

Cite this: *RSC Adv.*, 2017, 7, 18793

# Bioremoval of $\text{Cu}^{2+}$ from CMP wastewater by a novel copper-resistant bacterium *Cupriavidus gilardii* CR3: characteristics and mechanisms†

Yushuang Yang,<sup>a</sup> Mingzhong Hu,<sup>c</sup> Dandan Zhou,<sup>b</sup> Wei Fan,<sup>\*b</sup> Xiaoyu Wang<sup>ID</sup> <sup>\*ab</sup> and Mingxin Huo<sup>b</sup>

Bacteria of the genus *Cupriavidus* are known for the ability of resistance to various heavy metals and metal-binding capability. Herein, we investigated the bioremoval of  $\text{Cu}^{2+}$  from synthesized chemical–mechanical polishing (CMP) wastewater by living cells of *Cupriavidus gilardii* CR3, a novel copper-resistant bacterium isolated in our previous study. The surface topography changes of strain CR3 were observed by SEM-EDX, where images showed that binding took place on the bacterial cell surface. FTIR spectra provided evidence that carboxyl, hydroxyl, amino, and phosphate groups on the surface of strain CR3 could be available for characteristic coordination bonding with  $\text{Cu}^{2+}$ . Zeta potential confirmed that electrostatic interaction was involved in  $\text{Cu}^{2+}$  binding. The biosorption and bioaccumulation of  $\text{Cu}^{2+}$  by strain CR3 was highly pH-dependent, and the optimum pH value was 5.0. The maximum binding capacity for  $\text{Cu}^{2+}$  was  $18.33 \text{ mg g}^{-1}$  and the bioremoval efficiency was 27% under optimal conditions. The  $\text{Cu}^{2+}$  binding process obeyed the Langmuir isotherm ( $R^2 = 0.99$ ). Kinetic data were properly fitted with both pseudo-second order kinetic model ( $R^2 = 0.99$ ) and an intraparticle diffusion model ( $R^2 = 0.98$ ). It can be concluded that living cells of *C. gilardii* CR3 have the potential to be utilized for the removal of  $\text{Cu}^{2+}$  from CMP wastewater.

Received 26th January 2017  
Accepted 10th March 2017

DOI: 10.1039/c7ra01163f

rsc.li/rsc-advances

## 1. Introduction

Chemical–mechanical polishing/planarization (CMP) is a critical step in semiconductor circuits, which is employed after the metal deposition step to eliminate any topography on a wafer's surface with the combination of chemical reactions and mechanical forces.<sup>1</sup> Copper metallization is rapidly replacing the aluminum-based processes in the CMP process. Copper is high resistance to electromigration effects and low electrical compared to early used aluminum. This transition, however, poses new challenges to wastewater treatment.<sup>2</sup> Copper CMP effluents can account for 30–40% of the water discharge in the semiconductor manufacturing and contain high concentrations of soluble copper and a complex mixture of organic constituents.<sup>3</sup> Copper is toxic to aquatic

organisms, which has been reported to cause toxic effects on algae and fish or amphibians at as low as 5–10 ppb.<sup>4</sup> The dissolved copper in CMP wastewater is in the range of 5–100  $\text{mg L}^{-1}$ .<sup>5</sup> The 100  $\text{mg L}^{-1}$  concentration of copper in CMP wastewater has also been reported.<sup>6</sup> It is thus evident that the copper concentration range of CMP wastewater is by far higher than copper effective concentration towards aquatic faunas and its concentration is strictly regulated in many countries both for discharges to municipal wastewater treatment facilities and for direct discharge to receiving waters. In China, the integrated circuit has been designated as a pillar industry in the 10<sup>th</sup> Five Year Economic plan since 2000. This has certainly driven large scale domestic and foreign semiconductor manufacturing plants in the past decade. High production volumes of semiconductor circuits bring forth a large amount of water consumption and increase the amount of wastewater discharged into the local environment. Therefore, an appropriate treatment of copper CMP wastewater is indispensable to meet stringent discharge regulations.

Various technologies have been employed for the removal of copper from copper CMP wastewater.<sup>7</sup> Flocculation/clarification is a conventional treatment for the removal of copper ions. However, this process requires large tanks and generates an undesirable copper-rich sludge and secondary environmental pollution. Other methods such as those employing membrane,<sup>8</sup> ion exchange,<sup>9</sup> and electrochemical technologies<sup>10</sup> have also been considered. Drawbacks associated with the abovementioned methods including the

<sup>a</sup>School of Environment Sciences, Key Laboratory of Wetland Ecology and Vegetation Restoration of National Environmental Protection, Northeast Normal University, Changchun 130117, China. E-mail: xywang@nenu.edu.cn; yangys990@nenu.edu.cn; Fax: +86-431-89165610; Tel: +86-135-04403055

<sup>b</sup>School of Environment Sciences, Science and Technology Innovation Center for Municipal Wastewater Treatment and Water Quality Protection of Jilin Province, Northeast Normal University, Changchun 130117, China. E-mail: fanw100@nenu.edu.cn; huomx097@nenu.edu.cn; zhoud100@nenu.edu.cn

<sup>c</sup>School of Chemical Engineering, Changchun University of Technology, Changchun 130012, China. E-mail: humingzhong@ccut.edu.cn

† Electronic supplementary information (ESI) available. See DOI: 10.1039/c7ra01163f

addition of chemicals or high-cost limit their application. Accordingly, the need for the development of alternatives to copper CMP wastewater treatment has led to an increasing interest in sorption processes.<sup>5,11</sup> Among various sorbents, bacterial biomass might be an efficient class of biosorbents for the removal of metal ions because of their high surface to volume ratios and presence of potentially active chemisorption sites.<sup>12,13</sup> Both the living and non-living cells of bacteria can be used as biosorbents and have their own advantages and disadvantages.<sup>14</sup> However, it is a controversial issue to choose living and non-living bacterial cells to remove heavy metals. Living cells might be preferential in the continuous treatment of heavy metal-bearing effluent<sup>15</sup> and have been successful in the treatment of heavy metals in genuine industrial and municipal effluents.<sup>16</sup> Biosorption and bioaccumulation often simultaneously participate in heavy metal removal by living bacterial cells.<sup>17</sup> Hence, the use of heavy metal-resistant bacteria has gained extensive attention in the removal of heavy metals from aqueous solution because they can resist the toxicity of heavy metals even at high concentrations due to specific resistance systems, such as efflux and uptake mechanisms, and extracellular precipitation.<sup>18</sup> Several studies have explored the copper removal from model copper CMP wastewater using metal tolerant bacteria from the genus *Staphylococcus*<sup>7</sup> and *Lactobacillus casei*.<sup>19</sup> However, bacterial mechanisms for the removal of various heavy metals are often highly specific for certain metals due to the heterogeneity of bacterial cell constituents.<sup>13</sup> The high diversity and complex structure of bacteria imply that there are many ways for the metal to be bound by bacterial cells. Hence, there is much work that needs to be carried out in screening promising bacteria for their features and mechanisms for the bioremoval of heavy metal species.

