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Biosorption of Zn(II) by live and dead cells of *Streptomyces ciscaucasicus* strain CCNWHX 72-14

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ABSTRACT

The biosorption characteristics of Zn(II) using live and dead cells of *Streptomyces ciscaucasicus* strain CCNWHX 72-14 as biosorbents have been investigated in the present research. Optimum conditions for biosorption were determined to be: pH adjusted to 5.0, agitated at 90 rpm and at a dose of 2 g/L. For initial zinc concentrations of 1–150 mg/L, batch biosorption data of live biomass preferred to be simulated with Freundlich model while those of dead strain fit Langmuir isotherm well. Experimental maximum biosorption capacity turned out to be 42.75 mg/g (0.654 mmol/g) for living material and 54 mg/g (0.826 mmol/g) for dead sorbents, respectively. The pseudo-second-order equation, instead of the pseudo-first-order one, was chosen to describe the time course biosorption process. In contrast to live biosorbents, dead biomass seemed to have lower binding strength with higher desorption efficiency at pH 1.0. Competitive biosorption revealed the order of competing metal ion to be: $Cu^{2+} > Cd^{2+} > Ni^{+}$. FT-IR analysis indicated that more functional groups were involved in the biosorption process of dead adsorbents, compared with those linked to live biomass. Taken together, it can be concluded that dead cells of CCNWHX 72-14 were better and cheaper biosorbents than live ones.

1. Introduction

With tendency of accumulation in the food chain and extreme toxicity even in relatively low concentrations, heavy metals released into the environment are becoming a global concern [1,2]. Zinc, which often exists in industrial effluents, is one of the 13 metals found in the contamination list proposed by the United States Environmental Protection Agency (USEPA) [3]. The World Health Organization (WHO) recommends a 5.0 mg/L maximum acceptable concentration of zinc in drinking water [4]. Consequently there is a significant interest regarding zinc removal from wastewater [5].

Conventional physiochemical methods for metals remediation include precipitation, filtration, coagulation, evaporation, ion exchange, membrane separation and solvent extraction. However, application of such processes is always expensive and ineffective in terms of energy and chemical products consumption, especially at low metal concentrations of 1–100 mg/L [6]. Therefore, there is a great need for an alternative technique, which is both economical and efficient. Biosorption, based on live or dead biosorbents, has been regarded as a cost-effective biotechnology for the treatment of complex wastewater containing heavy metals at high volume and low concentration [7]. Biosorption can be defined as the property of certain biomass to bind and concentrate selected ions or other molecules from aqueous solutions [8]. A number of economic biological materials, such as fungi, bacteria, yeast and algae, have been used to eliminate heavy metals from contaminated water [9–11].

In literatures, more and more *Streptomyces* strains have been employed as inexpensive biosorbents in the removal of metal ions. The simultaneous biosorption of Cu^{2+}, Zn^{2+} and Cr^{6+} from wastewater by *Streptomyces rimosus* biomass was reported by Chergui et al. [12]. In another study, Yuan et al. [13] investigated the comparative biosorption of cadmium by two different *Streptomyces* strains K33 and HL-12. In recent research, dead *Streptomyces* strains were also widely used in the biosorption of metal ions, especially in a series of studies of Selatnia et al. [14,15] and Nacera et al. [16]. However, little work has been done to compare the biosorption of Zn(II) using live and dead cells of given strain.

Streptomyces ciscaucasicus CCNWHX 72-14 has been shown to have higher degree of resistance to 13 mmol/L Zn^{2+}, 0.8 mmol/L Cu^{2+}, 0.6 mmol/L Cd^{2+} and 0.6 mmol/L Ni^{+} than other isolates. The 16S rRNA gene sequence of CCNWHX 72-14 was amplified with primers P1 and P6, from which the phylogenic tree was constructed [17]. As revealed in Fig. 1, the strain CCNWHX 72-14 can be classified in the branch of *Streptomyces* genera, being 100% identical to the *S. ciscaucasicus* strain HBUM 82686. Its recti-flexible aerial chains, composed of crinkly and columnar spores observed with scanning electron microscope, were illustrated in Fig. 2 [18]. The tremendous specific surface and capacious intracellular space

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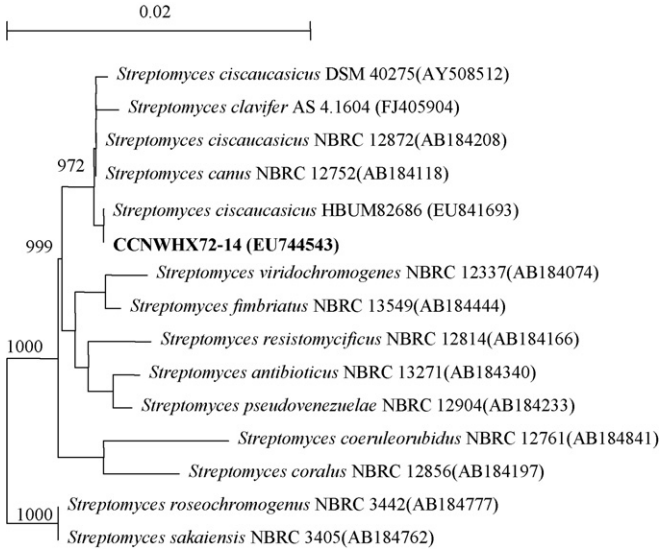

Fig. 1. Phylogenetic tree based on 16S rRNA gene of CCNWHX 72-14 and reference strains with the neighbour-joining method. (Numbers at nodes indicate bootstrap values. 0.02 denotes the genetic distance.)

produced by the strain, might play an important role in the removal of metal ions [19].

The objective of the present work is to investigate the biosorption potential of live and dead cells of *S. ciscaucasicus* CCNWHX 72-14 in the removal of Zn(II) from aqueous solution. The influence of different parameters on zinc biosorption, such as pH, agitation speed, biosorbent dose, initial metal concentration and contact time, were performed. Different equilibrium and kinetic models were applied to describe the biosorption process of live and dead biomass. The desorption experiments were implemented to identify the release of metal ions and recovery of biosorbents, while competitive biosorption and the Fourier transform infrared (FT-IR) analysis were also used in this study to look at potential binding sites and possible functional groups of live and dead biomass.

