Ni(II) and Cu(II) removal from aqueous solution by a heavy metal-resistance bacterium: kinetic, isotherm and mechanism studies
Haikun Zhang, Xiaoke Hu and Hong Lu

ABSTRACT
The potentiality of a heavy metal-resistance bacterium Acinetobacter sp. HK-1 for removing Ni(II) and Cu(II) ions from aqueous solution and the biosorption mechanism were investigated in this study. The effects of pH, contact time and Ni(II)/Cu(II) concentration on the adsorption process were evaluated and the maximum biosorption capacity of strain HK-1 was found to be 56.65 mg/g for Ni(II) and 157.2 mg/g for Cu(II), respectively. The experimental kinetic data fit well with the pseudo-second-order model ($R^2 > 0.98$) and the biosorption process was best explained by the Langmuir-Freundlich dual model ($R^2 > 0.97$). The morphologies of HK-1 before and after adsorption in a Ni(II)/Cu(II) supplemented system were compared using a scanning electron microscope. After adsorption, the valence state of Ni(II)/Cu(II) was not changed and the formation of nickel/copper phosphate was observed using X-ray photoelectron spectroscopy (XPS) and X-ray diffraction. The results of Fourier transform infrared spectroscopy and XPS further indicated that amine, phosphate and carboxyl groups were involved in the biosorption process. Cu(II) biosorption by Acinetobacter sp. was firstly reported. Based on the above results, it can be concluded that Acinetobacter sp. HK-1 has a promising application in Ni(II) and Cu(II) ion removal from industrial wastewater.

Key words | Acinetobacter sp., biosorption, Cu(II), mechanism, Ni(II)

INTRODUCTION
Heavy metal pollution caused by mining, metallurgical and electroplating industries has posed significant risks to the ecosystem (Kunhikrishnan et al. 2012; Mejias Carpio et al. 2014). Indeed, they could not be degraded by microbes in the environment, and thus can accumulate through the food chain, threatening human health (Aryal & Liakopoulou-Kyriakides 2015). Hence, the removal of heavy metals from wastewaters has received much attention. Chemical precipitation such as sulphides or hydroxides is the traditional treatment process for heavy metal polluted effluents (Chen et al. 2009; Wang & Chen 2009). However, the polluted sludge produced in this traditional treatment has to be disposed of in landfills and could result in huge environmental and economic costs. Particularly, the traditional methods are generally effective at metal concentrations greater than 100 mg/L and may not be suitable for dealing with dilute metal solutions, as excessive chemicals are required (Kratochvil & Volesky 1998).

Compared with chemical methods, bio-treatment methods are more suitable to treat the increasing volume of wastewater containing low metal concentrations due to their low cost and eco-friendliness advantages (Kratochvil & Volesky 1998; Wang & Chen 2009; Aryal & Liakopoulou-Kyriakides 2015). Thus, there is growing interest in bio-treatment for heavy metal removal by bio-sorbents. Among the diverse bio-sorbents, various genera such as Pseudomonas, Bacillus, Staphylococcus, Streptomyces, Ochrobactrum, Cupriavidus and Spirulina, etc., have been reported to be capable of removing heavy metals from aqueous solution (Chojnacka et al. 2005; Wang & Chen 2009; Fan et al. 2014). Although the biosorption process can be theoretically performed in both living and dead biomass, using living cells in hybrid technology is supposed to be a promising way for heavy metal removal (Aryal & Liakopoulou-Kyriakides 2015). However, heavy metals have a toxic effect on living microorganisms.
Therefore, bacterial biomass with high metal-resistant ability has more practical values in the treatment of heavy metal-containing wastewater (Lodeiro et al. 2006; Panda et al. 2006; Das et al. 2007). In our previous study, a Cr(VI)-reducing bacterium named Acinetobacter sp. HK-1 that can tolerate approximately 500 mg/L Cr(VI) was isolated (Zhang et al. 2014). Moreover, strain HK-1 was proven to be capable of secreting some glycolipids (Zhang et al. 2014). As reported by many researchers, extracellular secretion such as proteins, lipids and polysaccharides, with different functional groups, are usually the binding sites for ion exchange and complexion reactions with heavy metals (Errasquin & Vazquez 2003; Lodeiro et al. 2006; Wang & Chen 2009). Thus, examining the role of functional groups may provide robust information regarding the mechanism of heavy metals removal by biotreatment process.

