Sol–gel-immobilized recombinant *E. coli* for biosorption of Cd\(^{2+}\)

Jyh-Ping Chen\(^1\),* Yung-Sheng Lin\(^2\)

Department of Chemical and Materials Engineering, Chang Gung University, Kweisan, Taoyuan 333, Taiwan

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### Abstract

Recombinant *Escherichia coli* engineered with a metal-binding peptide was immobilized by entrapment in SiO\(_2\) gel beads using the sol–gel method. Biosorption of Cd\(^{2+}\) ions by the immobilized cells was studied in both batch and continuous systems. Adsorption equilibrium could be established within 3 h and the kinetics was well described by the pseudo-second-order kinetic model. The equilibrium data were best described by the Langmuir isotherm with the maximum uptake capacity being 79.9 mg/g cell at 25 °C. More than 95% of the adsorbed Cd\(^{2+}\) could be removed with 0.1 M CaCl\(_2\) during desorption. No loss in adsorption capacity was found up to five repeated adsorption/desorption cycles. From mass transfer analysis, only intraparticle diffusion effect was found to be important at low Cd\(^{2+}\) concentration (50 mg/dm\(^3\)), while at high concentration (250 mg/dm\(^3\)), both intraparticle and external mass transfer affected biosorption. Continuous removal and recovery of Cd\(^{2+}\) could be carried out by the immobilized cells in a packed-bed reactor.

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**Keywords:** Sol–gel; Immobilized cells; Biosorption; Cadmium

### 1. Introduction

Many industrial processes, such as mining, electroplating, dyeing, paper, and petroleum produce heavy metals-containing wastewaters, which are toxic to living organisms. Unlike organic pollutants, which in most cases can eventually be degraded, metallic pollutants released into the environment tend to persist indefinitely, circulating and eventually accumulating throughout the food chain thus posing a serious threat to animals and mankind. Cd\(^{2+}\) is attracting widespread attention of environmentalists as one of the most toxic heavy metals. Pollution by cadmium usually comes from several industrial process, such as electroplating, plastics manufacturing, nickel–cadmium batteries, fertilizers, pigments, mining, and metal-lurgical processes (Kaewsarn and Yu, 2001; Kefala et al., 1999). It has been recognized for adverse health effects by causing renal disturbances, lung insufficiency, bone lesions, cancer, and hypertension in humans (Bernard and Lauwerys, 1986; Nriagu, 1988).

Conventional processes for removing heavy metals from aqueous solution include chemical precipitation, sludge separation, chemical oxidation or reduction, ion exchange, reverse osmosis, electrochemical treatment, and evaporation recovery. However, these techniques may be ineffective or extremely expensive, especially when the metals are at relatively low concentrations (1–100 mg/dm\(^3\)) (Park et al., 1999). The use of biological materials as biosorbents for recovering heavy metals has emerged as a potential alternative method to conventional techniques (Kratochvil and Volesky, 1998). Several biological materials have been investigated for heavy metals removal, including bacteria, yeast, algae, and fungi (Veglio and Beolchini, 1997; Volesky, 2001). Commercial application of these microbial biomass as a biosorbent, however, has been hindered by problems associated with physical characteristics of these materials, such as small particle size with low density, poor mechanical strength and rigidity, and difficult solid–liquid separation (McHale and McHale, 1994; Volesky and Holan, 1995). Immobilization of the biomass within a suitable matrix can overcome these problems by offering ideal size, mechanical strength, rigidity, and porous characteristics to the biological material (Trujillo et al., 1995). For the application of immobilized cells in wastewater-treatment systems, the support materials should be insoluble in wastewater, non-biodegradable, insensitive to...
condensation and strengthen the network (Brinker and Scherer, 1990). The main chemical structure of the gel synthesized by the sol–gel method is a three-dimensional inorganic silica \( \text{SiO}_2 \) network, resembling ceramic materials. These matrices have several advantages over traditional materials, e.g., negligible swelling in solvents, chemical stability, and rigidity. The matrices are porous materials whose pores are large enough to allow the diffusion of substances. In addition, since the sol–gel process is performed at an ambient temperature from the gelation of molecular precursors, conditions during the sol–gel process is therefore mild enough to maintain the integrity of the entrapped cells (Branyik et al., 1998; Inama et al., 1993).

