

Comparative Study on the Ni²⁺ Biosorption Capacity and Properties of Living and Dead *Pseudomonas putida* Cells

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ABSTRACT: Microbial cells have been successfully used as biosorbents to remove heavy metals from wastewater. In some cases, dead cells appear to offer more advantages than living cells in the removal and recovery of heavy metal ions from industrial wastewater. Maintaining higher biosorption capability and understanding the biosorption properties of dead cells are the keys to heavy metal removal and recovery from wastewater using dead cells as biosorbents. The present experiment showed that the dead *Pseudomonas putida* 5-x cells killed by dilute HCl had a higher Ni²⁺ biosorption capacity due to the retention of a complete cell structure during the acid treatment process. The biosorption process of the dead cells was faster than that of the living cells. Metabolic-independent physical adsorption played a major role in the Ni²⁺ sorption by the dead cells. The pH obviously affected the biosorption capacity of the dead cells, because of the variation of the hydrogen ion concentration and cell surface property, as well as occurrence of micro-precipitation along with the change of solution pH. Considering both biosorption capacity and desorption efficiency, pH 6.5-7.0 is a suitable condition for Ni²⁺ biosorption by dead *P. putida* 5-x cells killed with dilute HCl.

KEY WORDS: Biosorption, Cell surface structure, Dead cell, Heavy metal.

INTRODUCTION

In general, all cell surfaces are anionic [1], and can adsorb metal cations because of the interaction between the metal cations and the anionic groups within the cell surface [2, 3]. In addition, metal ions can also be transported into the cell through its ion transportation system. Many studies have shown that soluble metal ions in the environment can be taken up by microbial cells, because of their surface adsorption capability and ion transportation system. During the last two decades,

bacteria, algae, and fungi have been used successfully as biosorbents for heavy metals [4-8]. Among the microbes, bacteria were of particular interest because of their high cell-surface area per unit volume in general.

Both living and dead cells can be used as biosorbents for heavy metal removal from wastewater. However, there are distinct advantages in using either living or dead biomass [9-11]. The main advantages of using living biomass are 1) it can be self-renewing resulting in

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an increase of cell mass enabling biosorption of more heavy metal ions, 2) cellular ion transportation system can transport metal ions into cells, and 3) some excreted metabolic products can contribute to the removal of heavy metal ions. All of these may lead to a higher level of metal ion uptake. However, in practical operations, living cell as biosorbents does not always qualify for heavy metal removal and recovery from toxic industrial wastewater. The use of dead biomass can avoid the problem of toxicity of heavy metals toward living cells (the toxicity of heavy metals is often the reason for removing it). In addition, the biosorption process involving dead biomass is often faster as only cell surface-based binding, rather than active transport into the cell, occurs [12]. Another advantage of using dead biomass is the easy and non-destructive recovery of adsorbed metal ions, which allows regeneration of the biosorbent for reuse. In contrast, metal ions accumulated inside living cells through a cellular ion transportation system are often recovered only when the cell is destroyed. So, in many cases, for example the removal and recovery of heavy metal ions from electroplating effluent, working with dead cells may offer more advantages than living cells.

In previous studies, a Gram-negative bacterium *Pseudomonas putida* 5-x with high heavy metal uptake capacity was obtained from local industrial effluent, and its optimal biosorption conditions, biosorption mechanism and cell immobilisation were clarified [13-15]. These experimental results indicated that *P. putida* 5-x was a better biosorbent for heavy metal uptake, due to the presence of negatively-charged groups within the cell surface providing the major contribution to heavy metal biosorption. However, the heavy metal ions contained wastewater, such as electroplating effluent, commonly has higher toxicity toward living cells. In addition, in order for *P. putida* 5-x cells to become a competitive biosorbent (compared with a physical adsorbent) for the removal and recovery of heavy metal ions from industrial effluent, the *P. putida* 5-x cells should be more regenerable and reusable. For avoiding the toxicity of heavy metal containing wastewater toward living *P. putida* 5-x cells and for reusing *P. putida* 5-x cells more times for reducing operational costs, dead *P. putida* 5-x cells may be a better choice for the removal and recovery of heavy metal ions from toxic industrial effluent.

