Chemical Engineering Journal 102 (2004) 249–253

Chemical Engineering Journal

www.elsevier.com/locate/cej

Biosorption of chromium(VI), cadmium(II) and copper(II) by *Pantoea* sp. TEM18

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Accepted 28 January 2004

Abstract

Microorganisms and microbial products can be highly efficient bioaccumulators of soluble and particulate forms of metals, especially from dilute external solutions, and microbe-related technologies may provide an alternative or adjunct to conventional techniques of metal removal/recovery. In this work, among microorganisms isolated from wastewater treatment of a petrochemical industry, a gram-negative bacterium *Pantoea* sp. TEM18 exhibited the greatest copper tolerance. It was able to survive in the medium containing copper at concentrations as high as 180 mg/l. The biosorption properties of bacterial biomass for cadmium and the effects of environmental factors (i.e. pH, metal concentration contact time) on the chromium, cadmium and copper biosorption were explored. Optimum adsorption pH values of chromium(VI), cadmium(II) and copper(II) were determined as 3.0, 6.0 and 5.0, respectively. Experimental results also showed the influence of initial metal concentration on the metal uptake for dried biomass. Both the Freundlich and Langmuir adsorption models were suitable for describing the short-term biosorption of chromium(VI), cadmium(II) and copper(II) by *Pantoea* sp. TEM18.

Keywords: Waste water treatment; *Pantoea* sp.; Biosorption; Chromium; Cadmium; Copper

1. Introduction

The production of heavy metals has increased rapidly since the industrial revolution [1]. Heavy metals usually form compounds that can be toxic, carcinogenic or mutagenic, even in very low concentrations [2]. The conventional methods of removing metals from wastewaters are generally expensive and have many limitations [3]. Alternative methods of metal removal and recovery based on biological materials have been considered. Certain types of microbial biomass can retain relatively high quantities of metals by means of passive processes known as biosorption, which is dependent on the affinity between the metallic species or its ionic forms and the binding sites on the molecular structure of the cellular membrane, cell wall and capsule [4]. Such processes are of industrial interest because the removal of potentially hazardous heavy metals and radionuclides from industrial effluents and wastewaters by microbial biomass can lead to detoxification and also to recovery of valuable elements such as gold and silver after appropriate treatment of the loaded biomass [5]. The employment of bacterial biomass for the metal removal treatment of effluents is a perspective suggested by many researchers dealing with metal-bacteria interactions [6]. The presence on bacterial surfaces, of polarizable groups capable of interacting with cations is responsible for their reversible metal binding capacity. Such groups (‘sites’) are mainly: phosphate, carboxyl, hydroxyl and amino-groups [7].

The objective of this investigation was to study capacity of a strain of *Pantoea* sp., having high tolerance to copper. Therefore, the copper tolerant bacterium was isolated from activated sludge of a petrochemical industry wastewater treatment plant. Also, the toxicity of cadmium, copper and chromium and biosorption capacity of the isolated bacterium were investigated.

2. Methods

2.1. Screening and isolation of microorganisms resistant to copper

To isolate the copper resistant bacterium, samples were obtained from wastewater treatment plant of a petrochemical

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doi:10.1016/j.cej.2004.01.032

Nomenclature

b	Langmiur adsorption constant ($l\,mg^{-1}$)
C_{eq}	residual metal ion concentration at equilibrium ($mg\,l^{-1}$)
C_0	initial metal ion concentration ($mg\,l^{-1}$)
K_F	Freundlich adsorption constant
n	Freundlich adsorption constant
q_{eq}	adsorbed metal ion quantity per gram of cell at equilibrium ($mg\,g^{-1}$)
Q^0	Langmiur adsorption constant ($mg\,g^{-1}$)
R^2	correlation coefficient
X	cell concentration ($g\,l^{-1}$)

plant, Izmir. The samples were passed aseptically through tenfold dilutions of Ringers solution to yield final concentrations of 10^{-4} and 10^{-5}. Spread plates were prepared from each dilution (0.1 ml) on brain heart infusion agar (BHI agar) containing 10 mM concentration of copper sulfate and incubated at 30 °C for a maximum of 3 days [8]. Discrete colonies were picked from each plate.