Naturally occurring metal-binding peptides, such as metallothioneins (MTs) are the main metal sequestering molecules used by cells to immobilize metal ions, offering selective, high-affinity binding sites (Mehra and Mulchandani, 1995). MTs are low molecular weight (6–7 kDa), cysteine-rich proteins found in animals, higher plants, eukaryotic microorganisms, and some prokaryotes (Hamer, 1986). Phytochelatins (PCs (\( \text{Glu–Cys} \)), \( n = 2 \)–11) are small, cysteine-rich peptides produced by plant, algae, and fungi (Cobbett, 2000; Zenk, 1996). They are capable of binding heavy metals via thiolate coordination and have higher metal-binding capacity (on a per cysteine basis) than MTs (Bae et al., 2000). Great success in heavy metal removal by microbial-based systems has been achieved by using microorganisms over-expressing either MTs or PCs (Kille et al., 1991; Sousa et al., 1998). Over-expression of metal-binding peptides or proteins by bacterial cells results in enhanced accumulation of \( \text{Cd}^{2+} \) and offers a promising strategy for the development of microbe-based biosorbents for the removal of heavy metal ions from wastewater (Huang et al., 2003; Yoshida et al., 2002).

In this study, recombinant *Escherichia coli* cells displaying functional synthetic PCs intracellularly were entrapped in silica gel beads prepared by the sol–gel process. The adsorption of \( \text{Cd}^{2+} \) in aqueous solution by the immobilized cells biosorbent was studied in details in both batch and continuous modes.

2. Materials and methods

2.1. Microorganism and media

Recombinant *E. coli* strain engineered with a phytochelatins-like metal-binding peptide (\( \text{Glu–Cys} \)) \( n = 20 \) using the pTrc expression vector was obtained from the Development Center for Biotechnology, Taipei, Taiwan (Sriprang et al., 2003). The cell growth medium contains (per liter): glucose, 10 g; yeast extract, 5 g; \( \text{NaCl} \), 5 g; tryptone, 10 g; \( \text{MgSO}_4 \), 1 g; \( \text{MnSO}_4 \), 0.01 g; \( \text{KH}_2\text{PO}_4 \), 2 g and \( \text{K}_2\text{HPO}_4 \), 2 g. The pH of the medium was adjusted to 7 by adding 0.1 N \( \text{NaOH} \).

2.2. Preparation of immobilized cells

Silica gels were prepared based on the sol–gel process where hydrolysis of the silicon alkoxide precursor was achieved under acidic conditions, and gelation was induced through the addition of base. Pre-hydrolyzed sols (20 cm\(^3\)) with high hydrolysis ratio were prepared by heating nitric acid aqueous...
solution (100:0.1 water to nitric acid molar ratio) to distillation temperature, followed by adding 7.6 cm³ of chilled (4 °C) tetraethylorthosilicate (TEOS, Fluka) to obtain a hydrolysis ratio (water to TEOS) of 33. After stirred for 10 min at 400 rpm, the turbid mixture became clear due to the hydrolysis of TEOS. The solution was continuously stirred for another 1 h at 85 °C to remove ethanol byproduct generated from the alcohol condensation step. After the distillation, the solution was cooled to room temperature and 4 cm³ of polyethylene glycol (PEG 600, Showa) was added. PEG was used as an additive to prevent collapse and excessive shrinkage of the mesopores formed within the gel beads after the polymerization step, which may be harmful to the bacteria cells.

