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Cadmium biosorption by polyvinyl alcohol immobilized recombinant *Escherichia coli*

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ABSTRACT

Recombinant *Escherichia coli* expressing human metallothionein protein was immobilized with polyvinyl alcohol (PVA) for the removal of cadmium from solution. The adsorption ability was strongly affected by pH with optimal performance at pH 5.0, while it was less sensitive to temperature over the range of 20–42 °C. The adsorption kinetics and equilibrium of PVA-immobilized cells was best described by pseudo-second order model and Langmuir isotherm, respectively. Over the Cd concentrations range of 10–150 mg/l, PVA-cells had the highest Cd removal percentage (82.7%) at 10 mg Cd/l and a biomass loading of 15.4 wt.%. Better adsorption ability was obtained when biomass loading was increased, as the highest adsorption capacity of 4.29 mg/g was achieved at 33.0 wt.% of biomass (initial Cd concentration = 100 mg/l). An aqueous solution of 0.01 M Na_3NTA displayed the best desorption efficiency (57–89%) for four A/D cycles, while 51–61% of the original adsorption capacity was retained after regeneration.

1. Introduction

Heavy metal pollution has received worldwide attention in the recent years due to the toxicological importance in the ecosystem, agriculture and human health [1–10]. Conventional methods for the removal dissolved heavy metal ions from the contaminated environments, such as chemical precipitation, chemical oxidation and reduction, ion exchange, filtration, electrochemical treatment, activated carbon adsorption, evaporative recovery [1,3–6,11,12], have a variety of disadvantages, including incomplete metal removal, high operation cost, high reagent or energy requirements, and generation of large quantity of toxic sludge or other wastes that require disposal [1–4,6,11–15]. Therefore, there is a need to develop more cost-effective and environmental-friendly technology for heavy metal treatment.

Biosorption, often referred to passive uptake and physicochemical binding of chemical species or ions to biomass or biopolymers, is one of the promising alternatives to the conventional wastewater treatments and plays an important role in pollution control of heavy-metal contaminated water [3–6,12,16,17]. Biomass of bacteria, fungi, algae, yeasts, and even plants was utilized as biosorbents to adsorb or accumulate metal ions [4,5,11,18]. However, the use of biomass whether they are dead or live in continuous biosorption operations needs to consider the problems like difficulty in separation of biomass after adsorption, biomass loss after regeneration, and poor physical strength [3,6,17]. The foregoing problems may be solved by applying immobilized cell technology [3,6,17,19–21]. Through immobilization, the mechanical strength of biomass and its resistance to various chemical constituents in aqueous environment could be enhanced. In addition, efficient biosorbent regeneration and metal recovery could be achieved by using immobilized-cell systems, thereby being suitable to be applied in continuous removal of heavy metal contaminants via an appropriate continuous bioprocess, such as packed bed, fluidized bed, and CSTR reactor [6,21].

Several support materials have been used for cell immobilization, such as natural gels (e.g., agar, carrageenan) and synthetic matrices (e.g., polyurethane, polyethylene glycol, polyacrylamide) [3,6,17,20–23]. These natural gel matrices are biodegradable and subject to abrasion whereas synthetic gels have better mechanical properties and are not biodegradable [17,21]. In this study, a synthetic gel, polyvinyl alcohol (PVA), is utilized as the cell immobilization matrix due to its merits of low cost, high durability, chemical stability, and non-toxicity to viable cells [17,21]. The aim of this work was to assess the potential of using PVA-immobilized recombinant *Escherichia coli* strain capable of expressing human

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metal-binding proteins (namely metallothioneins) for the removal of cadmium, which is commonly found in the industrial effluents in Taiwan.

2. Materials and methods

2.1. Plasmid and host strain

The recombinant *E. coli* strain harboring human metal-binding protein genes was constructed as described in our previous work [11]. The host strain (*E. coli* DH5α) employed plasmid *pMAL-p2X* that expressed maltose-binding protein (MBP) as the fusion partner to obtain the plasmid *pMAL-p2X-MT1A*, which could express the MBP-MT1A fusion proteins in the periplasmic compartment. The metal-binding proteins (metallothionein, MT) were originated from human hepatoma cell line HepG2 (*Homo sapiens MT1A*, genbank accession no. NM_005946). The resulting recombinant *E. coli* strain able to express periplasmic MBP-MT1A protein is designated as *E. coli* DH5α (*pMAL-p2X-MT1A*).

2.2. Strain cultivation and proteins expression in recombinant E. coli

Single colony from Luria Bertani (Difco) agar plate (Difco) supplemented with ampicillin (Sigma) was picked up and inoculated in 20 ml LB medium amended with 100 μg/ml of ampicillin at 37 °C with 200 rpm agitation for 12–14 h. Afterward, 2 ml (1.6% v/v) broth was transferred separately to several fresh 125 ml LB medium (in 500 ml shake flask) amended with ampicillin, and grow the cells at 37 °C with 200 rpm agitation. When cell density reached an optical density (at 600 nm) of 1.0 (i.e., OD_{600} = 1.0), 0.25 M of IPTG was added into the culture to induce the expression of fusion proteins. Eight hours after IPTG induction, cells were harvested by centrifugation (9050 × *g*, 10 min) from the culture, which had an OD_{600} of approximately 2.5 ± 0.3 (equivalent to 0.8 ± 0.1 g dry cells/l). After washing twice with 0.05 M Tris–HCl buffer (pH 5.0; Amresco), cells were re-suspended in approximate Tris–HCl buffer and stored at 4 °C for cell immobilization with PVA.

