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## Full Paper

# Biosorptive capacity of Cd(II) and Cu(II) by lyophilized cells of *Pseudomonas stutzeri*

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**The biosorptive capacity of Cd(II) and Cu(II) by lyophilized cells of *Pseudomonas stutzeri* was investigated based on Langmuir and Freundlich isotherms and biosorption kinetics were analyzed using first order kinetic with different initial metal concentrations. Biosorptive capacity for Cd(II) and Cu(II) decreased with an increment of metal concentration, reaching 43.5 and 36.2 mg/g at the initial concentration of 300 mg/L. Biosorption capacity for both metal ions was increased with increasing pH. The optimum pH for biosorption rate of Cu(II) and Cd(II) was pH 5; above pH 5.0 the metal cations came to be precipitated. The experimental data showed a better fit with the Langmuir model over the Freundlich model for both metal ions throughout the range of initial concentrations. The maximum sorptive capacity ($q_{max}$) obtained from the Langmuir equation for Cd(II) and Cu(II) were 47.86 ($r^2$=0.99) and 33.16 ($r^2$=0.99), respectively. The bacterial cells have more affinity to adsorb cadmium than copper. The first order kinetic was well fitted to the experimental data for initial concentrations from 30 to 100 mg/L during reaction times of 250 min. These results suggest that biosorption of Cu(II) and Cd(II) by lyophilized cells of *P. stutzeri* is a potential metal removal strategy.**

**Key Words**——biosorption, cadmium, copper, lyophilized cells, *Pseudomonas stutzeri*

## Introduction

Heavy metal bioremediation from human activities, such as mining operations and the discharge of industrial wastewater is one of the major methods, as it offers an effective alternative to physicochemical methods. The feasibility of using inert microorganisms as biosorbents has been studied widely in recent decades (Cho et al., 2001; Pardo et al., 2003; Sar et al., 1999). Physicochemical methods commonly using chemical adsorbents for heavy metal removal from industrial wastes have several disadvantages including both economic and environmental aspects (Eccles, 1999). In contrast to chemical sorbents, a wide variety of living and dead biomass of bacteria, algae, fungi, and plants is capable of sequestering toxic metals from waste streams, which offers an economical alternative for sorption technologies. Once the toxic metals are adsorbed and/or transferred within organic materials, they can be removed from wastewater (Smith and Collins, 2007).

The mechanisms associated with metal removal by microorganisms are rather complex compared with those associated with chemical absorbents and can be divided into three categories: (1) biosorption of metal ions on the cell surface, (2) intracellular uptake of metal ions, and (3) chemical transformation of metal ions by microorganisms (Pardo et al., 2003). Non-liv-

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ing biomass is involved in removal mechanisms of metal ions through adsorption and ion exchange (Volesky, 1990). The capacity of any biosorbent is mainly influenced by biomass characteristics, physicochemical properties of the target metals, and the microenvironment of contact solution including pH, temperature, and interaction with other ions (Chen and Wang, 2007). For example, increase in pH can cause precipitation of metal ions, generating in the order of $Fe^{3+} > Cu^{2+} > Pb^{2+} > Zn^{2+} > Cd^{2+}$ (Eccles, 1999). Considerable research has been conducted on metal removal by living microorganisms, generally suggesting that the use of chemical equilibrium modeling to predict biosorptive capacity for metal ions in aqueous conditions could provide predictions of the distribution of metals on cell surfaces (Fowle and Fein, 1999). Although living biomass has an additional capacity for metal ions due to metabolic entrapment, non-living biomass does not only depend on requirements for growth, metabolic energy, or transport yet also has a strong affinity for metal ions due to the lack of protons produced during metabolism (Pardo et al., 2003).