2. Materials and methods

2.1. Preparation of live and dead cells of CCNWHX 72-14 as biosorbents

The *S. ciscaucasicus* strain CCNWHX 72-14, isolated from a lead-zinc mine tailing in China, was tested in this study. Stationary-phase cells of CCNWHX 72-14 were typically inoculated into modified Gause's liquid (solute starch 16.0 g, glucose 4.0 g, NaCl 0.5 g, KNO_3 1.0 g, $KH_2PO_4{\cdot}3H_2O$ 0.5 g, $MgSO_4{\cdot}7H_2O$ 0.5 g, $FeSO_4{\cdot}7H_2O$ 0.01 g, water 1000 mL, pH 7.5 ± 0.1, MGL) medium and 120 rpm agitation was employed for shake-flask culturing at 28 °C. Then live cells were harvested by centrifugation (12,000 rpm, 10 min) at the end of the exponential phase, while dead cells were first subjected to autoclaving at 121 °C for 20 min as suggested by Kefala et al. [20] and Mameri et al. [21]. After rinsing with ddH_2O three times, live and dead cells of *S. ciscaucasicus* CCNWHX 72-14 were prepared as biosorbents for Zn(II) biosorption.

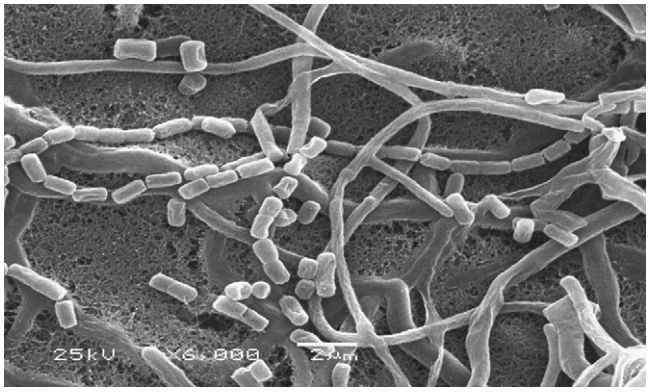

Fig. 2. Scanning electron micrograph of the recti-flexible aerial chains and crinkly spore surface of strain CCNWHX 72-14 after 14 days culture on modified Gause's synthetic agar medium. (Bar, 2 μm, JEOL-LV630.)

2.2. Effects of pH, agitation speed and biosorbent dose on biosorption

First of all, effects of pH, agitation speed and biosorbent dose on zinc biosorption and removal by live and dead CCNWHX 72-14 cells were examined to find optimum conditions. All the samples were incubated for 24 h in 150 mg/L of Zn^{2+}. Unless otherwise stated, standard conditions for biosorption experiments included an initial pH 5.0, being agitated at 120 rpm and having a dosage of 1.5 g/L. Effects of 3.0–7.0 pH values, agitation speeds from 60 to180 rpm and different biomass densities (0.5–2.5 g/L) were tested in parallel.

The residual zinc ions in the supernatant were measured by atomic absorption spectrophotometer (AAS) after centrifugation. Both the values of biosorption capacity and removal ratio of Zn^{2+} were evaluated as follows [22]:

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

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

where q_e is the equilibrium Zn^{2+} concentration on the biosorbent (mg/g dry cell); C_0 and C_e is the initial and residual metal concentration (mg/L); X is the biomass concentration (g dry cell/L).

2.3. Equilibrium and dynamic biosorption experiments

Live and dead biosorbents were suspended in Zn^{2+} solutions with an initial concentration ranging from 1 to 150 mg/L and gently agitated for 24 h. All the biosorption experiments were conducted under optimum conditions determined by experiments in advance.

To investigate the minimal contact time required for equilibrium, live and dead strains were suspended in solutions with specific initial concentration of Zn^{2+} (10, 50, 150 mg/L) and the residual metal ions were monitored at given time intervals within 8 h. The other conditions for biosorption were the same as those of earlier tests.

2.4. Effect of pH on desorption process

Live and dead biosorbents loaded with Zn^{2+} were obtained from earlier batch biosorption experiments at an initial concentration of 150 mg/L. The biosorbents were then re-suspended in metal free water after rinsing with ddH_2O. According to the research of Lu et al. [23], the pH values of the solutions were then adjusted to 1.0–7.0 with suitable amounts of 0.1 M HCl. After 24 h gentle agitation, the biomass was then harvested by centrifuging (12,000 rpm, 10 min) and the metal ions released into the supernatant were detected immediately with AAS. The desorption efficiency (%) was then calculated with the following equation:

$$\text{Desorption efficiency}\ (\%) = \frac{Dr}{Da} \times 100 \quad (3)$$

where Dr is the amount of metal ions released in the supernatant solution (mg) and Da represents the metal ions initially adsorbed on the biosorbent (mg).

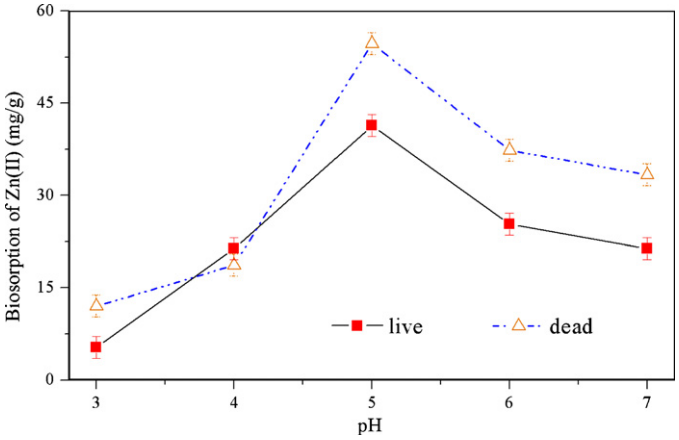

Fig. 3. Effect of pH on biosorption of Zn(II) by live and dead cells of CCNWHX 72-14. (Initial Zn^{2+} concentration: 150 mg/L; temperature: 28 °C; agitation speed: 120 rpm/min; biosorbent dose: 1.5 g/L.)

2.5. Competitive biosorption experiments

Competitive biosorption experiments were also performed with zinc in the presence of different groups of nickel, cadmium and copper. The solutions containing 0.5 mmol/L of each metal ion were incubated with live and dead cells of CCNWHX 72-14 and agitated for 24 h. The conditions for competing systems were set according to former results of optimum parameters. The removal ratios of Zn^{2+} for different combinations were then monitored by AAS.