The genus *Cupriavidus* is a Gram-negative  $\beta$ -proteobacteria that is well known for its resistance to heavy metals. Accordingly, *Cupriavidus* species is of great interest in the field of environmental biotechnology.<sup>20</sup> Some *Cupriavidus* sp. such as *C. metallidurans* CH34,<sup>21</sup> *C. necator* N-1,<sup>22</sup> *C. pinatubonensis* JMP134,<sup>23</sup> and *C. taiwanensis*<sup>24</sup> have been reported to resist various heavy metals. In particular, *C. metallidurans* CH34 has been employed in the removal of heavy metals by biosorption and bioprecipitation in a bioreactor.<sup>25,26</sup> To the best of our knowledge, the ability of *C. gilardii* in terms of removal of heavy metals has not been documented to date. In our previous study, we isolated a *C. gilardii* strain CR3 that was resistant to  $\text{Cd}^{2+}$ ,  $\text{Zn}^{2+}$ ,  $\text{Co}^{2+}$ ,  $\text{Cu}^{2+}$ ,  $\text{Cu}^+$ ,  $\text{Hg}^{2+}$ ,  $\text{Pb}^{2+}$ ,  $\text{Ni}^{2+}$ ,  $\text{CrO}_4^{2-}$ , and  $\text{Ag}^+$  at concentrations up to 4 mM.<sup>27</sup> Moreover, the whole genome of *C. gilardii* was first sequenced using strain CR3 as a reference. Sequence analysis showed that the genome of the bacterium consists of two chromosomes and does not possess a plasmid.<sup>27</sup> In addition, the genes responsible for heavy metal resistance in most bacteria, such as *Pseudomonas syringae*<sup>28</sup> and *Escherichia coli*, are located on either plasmid(s) or dispersed between the chromosome and plasmids.<sup>29</sup> There are several exceptions in which heavy metal resistant genes are only located in the bacterial chromosome, suggesting their heavy metal resistance ability.<sup>30</sup> *C. gilardii* CR3 is such a distinctive bacterium. It can be inferred that *C. gilardii* CR3 has the ability to accumulate heavy

metals at the outside of the cells and is thus bound to cell surface structures *via* binding heavy metals.

Herein, we further investigated the bioremoval of copper ions from synthetic Cu-CMP wastewater by living cells of *C. gilardii* CR3 as a biosorbent. The experiments were designed based on the hypothesis that biosorption and bioaccumulation were assumed to occur in the living biomass. The major objectives of this study are two fold: (1) to study the characteristics of the bioremoval of copper by living cells of *C. gilardii* CR3 and (2) to understand the mechanisms of biosorption and bioaccumulation of copper in *Cupriavidus gilardii*.

## 2. Materials and methods

### 2.1 Preparation of biosorbent and synthetic Cu-CMP wastewater

The bacterium strain of *C. gilardii* CR3 used in this study was isolated from a natural asphalt deposit in our previous study.<sup>27</sup> The GenBank accession number of *C. gilardii* CR3 is JX945578. *C. gilardii* CR3 was grown in a Luria broth (LB) medium that contained 10.0 g tryptone, 5.0 g yeast extract, and 10.0 g NaCl per liter. Cells of *C. gilardii* CR3 were initially inoculated into sterile LB and placed on a shaker at 28 °C for 24 h. The live cells were harvested at an early stationary phase by centrifugation at 10 000g for 5 min. The cells were rinsed with phosphate buffer solution (PBS) three times and were subsequently stored in PBS at 4 °C. Cell concentrations in the suspension were determined by drying an aliquot onto a pre-weighed aluminum foil container to a constant weight at 60 °C.

Synthetic Cu-CMP wastewater used in this study was similar to that described by Mosier *et al.* (2015).<sup>19</sup> Briefly, the stock synthetic Cu-CMP wastewater containing 5 mM  $\text{Cu}^{2+}$  was prepared with  $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$  and deionized water.

All chemicals used in this study were of analytical grade and were purchased from Sigma Aldrich Co., Ltd, USA. Biochemicals were obtained from Dingguo Biotechnology Co., Ltd., China.

### 2.2 Scanning electron microscopy and energy dispersive X-ray spectroscopy

The concentrations of  $\text{Cu}^{2+}$  in the supernatant were determined *via* atomic absorption spectrophotometer (AA6300, Shimadzu, Japan). Scanning electron microscopy equipped with energy dispersive X-ray spectroscopy (SEM-EDX) was used to study the chemical characteristics of *C. gilardii* CR3 before and after  $\text{Cu}^{2+}$  binding. The biosorbent samples were fixed using 2.5% glutaraldehyde for 12 h and dehydrated in graded concentrations by alcohol. Samples were freeze-dried for 24 h and the dried cells were coated with a thin layer of gold and examined using SEM-EDX.

### 2.3 Fourier transform infrared spectroscopy

The possible involvement in the functional groups of cells of strain CR3 during the removal of  $\text{Cu}^{2+}$  from aqueous solution was elucidated using the Fourier transform infrared spectroscopy (FTIR) analysis. FTIR spectra of the cells loaded with and without  $\text{Cu}^{2+}$  were obtained in KBr discs using an FTIR



spectrometer (Perkin Elmer 2000). The FTIR samples were analyzed in the range of 4000–400  $\text{cm}^{-1}$  with a resolution of 4  $\text{cm}^{-1}$ .

## 2.4 Zeta potential

The zeta potential for *C. gilardii* CR3 was measured before and after the  $\text{Cu}^{2+}$  binding using the Zeta Meter (Malvern, Nano ZS90). The concentration of  $\text{Cu}^{2+}$  was set at 64  $\text{mg L}^{-1}$  in the measurement of zeta potential *versus* pH of cells (2.0–7.0).

## 2.5 Batch experiments

All the batch experiments were carried out by mixing 1  $\text{g L}^{-1}$  biosorbent at ambient temperature ( $28 \pm 2^\circ\text{C}$ ) and the mixtures were agitated at the speed of 180 rpm for 24 h. Control experiments were carried out in the free-biosorbent in every test to investigate the removal of  $\text{Cu}^{2+}$ , which might occur *via* chemical precipitation and sorption on to vessel walls.

The effects of pH, initial  $\text{Cu}^{2+}$  concentration of CMP wastewater, and contact time on biosorption and bioaccumulation capacity were studied. The effect of wastewater pH on the binding capacity of bacteria for  $\text{Cu}^{2+}$  was determined in the pH range from 2.0 to 7.0 with the intervals of 1.0 using 50 mL solutions containing 64  $\text{mg L}^{-1}$  of  $\text{Cu}^{2+}$ . The Cu-CMP wastewater was adjusted to required pH values using 0.1  $\text{mol L}^{-1}$  HCl and 0.1  $\text{mol L}^{-1}$  NaOH. Biosorbent was added to Cu-CMP wastewater. Similarly, the effect of initial  $\text{Cu}^{2+}$  concentration on biosorption and bioaccumulation was conducted by changing the initial  $\text{Cu}^{2+}$  concentration to 6.4, 19.2, 32, 44.8, 64, 83.2, 96, 108.8, and 128  $\text{mg L}^{-1}$  (at optimum pH 5.0 for 24 h). In addition, the batch studies were carried out at room temperature ( $28 \pm 2^\circ\text{C}$ ) with 64  $\text{mg L}^{-1}$  of  $\text{Cu}^{2+}$  using 1  $\text{g L}^{-1}$  of biosorbent dosage at different time intervals between 5 and 400 min at pH 5.0. The  $\text{Cu}^{2+}$  binding capacity and removal efficiency of the tested bacterium were calculated according to the following equation:

$$q_e = \frac{v(C_0 - C_e)}{m} \quad (1)$$

$$\text{Removal efficiency (\%)} = \frac{C_0 - C_e}{C_0} \times 100 \quad (2)$$

where  $q_e$  is the amount of  $\text{Cu}^{2+}$  uptake by unit of sorbents in  $\text{mg g}^{-1}$ ,  $v$  is the volume of Cu-CMP wastewater in the reaction system in L,  $m$  is the dosage of added biosorbents in g, and  $C_0$  and  $C_e$  are the initial and equilibrium concentration of  $\text{Cu}^{2+}$  in the solution in  $\text{mg L}^{-1}$ , respectively.

