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# Effects of cell condition, pH, and temperature on lead, zinc, and copper sorption to *Acidithiobacillus caldus* strain BC13

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ABSTRACT

This study describes the effects of cell condition, pH, and temperature on lead, zinc, and copper sorption to *Acidithiobacillus caldus* strain BC13 with a Langmuir model. Copper exhibited the highest loading capacity, $4.76 \pm 0.28$ mmol $g^{-1}$, to viable cells at pH 5.5. The highest $k_L$ (binding-site affinity) observed was $61.2 \pm 3.0$ L $mmol^{-1}$ to dehydrated cells at pH 4.0. The pHs that maximized loading capacities and binding-site affinities were generally between 4.0 and 5.5, where the sum of free-proton and complexed-metal concentrations was near a minimum. Of additional importance, lead, zinc, and copper sorbed to viable cells at pH values as low as 1.5. Previous studies with other acidithiobacilli did not measure viable-cell sorption below pH 4.0. In separate experiments, desorption studies showed that far less copper was recovered from viable cells than any other metal or cell condition, suggesting that uptake may play an important role in copper sorption by *At. caldus* strain BC13. To reflect an applied system, the sorption of metal mixtures was also studied. In these experiments, lead, zinc, and copper sorption from a tertiary mixture were $40.2 \pm 4.3\%$, $28.7 \pm 3.8\%$, and $91.3 \pm 3.0\%$, respectively, of that sorbed in single-metal systems.



## 1. Introduction

*Acidithiobacillus caldus* is a thermophilic acidophile that oxidizes reduced sulfur compounds, fixes carbon dioxide [1], has been isolated from several acidic environments [2–4], and is believed to be important for leaching metals from mineral sulfides in acid-mine environments [5–9]. Despite its important interactions with metals in such systems, to our knowledge, there have been no metal sorption studies published to date with this organism.

Microbial and non-biogenic sorption has been used as a relatively inexpensive tool for the immobilization of heavy metals from many types of contaminated systems [10–22]. Relevant to the work presented here, studies have shown that *Acidithiobacillus thiooxidans* and *Acidithiobacillus ferrooxidans* can remove heavy metals from contaminated soil and sewage systems [23–25]. In addition, zinc and copper sorption to *At. thiooxidans* and *At. ferrooxidans* has been studied by several researchers [26–29].

*At. thiooxidans* and *At. ferrooxidans* thrive in acid-mine environments, and are important in metal mobilization and transport [30], lending interest to their study as biological sorbents. However, these organisms are typically found at temperatures below 35 °C, whereas *At. caldus* grows optimally at 45 °C [1]. This suggests that a metal sorption study using *At. caldus* is very relevant to moderate temperature systems.

Here, the sorption of lead, zinc, and copper to *At. caldus* strain BC13 is described with a Langmuir model. In contrast to most prior research, experiments were carried out using viable cells, between pH 1.5 and 7.0, and from 25 to 45 °C. Tests were also carried out with dehydrated cells for comparison to other's work. The effects of proton competition and metal speciation were investigated with changing pH. In addition, temperature effects were observed to calculate the heat of sorption for lead, zinc, and copper to *At. caldus* strain BC13. Finally, the sorption of metal mixtures to *At. caldus* strain BC13 was measured to determine how the presence of competing metals affected the sorption capacity of lead, zinc, and copper. The results from these experiments are compared to those using other acidophilic prokaryotes, the sorption of zinc and copper by *At. caldus* strain BC13 also compared favorably to sorption of other heavy metals to non-biotic material, as well as various eukaryotic microorganisms [31–36].

The use of viable cells and mixed-metal systems provides an important fundamental link between past results and the mixed-metal systems found in acid-mine drainage and many remediation applications. This study increases the understanding of *At. caldus*' role in the fate and mobilization of metals and

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may lead the way for more applied studies with this organism.

## 2. Materials and methods

### 2.1. Culture and cell preparation

A basal salts medium (Fisher Scientific, Pittsburg, PA, U.S.A.) [1] was prepared and autoclaved for 15 min at 121 °C and 22 psig. Filter sterilized (0.2 μm) potassium tetrathionate was added to a concentration of 5 mM to provide an electron donor. The medium pH was adjusted to 2.5 using 6N sulfuric acid, aliquoted into 500-mL serum bottles (350 mL medium volume), and capped with butyl-rubber stoppers. A 20:80 mixture of carbon dioxide:nitrogen gas (Gases Plus Norco, Belgrade, MT, U.S.A.) was sparged into the medium to a headspace pressure of 5 psig to provide a carbon source. *At. caldus* BC13 (ATCC 51757), from here on referred to as BC13, cells preserved at 4 °C in nanopure water (17.4 MΩ, with the pH adjusted to 3.0 using 6N sulfuric acid), provided the initial inoculum. Cultures were grown aerobically at 45 °C and shaken at 150 rotations per minute (rpm). Cells were harvested via centrifugation during the mid-exponential growth phase and washed using the pH 3.0 nanopure water. This wash was repeated three times to remove residual potassium tetrathionate.