On the basis of the above background, the potentiality of strain HK-1 cells for Ni(II)/Cu(II) removal from aqueous solution was investigated in this study. The kinetic and equilibrium studies of the Ni(II)/Cu(II) removal in a batch system were performed for a better understanding of the biosorption process. Fourier transform infrared (FTIR) spectroscopy, scanning electron microscope (SEM), X-ray photoelectron spectroscopy (XPS) and X-ray diffraction (XRD) analyses were used to examine the fate and mechanism of Ni(II)/Cu(II) removal from aqueous solution.

**MATERIALS AND METHODS**

**Media and culture conditions**

High metal tolerance bacterial strain Acinetobacter sp. HK-1 used in the present study was previously isolated from activated sludge from the Dalian Bio Chemical Co., Ltd (Liaoning, China) using a dilution plate method at 30°C (Zhang et al. 2014). Acinetobacter sp. HK-1 was grown in Luria-Bertani (LB) medium, which contained (per liter): 10 g NaCl, 5 g yeast extract, 10 g peptone, and maintained with minimal salt medium, which contained (per liter): 2 g glucose, 3 g NH4NO3, 0.5 g KH2PO4, 0.5 g Na2HPO4·2H2O, 0.008 g MgSO4·7H2O, 0.002 g MnSO4·H2O, 0.002 g FeSO4·7H2O, 0.002 g CaCl2·2H2O, and pH 6.2. The biomass was harvested at early stationary phase via centrifugation at 10,000 rpm, 25°C for 10 min and washed three times with phosphate buffer solution (PBS, pH 6.0). All flasks were acid cleaned and rinsed with distilled water prior to use.

**Metal solution and analysis**

Stock solutions (1,000 mg/L) of Ni(II) and Cu(II) were prepared by dissolving analytical grade NiCl2·6H2O and CuCl2·2H2O in double distilled water and diluted to the desired concentration. The concentrations were analyzed by inductive coupled plasma optical emission spectrometer (ICP-OES, ELAN DRC II, Hong Kong).

**Batch biosorption experiments**

Ni(II)/Cu(II) resistance assays were performed in the presence of strain HK-1. Strain HK-1 was aerobically grown in LB medium for 12 h, then 1 mL of the culture was added into the flask containing 100 mL LB medium and Ni(II)/Cu(II) (0–500 mg/L). Samples were periodically taken for the analysis of cell concentration. The cell concentration was measured from optical density at 600 nm using a UV-Vis spectrophotometer (V-560, JASCO, Japan).

The adsorption assays, adsorption kinetics, and equilibrium studies were carried out in 250 mL Erlenmeyer flasks containing 100 mL PBS. Strain HK-1 cells (0.27 g/L) and Ni(II)/Cu(II) (10–100 mg/L) were added and incubated with shaking (150 r/min) at 35°C. Periodically, the biomass was separated by centrifugation at 10,000 rpm for 5 min, and the metal concentration in the supernatant was measured by ICP-OES. The amount of Ni(II)/Cu(II) adsorbed by strain HK-1 was calculated according to the following Equation (1):

\[
Q_c = \frac{(C_0 - C_e) \cdot V}{W}
\]

where \(Q_c\) (mg/g) is the amount of Ni(II)/Cu(II) adsorbed by strain HK-1, \(C_0\) (mg/L) and \(C_e\) (mg/L) are the initial and residual Ni(II)/Cu(II) concentration, respectively, \(V\) (L) is the solution volume and \(W\) (g) is the weight of the strain HK-1.

The effects of optimum pH on the Ni(II)/Cu(II) adsorption process were determined by suspending 0.27 g/L HK-1 biomass in 50 mg/L metal solution for 24 h at pH values between 2.0 and 7.0 (varied by 0.1 M NaOH/HCl). The adsorption kinetics of Ni(II)/Cu(II) by Acinetobacter sp. HK-1 was followed at regular intervals of time up to 150 min. The equilibrium adsorption experiment was conducted by varying the concentration of Ni(II)/Cu(II) from 10 to 100 mg/L for 24 h at pH 6.2, 35°C. Desorption study was carried out as follows: adsorbed biomass was treated with 0.1 mol/L HNO3, washed by PBS and used again.
for adsorption study. All experiments were performed in triplicate and the mean values of data were presented.