For mass production of MT-expressing *E. coli* DH5α (*pMAL-p2X-MT1A*) cells, 50 ml of the preculture broth (at early stationary phase) was transferred into 5 l fermentor containing 2 l fresh LB medium amended with 100 μg/ml of ampicillin. The cultivation condition was 37 °C, 200 rpm agitation, and 1 vvm aeration. When cell density reached an optical density (at 600 nm) of 1.0 (i.e., OD_{600} = 1.0), 0.25 M of IPTG was added into the culture to induce the expression of fusion proteins. Eight hours after IPTG induction, cells were harvested by centrifugation (9050 × *g*, 10 min) from the culture, which had an OD_{600} of approximately 3.0 ± 0.3 (equivalent to 0.96 ± 0.1 g dry cells/l). After washing twice with 0.05 M Tris–HCl buffer (pH 5.0; Amresco), cells were re-suspended in approximate Tris–HCl buffer and stored at 4 °C for cell immobilization.

2.3. Cell immobilization with polyvinyl alcohol (PVA)

Polyvinyl alcohol (PVA) with a grade of 99.92% saponification and 2000 degree of polymerization was purchased from Chang Chun Petrochemical Co. (Miaoli, Taiwan). All other chemicals were reagent grade purchased from Showa (Japan) [19]. The recombinant strain *E. coli* DH5α (*pMAL-p2X-MT1A*) after induced with IPTG for expression of human metal-binding protein was co-immobilized in phosphorylated PVA gel beads according to the method described in the literature [19,24]. In general, a mixture containing different concentrations of concentrated pure cultures was thoroughly mixed with an equal volume of PVA (14%, w/w). This PVA-cell mixture was dropped into a saturated boric acid solution and gently stirred for 0.5–1.0 h to form spherical beads. The labile beads thus formed were then transferred to a 0.5 M sodium phosphate solution for 0.5–1.0 h for gel hardening through esterification. The subsequent beads were washed with tap water and then swelling in the tap water for the following heavy metal biosorption experiments.

2.4. Measurement of heavy metals

The heavy metal adsorbate used in this study was cadmium chloride ($CdCl_2$) obtained from Sigma (USA). The cadmium solution (ca. 5000 mg/l) was prepared with 0.05 M Tris–HCl buffer (pH 5.0; Amresco) as the stock solution for biosorption experiments. Cadmium concentrations in solutions were measured by a flame atomic absorption spectrometer (AAS; Model 932 plus, GBC Scientific Equipment, Dandenong, VIC, Australia). Prior to AAS measurement, the heavy metal solutions were appropriately diluted with 0.1N HCl to ensure that the metal concentration in the sample was linearly dependent on the detected absorbance.

2.5. Biosorption at different pH

Ten grams of PVA gel beads (with or without biomass) were added to 50 ml of 100 mg Cd^{2+}/l solutions whose pH value was adjusted to 3.0–6.0 ± 0.03. The biosorption experiments were conducted at 30 ± 1 °C with 75 rpm agitation for 24 h. The PVA gel beads were then removed through centrifugation (9050 × *g* for 10 min) and the supernatant was appropriately diluted with 0.1N HCl for AAS measurement to determine the residual cadmium ions concentration.

2.6. Biosorption at different temperature

Ten grams of PVA gel beads (with or without biomass) were added to 50 ml of 100 mg Cd^{2+}/l solution at pH 5.0 ± 0.03 for biosorption experiments for a temperature range of 20–42 °C. The rest of experimental conditions and the procedures for determination of residual metal concentration were identical to those described earlier.

2.7. Determination of adsorption isotherms

The biosorbents (PVA gel beads with or without biomass) were added in aqueous solutions containing Cd^{2+} ions at different initial concentrations over the range of 10–200 mg/l. The adsorption experiments were carried out under the conditions of 30 ± 1 °C, pH 5.0 and 75 rpm shaking. After adsorption equilibrium was reached (using 24-h incubation to ensure saturation of metal adsorption), samples were taken from the solutions, and the residual metal concentration in the supernatant was measured by AAS. The equilibrium metal adsorption capacity (*q*; mg/g) was plotted versus the corresponding equilibrium metal concentration in aqueous phase (C_e, mg/l) to establish the adsorption isotherm curves. The adsorption capacity (*q*) was calculated according to Eq. (1).

$$q = \frac{(C_o - C_e)}{X} \quad (1)$$

where, C_o denotes the initial metal concentration (mg/l) and *X* (mg/l) denotes the mass concentration of PVA-immobilized cells or cell-free PVA beads.

2.8. Time-course profile of biosorption

The biosorbents (PVA gel beads with or without biomass) were added into 50 ml aqueous solution containing 100 mg Cd^{2+}/l in a

glass shake flask. The mixtures were gently agitated (75 rpm) at 30 ± 1 °C. The pH of the solution was controlled at 5.0 by Tris buffer to avoid precipitation of metals in the form of metal hydroxides. Samples were taken from the solution at desired time intervals and were subsequently centrifuged at 9050 × *g* for 10 min. The Cd^{2+} concentration in the resulting supernatant was determined by AAS. The cumulative Cd ions uptake capacity (q_c, mg/g) was also plotted against the incubation time (t, h) to establish the cumulative uptake capacity curves.