Two cubic centimeters of the above mixture was chilled in an ice bath to reduce the rate of polycondensation, and 0.17 cm³ of 1 M KOH was immediately added to bring the solution pH to about 7. Cell suspension (80 mg dry weight/cm³) in 0.17 cm³ phosphate buffer (pH 7) was added to the sol–gel mixture and stirred. Spherical gel beads were prepared from the mixture by the drop-tower method, where aliquots of the well-mixed solution were extruded from a KDS-100 syringe pump (KD Scientific, Holliston, MA, USA) connected with an 18 gauge needle and dropped from the top of a 6-ft-height cylindrical glass container filled with paraffin oil (containing 2% Span 80 as the surfactant). Gelation occurred within 3 min at room temperature during the fall of droplets. Spherical silica gel beads with 2.0 ± 0.1 mm diameter and 1.42 g/cm³ density were collected from the bottom of the tower and washed thoroughly with phosphate buffer to completely remove the oil phase and stored at 4 °C for adsorption experiments.

2.3. Batch adsorption studies

Cd²⁺ solutions in the concentration range of 25–250 mg/dm³ were prepared from a stock solution in distilled deionized (DDI). Solution pH was not adjusted and controlled during the experiments and the initial pH value ranging from 5.7 to 6.2. Equilibrium adsorption experiments were conducted with 0.75 ± 0.02 g of gel beads were added to 50 cm³ Cd²⁺ solution (100 mg/dm³, pH 7) and shaken at 100 rpm for 24 h at 25 °C. To desorb Cd²⁺, the metal-laden gel beads were soaked in 0.1 M CaCl₂ solutions for 2 h to completely remove the metal ion, and then rinsed thoroughly with DDI water several times before starting the next biosorption cycle.

2.4. Repeated batch adsorption/desorption studies

In order to determine the reusability, consecutive biosorption/desorption cycles were repeated five times by using the same biosorbent. 0.75 ± 0.02 g of gel beads were added to 50 cm³ of Cd²⁺ solution (100 mg/dm³, pH 7) and shaken at 100 rpm for 24 h at 25 °C. To desorb Cd²⁺, the metal-laden gel beads were soaked in 0.1 M CaCl₂ solutions for 2 h to completely remove the metal ion, and then rinsed thoroughly with DDI water several times before starting the next biosorption cycle.

2.5. Continuous adsorption and desorption studies

Continuous adsorption were studied at 25 °C with 20 mg/dm³ Cd²⁺ solution being continuously pumped into a water-jacked column (1.0 cm diameter × 25 cm length) packed with the immobilized cells at a constant flow rate (0.3 and 0.5 cm³/min) from the bottom of the column. After adsorption, regeneration of the column was carried out with 0.1 M CaCl₂ flowing through the column at 0.5 cm³/min. The effluents were collected at regular intervals with a fraction collector for later measurements of Cd²⁺ concentrations by atomic absorption spectrometry.

3. Results and discussion

3.1. Adsorption kinetics of batch adsorption studies

Fig. 1 shows the changes in Cd²⁺ concentrations with time during the adsorption process with different initial ion concentrations. The kinetic of adsorption can be seen to be very rapid and occurs immediately after contacting the biosorbent with the metal ion solution. Equilibrium could be reached in 3 h in all cases.

![Fig. 1. Adsorption kinetics of Cd²⁺ by sol–gel-immobilized recombinant E. coli cells at different initial Cd²⁺ ions concentrations.](image-url)
In order to examine the controlling mechanism of adsorption process, experiment kinetic data in Fig. 1 were analyzed using two kinetic models. The pseudo-first-order model considers that the rate of occupation of adsorption sites is proportional to the number of unoccupied sites (Cruz et al., 2004). The rate of adsorption can thus be represented as follows,

\[
\frac{dq}{dt} = k_1(q_{eq} - q_t)
\]

(1)

By applying boundary conditions, \(q_t = 0\) at \(t = 0\) and \(q_t = q_t\) at \(t = t\), Eq. (1) can be integrated to give,

\[
\log \left( \frac{q_{eq}}{C_0} \right) = \log \left( \frac{q_{eq}}{C_0} \right) - \frac{k_1}{2.303} t
\]

(2)

A plot of \(\log \left( \frac{q_{eq}}{C_0} \right)\) versus \(t\) should give a straight line if this model applies to the experiment data. The value of \(q_{eq}\) needs to be estimated beforehand by extrapolating the experiment data to \(t = \infty\).