In order to clarify the adsorption mechanism of Ni(II)/Cu(II) onto strain HK-1 cells, pseudo-first-order and pseudo-second-order model were applied to the experimental data as illustrated in Equations (2) and (3), respectively (Laguerren 1898):

\[
\ln\left(\frac{Q_e}{Q_t}\right) = -K_1t + \ln Q_e
\]

\[
\frac{t}{Q_t} = \frac{t}{Q_e} + \frac{1}{K_2Q_e^2}
\]

where \(Q_e\) (mg/g) and \(Q_t\) (mg/g) are the amounts of the Ni(II)/Cu(II) adsorbed at equilibrium and \(t\) (min), respectively; \(K_1\) (min\(^{-1}\)) and \(K_2\) (g/mg-min) are the rate constants of the first and second order rate equations, respectively.

The equilibrium study was performed by different isotherm models, including Freundlich (Equation (4)) (Freundlich 1906), Langmuir (Equation (5)) (Langmuir 1918) and Langmuir-Freundlich dual (Equation (6)) (Sips 1948) models.

\[
Q_e = K_F C_1^{1/n1}
\]

\[
Q_e = \frac{Q_{max} K_L C_e}{(1 + K_L C_e)}
\]

\[
Q_e = \frac{K_{LF} C_1^{1/n}}{(1 + a C_e^{1/n})}
\]

where \(Q_e\) is the equilibrium Ni(II)/Cu(II) concentration of biomass (mg/g), \(C_e\) is the equilibrium Ni(II)/Cu(II) concentration in solution, \(K_F\) and \(1/n_1\) are Freundlich adsorption constants, \(Q_{max}\) is the monolayer biosorption capacity of adsorbent (mg/g), \(K_L\) is the Langmuir adsorption constant (L/mg), \(K_{LF}, 1/n\) and \(a\) are Langmuir-Freundlich adsorption constants.

**Characterization of adsorption products**

The morphologies of strain HK-1 before and after adsorption in the Ni(II)/Cu(II) supplemented system were analyzed using an SEM (KYKY-AMRAY-100B, USA). XRD (X'pert PRO, The Netherlands) and XPS (ESCALAB 250Xi, England) were used to investigate the valence state changes and phase transformation of Ni(II)/Cu(II) before and after adsorption. FTIR (EQUINOX55, Germany) was used to investigate the chemical compositional changes of the surface of stain HK-1 cells.

For the SEM analysis, the cells associated with Ni(II)/Cu(II) were washed with phosphate buffer (10 mM, pH 7.5) and fixed in 3% (v/v) glutaraldehyde. The sample was then dried with ethanol in ambient conditions, mounted on an aluminum stub and then coated with gold. For the XPS, XRD and FTIR analyses, the cells were exposed to 50 mg/L Ni(II)/Cu(II) for 24 h under the optimal conditions (pH 6.2, 35 °C). Then, the cells were separated by centrifugation at 10,000 rpm at 4 °C for 5 min. The pellets were washed with deionized water and dried overnight in an oven at 50 °C. Simultaneously, cells without treatment with Ni(II)/Cu(II) were also prepared for comparison.

**RESULTS AND DISCUSSION**

Ni(II)/Cu(II) resistance assays and effects of pH, contact time and initial metal concentration

Ni(II) resistance assays showed that the aerobic growth in the LB medium had only a slight delay below the concentration of 100 mg/L. Cu(II) resistance assays showed that the aerobic growth in the LB medium had no delay less than or equal to the concentration of 100 mg/L, while the strain HK-1 could recover growth after 24 h incubation when 200 mg/L Cu(II) was added. However, the growth of strain HK-1 was inhibited severely when the Ni(II)/or Cu(II) concentration was greater than or equal to 200 or 300 mg/L, (Figure 1(a) and (b)). Our previous study demonstrated that only when the Cr(VI) concentration was up to 500 mg/L, the growth of strain HK-1 was inhibited severely (Zhang et al. 2014). Moreover, the toxic effect on HK-1 growth was, in descending order of damage, Ni(II) > Cu(II) > Cr(VI).