## 2.6 Isotherms and kinetics

The experimental data were evaluated using a Langmuir isotherm and Freundlich isotherm equations. The linear form of the Langmuir isotherm and Freundlich isotherm equations can be expressed as follows:

$$\frac{C_e}{q_e} = \frac{1}{q_m b} + \frac{C_e}{q_m} \quad (3)$$

$$\ln q_e = \ln K_F + \frac{1}{n} \ln C_e \quad (4)$$

where  $q_e$  is the amount of  $\text{Cu}^{2+}$  uptake by unit of sorbents in  $\text{mg g}^{-1}$ ,  $C_e$  is the equilibrium concentration of  $\text{Cu}^{2+}$  in the solution in  $\text{mg L}^{-1}$ ,  $q_m$  is the theoretical monolayer sorption capacity in  $\text{mg g}^{-1}$ ,  $n$  and  $K_F$  are Freundlich equilibrium constants, and  $b$  is the Langmuir equilibrium constant.

To analyze the kinetics of  $\text{Cu}^{2+}$  using *C. gilardii* CR3, the linear form of the pseudo-second order kinetics and the intra-particle diffusion model were applied to the experimental data:

$$\frac{t}{q_t} = \frac{1}{k q_e^2} + \frac{1}{q_e} t \quad (5)$$

$$q_t = k_d t^{\frac{1}{2}} + d \quad (6)$$

where  $k$ ,  $k_d$ , and  $d$  are the rate constant of kinetic sorption,  $q_e$  is the amount of  $\text{Cu}^{2+}$  uptake by unit of sorbents in  $\text{mg g}^{-1}$ , and  $q_t$  ( $\text{mg g}^{-1}$ ) is the amount of  $\text{Cu}^{2+}$  uptake at any given time  $t$  (min).

## 2.7 Statistical analysis

All the experiments were performed in triplicate. The data is shown as mean values with the error bars representing the 95 percent confidence intervals around the average value.

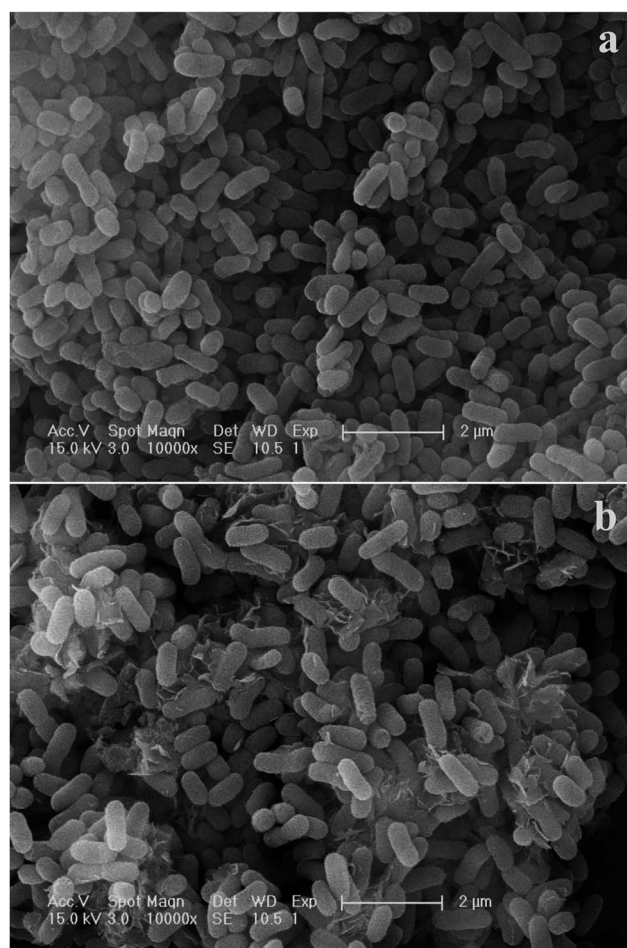


Fig. 1 Scanning electron microscopy (SEM) images of *Cupriavidus gilardii* CR3 in the absence (a) and presence (b) of  $\text{Cu}^{2+}$  concentration of 64  $\text{mg L}^{-1}$  at pH 5.0 for 24 h.



### 3. Results and discussion

#### 3.1 SEM-EDX analysis

Cell surface properties govern the metal–microorganism interactions to a large extent.<sup>31</sup> The morphological characterization of *C. gilardii* CR3 was studied before and after  $\text{Cu}^{2+}$  was loaded. After  $\text{Cu}^{2+}$  was loaded, the morphology of the bacterial cells did not show a greater variation in cell size and surface roughness. Moreover, the structural integrity of the cell was clear, without appearance of cell damage. Interestingly, reticulo-filamentous substances were obviously observed in the outer cells after  $\text{Cu}^{2+}$  was loaded (Fig. 1b), which were mostly like those secreted

by *C. gilardii* CR3 in the presence of ions from copper stress. This might reflect a self-protection of the strain CR3. Extracellular polymeric substances released by bacteria have been recommended as surface active agents and have an important role in binding heavy metals.<sup>32,33</sup> The present results might be associated with the genetic characteristics of *C. gilardii* CR3. Strain CR3 was isolated from a natural asphalt seep located at the Rancho La Brea Tar Pits (Los Angeles, USA), in which these natural asphalt seeps have existed for at least 40 000 year.<sup>34</sup> The distinctive echo niche caused bacterium CR3 to develop intrinsic heavy metal resistance mechanisms including self-detoxification through ion efflux, metal-complexation and