2.9. Biosorption using PVA-immobilized cells with different biomass loading

The PVA-immobilized cells were prepared following similar procedures described in previous sections, except that the cell concentrations were adjusted to obtain different biomass loading in the PVA beads (ranging from 8.4 to 45.8 wt.%). The biosorbents (PVA gel beads with or without biomass) were added in aqueous solutions containing Cd ions at an initial concentration of 100 mg/l. The adsorption experiments were carried out under the conditions of 30 ± 1 °C, pH 5.0 and 75 rpm shaking. After equilibrium adsorption, samples were taken from the solutions, and the residual metal concentration in the supernatant was measured by AAS. The percentage removal of Cd ions (R) was calculated according to Eq. (2).

$$R = \left[\frac{C_o - C_e}{C_o}\right] \times 100 \quad (2)$$

where, C_o denotes the initial metal concentration (mg/l), C_e denotes the equilibrium metal concentration in aqueous phase (mg/l), and R denotes percentage removal of Cd ions (%).

2.10. Desorption of Cd-loaded immobilized biosorbents

The biosorbents (PVA-immobilized cells, biomass loading = 33.0 wt.%) were added in aqueous solutions containing Cd ions at an initial concentration of 100 mg/l. The adsorption was carried out under the conditions of 30 ± 1 °C, pH 5.0 and 75 rpm shaking. After equilibrium adsorption, samples were taken from the solutions by centrifugation at 9050 × *g* for 10 min. Prior to desorption operations, the metal-loaded biosorbents were rinsed twice with deioned distilled water and were then added to solutions containing different desorption agents, which included 0.01 M Na_2EDTA, 0.01 M Na_3NTA, 0.1 M Na_3NTA (pH 5.0), and 0.1 M Na_2SO_3 (pH 5.0). The incubation conditions of desorption were the same as that of adsorption (i.e., 30 ± 1 °C and 75 rpm agitation). After desorption for 24 h, the supernatant was again taken from the solutions by centrifugation at 9050 × *g* for 10 min. The Cd^{2+} concentration in the supernatant was measured by AAS.

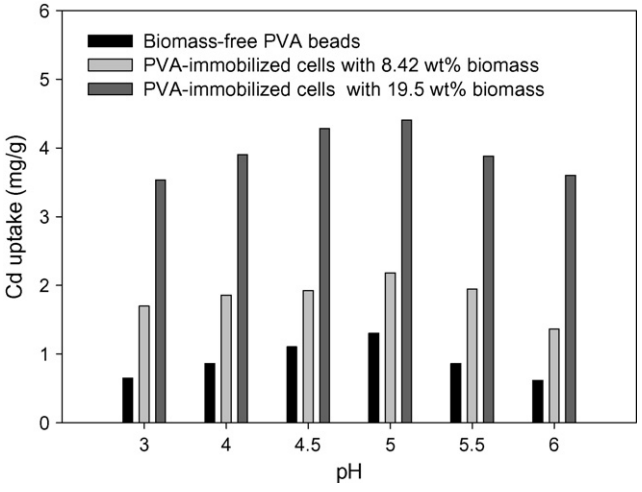

Fig. 1. Effect of pH on biosorption of Cd^{2+} using cell-free and cell-entrapped PVA gel beads (biomass loading was 8.42 and 19.5 wt.%, respectively). Initial Cd^{2+} concentration was 100 mg/l. The incubation temperature and agitation rate was 30 ± 1 °C and 75 rpm, respectively.

2.11. Repeated adsorption/desorption operations in shake flasks

The biosorbents (PVA-immobilized cells, biomass loading = 35.7 wt.%) were added in aqueous solutions containing Cd ions at initial concentrations of 100 mg/l. The adsorptions were carried out following the procedures shown above. After equilibrium adsorption, samples were taken from the solutions by centrifugation and the residual metal concentration was measured by AAS. The metal-loaded biosorbents were rinsed twice with deioned distilled water and was subjected to desorption operation with different desorption agents using the same procedures described earlier. After desorption for 24 h, the supernatant was again taken from the solutions by centrifugation and the metal concentration in the supernatant was detected by AAS. After desorption, the resulting biosorbents were rinsed with a large amount of deioned distilled water to remove the remaining desorption agents and were then used for next adsorption/desorption (A/D) cycle. The A/D cycles were repeated four times under identical conditions.