Cell pellets for use in viable-cell sorption studies were re-suspended in a small aliquot of the basal salts medium at a concentration of 1 g dry-cell weight $L^{-1}$. Cell pellets for use in dehydrated-cell sorption studies were dried in a critical point dryer (Samdri-795, Tousimis, Rockville, MD, U.S.A.). Sorption experiments were done immediately to avoid potential effects of cell storage.

The sorption experiments were carried out in the same basal salts medium, but without potassium tetrathionate. A filter sterilized (0.2 μm) metal solution (lead, zinc, or copper sulfate) was added from a stock solution prepared in the medium. The pH was adjusted to 1.5, 2.5, 4.0, 5.5, or 7.0, using either 6N sulfuric acid or 10N sodium hydroxide. Following cell preparation, aliquots that provided an initial cell density of 100 mg dry-cell weight $L^{-1}$ were added to 125-mL Erlenmeyer flasks (75 mL medium volume) fitted with foam stoppers. Samples were shaken at 150 rpm at 25, 35, or 45 °C in a temperature controlled incubator.

### 2.2. Measurement of aqueous-metal concentrations

Filter sterilized (0.2 μm) samples were taken and preserved at 4 °C for analysis. Inductively coupled plasma mass spectrometry (ICP-MS) (Agilent 7500ce ORS, Foster City, CA, U.S.A.) with an Octopole Reaction System and a MicroMist glass concentric nebulizer was used to measure metal concentrations. The sample temperature was set at 2 °C and the radio frequency power was set to 1500 W. Argon was used as the carrier gas, at a flow rate of 0.64 L $min^{-1}$. The nebulizer pump was operated at 0.15 rotations per second (rps) and the sample pump was set to 0.1 rps. Measurements were compared with standards to calculate metal concentrations. Germanium and indium were used as internal standards to correct for drift over a series of runs.

### 2.3. Calculation of sorption parameters

The Langmuir equation (Eq. (1)) is derived for monolayer sorption onto a surface with a finite number of homogenous binding sites, where the occupation of one site does not affect the binding affinity of adjacent sites.

$$Q_e = \frac{Q_o k_L C_e}{1 + k_L C_e} \tag{1}$$

where $Q_e$ is the concentration of sorbed metal at equilibrium, $Q_o$ is the maximum amount of metal per unit sorbent required for a complete monolayer (loading capacity), $C_e$ is the equilibrium metal concentration in solution, and $k_L$ describes binding-site affinity by Eq. (2):

$$k_L = \frac{\Phi}{(1 - \Phi)C_e} \tag{2}$$

where $\Phi$ is defined as the percent coverage of binding sites.

Eq. (1) may then be re-written in the linear form:

$$\frac{1}{Q_e} = \frac{1}{Q_o k_L C_e} + \frac{1}{Q_o} \tag{3}$$

and Lineweaver–Burk plots of $1/Q_e$ versus $1/C_e$ can be used to calculate values for $Q_o$ and $k_L$.

### 2.4. Calculating the heat of sorption

The Arrhenius equation (Eq. (4)) was used to calculate the heat of lead, zinc, and copper sorption to BC13.

$$k_L = A\ e^{[-E_a(RT)^{-1}]} \tag{4}$$

where $A$ is the Arrhenius pre-exponential factor, $E_a$ is the activation energy, $R$ is the universal gas constant, and $T$ is the absolute temperature. Because this is an equilibrium calculation, $E_a$ represents the difference between the activation energy required for sorption to occur, and the activation energy required for desorption to occur. This difference is equal to the enthalpy of sorption ($\Delta H_{sorption}$), assuming the thermal expansivity of the solution is negligible. Therefore:

$$E_a = \Delta H_{sorption} \tag{5}$$

Because Eq. (4) may be re-written in the linear form:

$$\ln(k_L) = \ln(A) - \frac{\Delta H_{sorption}}{RT} \tag{6}$$

A plot of $\ln(k_L)$ versus $1/RT$ has a slope equal to the negative heat of sorption, and a $y$-intercept equal to the natural logarithm of the Arrhenius pre-exponential factor.

### 2.5. Desorption experiments

Separate sorption experiments were done at 45 °C and either pH 1.5, 4.0, or 7.0. At equilibrium, cells were collected via centrifugation, and re-suspended in fresh basal salts medium containing 5 mM nitrilotriacetic acid (NTA). This solution was placed in a temperature controlled incubator at 45 °C and shaken at 150 rpm until the system equilibrated. Filter sterilized (0.2 μm) samples were taken and preserved at 4 °C for analysis using ICP-MS. In these experiments results were compared between whole and lysed cells. To lyse cells, cultures were autoclaved for 15 min at 121 °C and 22 psig, then sonicated for 1 min (Bronson 1020, Danbury, CT, U.S.A.). Cell lysis was confirmed visually using transmitted-light microscopy (Zeiss, San Marcos, CA, U.S.A.).

