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Biosorption of Zn(II) and Cu(II) by the indigenous *Thiobacillus thiooxidans*

Hsuan-Liang Liu [a,*], Bor-Yann Chen [b], Yann-Wen Lan [a], Yang-Chu Cheng [a]

[a] *Department of Chemical Engineering, National Taipei University of Technology, No. 1, Sec. 3, Chung-Hsiao E. Road, Taipei 10643, Taiwan, ROC*
[b] *Department of Chemical Engineering, National I-Lan Institute of Technology, I-Lan 260, Taiwan, ROC*

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Abstract

Biosorption of each of the heavy metals, Zn(II) and Cu(II), and of the binary mixture of these two metal ions by the indigenous *Thiobacillus thiooxidans* was investigated in this study. Equilibrium concentration (q_m) and dissociation constant (K_d) were calculated by fitting the experimental data with the Langmuir isotherms. The effects of pH, pretreatment of biomass, and temperature on the amount of metal uptake by this organism were also determined. Typically, the adsorption capacity increases with increasing pH in the ranges of 2.0–6.0 and 4.0–5.0 for Zn(II) and Cu(II), respectively. Chemical pretreatment of the biomass with 0.075 M NaOH has positive effects on its capacity for metal biosorption. Higher temperature yields higher biosorption capacity for both metals. The indigenous *T. thiooxidans* is in favor of Zn(II) uptake in the binary mixture. Biosorption of Cu(II) is inhibited by the existence of Zn(II). The total amount of metal adsorbed in the binary mixture decreases in comparison with biosorption of only one kind of metal ion.

Keywords: Biosorption; Heavy metal; *Thiobacillus thiooxidans*; Equilibrium concentration; Dissociation constant; Langmuir isotherms; Pretreatment

1. Introduction

The traditional approaches for removing or recovering metals, such as precipitation, oxidation/reduction, ion exchange, filtration, electrochemical processes, membrane separations, and evaporation, all exhibit several disadvantages, such as high cost, incomplete removal, low selectivity, high energy consumption, and generation of toxic slurries that are difficult to be eliminated [1]. Therefore, much attention has been paid to the removal of metal ions by microorganisms due to its potential applications in environmental protection, and recovery of toxic or strategic heavy metals [2–7]. Certain types of microbial biomass are considered to retain relatively high quantities of metals by means of passive process known as biosorption. The process is relatively fast and the fact that it is a surface phenomenon facilitates the removal of metal ions from solutions and the subsequent application of the material as biosorbent [1].

Biosorption is either metabolism independent, such as physical or chemical sorption onto the microbial cell walls, or metabolism associated, such as transport, internal compartmentalization, and extracellular precipitation by metabolites [8]. In addition, an important aspect of biosorption is that it can be carried out either with metabolically active or inactive cells. Many microorganisms have been intensively examined for their abilities to be applied in biosorption of heavy metals, such as bacteria, fungi, yeast, and algae [9–12]. Both chemical pretreatments, such as contacting cells with acids, alkali, and organic compounds [13,14], and physical pretreatments, such as heat treatment, autoclaving, freeze drying, and boiling [13,15], showed enhancement in metal biosorption by microorganisms.

There is a current need to find alternative technologies to substitute or improve the existing processes of removal of heavy metals in solution. This constitutes in itself a sufficient justification to carry out research that could lead to basic information for the development of new processes [1,16]. *Thiobacillus thiooxidans* is a chemolithotrophic acidophilic bacterium that grows on elemental sulfur as energy source and is important in the microbial catalysis of sulfide oxidation. Since it oxidizes both elemental sulfur and sulfide to sulfuric acid, *T. thiooxidans* plays a significant role in bioleaching of metals from sulfide ores [17,18]. It is of great importance to examine the ability of the indigenous *T. thiooxidans* for removal of heavy metals. Therefore, the present study aimed to investigate the effects of cell

* Corresponding author. Tel.: +886-2-2771-2171x2542; fax: +886-2-2731-7117.
E-mail address: f10894@ntut.edu.tw (H.-L. Liu).

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pretreatment, pH and temperatures on the selective biosorption of Zn(II) and Cu(II) by the indigenous *T. thiooxidans*.