2.2. Determination of MICs and identification of microorganism

The MICs of Cr, Cd and Cu for selected microorganisms were determined with microtiter plates containing 160 μl of nutrient broth (Difco), 20 μl of distilled water and increasing amounts (0–3000 μg/ml) of Cr, Cd and Cu [9]. The bacterium (strain TEM18) growing in the most copper concentration was selected for the study. Pure colony of the strain TEM18 was identified presumptively on the following features: colony morphology, colonial pigmentation, cell morphology, Gram-staining reaction, oxidase positivity. Isolates were further characterized biochemically using the API 20E Kits (Analytical Profile Index, Bio Merieux, SA, France).

2.3. Preparation of the strain TEM18 for biosorption

In this study, strain TEM18, which was selected for the study, was cultivated aerobically in 500 ml conical flasks containing sterile nutrient broth on a rotary shaker (100 revolutions per min) at 30 °C. Cells were harvested at the end of exponential phase, i.e. after 48 h incubation. After cultivation, the cells were centrifuged at 10,000 × g for 20 min and autoclaved at 121 °C, 20 min and then washed twice with deionized water and finally freeze dried

2.4. Biosorption studies

Ten milligrams dried cells were mixed 10 ml of the metal solutions in an flask. The flasks were agitated on a shaker (150 rpm) at room temperature for 1 h. The effects of contact time, metal ion concentration and pH on the biosorption of Cr(VI), Cd(II) and Cu(II) were studied.

All pH adjustment were made using reagent grade HCl and NaOH. The samples were centrifuged at 10,000 × g for 20 min and supernatant liquid was used to estimate metal ion concentrations.

2.5. Analysis of metal ions

The concentration of unadsorbed Cr(VI), Cd(II) and Cu(II) ions in the biosorption medium were determined by using a ICP-AES (model DRE, Leeman Labs Inc.). Each experiment was repeated three times and the results given are the average values.

3. Results and discussion

3.1. Identification of the most metal resistant bacterium and determination MICs

Based on light-microscopy observation, strain TEM18 was gram-negative, motile, rod-shaped and 0.8–1.4 μm size. The API 20E Kit was used for physiological characterization of isolated strain. The API 20 E biotype of the most metal resistant bacterium was *Pantoea* sp. TEM18. The cells were cultivated in nutrient broth media amended with various concentrations of Cr(VI), Cd(II) and Cu(II). It appears that the cells were able to survive metal concentrations as high as 50 $mg\,l^{-1}$ for Cr(VI), 140 $mg\,l^{-1}$ for Cd(II) and 180 $mg\,l^{-1}$ for Cu(II).

3.2. Effect of pH on the biosorption capacity

As pH values in metal-containing water and wastewater can vary, solutions of different pH values were used to examine the effect of on heavy metal sorption by *Pantoea* sp. TEM18. In these tests, initial pH values of 100 $mg\,l^{-1}$ aqueous metal solutions were adjusted in the range 1.0–8.0, before addition of the biosorbent. The results are shown in Fig. 1. The greatest capacity of biosorption was obtained at

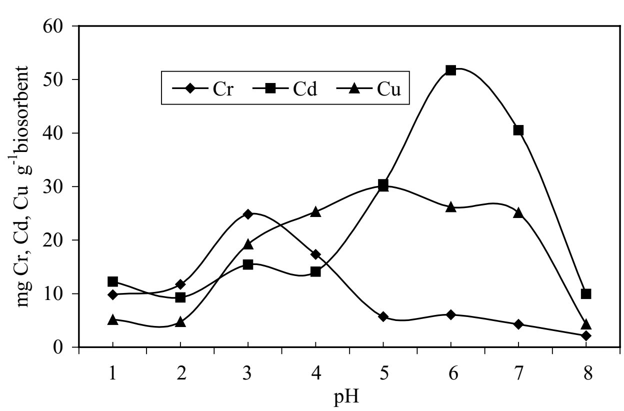

Fig. 1. Effect of pH on Cr(VI), Cd(II) and Cu(II) by *Pantoea* sp. TEM18 (C_0: 100 $mg\,l^{-1}$; X: 1.0 $g\,l^{-1}$; temperature: 25 °C; agitation rate: 150 rpm).

pH 3.0 for Cr(VI) ions, pH 6.0 for Cd(II), and pH 5.0 for Cu(II) ions. These results seem to suggest that the adsorption of Cr(VI), Cd(II) and Cu(II) to biomass is mainly due to ionic attraction. The medium pH affects the solubility of metals and the ionization state of the functional groups like carboxylate, phosphate and amino groups of the cell wall. The carboxylate and phosphate groups carry negative charges that allow the cell wall components to be potent scavengers of cations.