*Pseudomonas stutzeri* is Gram-negative and aerobic and resides in soil and water (Singleton and Sainsbury, 1987). The living biomass has been found in mine waste waters polluted with various heavy metals and has shown Cu tolerance at high concentrations, 1,000 mg/L (Cho et al., 2001). However, there is little research to estimate the biosorptive capacity of metal ions by lyophilized *P. stutzeri* strains. The objective of this research was to evaluate the biosorptive capacity of Cu(II) and Cd(II) by lyophilized bacteria (*P. stutzeri* stain KCCM 34719) in various conditions including initial metal concentration, pH, and time.

## Materials and Methods

*Bacterial species and preparation for biosorption.* The microorganism used for this experiment was *Pseudomonas stutzeri* strain KCCM 34719 which was obtained from Korean Culture Center of Microorganism (KCCM), Seoul, Korea. The bacteria was cultured in Nutrient broth for 48 h then harvested by centrifugation for 30 min at 5,000 rpm/min using MF 550 (Hanil, Korea) and the suspension was rinsed three times with sterile water, then freezed dried using a lyophilizer (FD5505 Ilshin, Korea).

*Metal solution for biosorption.* The nitrate salts of Cd(II) and Cu(II) were used to prepare stock solution with autoclaved distilled water. The stock solution (1,000 mg/L) was diluted to a desired value with autoclaved distilled water before experiments.

*Biosorption experiments.* The freeze-dried cells (0.05 g) were added to a series of Erlenmeyer flasks containing the diluted solution (50 ml) with different initial concentrations (30, 50, 100 mg/L). The flasks were shaken (130 rpm/min) at 30°C for a certain time (0–250 min). Cell solution (3 ml) at the time intervals was filtered through 0.2-μm filter membranes and the supernatant was analyzed for metal ions, using ICP-AES (Perkin Elmer XL3100). To study the effect of different concentrations on metal and pH on biosorption, initial concentrations (50, 100, 150, 200, 300 mg/L of each metal at pH 5) and pH (2.0, 3.0, 4.0, 5.0) at the initial concentration (200 mg/L) were tested using 50 mg of lyophilized cells at 30°C, at the equilibrium time. All the biosorption experiments were repeated three times to confirm the results.

*Biosorption kinetics and isotherm.* The biosorption equilibrium isotherm was obtained by the Freundlich model [Eq. (1)] and the Langmuir model [Eq. (2)] (Volesky, 1990).

$$q = K_f C_e^{1/n} \quad (1)$$

where $K_f$ and $n$ are the distribution coefficient and a correction factor, respectively. By plotting the linear form of Eq. (1), $\log q = 1/n \log C_e + \log K_f$, the slope is the value of $1/n$ and the intercept is equal to $\log K_f$.

$$q = kC_e b/(1 + kC_e) \quad (2)$$

where $k$ is a constant related to the adsorption capacity and $b$ is the maximum metal adsorption, $q_{max}$. Rearranging to a linear form, Eq. (2) becomes $C_e/q = 1/kb + C_e/b$. Plotting $C_e/q$ vs. $C_e$, the slope is $1/b$ and the intercept is $1/kb$.

Kinetic models were used for metal adsorption by lyophilized cells of *P. stutzeri*. Zero-[Eq. (3)], first-[Eq. (4)], and second-[Eq. (5)] order kinetic equations of each metal were calculated with following equations:

$$[Me]_t = [Me]_0 - kt \quad (3)$$

$$\ln [Me]_t = \ln [Me]_0 - kt \quad (4)$$

$$1/[Me]_t = 1/[Me]_0 + kt \quad (5)$$

where $[Me]_0$ and $[Me]_t$ are initial metal concentrations and metal concentrations retained at reaction time $t$ (min), and $k$ is rate constant.