If adsorption is by a mechanism where \(\text{Cd}^{2+}\) can displace any existing ions from the binding sites, the pseudo-second-order kinetic model can be used and the kinetics can be expressed as (De Franca et al., 2002),

\[
\frac{dq}{dt} = k_2(q_{eq} - q_t)^2
\]

(3)

After integrating and applying the same boundary conditions as before, a linearized form of the second-order rate equation could be obtained.

\[
\frac{t}{q_t} = \frac{1}{k_2 q_{eq}} + \frac{1}{q_{eq}} t
\]

(4)

The plot \(t/q_t\) versus \(t\) should give a straight line if the second-order kinetic model is applicable.

Aiming at evaluating the biosorption kinetics of cadmium ions, both pseudo-first-order and pseudo-second-order kinetic models were used to fit the data (up to 160 min). For the pseudo-first-order model, theoretical \(q_{eq}\) values could be obtained from the \(y\)-intercepts of Eq. (2) with the experiment \(q_{eq}\) values estimated from the equilibrium data (24 h) in Fig. 1 (not shown due to lack of fits). The linearized equation, Eq. (4), of the pseudo-second-order model was similarly plotted and theoretical \(q_{eq}\) values were obtained from the slopes (Fig. 2). A comparison of the validity of these two models is presented in Table 1, where theoretical \(q_{eq}\) values obtained from both models are compared with the experiment values together with coefficient of determination \((R^2)\) from linear regression. The theoretical \(q_{eq}\) values estimated from the pseudo-first-order model show significant deviation from the experiment values with \(R^2\) as low as 0.8. On the other hand, the pseudo-second-order results give theoretical \(q_{eq}\) values very close to the experiment values with \(R^2\) above 0.999 in all cases. We therefore conclude that the kinetic of cadmium adsorption by the immobilized cells could be well represented by the pseudo-second-order kinetic model.

### 3.2. Mass transfer effects during batch adsorption

By ignoring the diffusion resistance of \(\text{Cd}^{2+}\) from bulk fluid to the liquid film surrounding the biosorbent, the diffusion of ions into the porous silica gel beads can be divided into a three-step process: boundary layer diffusion, intraparticle diffusion, and biosorption on binding sites. In many cases of biosorption, there is the possibility that intraparticle diffusion will be the rate limiting step and this is normally assessed by examining the relationship between the adsorption and square root of time (Keskinkan et al., 2004). An intraparticle rate constant can be

### Table 1

<table>
<thead>
<tr>
<th>Initial (\text{Cd}^{2+}) concentration (mg/dm³)</th>
<th>Experiment (q_{eq}) (mg/g)</th>
<th>First-order kinetic model (q_{eq}) (mg/g)</th>
<th>(R^2)</th>
<th>Second-order kinetic model (q_{eq}) (mg/g)</th>
<th>(R^2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>25</td>
<td>31.5 (1.6)</td>
<td>2.28 (0.32)</td>
<td>0.8853</td>
<td>30.1 (0.1)</td>
<td>0.9999</td>
</tr>
<tr>
<td>50</td>
<td>48.9 (1.0)</td>
<td>7.93 (0.11)</td>
<td>0.9959</td>
<td>49.6 (0.6)</td>
<td>0.9995</td>
</tr>
<tr>
<td>100</td>
<td>52.0 (1.9)</td>
<td>13.6 (0.3)</td>
<td>0.9780</td>
<td>51.5 (0.6)</td>
<td>0.9995</td>
</tr>
<tr>
<td>150</td>
<td>78.7 (2.2)</td>
<td>23.6 (7.9)</td>
<td>0.8850</td>
<td>80.9 (1.4)</td>
<td>0.9991</td>
</tr>
<tr>
<td>200</td>
<td>75.9 (0.2)</td>
<td>14.4 (8.2)</td>
<td>0.7972</td>
<td>77.4 (0.5)</td>
<td>0.9998</td>
</tr>
<tr>
<td>250</td>
<td>80.2 (2.6)</td>
<td>28.0 (1.5)</td>
<td>0.9561</td>
<td>83.1 (1.1)</td>
<td>0.9994</td>
</tr>
</tbody>
</table>