The inconsistency in literature regarding the influence of pH on biosorption seems to indicate that the way pH would alter the adsorption of metal ions to biomass varies with the type of adsorbents (biomass) and also the type of adsorbates (metal ions). The optimal pH for adsorption of Cr(VI), Cd(II) and Cu(II) by *Ochrobactrum anthropi* were 2.0, 8.0 and 3.0, respectively [10]. For the biosorption of Cu(II) and Cd(II) by inactivated cells of *Pseudomonas aeruginosa* PU 21, the maximal capacity occurred at pH 6.0 [11]. The optimal pH for adsorption of Cd(II) by *Sphingomonas paucimobilis* was in the range of 5.0–6.0 [12], and the optimal pH for adsorption of the same metal ion was 8.0 for fungal biomass [13]. An optimal pH of 4.0 for the adsorption of Cu was found using various marine algae as the biosorbent [14]. For the biosorption of Cr by green algae *Spirogyra* species, the maximal capacity occurred at pH 2.0 [15].

3.3. Effects of initial concentration of metals ions on the biosorption capacity

The biosorption capacities of Cr(VI), Cd(II) and Cu(II) onto *Pantoea* sp. TEM18 are presented in Table 1. As is shown in Fig. 2, the biosorption capacity of the biomass increased first with increasing of the initial concentration of metal ions and reached a saturated value. As is shown in figure, when the initial Chromium (VI) concentration was increased from 28.9 to 245.2 mg l^{-1} approximately, the loading capacity increased from 7.81 to 53.8 mg g^{-1} of *Pantoea* sp. TEM18. The maximum cadmium loading capacity of the biosorbent was also found 52.0 mg g^{-1} at 95.8 mg l^{-1} initial cadmium (II) concentration. Maximum equilibrium uptakes of copper (II) ions were also determined as 30.1 mg g^{-1} at 106.5 mg l^{-1} of initial copper (II) ion concentrations.

The magnitude of changes in metal ion binding capacity of *Pantoea* sp. TEM18 may be due to the properties of the

Table 1
Isotherm model constants for adsorption of Cr(VI), Cd(II) and Cu(II) on *Pantoea* sp. TEM18

	Langmuir			Freundlich		
	Q^0	b	R^2	K_F	n	R^2
Cr(VI)	204.1	0.002	0.965	1.80	0.88	0.998
Cd(II)	58.1	0.057	0.971	10.34	0.34	0.762
Cu(II)	31.3	0.112	0.996	13.09	0.16	0.821

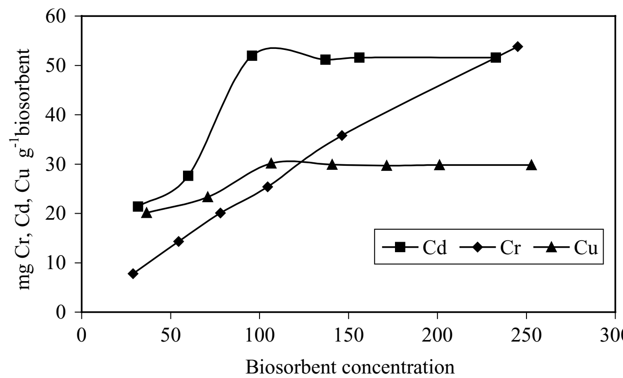

Fig. 2. Effect of biosorbent concentration on Cr(VI), Cd(II) and Cu(II) by *Ochrobactrum antropi* (X: 1.0 g l^{-1}, 25 °C, agitation rate: 150 rpm).

metal sorbates (e.g. ionic size, atomic weight, or reduction potential of the metal) and the properties of the bacterium (e.g. structure, functional groups, and surface area). Capsules and slime layers of bacteria contain polysaccharides as basic building blocks, which have ion exchange properties. They also contain proteins and lipids and therefore offer a host of functional groups capable of binding to heavy meals. These functional groups such as amino, carboxylic, sulphydryl and phosphate groups differ in their affinity and specificity for metal binding.