As shown in Figure 1(c), the biosorption of Ni(II)/Cu(II) by the strain HK-1 over time was significantly affected. During the process of biosorption, over 88% of Ni(II)/Cu(II) adsorption was completed within the first 30 min, then the adsorption process gradually reaches a dynamic equilibrium in Ni(II) or Cu(II) supplemented systems (Figure 1). Our previous study has demonstrated that strain HK-1 can secrete some sticky metabolites located on its cell surface (Zhang et al. 2014) and the abundance of binding sites on the surface of cells may be responsible for the initial high uptake rate (Panda et al. 2006). The maximum biosorption capacity of 0.27 g/L HK-1 cells were found to be 35.65, 29.20, 39.40, 50.87 and 56.66 mg/g for Ni(II) in the presence
of 10, 25, 50, 75 and 100 mg/L Ni(II), and 40.75, 93.77, 154.8, 154.8, 157.3 mg/g for Cu(II) in the presence of 10, 25, 50, 75 and 100 mg/L Cu(II), respectively (data not shown). Thus, these results indicated that surface binding was mainly involved in the Ni(II) and Cu(II) adsorption process.

The biosorption capacity between 10 °C and 45 °C was almost constant (data not shown). Thus, 35 °C was selected for further study due to the optimum temperature for HK-1 cell growth. As the acidity of the solution can directly affect the binding ability of metal ions to active sites on the cell surface, the effects of pH on Ni(II)/Cu(II) adsorption by strain HK-1 was studied. Figure 1(d) shows that the biosorption capacity of 0.27 g/L HK-1 cells was 11.60 mg/g for Ni(II) and 25.45 mg/g for Cu(II) at pH 2.41. The uptake of Ni(II) and Cu(II) increased with increasing pH of the solution. At a pH of 6.20, the adsorption of 39.40 mg Ni(II)/g dry weight biomass and 154.88 mg Cu(II)/g dry weight biomass could be achieved. Similar observation was also reported in the biosorption process of Ni(II) and Cu(II) by *Paenibacillus polymyxa* biomass (Colak et al. 2013). A possible explanation is that the increase in pH values in the solution further correlated with a negative charge of the cell surface, which was more favorable for Ni(II) or Cu(II) adsorption (Huang & Liu 2013).

Biological adsorption of Ni(II)/Cu(II) is a cost-effective approach in Ni(II)/Cu(II) remediation, especially for Ni(II)/Cu(II) wastewater with low concentration (<100 mg/L). Many species have been demonstrated to have potential application for Ni(II)/Cu(II) removal under various conditions, including *Paenibacillus* (Colak et al. 2013), *Lysinibacillus* (Prithviraja et al. 2014), *Bacillus* (Masood & Malik 2011; Rodriguez-Tirado et al. 2012; Oves et al. 2013), *Pseudomonas* (Gabr et al. 2012; Ni et al. 2012) and
Streptomyces (Veneu et al. 2013), etc. These studies collectively suggest that the biosorption capacity of biosorbents generally ranged from 8.8 to 508 mg Ni(II)/g dry weight biomass and from 1.884 to 381 mg Cu(II)/g dry weight biomass, respectively (Wang & Chen 2009; Aryan & Liakopoulos-Kyriakides 2015). In the present study, Acinetobacter sp. HK-1 resistance levels of Ni(II) and Cu(II) ions and Cu(II) biosorption by Acinetobacter sp. was firstly reported. The biosorption capacity of Acinetobacter sp. HK-1 can achieve 56.66 mg/g for Ni(II) and 157.3 mg/g for Cu(II) under the tested conditions. Comparatively, reports on the Ni(II)/Cu(II) adsorption by Acinetobacter sp. are rather scarce. To the best of our knowledge, Acinetobacter baumannii was once studied for nickel biosorption and the nickel adsorption rate was only 8.8 mg/g (Rodriguez et al. 2006).