Values in parenthesis are standard deviations.
defined using the equation described by Weber and Morris (1963),

\[ K_p = \frac{q}{t^{1/2}} \]  

The relationship between \( q \) and \( t^{1/2} \) is shown in Fig. 3. It can be seen that the plot is linear at 50 mg/L Cd\(^{2+}\), with \( K_p \) value calculated from the slope to be 0.61 mg/(g min\(^{1/2}\)) \( (R^2 = 0.993) \). At higher Cd\(^{2+}\) concentrations (100 and 250 mg/dm\(^3\)), Eq. (5) did not hold over the entire time range, implying that although intraparticle diffusion is significant, more than one process is affecting the adsorption. This type of behavior has been reported previously (Kandah, 2001; Pan et al., 2005), and the double nature of these relationships has been interpreted in terms of two processes: boundary diffusion, which gives the initial, curved portion, and intraparticle diffusion, which gives the final linear portion. Under these circumstances, the slope of the final linear portion can be used to obtain the rate parameters by regression analysis. Values of \( K_p \) thus obtained (using the last three data points) are 0.66 and 0.69 mg/(g min\(^{1/2}\)) for 100 mg/dm\(^3\) \( (R^2 = 0.972) \) and 250 mg/dm\(^3\) \( (R^2 = 0.965) \) initial ion concentrations, respectively. It can be seen that an increase in C\(_i\) brings about an increase in \( K_p \) values, which is consistent with previous work (McKay et al., 1980).

External mass transfer has rarely been investigated for metal ion adsorption to spherical biosorbent. To determine the extent of external mass transfer resistance around a spherical gel bead, a simple approach has been proposed for estimating the mass transfer coefficient in the boundary layer during the biosorption process by using the initial adsorption rate data (Chen et al., 1996). The model took into account the metal ion concentration decrease in the bulk phase, the mass transfer resistance external to the particles, and the metal ion diffusion inside the particle. During the initial stage of adsorption, it can be assumed that the intraparticle metal ion diffusion is negligible and the adsorption isotherm is linear (Crank, 1975) and the model gives,

\[
\ln \left( \frac{C}{C_i} - \frac{1}{1 + \rho_p K} \right) = -\left( \frac{1 + \rho_p K}{\rho_p K} k_{L,a} \right) t + \ln \left( \frac{\rho_p K}{1 + \rho_p K} \right)
\]

A plot of \( \ln[C/C_i - 1/(1 + \rho_p K)] \) against \( t \) will yield a straight line from which the mass transfer coefficient can be calculated from the slope. The value of \( K \) was calculated as \( q_{\text{max}} K_L \) and can be obtained from the parameters of the Langmuir isotherm in Table 2 (see next section). Eq. (6) was used to analyze the initial adsorption data in Fig. 1 up to 40 min. The values of \( k_{L,a} \) were found to be 6.44, 2.03, and \( 1 \times 10^{-4} \) \( \text{h}^{-1} \) for \( C_i \) of 50, 100, and 250 mg/dm\(^3\), respectively. Assuming that the Sherwood number \( (d k_{L}/D) \) for metal ion diffusion through stagnant thin film around the gel bead is 2.0, we can calculate the diffusion coefficients of Cd\(^{2+}\) to be \( 0.60 \times 10^{-9} \), \( 0.19 \times 10^{-9} \), and \( 0.10 \times 10^{-9} \) \( \text{m}^2/\text{s} \), for \( C_i \) of 50, 100, and 250 mg/dm\(^3\), respectively. This value is of the same order of magnitude but smaller than that for Cd\(^{2+}\) in water \( (1.23 \times 10^{-9} \text{ m}^2/\text{s}) \) (Pepelis et al., 1995). The high diffusion coefficient in the boundary layer at 50 mg/dm\(^3\) also agrees with previous analysis where intraparticle diffusion was found to be the rate-determining step at \( C_i = 50 \text{ mg/dm}^3 \) but not at 100 and 250 mg/dm\(^3\). It is conceivable that external mass transfer rate around a gel particle for biosorption in a packed-bed reactor will be reduced further without the vigorous shaking action experienced in a batch adsorption study. Surface film resistance related to fluid flow is therefore an important factor to consider for continuous biosorption in a column reactor.