3. Results and discussion

3.1. Effect of pH on Cd^{2+} uptake by PVA-immobilized cells

Heavy metal biosorption from aqueous solutions is generally known to be greatly dependent on solution pH since the pH value may affect the surface charge and degree of ionization of the biosorbents. In addition, the pH value not only influences the metal binding sites on the cell surface but also the chemistry of metal adsorbates in solution [1,2,4,5,25,26]. Therefore, the effect of pH on biosorption of Cd^{2+} onto the PVA gel beads with or without biomass loading was investigated under different initial pH values ranging from 3.0 to 6.0. Note that biosorption was not conducted at a pH beyond pH 6.0 to avoid precipitation of heavy metal ions [26–28]. As shown in Fig. 1, it was observed that as the pH was increased from 3.0 to 5.0, the Cd^{2+} uptake ability of the PVA gel beads (with or without biomass) also increased, reaching a maximum adsorption capacity at pH 5.0. However, the metal uptake ability decreased when the pH was increased from pH 5.0 to 6.0. At pH 5.0, the Cd^{2+} biosorption capacity of the biomass-free PVA beads was 1.30 mg/g, which is significantly lower than the adsorption capacity of PVA-immobilized cells, displaying a capacity of 2.18 and 4.41 mg/g for biomass loading of 8.42 and 19.5 wt.%, respectively. It is observed that under highly acidic conditions (i.e., at low pH values), the PVA immobilized cells obtained low adsorption capacities, probably due to protonation of the biomass components inside the PVA particles. Moreover, low pH environments may lead to a high concentration of H_3O^+, thereby intensifying the competition between H_3O^+ and heavy metal ions for negatively charged adsorption sites [5,25]. Thus, the increase in the biosorption capacity for a pH increase from 3.0 to 5.0 may indicate the increase in electrostatic attractions between positively charged Cd^{2+} and negatively charged binding sites [25], as the competing effect of H_3O^+ against metal ions decreases, leading to the enhanced metal uptake capacity. On the other hand, the decrease in adsorption capacity for a pH increase from 5.0 to 6.0 was due probably to the decrease in the degree of protonation of biosorbents surfaces and the slowly increase of the OH^- concentration in the solutions [29].

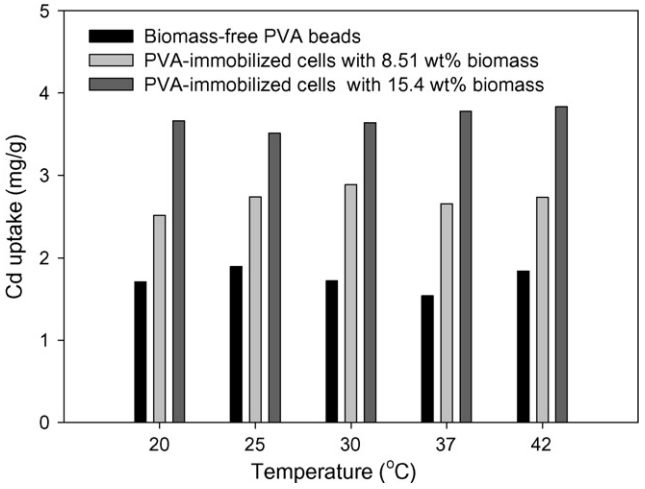

Fig. 2. Effect of temperature on biosorption of Cd^{2+} using cell-free and cell-entrapped PVA gel beads (biomass loading was 8.51 and 15.36 wt.%, respectively). Initial Cd^{2+} concentration was 100 mg/l. The incubation pH and agitation rate was 5.0 and 75 rpm, respectively.

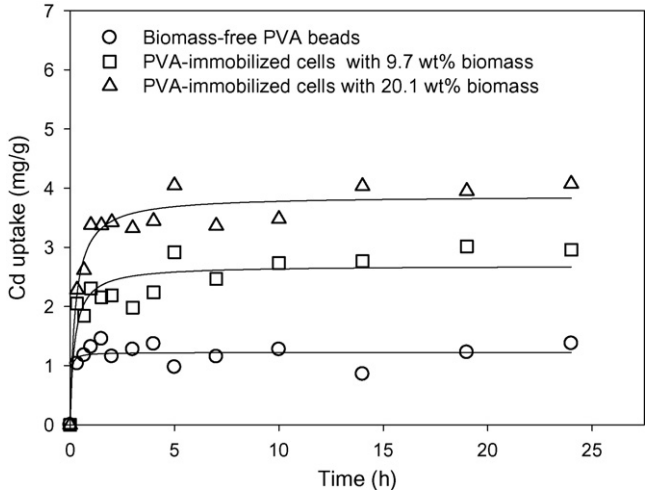

Fig. 3. Time-course cumulative Cd^{2+} uptake ability of cell-free and cell-entrapped PVA gel beads (biomass loading was 9.70 and 20.1 wt.%, respectively). Initial Cd^{2+} concentration was 100 mg/l. The incubation temperature, pH, and agitation rate was $30 \pm 1\,^{\circ}C$, 5.0, and 75 rpm, respectively.

3.2. Effect of temperature on Cd^{2+} uptake by PVA-immobilized cells

The temperature of the adsorption medium could be important for energy dependent mechanisms in metal removal by microorganisms and biosorbents. Furthermore, temperature is also known to affect the stability of the cell wall, its configuration and can also cause ionization of chemical moieties. These factors may cause positive or negative effects on heavy metal removal of the microbial biosorbents [30,31]. On the other hand, energy-independent mechanisms are less sensitive to temperature since the processes responsible for metal removal are largely physicochemical in nature [31]. As shown in Fig. 2, the effect of temperature on the metal uptake capacity (i.e., q value) of PVA gel beads (with or without biomass loading) hardly changed over the tested temperature of 20–42 °C. The Cd^{2+} biosorption capacity of the biomass-free PVA beads and PVA-immobilized cells with biomass loading of 8.51 and 15.4 wt.% (pH 5.0, 100 mg/l initial Cd^{2+} concentration, 75 rpm agitation) was between 1.54 and 1.90, 2.51 and 2.89 and 3.51 and 3.83 mg/g, respectively. This suggests that Cd adsorption by PVA gel beads (with or without biomass) may not be significantly dominated by temperature-sensitive physical adsorption [5]. The temperature-effect results obtained from this study are consistent with that observed by Mack et al. [13].