3.4. Biosorption time

As is shown in Fig. 3, the biosorption times of heavy metal ion species [245.2 mg l^{-1} for Cr(VI), 95.8 mg l^{-1} for Cd(II) and 106.5 mg l^{-1} for Cu(II)] on the biomass were obtained by following the decrease of concentration Cr, Cd and Cu within the adsorption medium with time. The metal adsorption increased rapidly during the first 5 min and remained nearly constant after 15 min. After this equilibrium period, the amount of adsorbed metals ions did not significantly change with time.

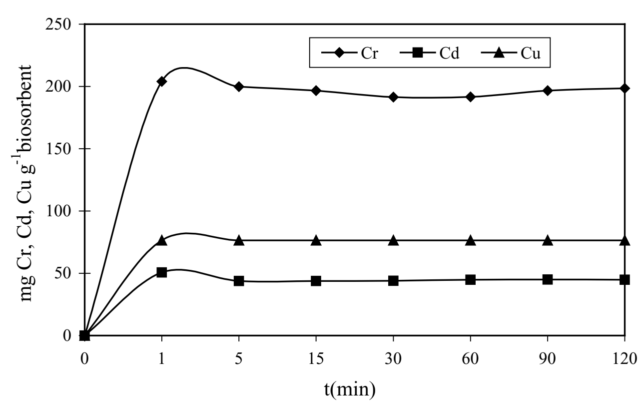

Fig. 3. Effect of initial time on Cr(VI), Cd(II) and Cu(II) by *Pantoea* sp. (C_0: 245.2 mg l^{-1} for Cr(VI), 95.8 mg l^{-1} for Cd(II), 106.5 mg l^{-1} for Cu(II); X: 1.0 g l^{-1}, 25 °C, agitation rate: 150 rpm).

3.5. Freundlich and Langmuir adsorption isotherms

The equlibria of biosorption of heavy metals were modeled using adsorption-type isotherms. The Freundlich and Langmuir models were used to describe the biosorption equlibrium.

The Langmuir equation which is valid for monolayer sorption onto a surface a finite number of identical sites and is given by Eq. (1).

$$q_{\mathrm{eq}} = \frac{Q^0 b C_{\mathrm{eq}}}{1 + b C_{\mathrm{eq}}} \tag{1}$$

where Q^0 is the maximum amount of the metal ion per unit weight of cell to form a complete monolayer on the surface bound at high C_{eq} ($\mathrm{mg\,g^{-1}}$) and b is the constant related to the affinity of the binding sites, Q^0 represents a practical limiting adsorption capacity when the surface is fully covered with metal ions and assists in the comparison of adsorption performance, particularly in cases where the sorbent did not reach its full saturation in experiments. Q^0 and b can be determined from the linear plot of $C_{\mathrm{eq}}/q_{\mathrm{eq}}$ versus C_{eq}.

The empirical Freundlich equation based on sorption on a heterogeneous surface is given below by Eq. (2).

$$q_{\mathrm{eq}} = K_{\mathrm{F}} C_{\mathrm{eq}}^{1/n} \tag{2}$$

where K_{F} and n are Freundlich constants characteristic of the system. K_{F} and n are indicators of adsorption capacity and intensity, respectively. Eq. (2) can be linearized in logarithmic form and Freundlich constants can be determined. The Freundlich isotherm is also more widely used but provides no information on the monolayer adsorption capacity, in contrast to the Langmuir model [16].

The linearized Freundlich and Langmuir adsorption isotherms of each metal ion for *Pantoea* sp. TEM18 were shown in Figs. 4 and 5. The Freundlich and Langmuir adsorption constants evaluated from the isotherms with the correlation coefficients are also give in Table 1. As seen in table, regression correlation coefficients for all the metal ions–bacterium systems are very high. The magnitude of K_{F} and n (the Freundlich constants) showed easy uptake of cadmium(II) and copper(II) from wastewater with a high adsorptive capacity of *Pantoea* sp. TEM18.