2.6. FT-IR analysis

Infrared spectra of live and dead biosorbents loaded with and without zinc ions were obtained using a 330 spectrometer (Nicolet Avatar, USA). The dried samples mixed with KBr were immediately analyzed with a spectrophotometer in the range of 4000–400 cm^{-1} with a resolution of 4 cm^{-1}. The background was automatically subtracted from the sample spectra.

3. Results and discussion

3.1. Conventional parameters

3.1.1. Effect of pH

Fig. 3 showed that pH had a significant effect on the biosorption of Zn(II) not only for live but also for dead cells of CCNWHX 72-14. For live strain, Zn^{2+} uptake increased steadily with the increase of pH from 3.0 to 5.0, while that for dead cells was restricted at lower pH ($\leq$4.0) and went up to 54.7 mg/g at pH 5.0. Both biosorption capacities began to decrease as pH increased beyond 5.0.

As reported by Sheng et al. [24] and Vasquez et al. [25], pH has a significant effect on the solubility, speciation and biosorption capacity of heavy metals. The dependence of metal uptake on pH is related to both the surface functional groups on the biomass and the metal chemistry in solution. Kiran et al. [26] reported that as the pH increased to 5.0, more functional groups with negative charge such as carboxyl, amine or hydroxyl became exposed with subsequent increase of attraction sites to positively charged ions, and thus enhanced the biosorption capacity. At lower pH in this research, the effect of pH could be explained as the competition for active sites between H_3O^+ and positive zinc ions. The hydrolysis and precipitation of metal ions would affect adsorption by changing the concentration through formation of soluble metal species. The decrease of biosorption at higher pH might be attributed to the speciation of other metal species, such as the occurrence of $Zn(OH)_3$ ions as a result of the dissolution of $Zn(OH)_2$.

Similar studies were carried out for the biosorption of Cu and Ni by Bueno et al. [27] and Cayllahua et al. [28]. Here the optimum pH for Zn^{2+} uptake by live and dead biosorbents of CCNWHX 72-14 was determined to be 5.0 and all the following biosorption experiments were conducted at this most favorable pH value.

3.1.2. Effect of agitation speed

As can be seen in Fig. 4, the effect of agitation speed on Zn(II) biosorption by live and dead CCNWHX 72-14 had similar trends, where maximum biosorption capability was found at 90 rpm. The monitored biosorption capacity increased to 48 mg/g for live cells and 58.7 mg/g for dead ones as the agitation speed changed from 60 to 90 rpm. It has been reported that in the biosorption system, agitation brought more contact between the metal ions and biosorbent binding sites, which promoted the transference of sorbate ions to biosorbents. The results indicated that biosorption of Zn(II) by live and dead biomass was more effective with moderate agitation, showing good agreement with former reports of Bai et al. [29] and Ucun et al. [30].

3.1.3. Effect of biosorbent dose

Earlier studies of Yao et al. [31] and Acharya et al. [32] have indicated that biosorbent dose was also an important parameter affecting biosorption capacity as well as removal efficiency. As clearly depicted in Fig. 5, the removal ratio increased gradually as the biosorbent concentration increased from 0.5 to 2.5 g/L, simultaneously the biosorption capacity fell inversely. When using live strain, a maximum Zn(II) biosorption of 52 mg/g was observed at a dosage of 0.5 g/L, while the corresponding removal ratio was just 17.3%. As shown at the cross point of the two lines for live cells, 53.3% of Zn^{2+} was removed at a 2.0 g/L dosage, where the biosorption capacity was 40 mg/g, not much lower compared with 42 mg/g at 1.0 g/L or 41.3 mg/g at 1.5 g/L. As to dead biosorbents, the removal efficiency was only 20% whilst the maximum capability of 60 mg/g was attained at the lowest dosage. At the dose of 2.0 g/L, 52 mg/g of biosorption capacity was achieved with 69.3% zinc removal, being better than 53.3 mg/g biosorption and 53.3% removal values at 1.5 g/L dosage. It's obvious that with the increase of biosorbents, more binding sites were available and thus the removal efficiency went up. However, under specific initial concentration, redundant biosorbents were not necessary for an efficient biosorption process, as the biosorption efficiency began to reduce with the decrease

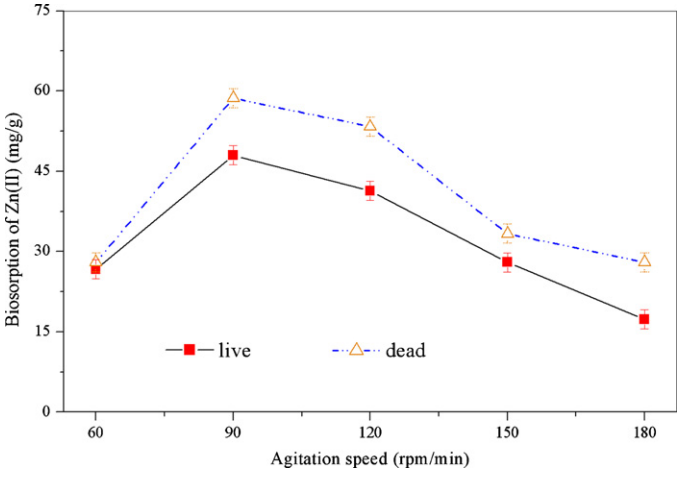

Fig. 4. Effect of agitation speed on biosorption of Zn(II) by live and dead cells of CCNWHX 72-14. (Initial Zn^{2+} concentration: 150 mg/L; temperature: 28 °C; pH: 5.0; biosorbent dose: 1.5 g/L.)