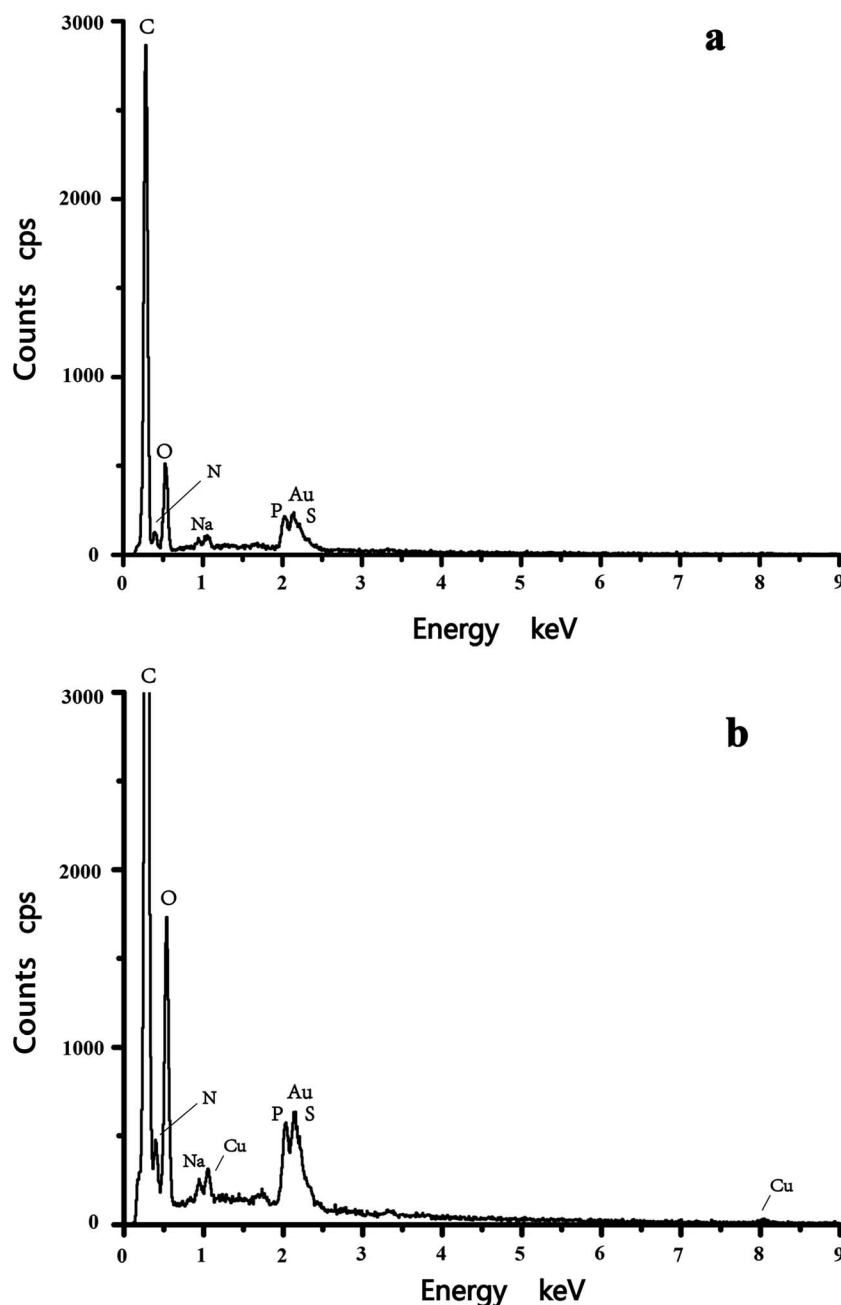


Fig. 2 Energy dispersive X-ray spectroscopy (EDX) of *Cupriavidus gilardii* CR3 in the absence (a) and presence (b) for  $\text{Cu}^{2+}$  concentration of  $64 \text{ mg L}^{-1}$  at pH 5.0 for 24 h.





metal-reduction, and a powerful DNA self-repair.<sup>27</sup> Moreover, the EDX analysis confirmed that  $\text{Cu}^{2+}$  combined with the biomass of *C. gilardii* CR3 (Fig. 2). There was no copper peak appearing on the cells before  $\text{Cu}^{2+}$  was loaded (Fig. 2a). In contrast, two additional signals of copper existed in the EDX profile after  $\text{Cu}^{2+}$  was loaded on the cells (Fig. 2b).

### 3.2 FTIR analysis

A number of sorption peaks that represented various functional groups of the cell surface were observed in the FTIR spectra (Fig. 3). The major sorption bands of *C. gilardii* CR3 were as follows:  $3307\text{ cm}^{-1}$ , corresponding to  $-\text{OH}$  and  $-\text{NH}$  stretching associated with protein;  $2927\text{ cm}^{-1}$ , for asymmetric/symmetric stretching vibration of  $\text{CH}_2$ ;  $1654$ ,  $1390$ , and  $1072\text{ cm}^{-1}$ , for the  $\text{C}=\text{O}$  stretching of amide I, the symmetric stretching  $\text{C}=\text{O}$  peptidic bond of carboxyl groups, and the stretching of  $\text{PO}_2^-$  in phosphodiester and phosphates, respectively; and  $545\text{ cm}^{-1}$ , for phosphate or sulphate functional groups. In a comparison with the spectra of *C. gilardii* CR3 prior to being treated with  $\text{Cu}^{2+}$ , a minute shift in the peak position was observed ( $<15\text{ cm}^{-1}$ ) after  $\text{Cu}^{2+}$  binding. The band shifts were from  $3307\text{ cm}^{-1}$  to  $3309\text{ cm}^{-1}$  for  $-\text{OH}$  and  $-\text{NH}$ , from  $1390\text{ cm}^{-1}$  to  $1391\text{ cm}^{-1}$  for  $-\text{COOH}$ , and from  $1072\text{ cm}^{-1}$  to  $1069\text{ cm}^{-1}$  for  $\text{PO}_2^-$  in the presence of  $\text{Cu}^{2+}$ . Functional groups such as carboxyl, hydroxyl, amino, and phosphate groups played a key role during the metal ion binding process.<sup>35,36</sup> The present FTIR analysis also provided evidence that these functional groups were involved in the binding of copper for *C. gilardii* CR3.

### 3.3 Zeta potential analysis

Bacterial surface charge is usually characterized by the zeta potential.<sup>37</sup> To elucidate the mechanism that *C. gilardii* CR3 sorbs

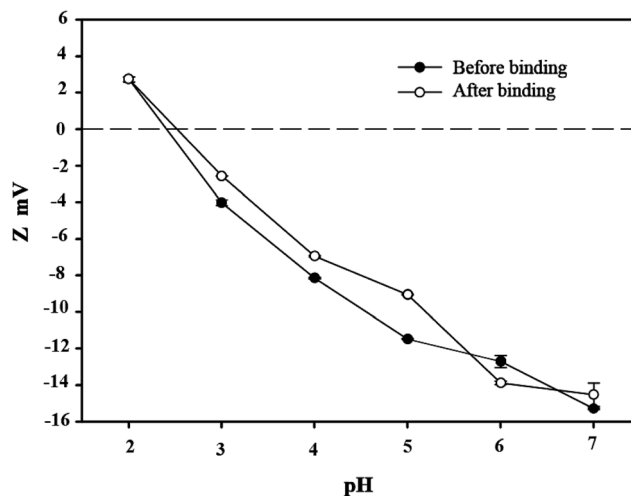


Fig. 4 Zeta potential of *Cupriavidus gilardii* CR3 before and after binding initial  $\text{Cu}^{2+}$  concentration of  $64\text{ mg L}^{-1}$  at different pH values.

$\text{Cu}^{2+}$ , the zeta potentials of *C. gilardii* CR3 were measured at different pH values with and without  $\text{Cu}^{2+}$  binding (Fig. 4). The isoelectric point of *C. gilardii* CR3 was around 2.2. The zeta potential of cells changed from positive to negative once the pH exceeded 2.2, and the negative values of zeta potential increased with continuously increasing pH. The values of the zeta potential of the cell ranged from about  $+3\text{ mV}$  to about  $-15\text{ mV}$ . The variations of zeta potential in strain CR3 with pH revealed the electrostatic interaction between bacteria and  $\text{Cu}^{2+}$  in an aqueous system, which is consistent with the previous observations that most bacteria carry a net negative charge.<sup>35</sup> Additionally, the obtained results indicated that the zeta potentials of the cell with

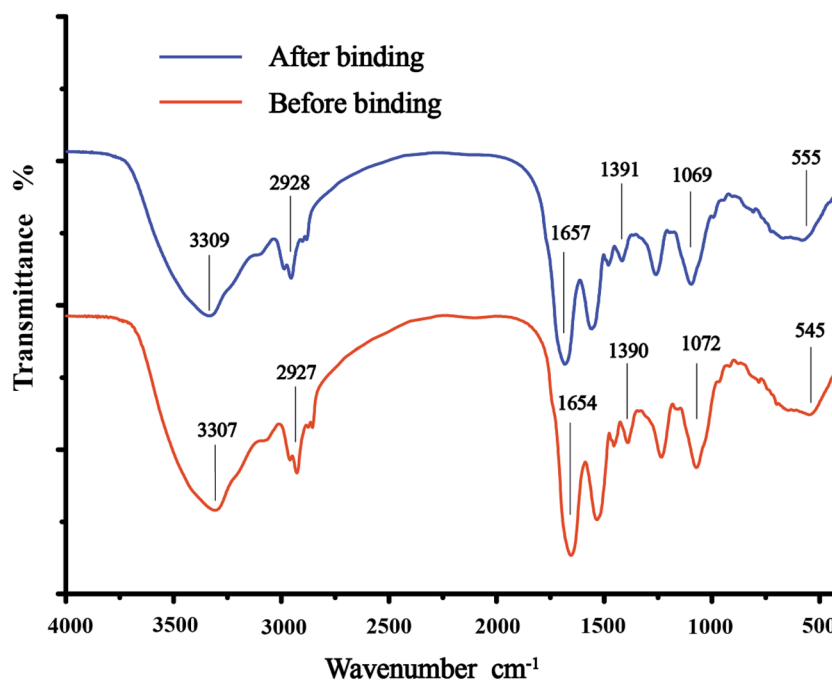


Fig. 3 Fourier transform infrared spectroscopy (FTIR) of *Cupriavidus gilardii* CR3 before and after binding the initial  $\text{Cu}^{2+}$  concentration of  $64\text{ mg L}^{-1}$  at pH 5.0 for 24 h.



and without binding  $\text{Cu}^{2+}$  exhibited similar trends over the pH range from 2.0 to 7.0. The absolute values of the zeta potential of the cells after binding with  $\text{Cu}^{2+}$  were lower than those prior to binding at the pH range from 2.2 to 7.0.