*Data evaluations.* The effect of initial concentration and pH on metal adsorption was calculated using the following equation:

$$\text{Metal adsorbed (\%)} = (C_e/C_i) \times 100 \quad (6)$$

The specific metal biosorption $q$ was calculated using

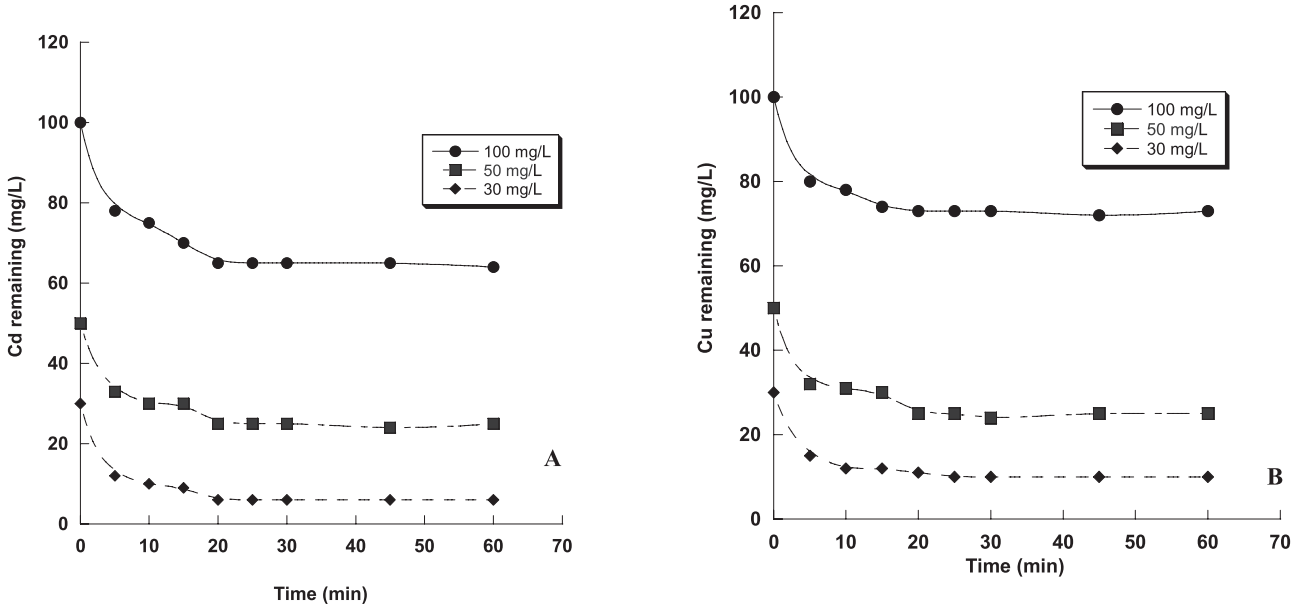


Fig. 1. The sorption of Cd (A) and Cu (B) by *Pseudomonas stutzeri* over the reaction time at different initial concentrations, pH 5.0 and 30°C.

the following equation (7):

$$q=V(C_i-C_e)/m \quad (7)$$

where $q$ is the specific metal biosorption (mg metal /g biomass), $V$ is the volume of metal solution (ml), $C_i$ and $C_e$ are the initial and equilibrium concentration of metal (mg metal /L), respectively, and $m$ is the dry weight of the biomass (g).

## Results and Discussion

*Effect of contact time on biosorption process*

Contact time is one of the important parameters for successful biosorption application. Figure 1 A, B shows the effect of contact time on the extent of adsorption of cadmium and copper on bacterial biomass. Figure 1 A, B showed that the rate of metal uptake increases rapidly in the first part within 5 min of contact. After that the rate decreases till we reach a constant value of metal concentration after 30 min. Therefore, one can conclude that the appropriate equilibrium time for measurements was at 30 min. This represents the equilibrium time at which an equilibrium metal ion concentration is presumed to have been attained. The data obtained from this experiment was further used successfully to evaluate the kinetics of the adsorption process. This short time required for biosorption is in accordance with the result given by other authors (Sar et al., 1999; Volesky, 1990; Zouboulis et al., 2004). Gabr et al. (2008) showed that the maximum biosorption of lead and nickel was reached after 30 min.