However, prior to implementing dead *P. putida* 5-x cells as a biosorbent, simple and practical methods for killing cells while still maintaining high biosorption capability should be developed. In addition, understanding the properties of dead *P. putida* 5-x cells for biosorbing heavy metal ions, and comparing with that of living cells, may serve as a useful aid for designing and optimizing the biosorption processes of dead *P. putida* 5-x cells.

Ni^{2+} is a major heavy metal ions in electroplating effluent in China [16], which toxicity to human and fish has been well documented [17,18]. So in the experiment, the Ni^{2+} was used as example to study the biosorption capacity of the *P. putida* 5-x cells killed by different techniques in order to determine a better cell-killing method. In addition, the differences in the properties between living and dead *P. putida* 5-x cells for Ni^{2+} biosorption were studied and compared.

EXPERIMENTAL SECTION

Cell cultivation

The bacterial cell used as biosorbent in this study was *P. putida* 5-x, which was isolated from local industrial effluents. The *P. putida* 5-x cells were cultured in SL medium according to the method of Sze et al. [14]. The bacterial cells cultured for 34~36 h were harvested for the following experiments.

Killing cell

Acid, alkali, and heat treatment were used to kill *P. putida* 5-x cells. The cells harvested under the optimal growth phase were resuspended in HCl, H_2SO_4 or NaOH solution with different concentration for 30 min, or treated at 100°C for 10 min. Then the treated cells were recovered and washed twice with 2-(*N*-morpholino)-ethanesulfonic acid (MES) buffer and were used for the following experiments. Culturing for 12 h under sterilized condition was used to identify the activity of living and dead bacterial cell. Before and after treatment, cell dry weight was tested to determine the biomass loss ratio (BLR) during the treating process according to Eq. (1):

$$\text{BLR}(\%) = \frac{W_{\text{B-cdw}} - W_{\text{A-cdw}}}{W_{\text{B-cdw}}} \quad (1)$$

where $W_{\text{B-cdw}}$ and $W_{\text{A-cdw}}$ are cell dry weight before and after treatment with different methods. The dry cell weight was determined by weighing after balancing 2-3 g wet cell for 4 h at 105°C.

Biosorption and desorption process

Owing to the concentration of Ni²⁺ in the electroplating effluent in China generally range from several to several ten milligram per liter, so batch experiments for determining the Ni²⁺ biosorption properties were carried out with the initial Ni²⁺ concentrations of 46 mg L⁻¹ (1 mmol L⁻¹) and some cell biomass (corresponding to 0.5 g dry cell L⁻¹). The cell biomass was added into a 50-mL beaker containing 25 mL Ni²⁺ laden MES buffer solution with different pH values. All experiments were performed in duplicate. Control experiments were carried out under identical conditions, but without any biomass. The biosorption process was conducted by agitation using a magnetic stirrer at 200 rpm for different lengths of time, then the biomass was separated using a centrifuge. The concentrations of Ni²⁺ in the supernatants were determined using a Perkin Elmer 3300 atomic absorption spectrophotometer. The Ni²⁺ adsorption capacity of biosorbent can be calculated using Eq. (2):

$$Q = (C_c - C_e) V / W \quad (2)$$

where C_c is the final Ni²⁺ concentration in the control (mg L⁻¹),

C_e is the residual Ni²⁺ concentration in the supernatant after biosorption (mg L⁻¹),

W is the dry cell weight (g),

V is the volume of the Ni²⁺-containing buffer solution (L).

For determining adsorption isotherm of biosorbents for Ni²⁺, adsorption experiments were carried in the same conditions as above, but with the different Ni²⁺ concentrations, such as 9.2, 23, 37 and 46 mg L⁻¹. After adsorption, according to the residual Ni²⁺ concentrations in the supernatant and Ni²⁺ adsorption capacities of biosorbents, the adsorption isotherms of biosorbents were determined using residual concentration of Ni²⁺ in supernatant plotted against the adsorption capacity.

Biosorbent bound with heavy metal ions was separated by centrifugation, and was desorbed and regenerated by 0.1 mol L⁻¹ HCl with the ratio of 1:10 (w/V) between biosorbents and desorbent for 10 min. The recovery ratio of heavy metal ions from the biosorbent was calculated according to the concentration of Ni²⁺ in the desorbed solution. The regenerated biosorbent was reused for another biosorption cycle after being washed with MES buffer.