2. Materials and methods

2.1. Isolation and growth of microorganisms

The indigenous *T. thiooxidans* used throughout this study was obtained from sewerage samples from a suspected lead-contaminated site near Keelung, Taiwan. This site was originally a collection site for used lead storage batteries and thus heavily contaminated with sulfuric acid and likely lead and related compounds (e.g. Pb, PbO_2 and $PbSO_4$) as well. The electrodes in cells are suspended in dilute sulfuric acid solution (ca. 6 M). Residual acid solutions in batteries likely produced a high level of sulfate contamination on site. It was confirmed through chemical analysis (i.e. pH $= 3.7 \pm 0.2$, and $[SO_4^{2-}] = 3.77$ g/l) of site characterization after filtering out solid particles in sewerage. Isolation experiments were thus undertaken at pH 4.0 to imitate a favorable in situ condition from an ecological perspective. Since *T. thiooxidans* is so-called an extremely acidophilic bacterium, it is remarkably tolerant in acid environments at pH 1 or below, which is different from the characteristics of *Thiobacillus ferrooxidans*. It indicates that successful enrichment cultures on elemental sulfur should exhibit a decreasing profile in pH and biased amplification in sulfur-oxidizing bacteria, leading to high purity of *T. thiooxidans*. After all, the pH level in enrichment cultures of *T. thiooxidans* with elemental sulfur can fall to 1.0 or below [19]. The abundance of *T. thiooxidans* was obtained by inoculating sewerage dilutions into *T. thiooxidans* optimum growth medium (*T. thiooxidans* OGM) (N:P = 5:1; composition (g/l): KH_2PO_4, 1.0; $(NH_4)_2SO_4$, 2.54; $MnSO_4$, 0.02; $MgSO_4$, 0.1; $CaCl_2$, 0.03; $FeCl_3$, 0.02; powdered S^0, 2.0; nystatin, 0.1; pH 4.0). The inoculated culture was then incubated in a water-bath shaker at 30 °C, 15.7 rad/s. The sulfate, biomass concentrations and pH level were measured over time for consecutive subcultures. Experiments were undertaken in triplicate to guarantee the symmetrical nature with respect to space and time for data reproducibility. Pure *T. thiooxidans* isolates were obtained after seven consecutive subcultures and maintained for subculture in shaker flasks at 30 °C, 15.7 rad/s for 14 days before being used in the following biosorption experiments.

2.2. Chemical pretreatment

For the experiments where the effect of pretreatment was analyzed, 300 ml of cultivated cells were treated with 30 ml of 0.075 M NaOH for a period of 10 min. The cells were then centrifuged (KR-20000S from Kubota) for 10 min at 628 rad/s. The resulting cells were resuspended in deionized water and centrifuged again. This operation was repeated until a neutral pH was obtained.

2.3. Solutions

For the preparation of Zn(II) and Cu(II) solutions, $ZnSO_4$ and $CuSO_4$ from Wako Pure Chemical Industries Ltd. were used, respectively. These solutions were usually prepared at concentrations of 25, 50, 75, 100 and 150 mg/l. These solutions were made using deionized water and the pH was adjusted using either 0.1 M NaOH or 0.01 M HCl.

2.4. Dry cell weight

Dry cell weight measurements were carried out by passing a volume of 50 ml cell culture through a previously weighted Millipore filters (White GSWP, 0.22 ± 0.2 μm, 47 mm diameter) using a reusable filter unit (Nalgene). Cell pellets were also washed twice with filtered deionized/distilled water to remove non-biomass ash. Filtered and collected cells were dried in an oven (Precision Thelco, model 26, Precision Scientific P/S) set at temperature 110 ± 5 °C and weight for every 24 h until constant weight was obtained.

2.5. Metal biosorption

Metal biosorption experiments were carried out in a 250 ml flask at both 30 and 40 °C in a water-bath shaker with 13.1 rad/s. The flask was filled with 100 ml of previously prepared solution with a different initial Zn(II) or Cu(II) concentration at each run (25, 50, 75, 100 and 150 mg of metal ion/l of solution). A predetermined concentration of the indigenous *T. thiooxidans* biomass (0.3 g of dry cell weight/ml) was used. Each experiment was conducted for 2 h, which was enough time to achieve steady state biosorption. The pH was controlled throughout the experiment. Each experiment was performed in triplicate.