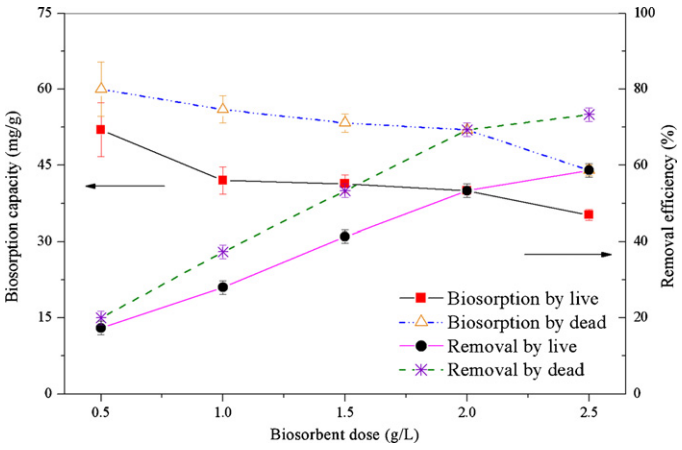

Fig. 5. Effect of biosorbent dose on Biosorption capacity and removal efficiency of Zn(II) by live and dead cells of CCNWHX 72-14. (Initial Zn^{2+} concentration: 150 mg/L; temperature: 28 °C; pH: 5.0; agitation speed: 120 rpm/min.)

of metal ions. Hence, it could be stated that 2.0 g/L was the optimum biosorbent dose for further tests, where adequate biosorption capability could be obtained with a high removal ratio.

So far, the optimum conditions for zinc biosorption and removal were determined as pH 5.0, agitated at 90 rpm and at a dose of 2 g/L, and all the following biosorption experiments were conducted under these conditions.

3.2. Batch experiments

3.2.1. Effect of initial concentration

As shown in Fig. 6, with the increase of initial metal ions, biosorption capacity and removal ratio changed inversely. The Zn(II) biosorption process of live and dead cells of CCNWHX 72-14 exhibited similar trends over the initial concentration range of 1–150 mg/L. However, the removal ratio of live biosorbents decreased more rapidly compared with that of dead ones. The equilibrium biosorption capability of live biomass was always lower than that of dead strain and the difference seemed to increase with increased initial concentration. The lowest removal ratio was achieved at the highest initial zinc concentration, where the equilibrium biosorption capacity for live biomass was 42.75 mg/g (0.654 mmol/g) while dead cells were able to uptake 54 mg (0.826 mmol) Zn^{2+} by each gram of dry cells.

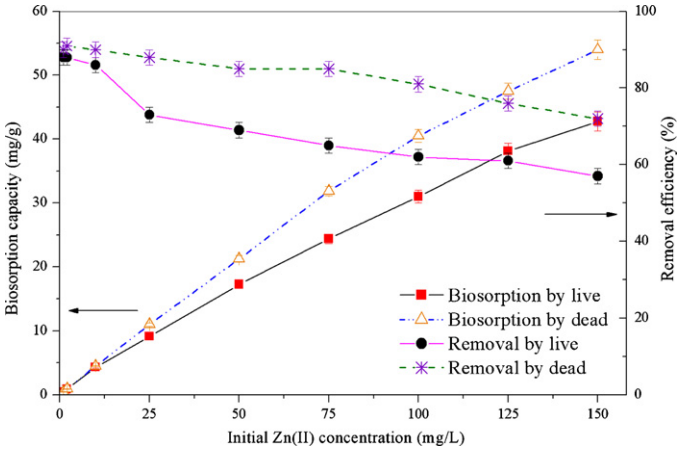

Fig. 6. Biosorption capacity and removal efficiency of Zn(II) by live and dead cells of CCNWHX 72-14 over initial concentration ranging from 1 to 150 mg/L.

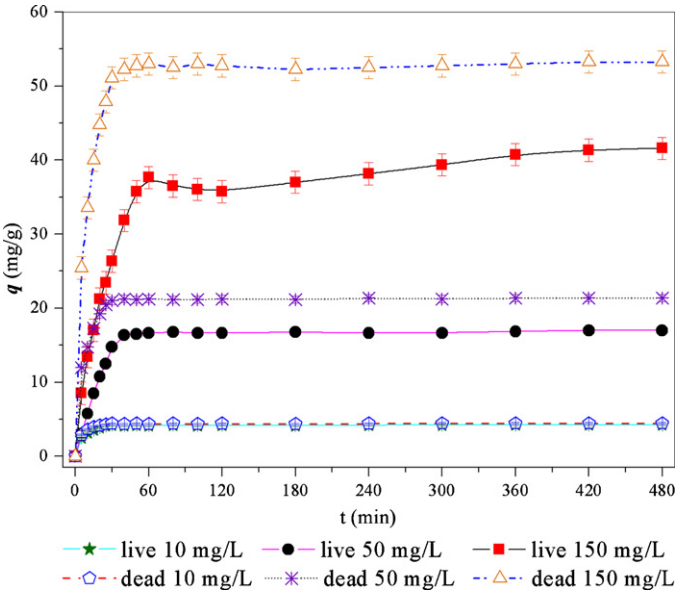

Fig. 7. Effect of contact time on biosorption of Zn(II) by live and dead cells of CCNWHX 72-14 at an initial zinc concentration of 10, 50 and 150 mg/L.

3.2.2. Effect of contact time

The effect of contact time on biosorption equilibrium by live and dead cells of CCNWHX 72-14 at initial concentrations of 10, 50 and 150 mg/L were represented in Fig. 7, from which the least time required for biosorption equilibrium could be concluded. In the case of dead biomass, the time required for equilibrium was 25–30 min, after that the q value was nearly constant. Volesky et al. [33] indicates that the first phase of biosorption is always rapid, and it is considered to be a spontaneous process with no energy consumed. When using live cells of CCNWHX 72-14 as biosorbents, the contact time necessary to reach equilibrium was different for different initial metal concentration. When the initial zinc concentration was 10 mg/L, 96% of total biosorption capacity was obtained within 25 min, while 40 min was needed for the process at 50 mg/L initial zinc ions. For the biosorption by live biomass at 150 mg/L of initial zinc, there seemed to be a second biosorption step except for the first one within 1 h, as the capacity began to increase slowly from the 2nd h and the equilibrium was achieved in 7–8 h. It suggested that the intracellular bioaccumulation might also contribute to the uptake of zinc ions, in addition to the rapid adsorption by the cell surface.