### 3.3. Adsorption isotherms

In order to optimize the design of a biosorption system, it is necessary to establish an appropriate correlation for the equilibrium data. Many isotherm equations have been used for the equilibrium modeling of biosorption systems, two of them are commonly used and have been applied in this study, i.e., the

<table>
<thead>
<tr>
<th>( T (\degree C) )</th>
<th>( q_{\text{max}} ) (mg/g)</th>
<th>( K_L ) (dm(^3)/mg)</th>
<th>( R^2 )</th>
<th>( K_F )</th>
<th>( n )</th>
<th>( R^2 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>68.0 (2.2)</td>
<td>0.098 (0.005)</td>
<td>0.995</td>
<td>22.2 (0.5)</td>
<td>0.204 (0.018)</td>
<td>0.965</td>
</tr>
<tr>
<td>25</td>
<td>79.9 (1.9)</td>
<td>0.136 (0.011)</td>
<td>0.998</td>
<td>25.3 (0.9)</td>
<td>0.224 (0.025)</td>
<td>0.940</td>
</tr>
<tr>
<td>40</td>
<td>61.5 (2.1)</td>
<td>0.151 (0.010)</td>
<td>0.994</td>
<td>23.9 (0.5)</td>
<td>0.176 (0.016)</td>
<td>0.962</td>
</tr>
</tbody>
</table>

Values in parenthesis are standard deviations.

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Fig. 3. Plot for analyzing intraparticle diffusion effect. Eq. (5) is used by plotting adsorption capacity \( (q) \) against the square root of the contact time \( (t^{1/2}) \) for different initial Cd\(^{2+}\) concentrations. Linear regression lines to y-axis are shown using the final linear portion of the data.
Langmuir and the Freundlich isotherms. The assumption of Langmuir isotherm for describing the adsorption process was that the adsorption occurred on a homogeneous surface with negligible interaction between adsorbed molecules (Langmuir, 1918). It has the following form,

$$q_{eq} = \frac{q_{\text{max}} K_L C_f}{1 + K_L C_f} \quad (7)$$

To calculate $q_{\text{max}}$, the Langmuir equation can be rearranged into a linearized form,

$$\frac{C_f}{q_{eq}} = \frac{C_f}{q_{\text{max}}} + \frac{1}{K_L q_{\text{max}}} \quad (8)$$

$q_{\text{max}}$ and equilibrium constant ($K_L$) can then be calculated from the slope and intercept of the regression line of $C_f/q_{eq}$ versus $C_f$.

The Freundlich isotherm is an empirical equation based on sorption on a heterogeneous surface (Freundlich, 1906). The Freundlich equation is expressed as,

$$q_{eq} = K_F C_f^n \quad (9)$$

$K_F$ and $n$ are empirical constants representing the adsorption capacity and adsorption intensity, respectively. The Freundlich equation can also be linearized by taking logarithms of both sides of Eq. (9),

$$\ln q_{eq} = \ln K_F + n \ln C_f \quad (10)$$

$K_F$ and $n$ can be similarly determined by linear plots of $\ln q_{eq}$ versus $\ln C_f$.

Figs. 4 and 5 show the results from batch equilibrium adsorption experiments conducted at different temperatures. The data are fitted with the linearized forms of Langmuir (Eq. (8)) and Freundlich (Eq. (10)) adsorption isotherms, respectively. The parameters along with the correlation coefficients have been estimated by linear regression and the results are summarized in Table 2. As can be seen from Table 2, the Langmuir isotherm fits the experimental results better than the Freundlich isotherm with $R^2$ above 0.99 in all cases. The optimum maximum uptake capacity ($q_{\text{max}}$) was 79.9 mg/g at 25 °C. The maximum uptake capacity of Cd$^{2+}$ for many biosorbents has been summarized and the value is between 7.8 and 125 mg/g with only three marine algae showing higher $q_{\text{max}}$ than the recombinant cell here (Cruz et al., 2004).