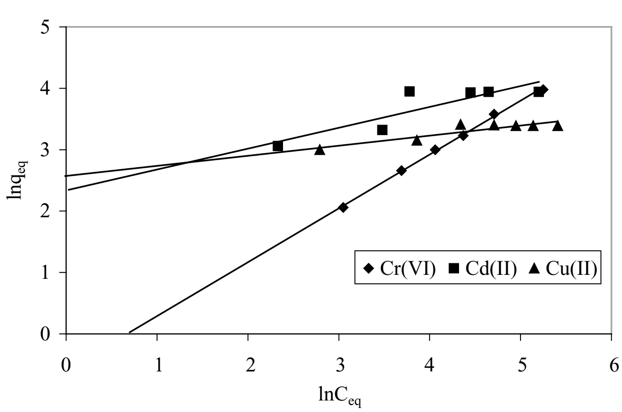

Fig. 4. The linearized Freundlich adsorption isotherms of Cr(VI), Cd(II) and Cu(II) by *Pantoea* sp.

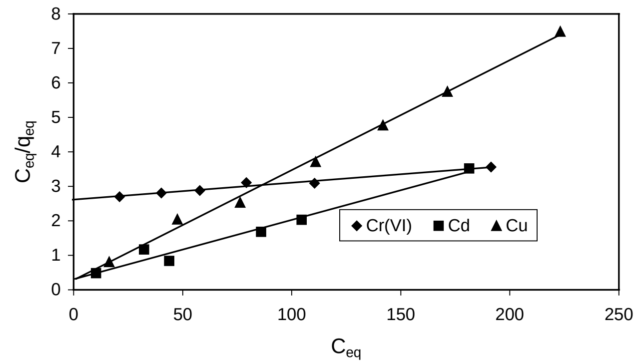

Fig. 5. The linearized Langmuir adsorption isotherms of Cr(VI), Cd(II) and Cu(II) by *Pantoea* sp.

The value of Q^0 appears to be significantly higher for the Cr(VI) *Pantoea* sp. TEM18. system. Langmuir parameters of *Pantoea* sp. TEM18 also indicated a maximum adsorption capacity of 204.1 $\mathrm{mg\,g^{-1}}$ for Cr(VI), 58.1 $\mathrm{mg\,g^{-1}}$ for Cd(II) and 31.3 $\mathrm{mg\,g^{-1}}$ for Cu(II).

In contrast, specific uptake of Cr(VI), Cd(II) and Cu(II) found in this study was comparable with values found in the literature. The biosorption capacity of Cr(VI) was 86.2 $\mathrm{mg\,g^{-1}}$ for *O. anthropi* [10], 23.6 $\mathrm{mg\,g^{-1}}$ for *Chlorella vulgaris* [17], 23.0 $\mathrm{mg\,g^{-1}}$ for *Chlorella crispata* [18]. The adsorption capacity of Cd(II) was 37.3 $\mathrm{mg\,g^{-1}}$ for *O. anthropi* [10], 62 $\mathrm{mg\,g^{-1}}$ for *Rhizopus arrhizus* [19]. The biosorption capacity of Cu was 32.6 $\mathrm{mg\,g^{-1}}$ for *O. anthropi* [10], 2.66 $\mathrm{mg\,g^{-1}}$ for *Aspergillus niger* [20].

4. Conclusion

The aim of this work was to find the biosorption characteristics of selected one of the most resistant bacteria against to heavy metals for the removal of chromium, cadmium and copper ions. Experiments were performed as a function of pH, initial metal ion concentration and time. The obtained results showed that *Pantoea* sp. TEM18 is good adsorbing medium for metal ions and had high adsorption yields for the treatment of wastewater containing chromium, cadmium and copper ions.

The Freundlich and Langmuir adsorption models were used for the mathematical description of the biosorption of chromium, cadmium and copper ions to dried *Pantoea* sp. TEM18 and the isotherms constants were evaluated to compare the biosorptive capacity dried *Pantoea* sp. TEM18 for metal ions. Consequently, bacteria biosorption technologies are still being developed and much more work is required.

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