2.6. Determination of Zn(II) and Cu(II) concentration

The samples were collected and filtered using Millipore filters of 0.22 μm. The filtrate was collected for Zn(II) and Cu(II) analysis. The concentration of Zn(II) and Cu(II) in solution was determined using a GBC932 atomic absorption spectrophotometer (Pantech Instruments, Victoria, Australia).

2.7. Langmuir isotherms

The uptake of the metals (in mg of metal/g of dry cell weight) was calculated according to the following formula:

$$q = \frac{C_0 - C_e}{X} \tag{1}$$

where q is the biosorption capacity, and X the biomass concentration. Kinetic model for metal biosorption is shown with the initial condition (at time $t = 0$, $C = C_0$):

$$\frac{dC}{dt} = -K_1(q_m - q)C + K_2 q \tag{2}$$

where K_1 and K_2 are the forward and reverse rate coefficients, respectively; C the concentration of metal ion; and q_m the maximum biosorption capacity. Parameters were determined by minimizing J, the sum of squared errors, defined by:

$$J = \sum (C_{\text{pred},j} - C_{\text{exp},j})^2 \quad \text{for all } j \tag{3}$$

where $C_{\text{pred},j}$ and $C_{\text{exp},j}$ are predicted and experimental concentrations of extracellular metal ion, respectively. Equilibrium parameters K_d ($=K_2/K_1$) and q_m for Langmuir isotherms (Eq. (4)) were determined from the values of slope ($1/q_m$) and intercept (K_d/q_m) through linear regression C_e/q versus C_e:

$$\frac{C_e}{q} = \frac{K_d}{q_m} + \frac{1}{q_m} C_e \tag{4}$$

3. Results and discussion

3.1. Type of biosorption

Biosorption of metal ions usually can be classified as two types: the Freundlich model, in which the amount of metal uptake by the biomass increases with time, and the Langmuir model, in which the amount of metal uptake by the biomass reaches equilibrium [5]. To evaluate the feasibility of metal uptake by the indigenous *T. thiooxidans*, these two models were tested for Zn(II) biosorption at pH 6.0 and 30 °C. As shown in Fig. 1, both models seemed to fit the experimental data well, except for the last data point at 250 mg Zn(II)/l of solution. Since most of the data given in this paper are below 250 mg metal/l of solution, it is likely that both models could fit the data. The Langmuir isotherm assumes that a monomolecular layer is formed when biosorption takes place and that there is no interaction between molecules (i.e. metals) adsorbed on adjacent binding sites. Therefore, both adsorption and desorption are independent of the total number of sites occupied. Adsorption is considered as a state of dynamic equilibrium, in

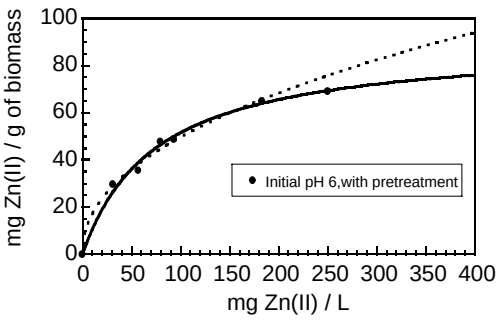

Fig. 1. The feasibility of the Langmuir model (—) and the Freundlich model (···). Zn(II) biosorption by the indigenous *T. thiooxidans* was conducted at pH 6.0 and 30 °C with pretreatment of the biomass using 0.075 M NaOH.