The equilibrium constant ($K_L$) increased with increasing temperature, indicating tighter affinities between binding sites and Cd$^{2+}$ at higher temperatures (endothermic reaction). The dependence of $K_L$ on temperature can be used to estimate both the enthalpy ($\Delta H$) and the entropy ($\Delta S$) change of the adsorption process from the thermodynamic relationship,

$$\ln K_L = -\frac{\Delta G}{RT} = -\frac{\Delta H}{RT} + \frac{\Delta S}{R} \quad (11)$$

By a linear plot of $\ln K_L$ and $1/T$, $\Delta H$ and $\Delta S$ were calculated from the slope and the intercept of the regression line, respectively ($R^2 = 0.951$). The $\Delta H$ was estimated to be 2.55 kcal/mol and $\Delta S$ was 27.57 cal/mol K. A positive value of $\Delta H$ demonstrates the endothermic nature, while the positive value of $\Delta S$ confirms the increased randomness at the solid–solution interface during biosorption. Cruz et al. (2004) reported an exothermic behavior (negative $\Delta H$ value) for Cd$^{2+}$ biosorption by dead Sargassum sp. biomass. This difference may probably arise from location of the adsorption site as the metal-binding peptide molecules are expressed intracellularly in the present case, which renders diffusion of ion through the cell membrane an energy barrier for adsorption.

Fig. 6 shows the effect of temperature on cadmium adsorption. The results show that temperature did not significantly affect cadmium adsorption, although the maximum adsorption capacity occurred at 25 °C. It is well known that biosorption of heavy metal ions depends on the pH of the solution (Kapoor et al., 1999; Rangsayatorn et al., 2004; Zhang et al., 1998). The solution pH affects the availability of metal ions in solution and binding sites. The pH value was found to influence biosorption only below 4 (Fig. 7). Adsorption was expected to be less favorable at pH values close or below the isoelectric point of the metal-binding peptide when it became

![Fig. 4. Linearized Langmuir adsorption isotherms of Cd$^{2+}$ by sol–gel-immoobilized recombinant E. coli cells at different temperatures. The lines are best-fits regression lines using the Langmuir model.](image1)

![Fig. 5. Linearized Freundlich adsorption isotherms of Cd$^{2+}$ by sol–gel-immoobilized recombinant E. coli cells at different temperatures. The lines are best-fits regression lines using the Freundlich model.](image2)
neutral or positively charged. This will invariably inhibit the adsorption of the positively charged Cd$^{2+}$. The result also suggest that Cd$^{2+}$ could be desorbed from the immobilized cells by lowering solution pH and an adjustment of solution pH before treatment will not be necessary if the pH is between 4.0 and 6.5.

3.4. Recovery and reuse efficiencies

Reusability of a biosorbent is of crucial importance in industrial practice for metal removal from wastewater. Cd$^{2+}$ adsorbed onto immobilized cells could be efficiently eluted with 0.1 M CaCl$_2$ due to ion-exchange effect. Fig. 8 shows the amount of Cd$^{2+}$ adsorbed and later recovered from batch adsorption/desorption experiments at different $C_i$. More than 95% of the adsorbed Cd$^{2+}$ was recovered irrespective of initial ion concentration used.

In order to examine the reusability of the biosorbent, the adsorption/desorption cycles were repeated five times with the same biosorbent preparation. The results in Fig. 9 indicated that no statistical difference ($p < 0.05$) existed between the adsorption capacity in each cycle and the elution was close to 100%. The immobilized cells can therefore be used repeatedly to remove Cd$^{2+}$ from wastewater following simple regeneration with 0.1 M CaCl$_2$. Acid solutions, such as 0.1 M HCl have also been used during the experiment to give good recovery of the adsorbed metal ion. Nonetheless, reusability of the biosorbent was poor with peptide molecules detected in desorption solution. This suggests that acid may lead to cell rupture and release of metal-binding peptide from the sol–gel matrix, which will result in diminished biosorption capacity after each reuse cycle.