### 3.4 Effect of pH on copper bioremoval

The pH value of the aqueous solution is an important variable in the binding of metals on the biosorbents. The influence of the initial pH value of the solution on the removal of  $\text{Cu}^{2+}$  was examined in the pH range from 2 to 7. The effects of pH on the binding of  $\text{Cu}^{2+}$  at various pH values (2.0–7.0) are shown in Fig. 5. The results showed that pH values significantly affect bacterial binding capacity to copper in the solution. The capacity of *C. gilardii* CR3 and removal efficiency of  $\text{Cu}^{2+}$  increased with the elevation of the pH value. At the pH range of 2.0–5.0, the binding capacity sharply increased from 2.08  $\text{mg g}^{-1}$  (removal 2%  $\text{Cu}^{2+}$ ) to 18.33  $\text{mg g}^{-1}$  (removal 27%  $\text{Cu}^{2+}$ ). A 30% removal of  $\text{Cu}^{2+}$  occurred at pH between 6.0 and 7.0.

The pH value affects the solution chemistry of metal ions and the activity of the functional groups of the cell wall.<sup>38</sup> Copper can be present in solution as three different species:  $\text{Cu}^{2+}$ ,  $\text{CuOH}^+$ , and  $\text{Cu(OH)}_2$ .<sup>39</sup> Under lower pH conditions,  $\text{Cu}^{2+}$  and  $\text{CuOH}^+$  were more favorable copper species. The competition of  $\text{Cu}^{2+}$  and protons in solution to the binding sites on the bacterium cell wall can explain the relationship between pH values and the uptake of metal ions.<sup>38</sup> In this study, the binding capacities of copper were greatly influenced by the pH value of the solution. At pH 2.0, the lowest binding capacity was obtained. The reason for this is that  $\text{H}_3\text{O}^+$  in the solution may more easily occupy the binding sites on the surface of *C. gilardii* CR3 than copper. With an increase in pH (2.0–5.0), the amount of negative charges on the surface of the strain CR3 increased (Fig. 4), which led to the enhancement in binding capacity. When the pH value was over 5.3, the precipitation of  $\text{Cu}^{2+}$  hydroxide occurred. The formation of metal hydroxide and other metal–ligand complexes significantly reduced the amount of metal ions bound at higher pH.<sup>40</sup> Therefore, the optimum pH condition was 5.0 in this study, and all the subsequent experiments were conducted at pH 5.0.

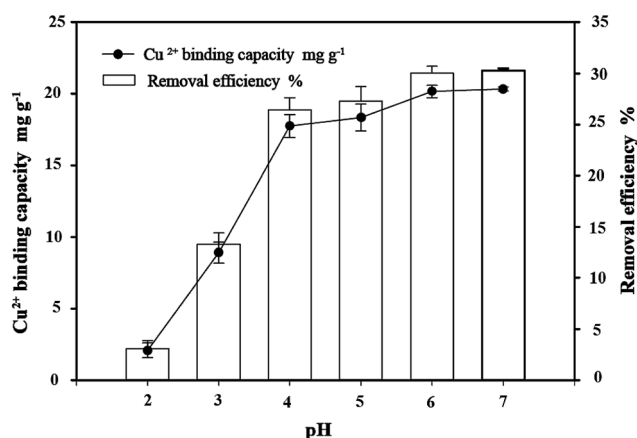


Fig. 5 Effect of pH on copper bioremoval of *Cupriavidus gilardii* CR3 with an initial  $\text{Cu}^{2+}$  concentration of 64  $\text{mg L}^{-1}$  for 24 h.

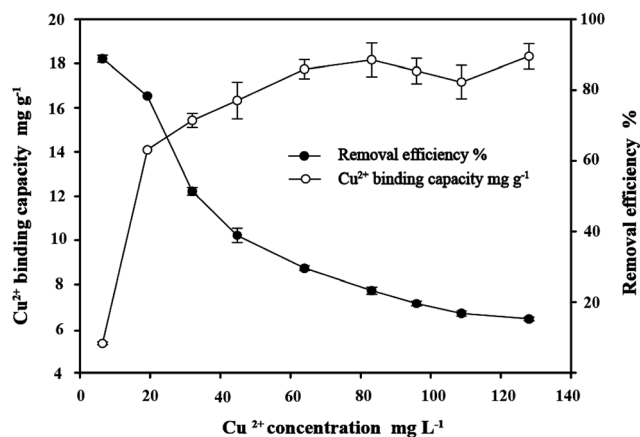


Fig. 6 Effect of initial  $\text{Cu}^{2+}$  concentration on copper bioremoval of *Cupriavidus gilardii* CR3 at pH 5.0 for 24 h.

### 3.5 Effect of initial $\text{Cu}^{2+}$ concentration on copper bioremoval

As depicted in Fig. 6, the  $\text{Cu}^{2+}$  binding capacity of *C. gilardii* CR3 increased with the increase of initial  $\text{Cu}^{2+}$  concentration from 6.4  $\text{mg L}^{-1}$  to 128  $\text{mg L}^{-1}$ , and the removal efficiency inversely changed. The rapid removal of  $\text{Cu}^{2+}$  was observed under the lower  $\text{Cu}^{2+}$  condition ( $<64 \text{ mg L}^{-1}$ ). With an increase in  $\text{Cu}^{2+}$  concentration, the removal of  $\text{Cu}^{2+}$  became gradually slow. The lowest removal ratio was achieved at the highest initial  $\text{Cu}^{2+}$  concentration, where the equilibrium binding capacity for the cells was 22  $\text{mg g}^{-1}$ . Therefore, the intersecting point of the two curves was the best ratio of the  $\text{Cu}^{2+}$  concentration and bacterial concentration.