Transmission electron microscopy analysis

P. putida 5-x cells were harvested by centrifugation, dehydrated through an ethanol series, and then embedded in SPUUR materials (EMS, Fort Washington, USA). Thin sections of 60 nm were prepared using a Reichert Ultracuts (Leica, Wien, Austria) equipped with a diamond knife (Diatome 45°, Fort Washington, USA). Sections were then examined by transmission electron microscopy (TEM) (JEM-1200 EX-II TEM JEOL Ltd., Tokyo, Japan). No fixative or stain was used in the preparation of the sections.

RESULTS AND DISCUSSION

Effect of treatment methods on the Ni²⁺ biosorption process of the *P. putida* 5-x cells

In order to choose a better technique for killing cells while retaining a high biosorption capability, the adsorption capacity of Ni²⁺ by the *P. putida* 5-x cells treated with HCl, H₂SO₄, NaOH and heating at 100°C were compared. The results are shown in Table 1. Obviously, after adsorbing for 3h, the cells treated with dilute HCl exhibited a relatively high Ni²⁺ biosorption capacity among the treated cells, but still lower than the living cells.

The biosorption of heavy metal ions based on the interaction between cations and anionic groups commonly occurs on the cell surface [6,7]. However, the serious destruction of the cell surface structure may affect the biosorption capacity [12]. Cell Biomass Loss Rate (BLR) can be used as a parameter for identifying the extent of destruction of cell structure during the treatment process [19]. For maintaining higher biosorption capability of the dead cells, a suitable method to kill cells should generally retain good cell surface structure, or rather a lower BLR. Table 1 shows that the BLRs of the cells treated with either dilute HCl (0.3 mol L⁻¹ or below) or heat at 100°C were < 8%. However, the BLRs of the cells treated with NaOH, H₂SO₄, and 0.6 mol L⁻¹ HCl were significantly high. This implied that the treatment with NaOH, H₂SO₄, and 0.6 mol L⁻¹ HCl caused the serious destruction of the cell structure, which resulted in a lower Ni²⁺ biosorption capability.

After treatment with 0.1 mol L⁻¹ HCl, cell still maintained a relatively complete cell surface structure (see Fig. 1 b), whereas no complete cell structures could be found with TEM after treated with NaOH, H₂SO₄, and

Tab. 1: Performance of the cells treated with different techniques¹.

Type of Cell	Ni ²⁺ Adsorption Capacity (mg g ⁻¹)	Decrease rate ² (%)	BLR ³ (%)
Living Cells	44.1 ± 6.3	/	/
Cells treated with 0.1 mol L ⁻¹ HCl	41.8 ± 5.3	5.2	7.8 ± 1.1
Cells treated with 0.3 mol L ⁻¹ HCl	41.9 ± 5.8	4.8	8.2 ± 1.3
Cells treated with 0.6 mol L ⁻¹ HCl	34.1 ± 4.7	22.7	14.2 ± 1.8
Cells treated with 0.1 mol L ⁻¹ H ₂ SO ₄	31.7 ± 4.4	28.1	16.1 ± 1.9
Cells treated with 0.3 mol L ⁻¹ H ₂ SO ₄	21.9 ± 2.6	50.3	17.9 ± 2.2
Cells treated with 0.6 mol L ⁻¹ H ₂ SO ₄	20.2 ± 2.7	54.2	27.8 ± 2.5
Cells treated with 0.1 mol L ⁻¹ NaOH	17.9 ± 2.1	59.4	27.4 ± 2.6
Cells treated with 0.3 mol L ⁻¹ NaOH	14.8 ± 1.8	66.4	37.8 ± 4.1
Cells treated by heating at 100°C	28.7 ± 2.9	34.9	8.0 ± 1.3

1) Ni²⁺ adsorption capacities were determined after adsorbing for 3 h in a pH 6.5 solution containing 46 mg L⁻¹ Ni²⁺ and 0.5 g L⁻¹ dry cells. All data was taken as the average of three experimental results.

2) decrease rate of Ni²⁺ adsorption capacity of biomass treating with different methods.