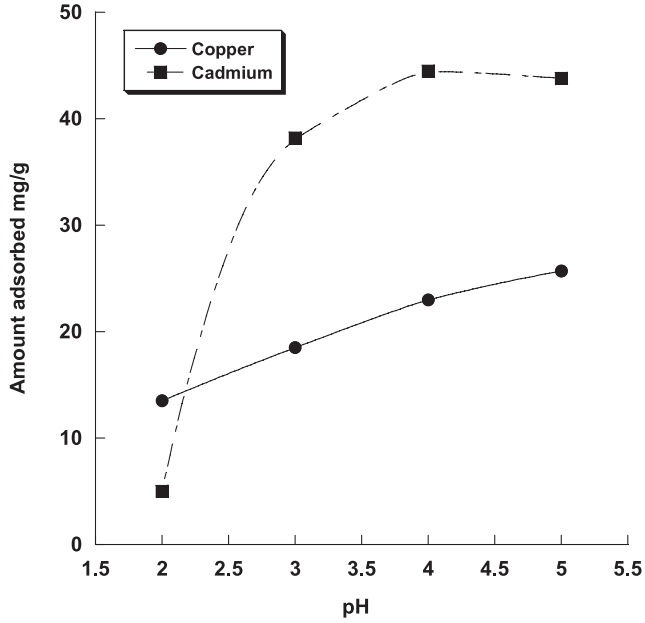


Fig. 2. Effect of pH on Cu and Cd biosorption by lyophilized cells of *P. stutzeri* at initial concentration 200 mg/L for each metal, and 30°C.

*Effect of pH*

The effect of pH on the biosorptive capacity of Cd(II) and Cu(II) was evaluated at an initial concentration of 200 mg/L at 30°C as shown in Fig. 2.

Biosorption capacities for both metal ions increased

with an increase in pH tell we reach the optimum at pH 5.0 for both metals. However, for more than pH 5.0 the metal cations begin to precipitate. At low pH, cell wall ligands were closely associated with the hydronium ions $H_3O^-$ and restricted the approach of metal cations as a result of the repulsive force. As the pH increased, more ligands such as carboxyl, phosphate, imidazole and amino groups would be exposed and carried negative charges with subsequent attraction of metallic ions with positive charge and biosorption onto the cell surface (Pardo et al., 2003). Previous research has shown that most living microorganisms have a limited absorption for heavy metals at lower pH, due to physiological properties (Fowle and Fein, 1999; Sar et al., 1999) as well as physical adsorbents such as zeolites steeply decreasing their adsorption capacity for both Cu and Cd with a decrease in pH (Ok et al., 2007).

*Effect of metal concentration*

The effect of initial metal concentration on metal biosorption by non-living biomass of *P. stutzeri* was evaluated under reaction conditions such as pH 5.0 and 30°C at an equilibrium time of 30 min as shown in Fig. 3. Biosorption rate of both metal ions decreased with an increase in initial concentration. The maximum biosorptions for Cd(II) and Cu(II) at the initial concentration of 300 mg/L were 43.5 and 36.2 mg/g dry mass, respectively. This result indicated that biosorptive capacity of non-living biomass for both Cu(II) and Cd(II) was similar at the range of initial concentrations, which was higher than those of living microorganisms as previously reported (Chen and Wang, 2007; Cho et al., 2001; Fowle and Fein, 1999). A previous study directly compared two Gram-positive bacteria for their biosorptive capacity of Cr(VI), indicating non-living biomass had higher maximum adsorption than living biomass (Zouboulis et al., 2004).

*Biosorption isotherm*

The isotherms indicate that the adsorption increases with an increase in equilibrium concentration of the sorbate. Figure 4 showed that the equilibrium adsorption isotherm of metal uptake by bacterial biomass was a chemically equilibrated and saturable mechanism. Thus, there was an increase in metal uptake as long as binding sites were free.