### 3.6 Effect of contact time on copper bioremoval

The equilibrium time is of crucial importance because it is one of the parameters for economical wastewater treatment plant application. The effect of contact time on bioremoval of *C. gilardii* CR3 at the initial concentration of 64  $\text{mg L}^{-1}$  is shown in Fig. 7. The results revealed that the binding process as a function of contact time occurs in two steps. The first step involves a rapid metal uptake within the first 50 min of contact time, suggesting that this step was a rapid process. Within the first 50 minutes of binding, 76% of the total binding capacity was achieved. Rapid binding during the initial stage of the process was attributed to the larger surface area that was available for the binding of metal ions.<sup>41</sup> The second step was characterized by the subsequent slower binding capacity of the metal. The exhaustion of binding sites during the process resulted in lower sorption rates during the second stage.<sup>42</sup>

### 3.7 Isotherms study

Several sorption isotherms can be used to describe the equilibrium concentration of the sorbed species as a function of the fluid phase concentration. The Langmuir isotherm assumes monolayer sorption and the Freundlich isotherm is an empirical equation based on sorption for a heterogeneous surface.<sup>43,44</sup> In the present study, the linearized Langmuir and Freundlich



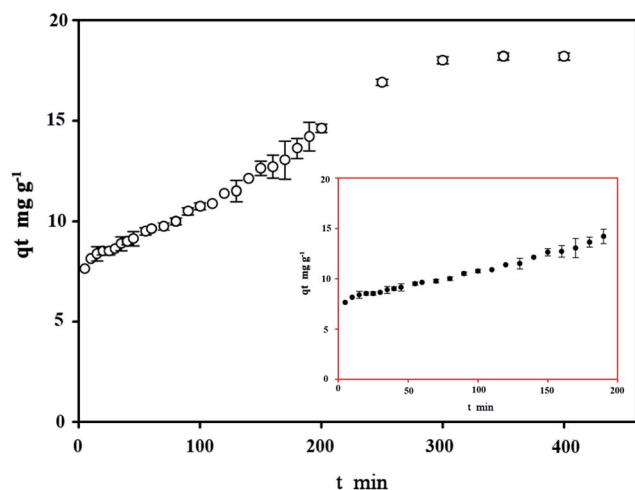


Fig. 7 Effect of contact time on copper bioremoval of *Cupriavidus gilardii* CR3 with an initial  $\text{Cu}^{2+}$  concentration of  $64 \text{ mg L}^{-1}$  at pH 5.0.

isotherms at various initial concentrations of copper ions were employed to determine the sorption isotherm data (Fig. 8). The correlation coefficient and other parameters obtained from Langmuir and Freundlich sorption isotherms for the sorption

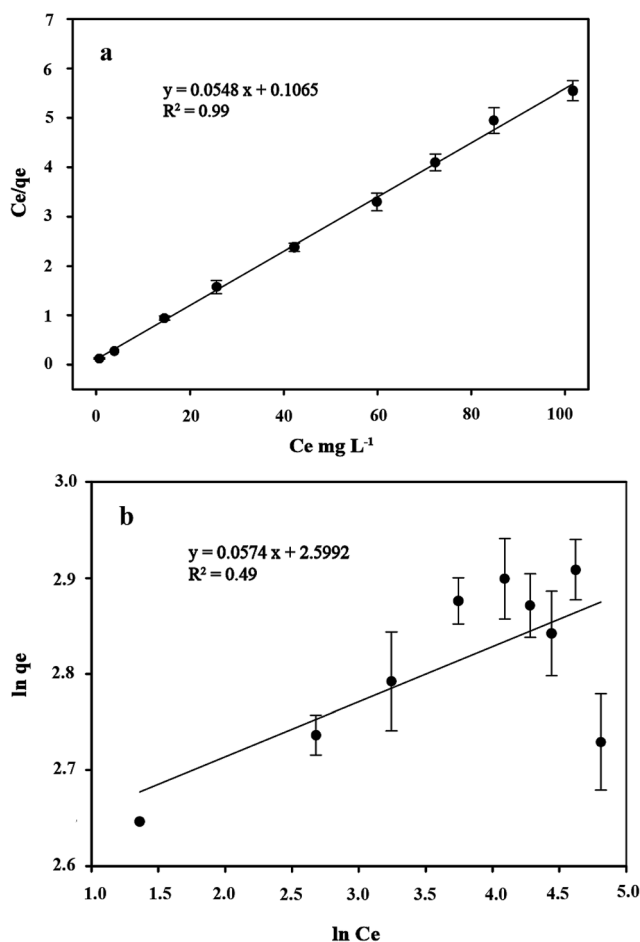


Fig. 8 The linearized form of a Langmuir isotherm (a) and Freundlich isotherm (b) of *Cupriavidus gilardii* CR3 for  $\text{Cu}^{2+}$  at pH 5.0 for 24 h.

Table 1 Isotherms of *Cupriavidus gilardii* CR3 for  $\text{Cu}^{2+}$  at pH 5.0 for 24 h

Equilibrium model	Langmuir			Freundlich		
	Parameter	$q_m^a$	$b^b$	$R^2$	$K_F^c$	$1/n^c$
Value		18.25	0.51	0.99	13.45	0.05

<sup>a</sup> The academic maximum amount of  $\text{Cu}^{2+}$  uptake per gram sorbents in  $\text{mg g}^{-1}$ . <sup>b</sup> Langmuir equilibrium constant. <sup>c</sup> Freundlich equilibrium constant.

of  $\text{Cu}^{2+}$  are shown in Table 1. The Langmuir model ( $R^2 = 0.99$ ) exhibited a better fit to the data of copper than the Freundlich model ( $R^2 = 0.49$ ) in the studied initial  $\text{Cu}^{2+}$  concentration range. It could be inferred that the binding of the cells of *C. gilardii* CR3 to  $\text{Cu}^{2+}$  is monolayer sorption. Most of bacterial binding for metals follow the Langmuir isotherm.<sup>45–47</sup> According to Langmuir isotherm, the predicted maximum biosorption capacity for *C. gilardii* CR3 was  $18.25 \text{ mg g}^{-1}$ , which is very close to the experimental binding capacity  $q_e$  value ( $18.33 \text{ mg g}^{-1}$ ). These results suggest that the binding of copper by strain CR3 principally occurred at cell sorption rather than accumulation because the maximum binding capacity calculated by the Langmuir isotherm did not consider the amount of metal uptake by bacteria.<sup>48</sup> Although both biosorption and bio-accumulation are involved in living cells for the removal of various heavy metals from aqueous solution, biosorption usually accounts for the main part in the binding capacity of heavy metals.<sup>49–51</sup> For instance, the cell surface of a *Lactococcus lactis* strain accounted for over 95% of the amount of cadmium binding.<sup>52</sup>

Additionally, a comparison within *Cupriavidus* strains showed that the copper biosorption capacity of *C. gilardii* CR3 was almost the same as that of the *C. taiwanensis* TJ208 ( $19.0 \text{ mg g}^{-1}$ ), whereas lower than that of *C. metallidurans* CH34 ( $26.69 \text{ mg g}^{-1}$ ) under the same experiment conditions.<sup>53</sup> Therefore, it is still efficient compared to other bacteria in terms of copper biosorption capacity: the copper biosorption capacity of *Bacillus* sp. is  $16.25 \text{ mg g}^{-1}$  (ref. 54) and *Sphaerotilus natans* is  $5.4 \text{ mg g}^{-1}$ .<sup>55</sup> Thus, *C. gilardii* CR3 can be an interesting alternative for the bioremoval of  $\text{Cu}^{2+}$  from Cu-CMP wastewater.

### 3.8 Kinetics study

Kinetic studies are important for the practical application of binding process because they can provide insight into the reaction pathways and the mechanisms of the sorption reaction.<sup>56</sup> To investigate the kinetic patterns of copper on *C. gilardii* CR3, the pseudo-second order kinetic model and intraparticle diffusion model were applied in this study (Fig. 9). The kinetics of copper binding by strain CR3 was well described by the pseudo-second order kinetic model ( $R^2 = 0.99$ ) and intraparticle diffusion model ( $R^2 = 0.98$ ) (Table 2). The experimental data fitting the pseudo-second order model demonstrated that the mechanism of the sorption process was controlled by chemical



