 ± 100	3	No buffer
	5,700 ± 600	5	Acetate (5 mM – NaOH)
	8,000 ± 500	7	Phosphate (10-40 mM)
Cu	1,700 ± 400	3	No buffer
	10,100 ± 800	5	Acetate (5 mM – NaOH)
	4,800 ± 100	7	Acetate (10-40 mM)

Table 2. Minimum residual concentration.

Metal	pH	Initial metal conc. (mg/L)	Residual metal conc. (mg/L)	Specific adsorption (mg/L)	EPA Fresh water quality criteria[1] (mg/L)	MDL[2] (mg/L)
Cd	5	0.21 ± 0.01	0.008 ± 0.001	257 ± 13	0.0011	0.0003
Zn	5	0.23 ± 0.01	0.010 ± 0.004	284 ± 14	0.047	0.01
Pb	5	0.17 ± 0.00	< 0.004	219 ± 10	0.0032	0.004
Ni	5	0.24 ± 0.00	0.011 ± 0.002	295 ± 14	0.096	0.002
Cu	5	0.21 ± 0.00	0.009 ± 0.007	265 ± 14	0.012	0.007

[1] Chronic value at 100/mg/L water hardness

[2] Minimum detection limits for Inductively Coupled Plasma Spectrometer

Table 3. Comparison of acetate buffer concentrations on metal adsorption by *Phormidium* at pH5.

Metal	Buffer Concentration	Y_m Obtained (mg/kg)
Pb	NaOH – 5 mM	13,600 ± 1,900
	10 mM – 40 mM	9,300 ± 600
Cu	NaOH – 5 mM	10,100 ± 800
	10 mM – 40 mM	6,800 ± 300

Table 4. Reproducibility of experimental run on metal adsorption by *Phormidium.*

Metal	Run No.	pH	Biomass Density (mg/L)	Buffer Concentrations	Y_m (mg/kg)
Cd	1	7	71	Phosphate (10-40 mM)	8,800 ± 1,300
	2	7	190	Phosphate (10-40 mM)	6,600 ± 600
	3	5	520	NaOH–Acetate (5 mM)	7,300 ± 700
	4	5	260	NaOH–Acetate (5 mM)	9,600 ± 1,200
Pb	1	3	94	No buffer	5,400 ± 1,300
	2	3	128	No buffer	6,800 ± 800
	3	5	280	Acetate (10-40 mM)	9,300 ± 600
	4	5	240	Acetate (10-40 mM)	10,300 ± 1,900

Table 5. Comparisons of specific adsorption between experimental measurement and Langmuir calculations.

Metal	From Langmuir Isotherm			From Experimental Data
	Y_m (mg/kg)	Corr. Coeff. r	k	Highest specific adsorption (mg/kg)
Cd	9,600 ± 1,200	0.96	0.40	9,300 ± 300
Zn	9,400 ± 1,100	0.98	0.23	7,700 ± 20
Pb	13,600 ± 1,900	0.97	0.96	$14x10^3$ ± 300
Ni	5,700 ± 600	0.98	0.40	5,100 ± 300
Cu	10,100 ± 800	0.99	0.73	9,500 ± 300

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