3.2.3. Desorption efficiency

Both the recovery of metal ions and the regeneration of biomass are based on an efficient desorption process, which can complement biosorption research by considering the optimum conditions to release the metal ions already adsorbed onto biosorbents. Earlier studies show that there are plenty of eluent agents and complexing desorption agents, such as H_2SO_4, HCl, HNO_3, Na_2CO_3, EDTA, citric acid or β-mercaptoethanol reported by Gong et al. [34] and Mata et al. [35]. Having a good desorption efficiency, HCl was selected to regulate the pH value in this investigation. As revealed in Fig. 8, the desorption efficiencies for both live and dead cells of CCNWHX 72-14 were very low when the pH value was higher than 5.0. In the case of live biosorbents, more and more metal ions were released as the pH decreased from 4.0, until the desorption process was almost completed at pH 2.0. For the dead material, the biosorption efficiency increased to 87.0% when the pH decreased to 1.0. In addition, with higher desorption efficiency, the binding strength of metal ions with dead biosorbents seemed to be a little weaker than

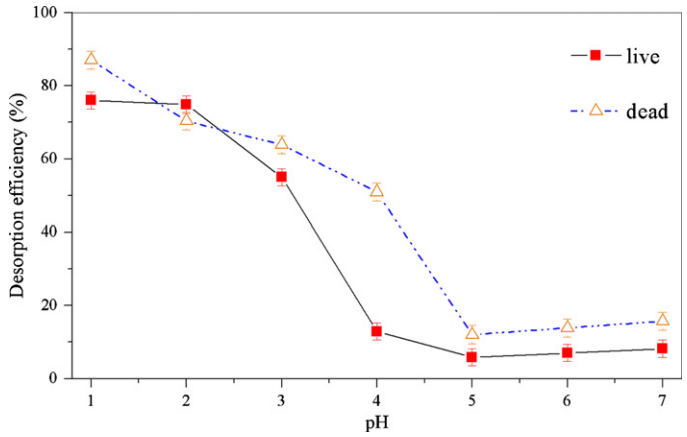

Fig. 8. Effect of pH on desorption efficiency of Zn(II) from metal-loaded biomass of live and dead cells of CCNWHX 72-14.

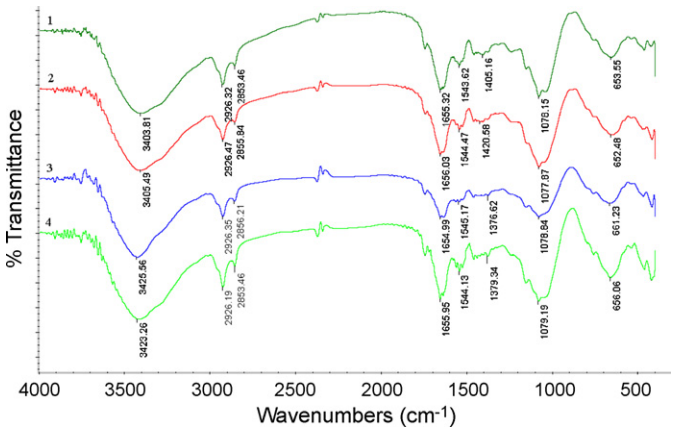

Fig. 10. FT-IR spectrum of live and dead biosorbents loaded with and without Zn(II) (1: natural live CCNWHX 72-14; 2: Zn(II)-loaded live CCNWHX 72-14; 3: Natural dead CCNWHX 72-14; 4: Zn(II)-loaded dead CCNWHX 72-14; Zn^{2+}: 150 mg/L).

that of living material. So it can be concluded that the desorption process differs with pH as well as the type of biomass.

3.2.4. Competitive biosorption

As shown in Fig. 9, the removal ratio of Zn^{2+} decreased in the presence of different groups of competing ions. In the case of live biosorbents, the removal of zinc decreased significantly when there was only one competing metal ion and copper lowered the efficiency furthest. A much lower removal ratio of zinc was achieved for the combination of $Cd^{2+}+Cu^{2+}$ and $Ni^{+}+Cd^{2+}$, while the cooperative effects of Ni^{+} and Cu^{2+} were not so strong. The value of removal efficiency decreased to 29.7% from 72.5% in a solution containing all the four metal ions. In the presence of one competitive ion, a little less zinc was removed by dead cells, while copper showed the biggest influence in the same way. In addition, both Cd^{2+} and Cu^{2+} lowered the zinc removal more markedly than the other two groups. The removal ratio of Zn^{2+} by dead biosorbents went down to 31.2% from 86.2% when four kinds of metal ions appeared together. As reported in former research of Tunali et al. [36], Pavasant et al. [37] and Aksu et al. [38], the metal binding sites on biosorbents were restricted, that's why the removal of one metal ion would decrease in the presence of other ions. Compared with live biomass, the removal ratio by dead biosorbents decreased to a greater extent in presence of all the competing ions. And the effect

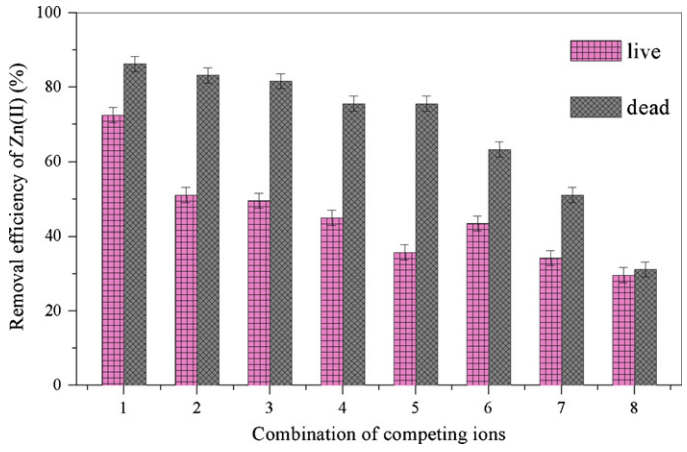

Fig. 9. Effect of competing ions on removal efficiency of Zn(II) by the live and dead cells of CCNWHX 72-14. (The concentration of each metal ion was 0.5 mmol/L; combination of competing metal ions: 1: Zn^{2+}; 2: $Zn^{2+}+Ni^{+}$; 3: $Zn^{2+}+Cd^{2+}$; 4: $Zn^{2+}+Cu^{2+}$; 5: $Zn^{2+}+Ni^{+}+Cd^{2+}$; 6: $Zn^{2+}+Ni^{+}+Cu^{2+}$; 7: $Zn^{2+}+Cd^{2+}+Cu^{2+}$; 8: $Zn^{2+}+Ni^{+}+Cd^{2+}+Cu^{2+}$.)

order of competing metal ion on zinc removal in this study seemed to be: $Cu^{2+} > Cd^{2+} > Ni^{+}$.