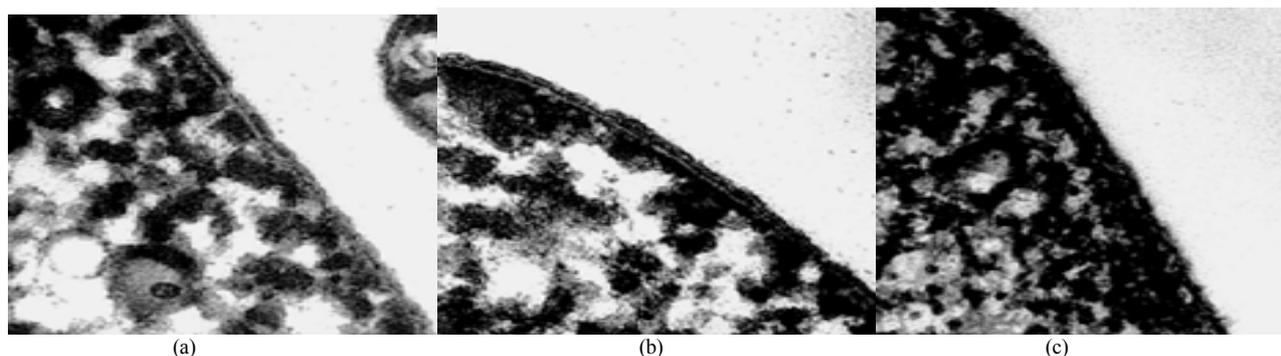


Fig. 1: TEM pictures of the cell structure of *P. putida* 5-x.

(a) Living Cells; (b) Cells treated with 0.1 mol L⁻¹ HCl; (c) Cells treated by heating at 100 °C.

0.6 mol L⁻¹ HCl. This further confirmed that a complete cell surface structure is the basic requirement for retaining higher heavy metal biosorption capability of dead cells. It is known that the bacterial cell surface is mainly composed of peptidoglycan (PEG), phospholipids, and proteins. Some negatively-charged groups, such as carboxyl and phosphodiester groups, attached to PEGs, phospholipids and proteins, provide the main binding sites for heavy metal ions [12]. Strong treatment factors destroy the cell structure because of the degradation of the PEGs, lipids, and proteins, leading to the loss of some of the negatively-charged groups. Therefore, the serious destruction of the cell surface structure is commonly accompanied by the loss of heavy metal adsorption capacity.

As seen in Fig. 1, although the cells treated with heat

at 100 °C also had a complete cell structure, the structure is denser than that of the living cells and the cells treated with dilute HCl. A dense cell structure resulted from the thermo-denaturation of the PEGs, lipids, and proteins would lead to some metal ion-binding sites within the cell surface becoming inaccessible to the heavy metal ions, thus the Ni²⁺ biosorption capacity of the cells treated with heat at 100 °C was lower than that of the cells treated with dilute HCl. Hu et al also found that the penetration of heavy metal ions into the cell surface structure is the key for their adsorption by cell biomass [20].

Obviously, from the point of biosorption capacity and BLR, the dilute HCl treatment is a better method for killing *P. putida* 5-x cells. Therefore, the cells treated with dilute HCl were used for the following experiments.

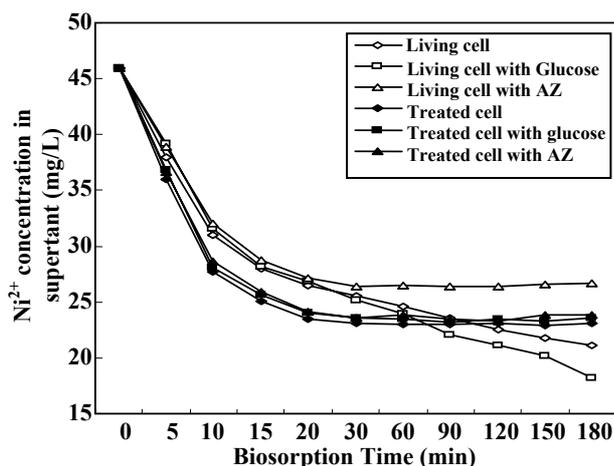


Fig 2: Ni^{2+} biosorption course with living cells and 0.1 mol L^{-1} HCl-treated cells in different conditions (AZ means sodium azide)

Metabolism-dependency of biosorption processes with living and dilute HCl treated *P. putida* 5-x cells

In broad terms, biosorption includes a metabolism-independent passive adsorption process by living/dead cells, which commonly occurs on the cell surface [9], and a metabolism-dependent bioaccumulation process only by living cells. Metabolism-independent biosorption can simplify the operation procedure; for example, it can shorten the adsorbing process, the toxicity of heavy metal ions in wastewater toward the cells cannot be considered, and heavy metal ions adsorbed on cell surface can be desorbed easily. For designing and optimizing the biosorption process for efficient removal of heavy metal ions from wastewater, the metabolism-dependency of the biosorption process with dilute HCl-treated cells should be studied.