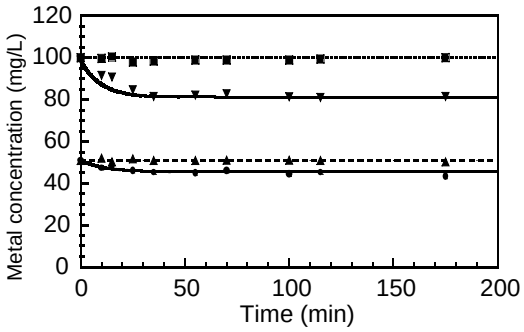

Fig. 2. Transient dynamics of extracellular Zn(II) concentration at various times. Experimental data for blank (■) and Zn(II) sorption (▼) with the initial Zn(II) concentration of 100 mg/l, and for blank (▲) and Zn(II) sorption (●) with the initial Zn(II) concentration of 50 mg/l are shown.

which the rate at which metals are adsorbed equals the rate at which metals are desorbed. In the early stage, the rate of biosorption is fast since most of the binding sites on cell surface are freely available, whereas the rate of biosorption decreases when the cell surface is occupied with bound metal molecules. In other words, the rate of biosorption decreases with decreasing accessible surface area on the cell walls.

In order to investigate the approximate time to reach the maximum amount of metal attached to the cell surface, biosorptions with and without cells were conducted using 50 and 100 mg/l Zn(II) solution at pH 6.0 and 30 °C. Fig. 2 shows the concentration–time profiles for Zn(II) biosorption, indicating transient dynamics for metal uptake by the indigenous *T. thiooxidans*. Equilibrium biosorption was reached within 50 min. However, the transient data of biosorption for initial Zn(II) concentrations of 50 and 100 mg/l were lower and higher than expected, respectively. It might suggest that biosorption at initial Zn(II) concentration of 50 mg/l has been attenuated by slightly different complementary geometry to bind metals. In contrast, some degree of interactions (e.g. bimolecular layer coverage) between metals adsorbed on adjacent binding sites might occur at initial Zn(II) concentration of 100 mg/l to enhance biosorption. However, the Langmuir model seems to be satisfied due to the rearrangement of adsorption capacity and binding conformation [12]. Furthermore, no significant sorption was found when cell was absent in the solution both with the initial Zn(II) concentrations of 50 and 100 mg/l, indicating that the indigenous *T. thiooxidant* was the only biosorbent in our experiments.

3.2. Biosorption of Zn(II): the effects of pretreatment and pH

Some metabolites and certain components of cell culture media might inhibit the biosorption of metal ions and reduce the maximum amount of biosorption. Thus, the effect of pretreatment on the capacity for Zn(II) biosorption by the indigenous *T. thiooxidans* biomass was evaluated with

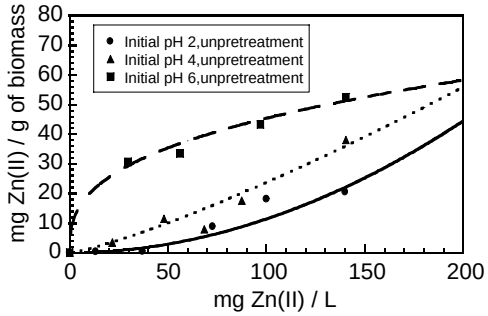

Fig. 3. Biosorption isotherms of Zn(II) at 30 °C without pretreatment of the biomass. The Freundlich model was used to fit the experimental data at pH values of 2.0 and 4.0, and the Langmuir model was adopted to fit the experimental data at pH 6.0.

respect to untreated biomass. Comparing Figs. 3 and 4, the difference in the capacity for Zn(II) biosorption is given for favorable against unfavorable behavior in favor of pretreated biomass with 0.075 M NaOH. The results were in good agreement with what has been reported, an appropriate physical or chemical pretreatment of the biomass has positive effects on its capacity for metal biosorption. This effect can be due to an increase in the availability of binding sites or to the removal of polysaccharides that presumably block the access of metals to the binding sites. It can also be due to a change of permeability on the cellular wall, in the case of bioaccumulation [1].

The effect of pH on the capacity for Zn(II) biosorption by the indigenous *T. thiooxidans* was also studied with and without pretreatment of the biomass. In the case of biomass without pretreatment, the results are shown in Fig. 3. The experiments were conducted at pH values of 2.0, 4.0 and 6.0. It is worth mentioning that the Freundlich model described the experimental data better than the Langmuir model for Zn(II) biosorption at pH values of 2.0 and 4.0 without pretreatment of the biomass as shown in Fig. 3. It was suggested that desorption occurs at these low pH values, resulting in relatively low adsorption capacity [7]. As shown in Fig. 4, all the adsorption isotherms fitted well with the Langmuir model. In the case of pretreated biomass, a maximum capac-