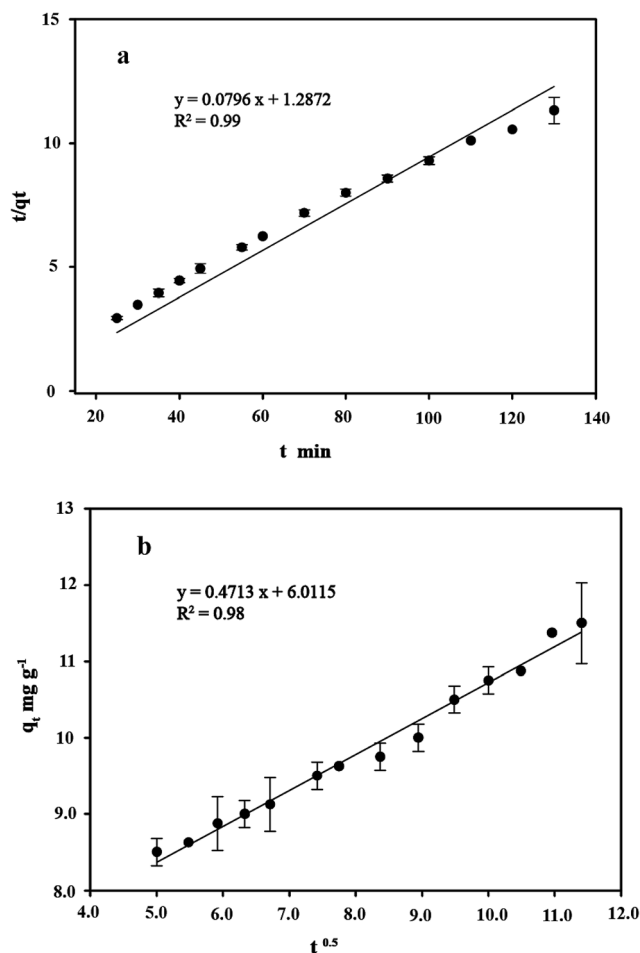


Fig. 9 The linearized form of the pseudo-second order kinetic model (a) and the intraparticle diffusion model (b) for the  $\text{Cu}^{2+}$  concentration of  $64 \text{ mg L}^{-1}$  bound by *Cupriavidus gilardii* CR3 at pH 5.0.

Table 2 Kinetics for  $\text{Cu}^{2+}$  concentration of  $64 \text{ mg L}^{-1}$  by *Cupriavidus gilardii* CR3 at pH 5.0

Kinetics model	The pseudo-second order kinetics			The intraparticle diffusion		
	Parameter	$q_e^a$	$k^b$	$R^2$	$k_d^b$	$d^b$
Value		12.56	0.004	0.99	0.47	6.01

<sup>a</sup> The amount of  $\text{Cu}^{2+}$  uptake by unit sorbents in  $\text{mg g}^{-1}$ . <sup>b</sup> The rate constant of kinetic sorption.

sorption involved in exchange sorption of metal ions and a surface proton.<sup>57,58</sup> Moreover, the fit line with an intraparticle diffusion model did not pass through the origin, suggesting that intraparticle diffusion occurred in the binding process, but it was not the sole rate-controlling step.<sup>40</sup> Furthermore, the amount of negative charges on the surface of strain CR3 increased with an increase in pH value based on the measurement of the zeta potential (Fig. 4), which confirmed that the electrostatic interaction contributed to the binding process. Therefore, chemisorption and physisorption might be

simultaneously involved in the binding process by means of ion exchange and electrostatic interactions between copper ions and functional groups in the cells of strain CR3.

## 4. Conclusions

The present study was carried out to use living cells of *C. gilardii* CR3 as a biosorbent for the removal  $\text{Cu}^{2+}$  from Cu-CMP wastewater. The binding ability was pH-dependent, and the highest binding capacity of copper ions ( $18.33 \text{ mg g}^{-1}$ ) was achieved at pH 5.0. The biosorption equilibrium was well described by the Langmuir isotherm. Both the pseudo-second order kinetic model and the intraparticle diffusion model were in good agreement with all the experimental data. The obtained results may provide pre-stage for an initial evaluation of binding mechanisms and operational conditions for the continuous removal of  $\text{Cu}^{2+}$  from CMP wastewater utilizing living cells of *C. gilardii* CR3 in a fixed-bed reactor.

## Authors' contributions

XW and FW conceived and designed the experiments. YY and XW performed the experiments. MH and DZ analyzed the data. MH and MH contributed reagents, materials, and analysis tools. XW and YY wrote the paper. XW and FW revised the paper.

## Ethics approval and consent to participate

Not applicable.

## Conflict of interest

The authors declare that they have no competing interests.

## Abbreviations

CMP	Chemical-mechanical polishing/planarization
LB	Luria broth
PBS	Phosphate buffer solution
SEM-EDX	Scanning electron microscopy equipped with energy dispersive X-ray spectroscopy
FTIR	Fourier transform infrared spectroscopy

## Acknowledgements

Analyses of SEM-EDX, FTIR, and zeta potential were carried out by the Analysis and Test Center, Northeast Normal University. The skillful technical assistance of Li Chen is gratefully acknowledged. We thank Prof. Fayek Negm, Dept. of Botany and Plant Sci. University California Riverside, USA, kindly helped us to improve the quality of our manuscript. This study was funded by the National Natural Science Foundation of China (No. 51678122), and Jilin Provincial Science and Technology Department Project, China (No. 20150414046GH).