3.3. Kinetics of biosorption

The time-course Cd^{2+} uptake profile was determined to identify the biosorption kinetics. As indicated in Fig. 3, where the cumulative Cd^{2+} adsorption was plotted as a function of time, the saturation capacity for biomass-free PVA beads, and PVA-immobilized cells (biomass loading of 9.7 and 20.1 wt.%) were 1.22, 2.69 and 3.87 mg/g, respectively, which was reached within one hour of incubation time. The initial adsorption rate was 3.40, 4.94, and 6.66 mg/g/h for cell-free PVA gel and PVA-immobilized cells with biomass loading of 9.7 and 20.1 wt.%, respectively. It is noted that the metal uptake ability (both capacity and rate) increased with an increase in biomass loading. The foregoing results show that the presence of biomass had a substantial impact on Cd biosorption even though PVA gel itself also possessed a minor metal uptake capacity. The time-course biosorption profile was fitted with Lagergren pseudo-first order model (Eq. (3)) and pseudo-second order model (Eq. (4)) [32–34]. The results show that pseudo-second order model (r^2 = 0.989–0.995) had a better description of the biosorption kinetics (Table 1). This result is consistent with previous findings on Cd biosorption using different biosorbents, such as hybrid biosorbent (fungal biomass of *Phanerochaete chrysosporium* with fibrous network of papaya wood [33], *Penicillium simplicissimum* [35], and olive stone [36].

$$\frac{dq_t}{dt} = k_1(q_e - q_t) \tag{3}$$

$$\frac{dq_t}{dt} = k_2(q_e - q_t)^2 \tag{4}$$

where, q_t and q_e denotes the amount of metal adsorbed per unit weight of adsorbent (mg/g) at time t, and at equilibrium, respectively, and k_1 and k_2 denotes the pseudo-first order and pseudo-second order adsorption rate equilibrium constants (h^{-1}), respectively.

3.4. Percentage Cd^{2+} removal under different initial concentrations

The percentage removal of Cd^{2+} by biomass-free PVA beads and PVA-immobilized cells was determined over a concentration range of 10–200 mg/l under the condition of 24-h adsorption, $30 \pm 1\,^{\circ}C$, and 75 rpm agitation. The results represented in Fig. 4 show that for the PVA-immobilized cells with a biomass loading of 9.71 and 15.4 wt.%, the removal efficiency was higher at lower initial Cd^{2+} concentration with a removal percentage of 25–58% and 31–83%, respectively. As expected, the PVA-immobilized cells with a higher biomass loading gave a higher removal percentage. Surprisingly, for cell-free PVA beads, the initial Cd^{2+} concentration had less significant effects on the removal efficiency (from 13.5 to 21.2%), which

Table 1
The correlation coefficient (r^2) of the time-course biosorption profile simulated with Lagergren pseudo-first order model and pseudo-second order model.

Adsorbents	Correlation coefficients (r^2)	
	Pseudo-first order model	Pseudo-second order model
Biomass-free PVA beads	9.527×10^{-3}	0.989
PVA cells with 9.7 wt.% biomass	0.481	0.995
PVA cells with 20.1 wt.% biomass	0.339	0.995

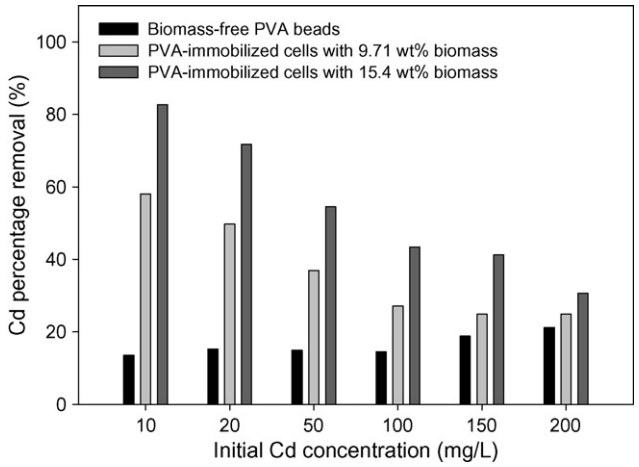

Fig. 4. Percentage Cd^{2+} removal by cell-free and cell-entrapped PVA gel beads (biomass loading was 9.71 and 15.4 wt.%, respectively) at different initial Cd^{2+} concentrations. The incubation temperature, pH, and agitation rate was 30 ± 1 °C, 5.0, and 75 rpm, respectively.