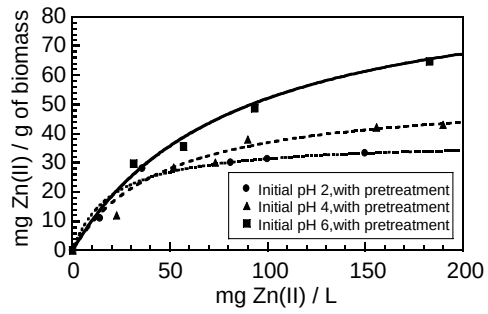

Fig. 4. Langmuir isotherms of Zn(II) biosorption at 30 °C with pretreatment of the biomass using 0.075 M NaOH.

Table 1
Values of equilibrium constants by fitting the experimental data with the Langmuir model at various conditions

Metal ion	Initial pH	T (°C)	NaOH pretreatment	K_d (mg/l)	q_{max} (mg/g)
Zn(II)	2.0 ± 0.1	30	No	ND[a]	ND
Zn(II)	4.0 ± 0.1	30	No	ND	ND
Zn(II)	6.0 ± 0.1	30	No	40.93 ± 1.33[b]	43.29 ± 0.94
Zn(II)	2.0 ± 0.1	30	Yes	20.20 ± 0.81	37.74 ± 1.23
Zn(II)	4.0 ± 0.1	30	Yes	46.47 ± 1.41	54.05 ± 2.22
Zn(II)	6.0 ± 0.1	30	Yes	82.94 ± 1.95	95.24 ± 3.56
Zn(II)	6.0 ± 0.1	40	Yes	213.8 ± 4.5	172.4 ± 4.4
Cu(II)	4.0 ± 0.1	30	No	22.94 ± 1.01	23.47 ± 0.89
Cu(II)	5.0 ± 0.1	30	No	38.52 ± 1.77	30.77 ± 1.15
Cu(II)	4.0 ± 0.1	30	Yes	11.24 ± 0.55	32.57 ± 1.21
Cu(II)	5.0 ± 0.1	30	Yes	16.75 ± 0.38	32.36 ± 1.63
Cu(II)	5.0 ± 0.1	40	Yes	68.46 ± 3.21	39.84 ± 0.96

[a] Not determined since the experimental data fit with the Freundlich model better than with the Langmuir model at these conditions.
[b] Standard error.

ity of 95.24 mg of Zn(II)/g of dry biomass was obtained at pH 6.0 (Table 1). Both the dissociation constant (K_d) and the maximum adsorption capacity (q_m) increased with respect to an increase in pH. At low pH values, the strong affinity of protons onto metal binding sites on cell walls of biomass results in a competitive inhibition for Zn(II) biosorption. Furthermore, the rate of desorption increases significantly as pH increases compared with the rate of adsorption, leading to reaching the transient dynamics [12].

3.3. Biosorption of Cu(II)

The pH values selected for biosorption of Cu(II) were 4.0 and 5.0 in our experiments, since no significant adsorption was observed at pH 2.0 and precipitation occurred when the $CuSO_4$ solution at pH 6.0 was prepared. Similar to the results observed for Zn(II) biosorption, the capacity for Cu(II) biosorption increased with respect to an increase in pH but the difference was not as significant as for Zn(II) biosorption. The pH dependence of metal uptake is due to various functional groups on bacterial cell walls and also due to the metal chemistry. The functional groups capable of metal sorption are usually basic (e.g. carboxyl, phosphate, and amine groups), which are deprotonated at high pH values. As the pH value increases, more functional groups are dissociated and become available for metal uptake due to much less competition from protons. However, due to higher K_d values at higher pH values, metals may have a weaker affinity than protons for deprotonated functional groups on the binding sites. Biosorption of Cu(II) reached a maximal capacity of 39.84 mg Cu(II)/g dry cell weight at pH 5.0 as shown in Table 1, which was relative low compared to Zn(II) biosorption. Comparing Figs. 5 and 6, we found that pretreatment of the biomass with 0.075 M NaOH also resulted in a more favorable adsorption behavior for Cu(II) biosorption.