Adsorption follows both Langmuir and Freundlich isotherms (Fig. 5). However, the equilibrium data fitted well with the Langmuir adsorption isotherm for Cu(II) and Cd(II) biosorption at various initial metal concentrations. Values of Langmuir and Freundlich parameters are summarized in Table 1.

These data showed that the $q_{max}$ obtained for cadmium uptake by lyophilized of *P. stutzeri*, was 47.86 which was higher than that obtained for copper: 33.2 mg metal/g biomass. The *b* values obtained for cadmium and copper were found to be 0.055 and

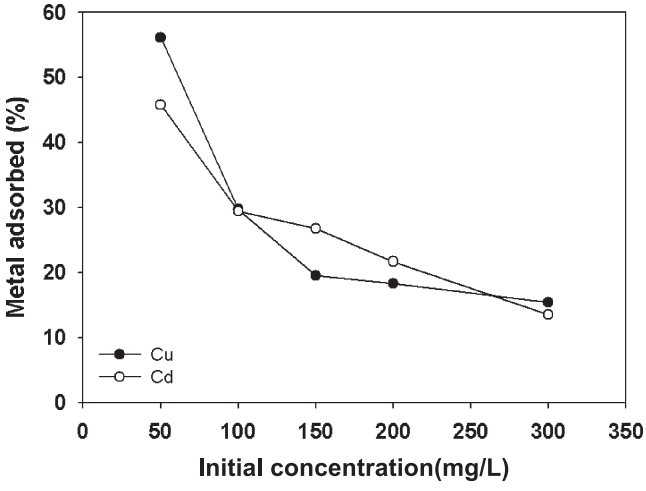


Fig. 3. Effect of initial concentration on biosorption Cu and Cd by *Pseudomonas stutzeri* at operating conditions (Biomass conc. 1 g/L, pH 5.0, temp. 30°C and time 30 min).

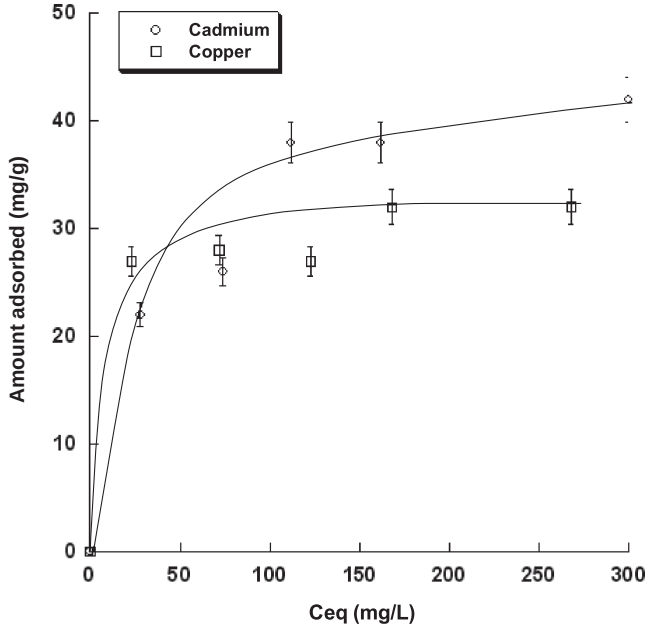


Fig. 4. Adsorption isotherm of cadmium and copper by lyophilized cells of *P. stutzeri* at operating conditions (Biomass conc. 1 g/L, pH 5.0, temp. 30°C and time 30 min).