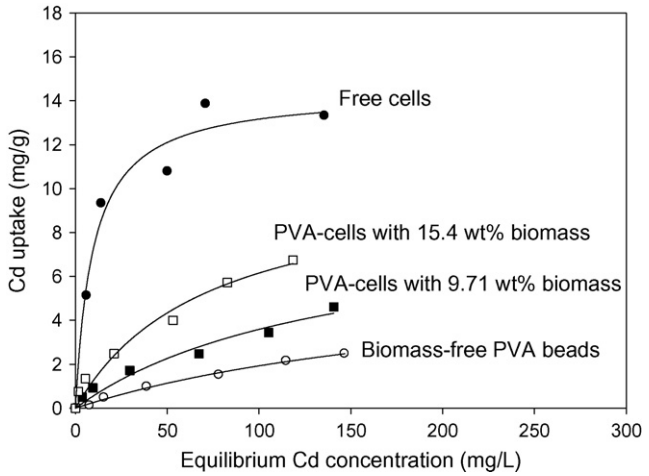

Fig. 5. Adsorption isotherms of PVA gel beads with and without biomass loading at different initial Cd^{2+} concentrations (biomass loading was 9.71 and 15.35 wt.%, respectively). The incubation temperature, pH, and agitation rate was 30 ± 1 °C, 5.0, and 75 rpm, respectively.

is much lower than that for immobilized cells. The removal efficiency of the immobilized cells did not vary considerably when initial metal concentration was higher than 100 mg/l (Fig. 4). The reason for a lower removal percentage of immobilized cells at a higher initial metal concentration could be due to competition of more metal ions for a fixed amount of available binding sites [6] or due to a lower metal ion to adsorption site ratio when initial metal concentration was higher [17]. Nevertheless, for low initial metal concentration (i.e., 10 mg/l), the PVA-immobilized cells had an excellent removal efficiency of 83 and 58% for a biomass loading of 15.4 and 9.71 wt.%, respectively (Fig. 4). The feature of having better removal efficiency at a low metal ion concentration suggests the feasibility of using the PVA-immobilized cells to remove metal ions from low-metal-concentration aqueous solutions, addressing one of the advantages of using biosorption methods for metal removal over using chemical or physical methods [3,4,30].

3.5. Biosorption equilibrium

An adsorption isotherm is characterized by certain constants, and the values of which express the adsorption properties and affinity of the adsorbate to biosorbent [25,26]. The Langmuir isotherm (Eq. (5)), one of the most commonly used adsorption isotherms, was used to describe the resulting adsorption equilibrium of the PVA gel beads (with or without biomass).

$$q = \frac{q_{max} C_e}{K_d + C_e} \quad (5)$$

where, q_{max} represents the maximum adsorption capacity (mg/g) and K_d is the dissociation constant (mg/l). The isotherm was determined by fitting the experimental data to Eq. (5) and the optimal q_{max} and K_d values estimated from the model are given in Table 2. The high correlation coefficient ($r^2 > 0.97$) indicates that Langmuir isotherm could properly describe the adsorption equilibrium of Cd^{2+} ions onto PVA-immobilized cells as also observed in many metal biosorption reports [1,5,17,25,26,30]. From Fig. 5 and Table 2, the q_{max} of biomass-free PVA beads was 6.35 mg/g, while the q_{max} value increased to 8.67 and 10.6 mg/g, respectively, when 9.71 and 15.4 wt.% of biomass was immobilized into the PVA beads. Meanwhile, a significant decrease in K_d values when biomass was included indicates the enhancement of the affinity between the adsorbents and Cd ions, due probably to the expression of metal-binding proteins on the recombinant cells. Although the Cd^{2+} biosorption capacity was lower than that for free cells (Fig. 5), probably attributed to the additional mass transfer resistance arising from cell immobilization with PVA gel, the PVA-immobilized cells are still convenient and efficient biosorbents for metal adsorption and sequential adsorbent regeneration and metal recovery [21].

3.6. Effect of biomass loading on Cd^{2+} biosorption

To identify the appropriate biomass loading in immobilized cells, biosorption experiments were carried out with PVA-immobilized cells containing different quantities of the biomass [6,22]. Fig. 6 shows a general trend that metal adsorption capacity increased with an increase in the biomass loading in the PVA-immobilized cells. The metal uptake capacity increased from 2.31 to 4.29 mg/g when biomass loading increased from 8.4 to 33.0 wt.%, above which the metal uptake capacity was essentially leveled off at a biomass loading of 39.1 and 45.8 wt.%. In general, the effect of biomass loading on biosorption capacity would be highly depen-

Table 2
Optimal Langmuir isotherm parameters (i.e., maximal adsorption capacity (q_{max}) and dissociation constant (K_d)) estimated from biosorption of Cd^{2+} by PVA gel beads with and without biomass loading. Initial Cd^{2+} concentrations ranged from 10 to 200 mg/l. The adsorption was carried out under the conditions of 30 ± 1 °C, pH 5.0 and 75 rpm agitation.

Metal	Parameters	Biosorbents			
		Free cells	Biomass-free PVA beads	PVA cells with 9.71 wt.% biomass	PVA cells with 15.4 wt.% biomass
Cd^{2+}	q_{max} (mg/g)	14.43	6.35	8.67	10.58
	K_d (mg/l)	9.64	228.98	141.31	71.94
	r^{2a}	0.97	0.99	0.97	0.98

[a] Correlation coefficient.