## References

- 1 G. C. C. Yang, *Environ. Prog. Sustainable Energy*, 2002, **21**, 57–62.
- 2 B. Maag, *Global Environ. Polit.*, 2000, **2**, 134–136.
- 3 J. Hollingsworth, R. Sierra-Alvarez, M. Zhou, K. L. Ogden and J. A. Field, *Chemosphere*, 2005, **59**, 1219–1228.
- 4 H. Chua, P. H. Yu, S. N. Sin and M. W. Cheung, *Chemosphere*, 1999, **39**, 2681–2692.
- 5 W. Maketon, PhD thesis, University of Arizona, 2007.
- 6 G. C. C. Yang and C. M. Tsai, *J. Membr. Sci.*, 2006, **286**, 36–44.
- 7 L. C. Stanley and K. L. Ogden, *J. Environ. Manage.*, 2003, **69**, 289–297.
- 8 Y. N. Su, W. S. Lin, C. H. Hou and W. Den, *J. Water Process Eng.*, 2014, **4**, 149–158.
- 9 W. Maketon and K. L. Ogden, *Chemosphere*, 2009, **75**, 206–211.
- 10 G. C. C. Yang and C. M. Tsai, *J. Membr. Sci.*, 2008, **321**, 232–239.
- 11 W. Maketon, C. Z. Zenner and K. L. Ogden, *Environ. Sci. Technol.*, 2008, **42**, 2124–2129.
- 12 P. Kotrba, T. Macek, M. Mackova, G. Naja, B. Volesky and F. Pagnanelli, Bacterial biosorption and biosorbents, in *Microbial Biosorption of Metals*, ed. Y. S. Yun, K. Vijayaraghavan and S. W. Won, Springer Science and Business Media, New York, 2011, pp. 121–141.
- 13 P. S. Li and H. C. Tao, *Crit. Rev. Microbiol.*, 2013, **41**, 140–149.
- 14 A. Malik, *Environ. Int.*, 2004, **30**, 261–278.
- 15 G. M. Gadd, *J. Chem. Technol. Biotechnol.*, 2009, **84**, 13–28.
- 16 P. Kotrba, T. Macek, M. Mackova, G. Naja, B. Volesky and F. Pagnanelli, Biosorption and metal removal through living cells, in *Microbial Biosorption of Metals*, ed. P. Kotrba, M. Mackova, J. Fišer and T. Macek, Springer Science and Business Media, New York, 2011, pp. 197–233.
- 17 K. Chojnacka, *Environ. Int.*, 2010, **36**, 299–307.
- 18 I. Kamika and M. N. Momba, *BMC Microbiol.*, 2013, **13**, 1–14.
- 19 A. P. Mosier, J. Behnke, E. T. Jin and N. C. Cady, *J. Environ. Manage.*, 2015, **160**, 67–72.
- 20 W. M. Chen, C. H. Wu, E. K. James and J. X. Chang, *J. Hazard. Mater.*, 2008, **151**, 364–371.
- 21 P. J. Janssen, R. Van Houdt, H. Moors, P. Monsieurs, N. Morin, A. Michaux, M. A. Benotmane, N. Leys, T. Vallaeys, A. Lapidus, S. Monchy, C. Médigue, S. Taghavi, S. McCorkle, J. Dunn, D. Van der Lelie and M. Mergeay, *PLoS One*, 2010, **5**, e10433.
- 22 A. Poehlein, B. Kusian, B. Friedrich, R. Daniel and B. Bowien, *J. Bacteriol.*, 2011, **193**, 5017.
- 23 A. Lykidis, D. Perez-Pantoja, T. Ledger, K. Mavromatis, I. J. Anderson, N. N. Ivanova, S. D. Hooper, A. Lapidus, S. Lucas, B. González and N. C. Kyrpides, *PLoS One*, 2010, **5**, e9729.
- 24 C. Amadou, G. Pascal, S. Mangenot, M. Glew, B. Contemps, D. Capela, S. Carrère, S. Cruveiller, C. Dossat, A. Lajus, M. Marchetti, V. Poinot, Z. Rouy, B. Servin, M. Saa, C. Schenowitz, V. Barbe, J. Batut, C. Médigue and C. Masson-Boivin, *Genome Res.*, 2008, **18**, 1472–1483.
- 25 R. Paterson, Immobilization of bacteria in composite membranes and development of tubular membrane reactors for heavy metal recuperation, in *Effective membrane processes: new perspectives*, ed. L. Diels, S. V. Roy, M. Mergeay, W. Doyen, S. Taghavi and R. Leysen, Kluwer Academic Publishers, The Netherlands, 1993, pp. 275–293.
- 26 A. E. Torma, M. L. Apel and C. L. Brieley, The use of *Alcaligenes eutrophus* immobilized in a tubular membrane reactor for heavy metal recuperation, in *Biohydrometallurgical technologies*, ed. L. Diels, S. V. Roy, S. Taghavi, W. Doyen, R. Leysen and M. Mergeay, The Minerals, Metals and Materials Society, Warrendale, 1993, pp. 133–144.
- 27 X. Y. Wang, M. L. Chen, J. F. Xiao, L. R. Hao, E. David Crowley, Z. W. Zhang, J. Yu, N. Huang, M. X. Huo and J. Y. Wu, *PLoS One*, 2015, **10**, 1–21.
- 28 S. Puig, E. M. Rees and D. J. Thiele, *Structure*, 2002, **10**, 1292–1295.
- 29 D. H. Nies, *FEMS Microbiol. Rev.*, 2003, **27**, 313–339.
- 30 J. Valdés, I. Pedrosa, R. Quatrini, R. J. Dodson, H. Tettelin, R. Blake II, J. A. Eisen and D. S. Holme, *BMC Genomics*, 2008, **9**, 82–86.
- 31 E. Kordialik-Bogacka, *Cent. Eur. J. Chem.*, 2011, **9**, 348–351.
- 32 M. Watanabe, K. Kawahara, K. Sasaki and N. Noparatnaraporn, *J. Biosci. Bioeng.*, 2003, **95**, 374–378.
- 33 A. Iyer, K. Mody and B. Jha, *Mar. Pollut. Bull.*, 2005, **50**, 340–343.
- 34 J. S. Kim and D. E. Crowley, *Appl. Environ. Microbiol.*, 2007, **73**, 4579–4591.
- 35 F. Huang, Z. Dang, C. L. Guo, G. N. Lu, R. R. Gu, H. J. Liu and H. Zhang, *Colloids Surf., B*, 2013, **107**, 11–18.
- 36 L. Xia, X. J. Xu, W. Zhu, Q. Y. Huang and W. L. Chen, *Int. J. Mol. Sci.*, 2015, **16**, 15670–15687.
- 37 V. D. M. Hc and H. J. Busscher, *Appl. Environ. Microbiol.*, 2001, **67**, 491–494.
- 38 A. Esposito, F. Pagnanelli and F. Vegliò, *Chem. Eng. Sci.*, 2002, **57**, 307–313.
- 39 X. N. Li, A. R. Li, M. Z. Long and X. J. Tian, *J. Environ. Health Sci. Eng.*, 2015, **13**, 1–8.
- 40 H. Xie, Q. Zhao, Z. Zhou, Y. Wu, H. Wang and H. Xu, *RSC Adv.*, 2015, **5**, 33478–33488.
- 41 Y. Liu, G. Cui, C. Luo, L. Zhang, Y. Guo and S. Yan, *RSC Adv.*, 2014, **4**, 55162–55172.
- 42 M. Torab-Mostaedi, M. Asadollahzadeh, A. Hemmati and A. Khosravi, *J. Taiwan Inst. Chem. Eng.*, 2013, **44**, 295–302.
- 43 G. Bayramoğlu, S. Bektaş and M. Y. Arica, *J. Hazard. Mater.*, 2003, **101**, 285–300.
- 44 M. T. Veit, C. R. G. Tavares, S. M. Gomes-Da-Costa and T. A. Guedes, *Process Biochem.*, 2005, **40**, 3303–3308.
- 45 X. C. Chen, Y. P. Wang, Q. Lin, J. Y. Shi, W. X. Wu and Y. X. Chen, *Colloids Surf., B*, 2005, **46**, 101–107.
- 46 R. M. Gabr, S. H. A. Hassan and A. A. M. Shoreit, *Int. Biodeterior. Biodegrad.*, 2008, **62**, 195–203.
- 47 Z. Khan, A. Rehman, S. Z. Hussain, M. A. Nisar, S. Zulfiqar and A. R. Shakoory, *AMB Express*, 2016, **6**, 54.



- 48 S. S. Shaikat, Biosorption of metals: state of the art, general features, and potential applications for environmental and technological processes, in *Progress in Biomass and Bioenergy Production*, ed. R. C. Oliveira, M. C. Palmieri and O. Garcia Jr, In Tech, Rijeka, 2011, pp. 151–176.
- 49 L. Velásquez and J. Dussan, *J. Hazard. Mater.*, 2009, **167**, 713–716.
- 50 R. J. Yin, Q. X. Zhai, L. L. Yu, Y. Xiao, G. Wang, R. P. Yu, F. W. Tian and W. Chen, *Eur. Food Res. Technol.*, 2016, **242**, 1–9.
- 51 T. Srinath, T. Verma, P. W. Ramteke and S. K. Garg, *Chemosphere*, 2002, **48**, 427–435.
- 52 Y. Sheng, Y. Wang, X. Yang, B. Zhang, X. He, W. Xu and K. Huang, *Environ. Toxicol. Pharmacol.*, 2016, **48**, 183.
- 53 R. Biondo, S. F. Da, E. J. Vicente, J. E. Souza Sarkis and A. C. Schenberg, *Environ. Sci. Technol.*, 2012, **46**, 8325–8332.
- 54 S. Tunali, A. Çabuk and T. Akar, *Chem. Eng. J.*, 2006, **115**, 203–211.
- 55 F. Beolchini, R. Pagnanelli, L. Toro and F. Vegliò, *Water Res.*, 2006, **40**, 144–152.
- 56 Y. S. Ho and G. McKay, *Water Res.*, 2000, **34**, 735–742.
- 57 G. Padmapriya and A. G. Murugesan, *Desalin. Water Treat.*, 2015, **53**, 3513–3520.
- 58 S. Khosravihaftkhany, N. Morad, A. Z. Abdullah, T. T. Teng and N. Ismail, *RSC Adv.*, 2015, **5**, 106498–106508.