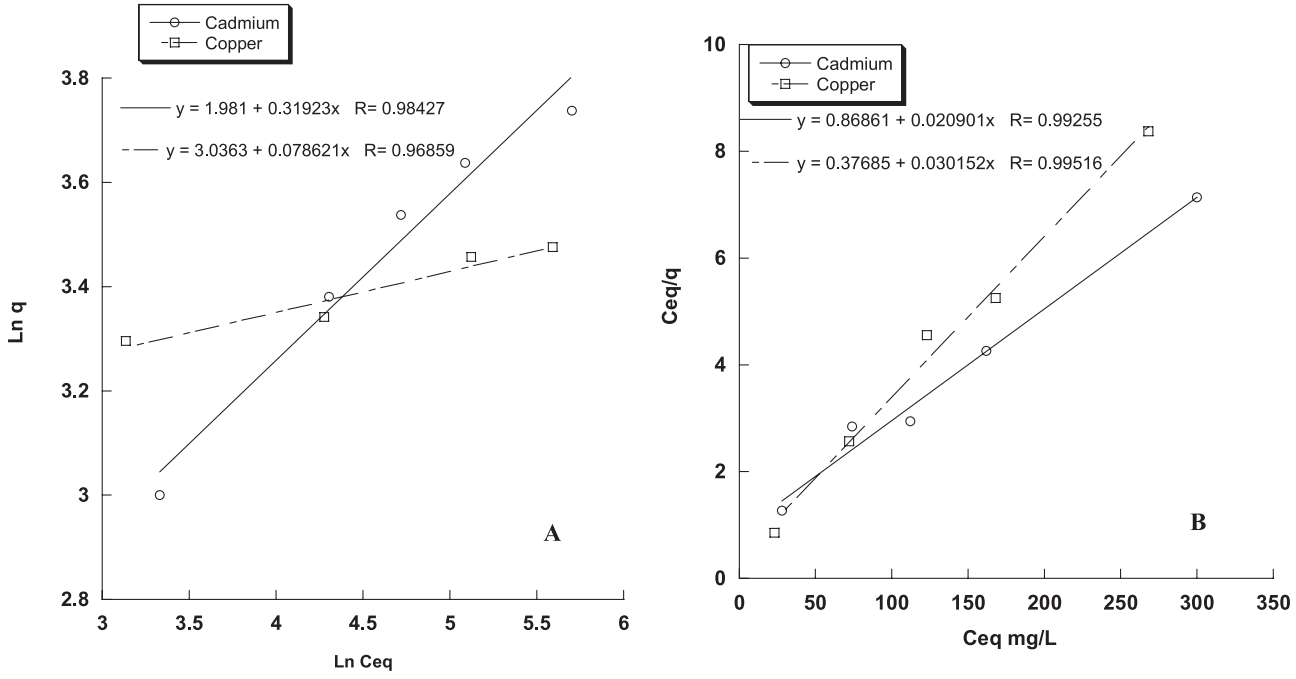


Fig. 5. The linear form of Freundlich (A) and Langmuir (B) adsorption isotherm of cadmium and copper by lyophilized cells of *P. stutzeri*.

Table 1. Freundlich and Langmuir isotherm parameters for metal biosorption by *Pseudomonas stutzeri*.

| Metal | Freundlich | | | Langmuir | | |
|---|---|---|---|---|---|---|
| | $K_f$ | $n$ | $r^2$ | $k$ | $b$ | $r^2$ |
| Cu[a] | 20.69 | 12.82 | 0.968*** | 0.035 | 33.16 | 0.999*** |
| Cd | 7.24 | 3.134 | 0.984*** | 0.055 | 47.860 | 0.999*** |

[a]Initial metal concentration ranged from 50 to 300 mg/L at pH 5.0 and 30°C.

***$p<0.01$.

Abbreviations: $K_f$ is the adsorption capacity, $n$ adsorption order, $r^2$ correlation coefficient, $k$ Langmuir constant and $b$ the maximum adsorption capacity.

0.035 L/mg respectively, which indicate that lyophilized cells of *P. stutzeri* possesses a high adsorption affinity for cadmium as compared to that for copper. Such correlations led us to conclude that the energy of adsorption is more favorable for cadmium than for copper.