**Kinetic and equilibrium studies**

The biosorption process of 25–75 mg/L Ni(II) and 10–50 mg/L Cu(II) were selected for kinetic and equilibrium studies. The kinetic study was performed using the pseudo-first-order model (Equation (2)) and pseudo-second-order model (Equation (3)). The fitted curves are shown in Figure S1 (available with the online version of this paper) and the related kinetic model parameters are listed in Table 1. It can be observed that the theoretical Qe (Qe1 and Qe2) values were closer to the experimental Qe (Qexp) values (Table 1). In addition, the adsorption rate constants K1 and K2 could be determined experimentally by plotting ln(Qe–Q) vs t and t/Qe vs t, respectively. Figure 2(a) and 2(b) show that the R2 values for the pseudo-first-order model (25–75 mg/L Ni(II) and 10–50 mg/L Cu(II)) were 0.69–0.81 and 0.48–0.79, respectively. Figure 2(c) and 2(d) further show that the R2 values for the pseudo-second-order model (25–75 mg/L Ni(II) and 10–50 mg/L Cu(II)) were all greater than 0.98. These results showed that the experimental kinetic data fit well with the pseudo-second-order model, indicating that chemisorption might be the rate-limiting step for Ni(II)/Cu(II) biosorption by strain HK-1 (Aryan & Liakopoulos-Kyriakides 2015). Similarly, previous studies reported that the experimental kinetic data of Ni(II)/Cu(II) biosorption by the genera Paenibacillus (Colak et al. 2013), Lysinibacillus (Prithviraja et al. 2014), Pseudomonas (Rodriguez-Tirado et al. 2012), Bacillus (Ni et al. 2012) and Streptomyces (Veneu et al. 2013), etc. can also be well described by the pseudo-second-order model.

The effects of initial Ni(II)/Cu(II) concentration (10–100 mg/L) on the biosorption capacity of strain HK-1 were investigated and the equilibrium adsorption data were correlated to different isotherm models, including Freundlich (Equation (4)), Langmuir (Equation (5)) and Langmuir-Freundlich dual (Equation (6)) model. The fitted curves are shown in Figure 3 and the related isotherm model parameters are listed in Table 2. It can be observed that the experimental data were best explained by using the Langmuir-Freundlich dual model in all three selected isotherm models. Similarly, previous studies also reported that Cd(II) biosorption by Acidiphilium sp. (Chakravarty & Banerjee 2012) and Pseudomonas sp. (Huang & Liu 2015) can be well described by the Langmuir-Freundlich dual model. In this study, the R2 values were 0.97 for Ni(II) biosorption and 0.98 for Cu(II) biosorption, respectively (Table 2). These results and previous study indicated that both physical and chemical adsorption took place simultaneously in the biosorption process of Ni(II)/Cu(II) by Acinetobacter sp. HK-1 cells (Huang & Liu 2015).

**Mechanism study**

To further elucidate the biosorption process of Ni(II)/Cu(II) by strain HK-1, the adsorption products were characterized using SEM, XPS, FTIR and XRD analyses. SEM analysis showed that the surfaces of Ni(II)/Cu(II)-treated cells were smooth (Figure 4). Moreover, XPS analysis of Ni(II)/Cu(II)-loaded cells showed that characteristic absorption peaks of the Ni element and Cu element were observed at 850–890 eV.

Table 1 | The adsorption constants of different kinetic models

<table>
<thead>
<tr>
<th>Metals</th>
<th>Concentration (mg/L)</th>
<th>$K_1$ (min$^{-1}$)</th>
<th>$Q_{e1}$ (mg/g)</th>
<th>$R^2$</th>
<th>$K_2$ (g/mg min)</th>
<th>$Q_{e2}$ (mg/g)</th>
<th>$R^2$</th>
<th>$Q_{exp}$ (mg/g)</th>
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</thead>
<tbody>
<tr>
<td>Ni(II)</td>
<td>25</td>
<td>$8.791 \times 10^{-2}$</td>
<td>27.06</td>
<td>0.92</td>
<td>$4.380 \times 10^{-3}$</td>
<td>29.96</td>
<td>0.96</td>
<td>29.20</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>$5.222 \times 10^{-2}$</td>
<td>39.07</td>
<td>0.97</td>
<td>$1.340 \times 10^{-3}$</td>
<td>45.75</td>
<td>0.94</td>
<td>39.40</td>
</tr>
<tr>
<td></td>
<td>75</td>
<td>$5.059 \times 10^{-2}$</td>
<td>47.48</td>
<td>0.98</td>
<td>$1.110 \times 10^{-3}$</td>
<td>55.33</td>
<td>0.98</td>
<td>50.87</td>
</tr>
<tr>
<td>Cu(II)</td>
<td>10</td>
<td>$1.307 \times 10^{-1}$</td>
<td>41.19</td>
<td>0.97</td>
<td>$4.660 \times 10^{-3}$</td>
<td>44.67</td>
<td>0.92</td>
<td>40.75</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>$6.392 \times 10^{-2}$</td>
<td>95.35</td>
<td>0.98</td>
<td>$7.300 \times 10^{-4}$</td>
<td>109.3</td>
<td>0.95</td>
<td>93.77</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>$7.228 \times 10^{-2}$</td>
<td>156.3</td>
<td>0.97</td>
<td>$5.260 \times 10^{-4}$</td>
<td>177.5</td>
<td>0.93</td>
<td>154.8</td>
</tr>
</tbody>
</table>
and 925–965 eV, respectively (Figure 5), indicating that the Ni/Cu element is adsorbed on the surface of HK-1 cells.