Fig. 2 depicts the time-course profiles for Ni^{2+} removal by 0.5 g L^{-1} biosorbents from pH 6.5 MES solution containing 46 mg L^{-1} Ni^{2+} . The results showed that Ni^{2+} removal by the living cells started with a rapid uptake process within the initial 20 min, followed by a slow uptake stage. About 80 % of the total Ni^{2+} taken up by the living cells during the biosorption process was removed within the initial 20 min. However, for 0.1 mol L^{-1} HCl-treated cells, Ni^{2+} removal was completed rapidly within the initial 20 min without the following slower stage.

Glucose is an excellent metabolism accelerant because it is the best carbon and energy source for bacterial growth and metabolism, whereas sodium azide (AZ) is a metabolism

inhibitor because of its inhibiting respiration [21]. In the experiment, 1 % glucose and 0.1 % AZ were added to the Ni^{2+} -containing solution for assessing the metabolism-dependency of the biosorption process with the living and 0.1 mol L^{-1} HCl-treated cells in the rapid and slow phases, respectively. The results in Figure 2 showed that the presence of glucose and AZ did not affect the Ni^{2+} biosorption process of the living cells in the rapid phase, but respectively improved or inhibited the Ni^{2+} uptake in the slow phase. It is well known that some cell metabolism actions are useful for heavy metal uptake. For example, some heavy metal ions can be transported into the cytoplasm via specific ion pumps; certain reactions of living cells can oxidize or reduce inorganic compounds to form insoluble compounds and accumulate them on the cell surface; cell metabolism can produce some compounds such as hydrogen sulfide, which react with heavy metal ions to form insoluble metal sulfides [22,23]. Glucose improved the Ni^{2+} biosorption (bioaccumulation) capability of the living cells in the slow stage, either by increasing the cell mass, or by activating the metabolism action. AZ on the other hand, inhibited the cell growth and metabolism of the living cells, thus decreasing its observed Ni^{2+} biosorption (bioaccumulation) capability in the slow stage. These results indicated that in the rapid phase, the biosorption process performed using living cell, is a metabolic-independent surface adsorption process, which is generally a fast process, while the slow uptake stage was metabolism-dependent.

However, experimental results as seen in Fig. 2 showed that glucose and AZ seemed to not affect the Ni^{2+} biosorption process of 0.1 mol L^{-1} HCl treated cells. This implied that the treatment with 0.1 mol L^{-1} HCl killed the cells completely, thus without cell metabolism. Therefore, the Ni^{2+} biosorption by 0.1 mol L^{-1} HCl treated cells was a metabolism-independent surface adsorption process.

The adsorption experiments with different Ni^{2+} concentrations showed that the Ni^{2+} adsorption processes of 0.1 mol L^{-1} HCl treated cell and of the living cells in the rapid phase (within 20 mins) could be expressed as $Q = 18.3 C_e^{0.29}$ and $Q = 15.7 C_e^{0.27}$, respectively, (Fig. 3). This illustrated that the Ni^{2+} uptake process of 0.1 mol L^{-1} HCl treated cells and the living cell in the rapid phase obeyed *Freundlich* isotherm of $Q = K_f C_e^n$.

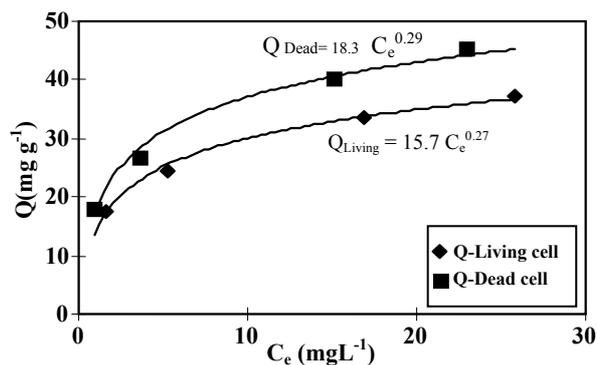


Fig 3: Ni^{2+} adsorption isotherms of 0.1 mol L^{-1} HCl-treated cell and living cell in the rapid phase.