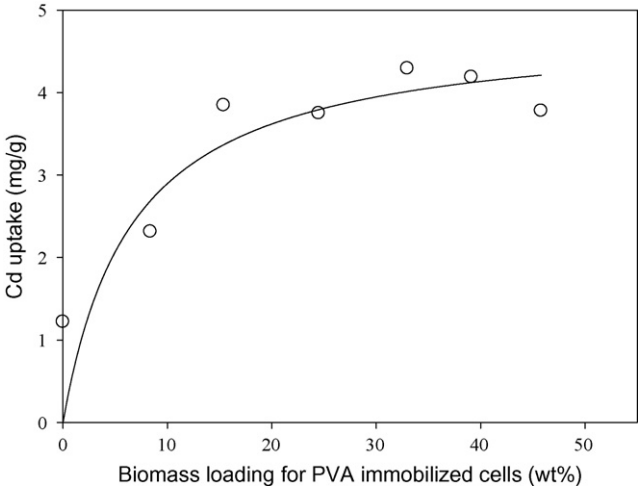

Fig. 6. Maximum uptake ability of Cd^{2+} by PVA gel beads with and without biomass loading (the biomass loading ranged from 8.38 to 45.8 wt.%). Initial Cd^{2+} concentration was 100 mg/l. The incubation temperature, pH, and agitation rate was 30 ± 1 °C, 5.0, and 75 rpm, respectively.

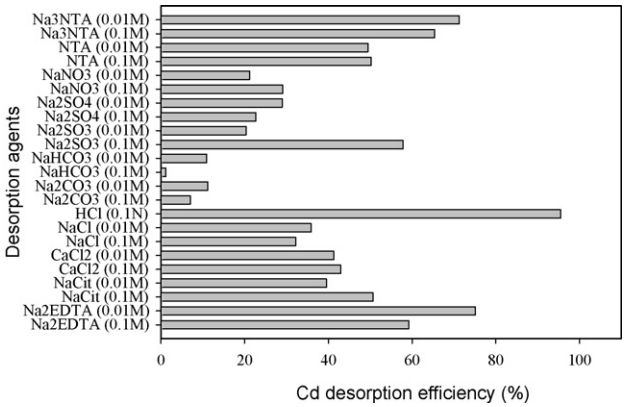

Fig. 7. Metal desorption efficiency of the suspension biomass of the recombinant *E. coli* cells (Cd adsorption capacity = 21.02 mg Cd/g cells) using different desorption agents.

dent on whether the biomass (acting as the adsorbent) could be effectively in contact with the adsorbates (e.g., metal ions). In particular, for the immobilized cells, how the biomass dispersed inside the immobilized beads play a major role in influencing the "averaged" adsorption capacity of the whole immobilized cells. As a result, the literature reports diversified biomass-loading effects on metal biosorption. Bai and Abraham [6] stated that for all matrices compared, increase in biomass load enhanced percentage of metal (Cr) adsorption, whereas a decrease in Cr adsorption was observed with a biomass loading beyond certain threshold value. However, Khoo and Ting [17] observed that an increase in the biomass loading in the PVA beads led to a decrease in the specific gold uptake. Fourest and Roux [37] also found that the specific uptake of zinc by Rhizopus arrhizus was inversely proportional to the mycelial concentration. It seems that the effect of biomass loading on biosorption capacity could vary according to what type of biosorbent was used and how the biosorbent was prepared.

3.7. Selection of desorption agents and repeated adsorption/desorption cycles

Regeneration and repeated uses of the biosorbent could markedly enhance the economical feasibility of a biosorption process [23]. The selection of appropriate desorption agents for efficient and non-damaged removal of the loaded metal ions from the biosorbent is the key to regeneration and reusability of biosorbents. Our preliminary screening of desorption agents for removing Cd^{2+} from the suspension biomass of the recombinant *E. coli* cells indicated that HCl, Na_2EDTA, Na_3NTA and Na_2SO_3 had better desorption efficiency over NaCit (sodium citrate), $CaCl_2$, NaCl, Na_2CO_3, $NaHCO_3$, Na_2SO_4, $NaNO_3$, and NTA (nitrilotriacetic acid) (Fig. 7). Although HCl could completely remove Cd^{2+} from the loaded biomass, we did not consider it as a good desorption agent because it severely damaged the biosorbent, leading to a poor biosorbent recovery efficiency (data not shown). Hence, we further examined the desorption efficiency of the better desorption agents at different pH and concentrations, including 0.01 M Na_2EDTA, 0.01 M Na_3NTA, 0.1 M Na_3NTA (pH 5.0), and 0.1 M Na_2SO_3 (pH 5.0). The foregoing desorption agents were used to treat Cd^{2+}-saturated PVA-immobilized cells with 35.7 wt.% biomass loading. The regenerated biosorbent was subjected to repeated adsorp-

Table 3
Cd adsorption and desorption performance in repeated adsorption and desorption (A/D) cycles for PVA-immobilized cells with 35.7 wt.% biomass loading. The adsorption was carried out under the conditions of 30 ± 1 °C, pH 5.0 and 75 rpm agitation. The desorption was carried out under the conditions of 30 ± 1 °C and 75 rpm agitation with different desorption agents at different pH and concentration.

Desorption agents	No. of A/D cycle	Relative adsorption capacity (%)[a]	Desorption efficiency (%)[b]	Adsorption capacity (mg Cd/g immobilized cells)
Na_2EDTA (0.01M)	1	100	72.9	5.84
	2	32.2	147.4	1.88
	3	45.7	60.6	2.67
	4	34.1	43.0	1.99
Na_3NTA (0.01M)	1	100	72.7	6.11
	2	60.9	89.3	3.72
	3	50.7	77.3	3.10
	4	61.1	57.2	3.73
Na_3NTA (0.1 M) pH 5	1	100	79.9	5.73
	2	15.2	202.1	0.87
	3	22.0	112.9	1.26
	4	25.8	68.1	1.48
Na_2SO_3 (0.1 M) pH 5	1	100	83.6	5.60
	2	43.4	99.7	2.43
	3	47.3	66.0	2.65
	4	54.3	43.0	3.04

[a] Setting adsorption capacity at cycle 1 as100%.
[b] The amount of Cd ions desorbed versus the amount of Cd ions adsorbed at each cycle.

tion/desorption (A/D) cycles to examine the reusability of the biosorbents.