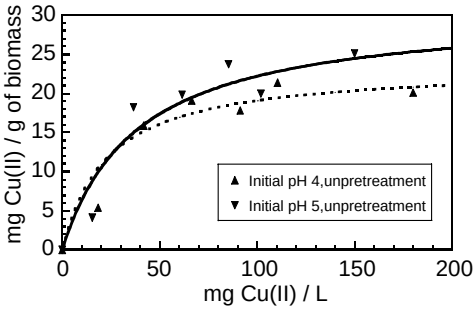

Fig. 5. Langmuir isotherms of Cu(II) biosorption at 30 °C without pretreatment of the biomass.

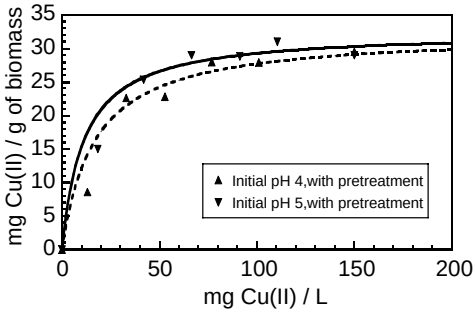

Fig. 6. Langmuir isotherms of Cu(II) biosorption at 30 °C with pretreatment of the biomass using 0.075 M NaOH.

3.4. The effect of temperature

As shown in Figs. 7 and 8, the biosorption capacities for both Zn(II) at pH 6.0 and for Cu(II) at pH 5.0 were higher at 40 °C than at 30 °C. The maximum capacities for Zn(II) biosorption were 95.24 and 172.4 mg/g at 30 and 40 °C, respectively, and those for Cu(II) biosorption were 32.36 and 39.84 mg/g at 30 and 40 °C, respectively (Table 1). Although temperature effect was not significant on the maximum capacity for Cu(II) biosorption, the amount of Cu(II) adsorbed at lower initial Cu(II) concentrations was increased at higher temperature (Fig. 8). Although higher temperature increases both the adsorption and desorption rates according to the Arrhenius equations, the equilibrium concentration of Langmuir isotherms still shift to a higher value since the adsorption rate is accelerated much more than the desorption rate. The Langmuir isotherm did not describe the experimental data well for Zn(II) biosorption at 40 °C as shown in Fig. 7, probably due to the quick increase of the adsorption rate.

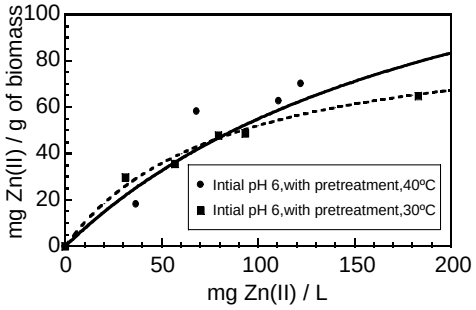

Fig. 7. Temperature effect on Langmuir isotherms of Zn(II) biosorption at pH 6.0 with pretreatment of the biomass using 0.075 M NaOH.

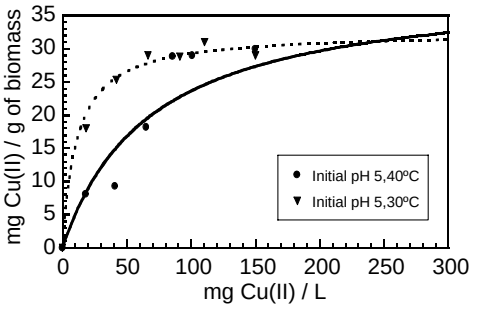

Fig. 8. Temperature effect on Langmuir isotherms of Cu(II) biosorption at pH 5.0 with pretreatment of the biomass using 0.075 M NaOH.