This further confirmed that the Ni^{2+} uptake of 0.1 mol L^{-1} HCl treated cells and of the living cells in the rapid phase were physical adsorption process. The K_f gives a measure of the adsorption capacity of biosorbent. The K_f Value of treated and living cell was 18.3 and 15.7, respectively, indicated that 0.1 mol L^{-1} HCl treated cell has higher Ni^{2+} adsorption capacity than living cell in a shorter adsorption process. This may be because the dilute HCl treatment can be considered as a biosorbent regeneration process, the removal of the impurities or native metal ions adsorbed on the living cells during cell culturing may enhance the biosorption capacity of the cells to Ni^{2+} .

Comprehensively considering of the biosorption capability, the biosorption time, the cell regeneration/reuse, and thus operating costs, dead *P. putida* 5-x cells killed with 0.1 mol L^{-1} HCl may be more effective than living cells in the removal of heavy metal ions from toxic industrial effluent.

Effect of pH on Ni^{2+} biosorption of living and dilute HCl treated *P. putida* 5-x Cells

The biosorption process is commonly influenced by the experimental conditions. It has been consistently reported that pH is the dominant parameter controlling biosorption [24]. In this experiment, the effects of pH on Ni^{2+} biosorption by dead and living *P. putida* 5-x cells were studied. As shown in Fig. 4, both the dead and living cells experienced a general increase in their Ni^{2+} biosorption capacity in accordance with ascending solution pH, either after adsorbing for 20 min or for 3h, but when the pH ascends to 9 and above, the biosorption capacity of both the dead and living cells does not obviously increase further.

Regarding the treated cells, the variation in Ni^{2+} biosorption capacity with changing solution pH after

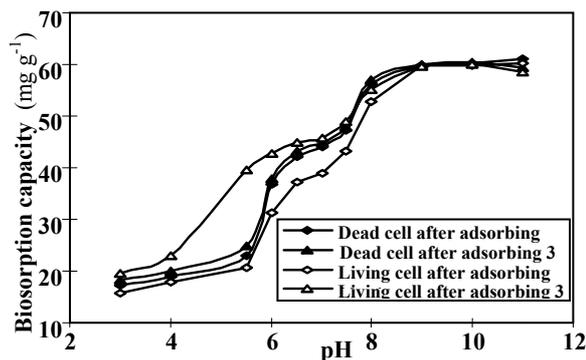


Fig 4: Ni^{2+} biosorption capacity of living and dead cells at different pH values.

adsorbing for 20 min or 3h was almost the same due to a lack of metabolism activity. When the pH ascended to about 5.5 and 7.5, the Ni^{2+} biosorption capacity of the dead cells increased sharply in both cases. Generally, surface adsorption based on the interaction between cations and the anionic groups within the cell surface plays an important part in the biosorption of bacterial cells, especially in dead cells [6,7]. At low pH conditions, the competition between hydrogen ions and Ni^{2+} for the anionic groups within the cell surface generally caused a low Ni^{2+} biosorption capacity. Along with the rise in pH, the hydrogen ion concentration decreased, thus improving the Ni^{2+} binding. In addition to the hydrogen ion concentration, the variation of pH might result in a change of the cell surface properties, thus affecting its biosorption capacity. For example, at low pH, some active groups related to metal ion binding, such as hydroxyls, amides, and the amines attached to proteins and polysaccharides, are electropositive, but at higher pH conditions, these groups become electronegative enabling efficient binding of heavy metal ions [19]. The Ni^{2+} biosorption capacity of the dead cells sharply increased when the pH ascended to above 5.5, implying that a large amount of active groups on the surface of the *P. putida* 5-x cells became too negatively-charged at this pH or above, thus providing many binding sites for efficient biosorption of Ni^{2+} . The observed Ni^{2+} biosorption capacity of the dead cells sharply increased again when the pH ascended to 7.5 and above may be due to the occurrence of micro-precipitation on the cell surface, which replace the surface adsorption and become the main contributor to heavy metal removal. However, it was found in Fig. 4 that the observed Ni^{2+} biosorption

Table 2: Desorption efficiency and BLRs of Ni²⁺-bound dead cells adsorbed at different pH conditions*.