Table 3 shows the relative adsorption and desorption efficiency after four A/D cycles. The PVA-immobilized cells exhibited good desorption efficiency at cycle 1 with a desroption efficiency of 73–84% (Table 3). However, the adsorption capacity markedly decreased at cycle 2 to cycle 4. When using 0.01 M Na_2EDTA and 0.1 M Na_3NTA as the desorption agent, the Cd^{2+} uptake capacity reduced to 32–45% and 15–26%, respectively, when compared to that of cycle 1, indicating a significant loss of adsorption capacity due to desorption operation. In contrast, using 0.01 M Na_3NTA and 0.1 M Na_2SO_3 as desorption agent, the regenerated biosorbents had better performance, as they attained 51–61% and 43–54%, respectively, of the original adsorption capacity at cycle 1 (Table 3). The desorption efficiency at cycle 2 was quite high and over 100% in some cases, whereas at cycle 3 to cycle 4 the desorption efficiency decreased to 43–61%, 57–77%, 68–113%, and 43–66% for using Na_2EDTA (0.01 M), 0.01 M Na_3NTA, 0.1 M Na_3NTA, and 0.1 M Na_2SO_3 as desorption agents, respectively (Table 3). The reason why the desorption efficiency of Cd^{2+} over 100% at cycle 2 was probably because some of the adsorbed Cd ions were located in the periplasmic space since the recombinant strain used in this study expresses the metal binding protein MT in the periplasmic compartment. The Cd^{2+} adsorbed in the periplasm may not be completely desorbed during the first desorption operation, but the residual Cd^{2+} in the periplasm could somehow be remove at cycle 2 or cycle 3 (for using 0.1 M Na_3NTA), resulting in an overshoot of over 100% in desorption efficiency. These results are in accordance with those reported by Vijayaraghavan and Yun [23], describing that the intracellular expression of MTs may prevent the recycling of the biosorbents, as the accumulated metals cannot be easily released. Moreover, the decrease in the desorption efficiency at cycle 3 to cycle 4 indicates a slightly damage of the desorption agents to the biosorbents or retention of residual desorption agents inside the PVA-immobilized cells, probably occupying the metal-binding sites.

Table 3 also compares the adsorption capacity of the PVA-immobilized cells treated with the four different desorption agents at each A/D cycle. All the PVA-immobilized cells gave a good Cd^{2+} uptake capacity at cycle 1 with a capacity of 5.60–6.11 mg Cd/g immobilized cells. For cycle 2 to cycle 4, the adsorption capacity was quite stable for the immobilized cells treated with different desorption agents, whereas the Cd uptake capacity significantly dropped after entering the second A/D cycle due probably to the destructive effect of the desorption agents on the immobilized cells, in particular on the expressed MT protein. Nevertheless, using Na_3NTA (0.01 M) as the desorption agent gave a better Cd uptake capacity of 3.1–3.7 mg Cd/g immobilized cells at cycle 2 to cycle 4. The capacity accounts for nearly 51–61% of the original adsorption capacity at cycle 1. In contrast, using a higher concentration of Na_3NTA (i.e., 0.1 M) resulted in a poor regeneration of the biosorbent with a low Cd uptake capacity of 0.87–1.48 mg Cd/g immobilized cells. These results indicate that the concentration of desorption agents is also an important parameter for the efficiency of metal recovery and biosorbent regeneration. Considering both desorption efficiency and the regeneration of the biosorbent, 0.01 M Na_3NTA seems to be the most preferable desorption agent for the repeated A/D operation using the immobilized recombinant cells as the biosorbent.

4. Conclusions

This work demonstrates that immobilized recombinant cells expressing human metallothionein (MT) proteins could be used to remove Cd ions from aqueous solution. The optimal adsorption ability of the immobilized cells occurred at pH 5.0, while the temperature effect was negligible within the experimental range. The adsorption kinetics could be described by pseudo-second order model, while the adsorption equilibrium followed Langmuir isotherm. The removal percentage of Cd ions of the PVA-immobilized cells was dependent on the initial concentrations, as up to 82.7% removal (with 15.4 wt.% biomass loading) was reached when the initial concentration was 10 mg/l, suggesting that the biosorbent could be best applied in removal of Cd ions at a low concentration range. The Cd uptake capacity increased with an increase in biomass loading of the PVA-immobilized cells over the biomass loading range of 8.4–33.0 wt.%. Among the 12 desorption agents examined, 0.01 M Na_3NTA displayed the best desorption efficiency and biosorbent regeneration of the PVA-immobilized cells. Using Na_3NTA (0.01 M) as the desorption agent, the PVA-immobilized cells were able to retain 51–61% of its original adsorption capacity after four adsorption/desorption cycles. The foregoing results indicate the feasibility of using PVA immobilized recombinant cells as the biosorbent for repeated batch or continuous removal of Cd ions in the contaminated effluent.

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