The Freundlich analysis determined the sorptive capacity ($K_f$) and the sorptive intensity ($n$) of the bacterium dry mass for different metals. The biosorptive capacity and intensity for Cd(II) were found to be 7.24; 313 while that for copper Cu(II) was 20.1; 12.2.

In general, these data indicate that the sorption capacity increased with increasing the initial metal-ion concentration for both metals on the biomass surface. This sorption characteristic indicates that the surface saturation is dependent on the initial metal-ion concentrations; at low concentrations, adsorption sites took up the available metal more quickly. However, at higher concentrations metals need to diffuse into the biomass surface by intra particular diffusion and greatly hydrolyzed ions will diffuse at a slower rate (Gabr et al., 2008). The overall observations from both Freundlich and Langmuir isotherms for metal biosorption were in agreement with many previous studies (Acosta et al., 2005; Pardo et al., 2003; Sar et al., 1999). The selectivity order for metal ion towards the studied biomass matrices is Cd>Cu for a given initial metal ion concentration. This preferential type of adsorption belonging to two different ions may be ascribed to the difference in their ionic radii (Gabr et al., 2008). The ionic radius of Cd is 0.97 Å, while that of Cu is 0.73 Å. The smaller the ionic radius, the greater its tendency

to be hydrolyzed, leading to reduced biosorption; for this reason the bacterial biomass has greater affinity for cadmium than copper. Our results agree with those of many authors; Horsfall and Spiff (2005) reported the biosorption of $Pb^{2+}$ and $Cd^{2+}$ by *Caladium bicolor* (wild cocoyam).

*Comparison with other biosorbents*

Table 2 compares maximum adsorption capacities obtained in this study with some other values reported in the literature. The adsorption capacity for cadmium and copper using the lyophilized cells of *P. stutzeri* is of the same order of magnitude or greater than what has been found using similar biosorbents.

*Biosorption kinetics*

Adsorption of Cu(II) and Cd(II) by lyophilized cells of *P. stutzeri* dry biomass followed first order kinetics to a significant extent (Table 3), indicating the adsorption of the metal onto strain KCCM 34719 dry biomass was dependent on the concentration of the reacting metal. Among the kinetic models, first order kinetics provided

Table 2. Comparison of other biosorbents from selected literature with the present work.

| Metal | Biosorbent | Operating conditions | | | | Amount adsorbed (mg/g) | Ref. |
|---|---|---|---|---|---|---|---|
| | | pH | Temp. (°C) | Biomass (g/L) | Time | | |
| Copper | *Bacillus* sp. (ATS-1) | 5 | 25 | 2 | 2 h | 16.3 | Tunali et al., 2006 |
| | *Bacillus subtilis* IAM 1026 | 5 | 25 | 0.5 | 30 min | 20.8 | Nakajima et al., 2001 |
| | *Enterobacter* sp. J1 | 5 | 25 | 1 | 24 h | 32.5 | Lu et al., 2006 |
| | *Micrococcus luteus* | 5 | 25 | 0.5 | 1 h | 33.5 | Nakajima et al., 2001 |
| | *Pseudomonas aeruginosa* PU21 | 5 | NA | 1 | 24 h | 23.1 | Chang et al., 1997 |
| | *Pseudomonas cepacia* | 7 | 25 | NA | 30 min | 65.3 | Savvaidis et al., 2003 |
| | *Pseudomonas putida* | 6.6 | 30 | NA | 10 min | 6.6 | Pardo et al., 2003 |
| | *Pseudomonas putida* CZ1 | 4.5 | 30 | 1 | 24 h | 15.8 | Chen et al., 2005 |
| | *Pseudomonas stutzeri* IAM 12097 | 5 | 25 | 1 | 30 min | 22.9 | Nakajima et al., 2001 |
| | *Sphaerotilus natans* | 5.5 | 30 | NA | NA | 5.4 | Beolchini et al., 2006 |
| | *P. stutzeri* KCCM 34719 | 5 | 30 | 1 | 30 | 36.2 | This study |
| Cadmium | *Bacillus circulans* | 7 | 20 | 0.5 | 2 | 26.5 | Yilmaz and Ensari, 2005 |
| | *Enterobacter* sp. J1 | 6 | 30 | 1 | 1 | 46.2 | Lu et al., 2006 |
| | *Pseudomonas aeruginosa* PU21 | 6 | 25 | 1.0–2.0 | 24 | 42.4 | Chang et al., 1997 |
| | *Pseudomonas putida* | 6 | 30 | NA | 1 | 8 | Pardo et al., 2003 |
| | *Streptomyces pimprina* | 5 | NA | 1 | 1 | 30.4 | Puranik et al., 1995 |
| | *P. stutzeri* KCCM 34719 | 5 | 30 | 1 | 30 | 43.5 | This study |