3.5. Continuous adsorption/desorption studies

From a processing point of view, a column reactor packed with the sol–gel-immobilized cells will be a desirable way for Cd$^{2+}$ removal during large-scale industrial operations. The resulting effluent concentration profile would have a typical S shape for the adsorption process in a packed-bed reactor, with...
an initial period of minimum solute followed by a gradual breakthrough that ultimately reached the feed concentration. The breakthrough curves developed under two flow rates are presented in Fig. 10. Increasing flow rate resulted in shortening of the breakthrough time and flattening of the breakthrough curve, which in turn resulted in decreasing difference between the breakthrough time (5% of feed concentration) and the saturation time (95% of feed concentration). Integrations of the effluent concentration over the elution volume up to the saturation point (Geankoplis, 2003) gave the overall adsorption capacity with respect to flow rate reflects little mass transfer limitation considering the fact that the adsorption process does not reach equilibrium in the column.

After the binding sites were exhausted, desorption was carried out to regenerate the biosorbent under the similar flow condition. Efficient desorption of Cd$^{2+}$ could be carried out by simply eluting the column with 0.1 M CaCl$_2$. The results are shown in Fig. 11. It is evident that the desorption process occurred five times faster than the adsorption process, which could be finished in 3 h in comparison with 15 h during adsorption. In addition, Cd$^{2+}$ was concentrated during the operation with the maximum effluent concentration during desorption being 6.5-fold that in the feed stream. Recovery was also good within experiment errors and accuracy of graphical integration. The total amount of Cd$^{2+}$ recovered was 98.3% of that adsorbed and the value was comparable to that in batch operation.

4. Conclusion

Our study shows that biosorption of Cd$^{2+}$ could be effectively carried out with sol–gel-immobilized recombinant E. coli cells engineered with a metal-binding peptide. The time required to reach equilibrium was relatively short and not significantly influenced by initial ion concentration. The adsorption kinetics was governed by intraparticle diffusion at low Cd$^{2+}$ concentrations, but this effect became less dominant at high ion concentrations as external mass transfer also influenced the kinetics. The overall adsorption kinetics showed better fit with the pseudo-second-order kinetic model and the equilibrium data were well described by the Langmuir adsorption isotherm. The biosorption capacity of the immobilized cells was only slightly affected by temperature and pH with the maximum adsorption capacity being 79.9 mg/g at 25 °C. The biosorbent could be regenerated efficiently with 0.1 M CaCl$_2$ and be used repeated in consecutive adsorption/desorption cycles without sacrificing adsorption capacity. Immobilizing recombinant E. coli cells within sol–gel processed silica gel beads is feasible to develop a continuous Cd$^{2+}$ adsorption/desorption process in a packed-bed reactor.

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References


以溶凝膠膜固定之基因重組細菌生物吸附重金屬鈉

陳志平 林永盛
長庚大學化工與材料工程學系

摘要

本研究以溶凝膠膜製備鈉球，固定含金屬離子結合強度大的基因重組大腸桿菌，以批次及連续方式進行鈉金屬離子的生物吸附。此吸附劑可在3小時內即達吸附平衡，而吸附動力可達二次吸附動力模式表示。Langmuir吸附等溫線曲線可最佳描述吸附熱動學數據，在25℃條件下每克鈉可吸附79.9 mg鈉離子。而被吸附的鈉離子可使用1m氯化鈉溶液回收95%以上。在重覆吸附/脫附5次實驗中亦未發現吸附量的降低。經質譜分析，在低鈉離子濃度時(50 mg/dm³)，只有內部擴散阻力較重要，而在高鈉離子濃度時(250 mg/dm³)，內外擴散阻力均影響吸附速率。以鈉金屬離子吸附鈉可進行連織鈉金屬離子自水溶液中的去除與回收。