3.3. FT-IR analysis

The FT-IR spectrum of natural and Zn(II)-loaded live and dead dried biosorbents were shown in Fig. 10, where the possible functional groups participating in the biosorption process were analyzed. The FT-IR spectra of natural live and dead biomass showed broad and strong bonds at 3403.81 cm^{-1} and 3425.56 cm^{-1}, respectively, indicating bounded hydroxyl (–OH) or amine (–NH) groups. The peaks at 2926.32 and 2853.46 cm^{-1} (live), 2926.35 and 2856.21 cm^{-1} (dead) were attributed to the stretching vibration of $–CH_2$ groups. The absorption bonds observed at 1655.32 cm^{-1} (live), 1654.99 cm^{-1} (dead) (mainly C=O stretch), 1543.62 cm^{-1} (live), 1545.17 cm^{-1} (dead) (mainly–NH,–CN stretch) and 1405.16 cm^{-1} (live), 1376.62 cm^{-1} (dead) (mainly C–N stretch) could be attributed to the amide I, II and III bonds of protein fractions. The peak at 1405.16 cm^{-1} might also be due to a symmetrical vibration of C=O for live biomass. The moderately strong bonds at 1078.15 cm^{-1} for living material and 1078.84 cm^{-1} for dead biosorbent could be assigned to the C–N stretching vibration of an amide bond or C–O stretching of alcohols and carboxylic acids. Particular absorption bonds for an aromatic structure were also obtained at 653.55 cm^{-1} (live) and 661.23 cm^{-1} (dead) as reported by Sarret et al. [39] and LinVien et al. [40]. It seemed that the FT-IR spectra of the two biosorbents were nearly the same except for the significant shifts at 3425.56 and 1376.62 cm^{-1} for dead biomass.

On the whole, the spectra of live biosorbent loaded with Zn(II) did not change a lot compared with that of the natural one. However, the peaks indicating C–N stretch or symmetrical C=O vibration were shifted to 1420.58 cm^{-1} for Zn(II)-loaded live strain. The amino (–NH) or carboxyl (–COOH) groups of live biosorbent might participate in the Zn(II) biosorption process. Similar FT-IR results were achieved in the report of Yuan et al. [13] concerning on cadmium biosorption by two *Streptomyces* strains. As for dead biomass, the spectral analysis before and after metal binding indicated that carbonyl (C=O), amino (–NH), carboxyl (–COOH) and aromatic ($–C_6H_5$) groups were involved in the Zn(II) biosorption process. The adsorption intensity of the above groups increased greatly for the Zn(II)-loaded dead material. Furthermore, another substantial increase of adsorption intensity occurred at 1079.19 cm^{-1}, from which it could be deduced that polysaccharides might also contribute to the Zn(II) biosorption of dead sorbents. In contrast to the FT-IR spectra of live biosorbents, more functional

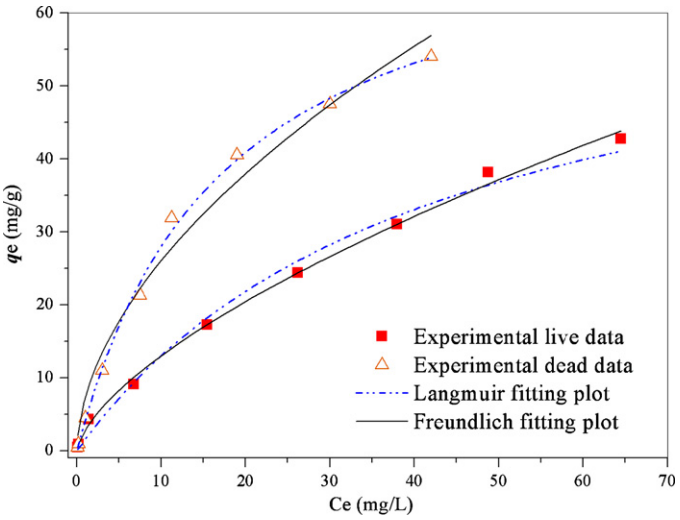

Fig. 11. Langmuir and Freundlich fitting plots of biosorption of Zn(II) onto live and dead cells of CCNWHX 72-14 (symbols: experimental data; lines: model prediction).

groups were involved in the Zn(II) biosorption process of dead biomass.

3.4. Biosorption equilibrium modeling

The adsorption isotherms are used to establish the ratio between equilibrium concentration of solute in the solution and equilibrium concentration of solute on the sorbent at constant temperature [41]. Langmuir and Freundlich models are widely applied in the equilibrium analysis to understand the sorption mechanisms.

The Langmuir model considers sorption by monolayer type and supposes that all the active sites on the sorbent surface have the same affinity by the sorbate [42]. This equation is used empirically to simulate favorable equilibrium uptake curves. The familiar form of the Langmuir isotherm is expressed as follows [43]:

$$q_e = \frac{q_{\max} K_L C_e}{1 + K_L C_e} \tag{4}$$

where $q_{\max}$ represents the maximum monolayer biosorption capacity of the biosorbent (mg/g) and K_L (L/mg) is related to the affinity of the binding sites.

The Freundlich isotherm is an empirical equation which assumes a heterogeneous biosorption system with different active sites. The general Freundlich equation is written as follows [44]:

$$q_e = K_F C_e^{1/n} \tag{5}$$

where K_F (L/g) is a constant related to the biosorption capacity and n is an empirical parameter representing the biosorption intensity.

The experimental isotherm data were fitted to Langmuir and Freundlich isotherms for nonlinear regression analysis. The fit plots were presented in Fig. 11 and the correlation parameters for each model were listed in Table 1.