NA: not available.

Table 3. The experimental kinetic parameters for the adsorption of Cu and Cd by *Pseudomonas stutzeri*.

| | Zero order kinetic | | | | First order kinetic | | | | Second order kinetic | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | $Y_0$[b] | $k$[c] | $r^{2***}$ | SE[d] | $Y_0$ | $k$ | $r^2$ | SE | $Y_0$ | $k$ | $r^2$ | SE |
| Cu | | | | | | | | | | | | |
| 30[a] | 0.235 | 0.000 | 0.893 | 0.083 | −1.501 | −0.002 | 0.976 | 0.271 | 4.660 | 0.007 | 0.969 | 0.975 |
| 50 | 0.474 | −0.001 | 0.955 | 0.107 | −0.770 | −0.001 | 0.961 | 0.182 | 0.782 | 0.000 | 0.996 | 0.058 |
| 100 | 1.288 | 0.000 | 0.994 | 0.106 | 0.249 | 0.000 | 0.900 | 0.078 | 0.782 | 0.000 | 0.996 | 0.058 |
| Cd | | | | | | | | | | | | |
| 30 | 0.106 | 0.000 | 0.760 | 0.058 | −2.377 | −0.003 | 0.977 | 0.424 | 11.805 | 0.027 | 0.928 | 4.022 |
| 50 | 0.293 | 0.000 | 0.967 | 0.055 | −1.247 | −0.002 | 0.987 | 0.165 | 3.531 | 0.006 | 0.985 | 0.511 |
| 100 | 0.694 | −0.001 | 0.990 | 0.075 | −0.372 | −0.001 | 0.951 | 0.103 | 1.460 | 0.001 | 0.993 | 0.413 |

[a]Metal-contaminated solution at 30°C, pH 7.0, and reaction time ranged from 1 to 250 min; [b]$Y_0$: intercept; [c]$k$: rate constant; [d]SE: standard error; $^{***}p < 0.01$.

the best fit (Table 3) based on the highest coefficient of determination ($r^2$) and the lowest standard error (Smith and Collins, 2007). The rate constants ($k$) for Cd(II) over the range of initial concentrations were higher than those for Cu(II). The patterns were observed in line within 50 min, indicating a correlation with the characteristics of inert biomass and its physicochemical interactions with the metal ion (Kim et al., 1996; Sar et al., 1999). The rapid metal sorption is also highly desirable for successful deployment of the biosorbents for practical applications (Sar et al., 1999; Volesky, 1990).

## Conclusions

- From the laboratory-based experiments, the following conclusions can be reached: The equilibrium time for adsorption of copper and cadmium is reached after 30 min at room temperature.
- The maximum pH for cadmium and copper biosorption was pH 5.0.
- The adsorption equilibrium data fitted well the Langmuir than Freundlich model for metal ions in the studied concentration range.
- The results demonstrate that lyophilized cells of *P. stutzeri* KCCM 34719 could be used as a promising biosorbent for the removal of Cu(II) and Cd(II) ions from aqueous solutions.

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