pH	6.5	7.0	7.5	8.0	9.0
Desorption Efficiency (%)	95.7 ± 5.3	95.5 ± 5.7	91.9 ± 4.7	79.6 ± 3.6	76.4 ± 3.5
BLR (%)	4.2 ± 0.3	4.4 ± 0.3	5.7 ± 0.4	11.6 ± 0.9	13.8 ± 1.1

*) 0.1 mol L⁻¹ HCl was used to desorb and regenerate Ni²⁺-bound cells. All data was taken as the average of three experimental results.

capacity in the solution containing 46 mg L⁻¹ Ni²⁺ and 0.5 g L⁻¹ cell biomass still maintained at about 60 mg g⁻¹ along with a further increase in the pH to 11. This indicated that even in alkaline solution, a part of the Ni²⁺ were still soluble, and could not therefore be removed from the solution by precipitation.

For living cell, after adsorbing for 20 min, the variation in the biosorption capacity with changing pH was similar to that of the dead cells, although the biosorption capacity at any pH value, excluding pH values from 8-11, was lower than that of the dead cells. This is because surface adsorption and micro-precipitation also play a major part in the Ni²⁺ biosorption by the living cells in the rapid biosorption phase. However, after adsorbing for 3h, the living cell exhibited much higher Ni²⁺ uptake capacity than that of the dead cells at lower pH conditions. This implied that at relatively low pH, *P. putida* 5-x cells still had some metabolic activity, and part of the Ni²⁺ were accumulated on the cell surfaces or inside the cells through metabolism-related oxidation-reduction reactions or ion transportation. At higher pH conditions, such as pH 8-11, the performance of the living cells after adsorbing for 3h was similar to that of the dead cells. This might be due to cell metabolism being inhibited by the high pH, such that micro-precipitation became the main contributor for the removal of Ni²⁺ from the solution.

The experimental results shown in Fig. 4 indicated that higher pH values seemed to be more advantageous in the Ni²⁺ biosorption process of the dead cells. However, from the point of operating costs, better operational conditions should not only lead to a biosorbent having a higher biosorption capacity, but should also make its regeneration and desorption more efficient. For assessing the effect of adsorbing pH on the desorption/regeneration of heavy metal bound biosorbent, the dead cells adsorbed at different pH values, such as pH 6.5, 7.0, 7.5, 8.0, and 9.0, were desorbed /regenerated by 0.1 mol L⁻¹ HCl, and the results shown in Table 2. It is clear that the Ni²⁺ desorption efficiency of the dead cells adsorbed at pH 8.0

above was obviously lower than that at pH 6.5-7.0, while the BLRs of the cells adsorbed at pH 8.0 and above were much higher than at pH 6.5-7.0 during the desorption/regeneration process. This implied that the dead cells adsorbed in alkaline conditions were more difficult to be regenerated, while the cell structure was seriously destroyed during the following desorption/regeneration process with 0.1 mol L⁻¹ HCl. This may be because alternation between acid and alkaline condition would result in the breakage of cell material easily, hence higher BLRs and lower observed desorption efficiency.

Although the adsorption of dead cells in alkaline conditions exhibit a higher biosorption capacity owing to the occurrence of precipitation, alkaline conditions were unfavourable from the view of desorption/regeneration and reuse of biosorbent. Therefore, biosorption using the dead cells at higher pH should be avoided in the practical applications.

CONCLUSIONS

The experimental results indicated that dilute HCl treatment was a suitable method to obtain dead *P. putida* 5-x cells as biosorbents for heavy metal ions, because of its retaining a complete cell structure during the treating process. The dead cells killed by 0.1 mol L⁻¹ HCl exhibited higher Ni²⁺ biosorption capacity than the living cells after biosorbing for 20 min, although the final biosorption capacity was lower than that of the living cells. Metabolic-independent surface adsorption played a major part in the Ni²⁺ biosorption of the dead cells. The pH obviously affected the Ni²⁺ biosorption capacity of the dead cells because of the variation in the hydrogen ion concentration in solution and the cell surface charge with change of solution pH, as well as occurrence of micro-precipitation. Considering both the biosorption capacity and desorption/regeneration efficiency, pH 6.5-7.0 was a suitable condition for Ni²⁺ biosorption by dead *P. putida* 5-x cells killed with 0.1 mol L⁻¹ HCl.

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