Former research of Davis et al. [7] and Hashim et al. [45] have showed that the biosorption process is defined by two Langmuir constants $q_{\max}$ and K_L together. The $q_{\max}$ estimated by Langmuir model normally could not be reached at experimental conditions, while K_L is an equilibrium constant representing the standard Gibbs energy of adsorption. Generally speaking, high $q_{\max}$ and high K_L are desirable for good biomass. However, sometimes at low metal concentration, a biosorbent with low $q_{\max}$ and high K_L could outperform that with high $q_{\max}$ and a low K_L. So K_L is also an important parameter which is related to the initial sorption isotherm slope. In the present work, the predicted $q_{\max}$ was 67.96 mg/g (1.04 mmol/g) for live biosorbents and 75.85 mg/g (1.16 mmol/g) for dead ones, respectively. The affinity constant K_L for dead biomass was 0.0584, much higher than that of live material (Table 1). Thus, with higher affinity towards Zn^{2+} than live ones, dead cells of CCNWHX 72-14 seemed to be better adsorbents, especially at low initial concentration.

Another essential factor of the Langmuir isotherm is R_L, which can be calculated according to the following equation:

$$R_L = \frac{1}{1 + K_L C_0} \tag{6}$$

where C_0 is the highest metal concentration (mg/L). As reported by Stephen et al. [46], the parameter R_L indicates the shape of the isotherm and nature of the biosorption process ($R_L > 1$: unfavorable; $R_L = 1$: linear; $0 < R_L < 1$: favorable; $R_L = 0$: irreversible). The R_L value was 0.22 for live biosorbents and 0.10 for dead ones (Table 1), indicating that biosorption of Zn(II) by dead biomass was much more favorable.

In the case of Freundlich model, the adsorption feature is defined by both K_F and n values, where K_F represents the adsorption coefficient and $1/n$ is related to the effect of concentration of metal ions. On average, a favorable adsorption tends to have the constant n between 1 and 10. Larger value of n implies stronger interaction between biosorbent and heavy metal [47]. The K_F value of dead biosorbents was 7.40, indicating a large biosorption capacity comparing to live ones, for which the K_F value was 2.89. The n value for live biomass was 1.53 while that for dead one was 1.83, from which it could be derived that the effect of metal ions on dead biomass was stronger than that on living material. Besides, with the n values between 1 and 10, the biosorption by both types of biomass were favorable under studied conditions.

As listed in Table 1, the correlation coefficient of biosorption data of live cells simulated by Freundlich model was 0.998, slightly higher than 0.992 by Langmuir one. In addition, the prediction of $q_{\max}$ by Langmuir equation for live biomass was much higher than actual results. Consequently, it could be concluded that the biosorption of live biosorbents might follow a heterogeneous model, and other mechanisms such as intracellular bioaccumulation would contribute to the uptake of Zn^{2+} except for surface binding [48].

For dead strain, with the R^2 value much higher than that of Freundlich model (Table 1), the Langmuir isotherm was more adequate to describe the biosorption data. It's suggested that the biosorption of zinc ions onto dead cells of CCNWHX 72-14 was considered as monolayer sorption. This result was consistent with a number of former research focusing on the adsorption of zinc ions [49–51].

Table 1
Constants simulated with Langmuir and Freundlich models for Zn(II) biosorption using live and dead CCNWHX 72-14 as biosorbents.

Strain	Langmuir model				Freundlich model		
	K_L (L/mg)	q_m (mmol/g mg/g)	R^2	R_L	n	K_F (L/g)	R^2
	$q = q_{\max} K_L C_e/(1 + K_L C_e)$				$q = K_F C_e^{1/n}$		
Live	0.0237	1.0396/67.96	0.9924	0.2195	1.5322	2.8906	0.9975
Dead	0.0584	1.1603/75.85	0.9977	0.1025	1.8316	7.3981	0.9823

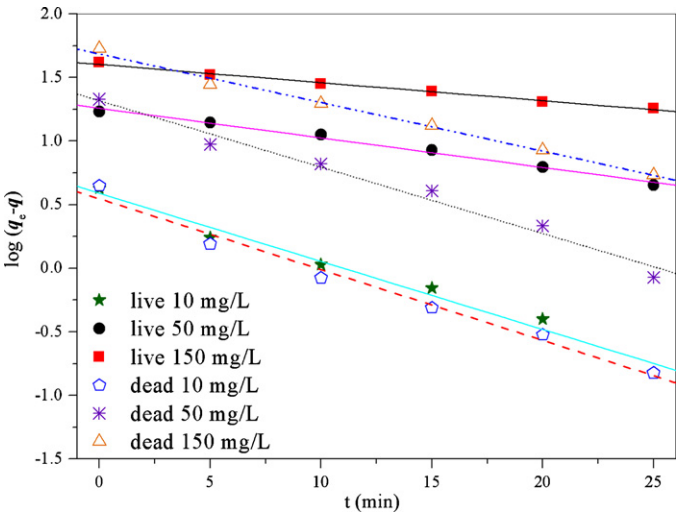

Fig. 12. Pseudo-first-order kinetic plots for Zn(II) biosorption by live and dead cells of CCNWHX 72-14 at an initial metal concentration of 10, 50 and 150 mg/L (symbols: experimental data; lines: model prediction).

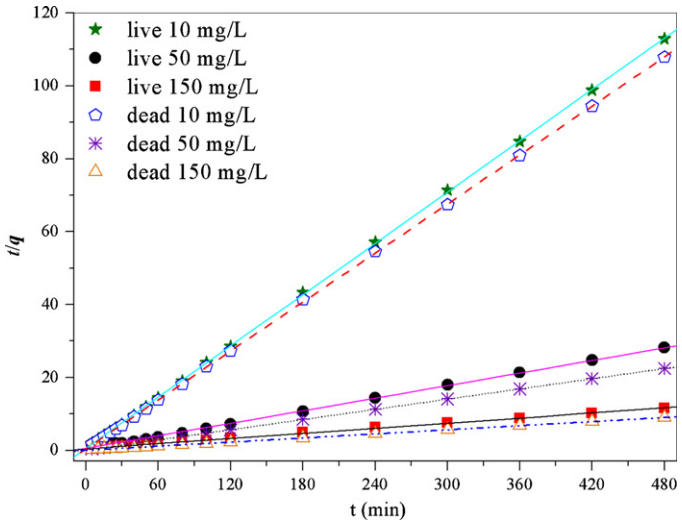

Fig. 13. Pseudo-second-order kinetic plots for Zn(II) biosorption by live and dead cells of CCNWHX 72-14 at an initial metal concentration of 10, 50 and 150 mg/L (symbols: experimental data; lines: model prediction).