In addition, the valence state changes, and phase transformation of Ni(II)/Cu(II) before and after adsorption were investigated by using XPS and XRD analysis. In the XPS spectrum of Ni-loaded cells, a pair of peaks at 853.28 and 871.48 eV representing Ni$^{2+}$ 2p$_{3/2}$ and Ni$^{2+}$ 2p$_{1/2}$, respectively, were observed (Figure S2a). In the XPS spectrum of Cu-loaded cells, a pair of peaks at 931.48 and 951.48 eV representing Cu$^{2+}$ 2p$_{3/2}$ and Cu$^{2+}$ 2p$_{1/2}$, respectively, were observed (Figure S2b). (Figure S2 is available with the online version of this paper.) Further analysis using XRD showed that Ni(II)/Cu(II)-loaded cells showed distinct peaks indicating the deposition of crystallized Ni/Cu (Figure 6). Specifically, the peaks corresponded to nickel phosphate (NH$_4$NiPO$_4$·6H$_2$O) and copper phosphate (Cu$_4$H(PO$_4$)$_3$·3H$_2$O), respectively. These observations indicated the metal-biomass complexation mainly resulting from interactions with anionic ligands like NH$_2$ and PO$_4^{3-}$, which were derived from cell membranes, proteins and detoxifying ligands of biomass. The formation of metal phosphides was also reported by Sar et al. (1999) during the nickel and copper biosorption process by lyophilized Pseudomonas aeruginosa cells.

Previous studies demonstrated that the carboxylic, hydroxyl, carbonyl, phosphate, phosphoryl, amine and...
amide sites of bacterial cells are key functional groups for metal ion interaction (Wang & Chen 2013; Aryal & Liakopoulou-Kyriakides 2015). As shown in Figures 5 and 6, phosphate or phosphoryl and amine or amide sites of strain HK-1 cell were probably involved in Ni(II)/Cu(II) biosorption. To further characterize the adsorption groups, FTIR and XPS analysis of Ni(II)/Cu(II)-loaded cells were performed. In the infrared spectra of the Ni(II)/Cu(II)-loaded cells, the characteristic absorption bands of -COOH at approximately 1,722 cm$^{-1}$ and the -COH stretching and bending vibrations at approximately 3,001 cm$^{-1}$ appeared (Figure 7(a)). Simultaneously, -CONH bonds at approximately 1,581 cm$^{-1}$ (mainly C=O stretching) and 1,485 cm$^{-1}$ (NH deformation coupled with C-N stretching) appeared in their infrared spectra (Figure 7(a)). XPS analysis showed that there are four types of carbon with different chemical valences in the C 1 s spectra of Ni(II)/Cu(II)-loaded cells (C–C/C=O, C=O, C=O, and O–C=O). Compared with the control cells, a significant decrease in the peak intensities of carbons combined with oxygen, especially for C=O and O–C=O, was observed in the spectra of Ni(II)/Cu(II)-loaded cells (Figure 2(b)–2(d)). These observations indicated that the mentioned groups were involved in the biosorption process of Ni(II)/Cu(II) by strain HK-1 cells and these results are in good agreement with those obtained by other researchers (Sar et al. 1999; Table 2 | The adsorption constants of different isotherm models