3.5. Competitive biosorption for Zn(II) and Cu(II)

Competitive biosorption is a common phenomenon examined with various microorganisms for metal uptake. The distinct characteristics of binding sites and certain functional groups on cell walls result in high selectivity towards metal biosorption. Fig. 9 shows the results of competitive biosorption for the binary mixture of Zn(II) and Cu(II) by the indigenous *T. thiooxidans* at 30 °C and pH 4.0. The indigenous *T. thiooxidans* selectively uptake Zn(II) during the entire course of biosorption with limited amount of Cu(II) adsorbed. The maximum capacity of Zn(II) adsorbed is about 30 times higher than that of Cu(II) adsorbed (Table 2), indicating that the indigenous *T. thiooxidans* is in favor of Zn(II) biosorption, which is in good agreement with previous report [20]. These results show that the indigenous *T. thiooxidans* might be a potential candidate for the separation of Zn(II) and Cu(II) from waste water or metal contaminated soil. Comparing Tables 1 and 2, we found that the coexistence of Zn(II) and Cu(II) reduced the maximum capacities of biosorption for both metals, with the uptake of Cu(II) being inhibited to a greater extent.

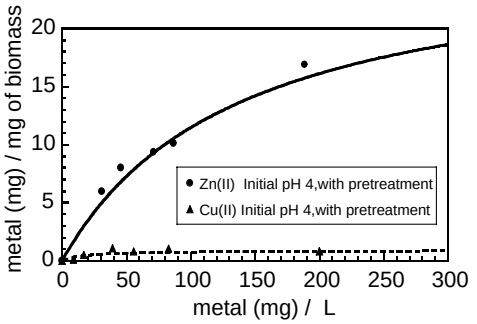

Fig. 9. Competitive biosorption in the binary mixture of Zn(II) and Cu(II) at pH 4.0 and 30 °C with pretreatment of the biomass using 0.075 M NaOH.

Table 2
Values of equilibrium constants for competitive biosorption by fitting the experimental data with the Langmuir model in the binary mixture of Zn(II) and Cu(II)

Metal ion	Initial pH	T (°C)	NaOH pretreatment	K_d (mg/l)	q_m (mg/g)
Zn(II)	4.0 ± 0.1	30	Yes	136.2 ± 7.1[a]	27.1 ± 0.5
Cu(II)	4.0 ± 0.1	30	Yes	18.40 ± 0.44	0.90 ± 0.08

[a] Standard error.

These results are in good agreement with the previous reports showing that the total amount of metal biosorption in a multiple metal system is lower than that in a single metal system [5,21,22], but are contrast to results from Chang and Chen [23].

The interference phenomenon for metal biosorption from binary mixture has been observed by many researchers. For example, Figueira et al. [21] have reported that Cd(II) affects the uptake of Fe(II) by non-living biomass of *Sargassum fluitans*, and vice versa. Chang and Chen [23] have found similar interference in metal uptake study involving *Pseudomonas aeruginosa* PU21 (RIP64) in a ternary system of Cu(II), Pb(II) and Cd(II). Although some microorganisms showed a slightly preference for Cu(II) adsorption over Zn(II) [23–25], our results showed that the indigenous *T. thiooxidans* is much more in favor of Zn(II) uptake over Cu(II). It indicates that the specific characteristics of the metal binding sites and the functional groups responsible for metal interaction on the cell walls of the microorganisms play major role in determining the selectivity of metal biosorption.

4. Conclusions

The indigenous *T. thiooxidans* is a microorganism that has extremely high capacity for Zn(II) biosorption (q_m = 172.4 mg of Zn(II)/g of dry biomass at 40 °C and pH 6.0) when pretreated with NaOH, whereas it shows relatively low capacity for Cu(II) uptake (q_m = 39.84 mg of Cu(II)/g of dry biomass at 40 °C and pH 5.0). The initial pH value of the solution has a significant effect on the capacities for both Zn(II) and Cu(II) uptake, principally, due to the protonization that occurs at low values of pH and due to the effect it has on the chemistry of both the solution and the functional groups on the cell walls. Typically, the adsorption capacity increases with increasing pH in the ranges of 2.0–6.0 and 4.0–5.0 for Zn(II) and Cu(II), respectively. Also, an appropriate physical or chemical pretreatment of the biomass shows positive effects on its capacity for metal biosorption. Higher temperature increases the capacity of metal biosorption more significantly for Zn(II) than Cu(II). The coexistence of both metals reduces the total amount of metal uptake by the indigenous *T. thiooxidans*, which shows strong preference for Zn(II) biosorption over Cu(II).

Acknowledgements

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