3.5. Biosorption kinetic modeling

In considering a proper model to describe the dynamic biosorption process of live and dead biosorbents, two different kinetic equations were used for correlation of the time course data. Lagergren's pseudo-first-order equation could correlate concentrations with time data for short times and it is generally expressed as follows [52]:

$$\log(q_e - q) = \log\ q_e - \frac{k_1 t}{2.303} \quad (7)$$

Pseudo-second-order model is derived on the basis of the sorption capacity of the solid phase, expressed in linear form [53]:

$$\frac{t}{q} = \frac{t}{q_e} + \frac{1}{k_2 q_e^2} \quad (8)$$

where k_1 is the rate constant of pseudo-first-order adsorption (1/min) and k_2 (g/mg min) is the rate parameter of second-order equation. q_e and q are the sorption capacities (mg/g) at equilibrium and at time t, respectively.

The applicability of the above two models can be examined by each linear plot of $\log(q_e - q)$ versus t, and t/q against t, respectively. The simulative plots were depicted in Figs. 12 and 13 while specific constants were represented in Table 2.

With the correlation coefficients ranging from 0.990 to 0.997, the pseudo-first-order model fit the actual data well for an initial period of the first reaction step. The rate constant k_1 also showed high agreement with former results, which were different for live or dead biomass at various initial zinc concentrations. However, the q_e evaluated by the first-order equations were lower or higher than the corresponding experimental values.

In comparison, with the calculated q_e being close to the actual data, the pseudo-second-order equation predicted equilibrium adsorption values more precisely than the first-order one. For the biosorption onto live and dead biosorbents at an initial zinc of 10 mg/L, the q_e were evaluated to be 4.26 mg/g and 4.46 mg/g with R^2-values up to 1.00, while the experimental q_e were 4.25 mg/g and 4.45 mg/g, respectively. Besides, with two distinct steps, the biosorption by live biomass at 150 mg/L initial zinc could not be described very well with two models. With the calculated q_e more close to experimental data, the prediction by the second-order equation was a little better than that of the first one.

In addition, the rate parameter k_2 listed in Table 2 also indicated that the biosorption of dead biomass was faster than that of live one at the same initial metal concentration. And the biosorption at higher metal concentration seemed to be slower due to the competition of binding sites. Similar conclusions were also obtained by former research reported by Aksu et al. [54] and Pamukoglu et al. [55].

Furthermore, the correlation coefficients of the pseudo-second-order equations were all higher than 0.999, suggesting that this model fit the biosorption data better than first-order one. Consequently, it suggested that the biosorption of live and dead cells of CCNWHX 72-14 followed the pseudo-second-order mechanism and corresponded to a chemisorption process [56].

3.6. Live-dead biosorbents comparison

Former researches of Selatnia et al. [57] and Gabr et al. [58] suggested that dead bacterial strain showed higher biosorption capability than live ones and seemed to be promising biosorbents

Table 2
Kinetic parameters obtained from pseudo-first-order and pseudo-second-order equations for the biosorption of Zn(II) by live and dead CCNWHX 72-14 at an initial metal concentration of 10, 50 and 150 mg/L.

Strain	Concentration (mg/L)	Experimental q (mg/g)	First-order kinetics			Second-order kinetics		
			q_e (mg/g)	k_1 (1/min)	R^2	q_e (mg/g)	k_2 (g/mg min)	R^2
Live	10	4.25	3.89	0.1233	0.9904	4.26	0.1085	1.0000
	50	17.00	18.10	0.0534	0.9949	17.39	0.0061	0.9992
	150	41.60	40.05	0.0328	0.9958	42.72	0.0012	0.9993
Dead	10	4.45	3.52	0.1281	0.9914	4.46	0.1276	1.0000
	50	21.35	20.78	0.1202	0.9903	21.45	0.0245	0.9999
	150	53.25	48.50	0.0880	0.9965	53.53	0.0064	0.9999

for bioremediation. As shown by the equilibrium and kinetic analysis in the present study, with higher biosorption capacity and faster adsorption process, dead biomass tended to be more efficient biosorbents in comparison with living material. Besides, the desorption efficiency of zinc ions by dead cells was a little higher than that of live cells, which indicated the recovery potential of dead adsorbents and the feasibility of reusing them [59]. With the advantage of high biosorption and desorption capacities, dead strains have the potential of becoming effective biosorbents for the removal and recovery of heavy metals.

Meanwhile, experiments focusing on competitive biosorption of zinc with other metal ions and FT-IR spectrum loaded with and without Zn(II) by live and dead biomass were also analyzed. In comparison, the removal of zinc by living material went down a lot more in the presence of one or two competitive ions while that of dead strain decreased to a greater extent in existence of all the competing ions. In addition, FT-IR analysis indicated that more functional groups might be involved in the biosorption process of dead biosorbents than those linked to live ones. Therefore, with more binding sites and functional groups, dead biomass seemed to be a good candidate for the elimination of multiple metal ions. At last, according to Bailey et al. [60], an adsorbent can be considered as low-cost if it is abundant in nature, requires little processing and is a byproduct of waste material. In the present research, dead biomass could be ideal adsorbents as they could be easily obtained by ordinary culture, and only need simple pretreatment at low cost.

To sum up, with more efficient biosorption process, higher probability for regeneration, more ions binding sites and more potential functional groups, dead cells of CCNWHX 72-14 would be better and cheaper biosorbents compared with live ones.

4. Conclusions

The biosorption of Zn(II) by live and dead cells of *S. ciscaucasicus* strain CCNWHX 72-14 has been investigated at optimum conditions determined in advance. Batch biosorption experiments with regard to initial metal concentration, contact time, desorption efficiency, competitive biosorption and FT-IR analysis were performed in this study. When live and dead cells of CCNWHX 72-14 were employed as biosorbents, the experimental maximum biosorption capacity turned out to be 42.75 mg/g (0.654 mmol/g) and 54 mg/g (0.826 mmol/g), respectively. At various initial zinc concentrations, batch biosorption data of live biosorbents preferred to be simulated with Freundlich model while those of dead strain fit Langmuir isotherm well. With much higher R^2-values and more accurate prediction of q_e, the pseudo-second-order equation described sorption kinetics better than the pseudo-first-order one. Taking into consideration of present findings, dead cells of CCNWHX 72-14 proved to be more efficient and low-cost biosorbents than live ones, which can be utilized as an alternative for the treatment of wastewater.

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