<table>
<thead>
<tr>
<th>Isotherm model</th>
<th>$K_F$</th>
<th>Standard error</th>
<th>$1/n_1$</th>
<th>Standard error</th>
<th>$R^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Frendullch model</td>
<td>Ni(II) 6.745</td>
<td>1.285</td>
<td>0.4605</td>
<td>4.727 × 10$^{-2}$</td>
<td>0.96</td>
</tr>
<tr>
<td></td>
<td>Cu(II) 11.92</td>
<td>3.611</td>
<td>0.5746</td>
<td>8.000 × 10$^{-2}$</td>
<td>0.91</td>
</tr>
<tr>
<td>Langmuir model</td>
<td>Ni(II) 0.02494</td>
<td>8.770 × 10$^{-3}$</td>
<td>76.29</td>
<td>0.1200</td>
<td>0.89</td>
</tr>
<tr>
<td></td>
<td>Cu(II) 0.02348</td>
<td>5.551 × 10$^{-3}$</td>
<td>228.0</td>
<td>29.86</td>
<td>0.97</td>
</tr>
<tr>
<td>Langmuir-Freundlich model</td>
<td>Ni(II) 2.669</td>
<td>7.172</td>
<td>0.03587</td>
<td>8.946 × 10$^{-2}$</td>
<td>0.97</td>
</tr>
<tr>
<td></td>
<td>Cu(II) 1.578</td>
<td>1.126</td>
<td>1.533</td>
<td>2.992 × 10$^{-1}$</td>
<td>0.98</td>
</tr>
</tbody>
</table>

Figure 4 | SEM images of control cells (a), Ni(II)-loaded cells (b) and Cu(II)-loaded cells (c).

Figure 5 | The whole XPS spectra of control cells, Ni(II)-loaded cells and Cu(II)-loaded cells.
In addition, in terms of molecular mechanism, the bacteria that can tolerate heavy metals often possess resistance genes (e.g. efflux pumps) with a broad substrate range (Pal et al. 2014). For Ni(II) and Cu(II), approximately 51 and 60 resistance genes were found in different microorganisms, respectively. Given that strain
HK-1 is a newly isolated bacterium and the whole genome of strain HK-1 is still unknown. Therefore, the resistance genes of strain HK-1 need to be further investigated in our following studies.

**Desorption study**

The efficiency of desorption of Ni(II) and Cu(II) were studied by using 0.1 mol/L HNO₃. As shown in Table S1 (available with the online version of this paper), 0.1 mol/L HNO₃ could remove most of the metal ions bound with HK-1 cells (~93% for Ni(II) and ~95% for Cu(II), respectively). The recyclability test showed that the decrease of biosorption capacity of Acinetobacter sp. HK-1 cells did not exceed 6.5% for both metals. In addition, 1 mol/L HNO₃ could remove all the metal ions bound by HK-1 cells; however, HK-1 cells can not recover the biosorption capacity because of the serious damage.

**CONCLUSION**

In this study, the potentiality of a heavy metal-resistance bacterium Acinetobacter sp. HK-1 in Ni(II) and Cu(II) ion removal from aqueous solution and the biosorption mechanism were investigated. The effects of pH, contact time and Ni(II)/Cu(II) concentration on the adsorption process were evaluated and the maximum biosorption capacity of strain HK-1 was 56.65 mg/g for Ni(II) and 157.2 mg/g for Cu(II), respectively, at pH of 6.2 and a dosage of 0.27 g/L. The experimental kinetic data were well described by the pseudo-second-order model ($R^2 > 0.98$) and the Langmuir-Freundlich dual model ($R^2 > 0.97$), indicating that both physical and chemical sorption took place simultaneously. XPS, XRD and FTIR analyses showed that amine, phosphate and carboxyl groups were involved in the biosorption process. Based on the above results, it can be concluded that Acinetobacter sp. HK-1 has promising application for Ni(II) and Cu(II) ion removal from industrial wastewater.

**ACKNOWLEDGEMENTS**

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**SUPPORTING INFORMATION**

Supporting information associated with this article can be found in the online version of this paper. This file includes Figures S1 and S2 and Table S1.

**REFERENCES**


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