### 2.6. Mixed-metal sorption

Mixed-metal sorption was studied with viable cells at physiologically relevant conditions (pH 2.5 and 45 °C) using the same techniques for cell preparation, experimental design, and measurement of aqueous-metal concentrations described in Section 2.2. The initial concentrations of lead, zinc, and copper were 0.24, 0.92, and 1.57 mM. For direct comparison with single-metal systems, these concentrations were equal to the highest initial metal concentrations used in the individual sorption studies.

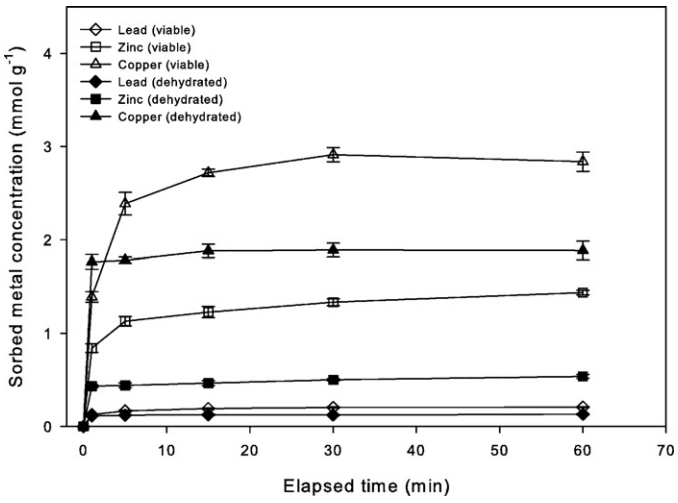


**Fig. 1.** Change in sorbed lead, zinc, and copper concentrations to viable and dehydrated *Acidithiobacillus caldus* strain BC13 cells with time at pH 2.5 and 45 °C. Error bars represent 95% confidence intervals.

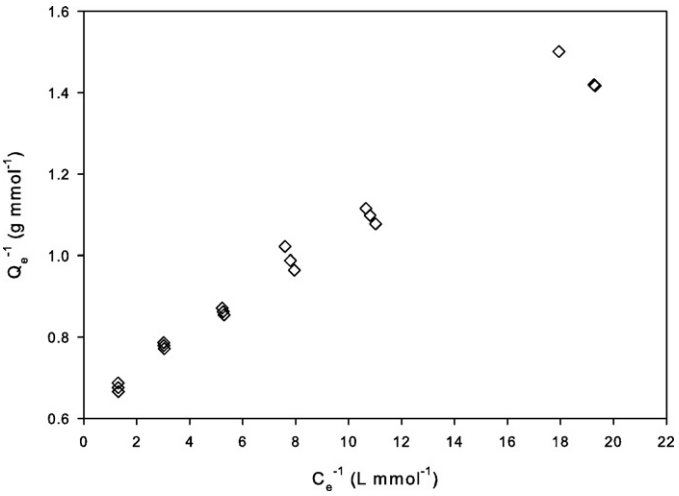


**Fig. 2.** A Lineweaver–Burk plot of the linearized Langmuir equation. Here, the sorption of zinc to viable *Acidithiobacillus caldus* strain BC13 cells, at pH 2.5 and 45 °C, is represented. The plotted values are the inverse of metal sorbed ($Q_e{}^{-1}$) and metal in solution ($C_e{}^{-1}$) at equilibrium.

### 2.7. Modeling metal speciation

Visual MINTEQ (version 2.53) was used to predict the speciation of metal using activities from the Debye–Huckel equation, and the default MINTEQA2 thermodynamic database. The temperature was set to 45 °C and the free-proton concentration was calculated from the pH, which was fixed at 1.5, 2.5, 4.0, 5.5, or 7.0. The lead, zinc, and copper concentrations used were 0.24, 0.92, and 1.57 mM, respectively, to match the highest metal concentrations used in the sorption experiments.

### 2.8. Statistical analysis and controls

In separate experiments, cells were cultured in the basal salts growth medium described in Section 2.1, with the pH adjusted to 1.5, 2.5, or 4.0 at 45 °C, covering the pH range over which *At. caldus* grows well [1]. These cells were then prepared for sorption experiments at the same pH they were grown at. The results were not statistically different from those observed when the growth medium pH differed from the sorption medium pH (data not shown).

All experiments were performed in triplicate, and average values and 95% confidence intervals were calculated. Langmuir and Arrhenius parameters, and corresponding 95% confidence intervals, were calculated with multiple linear regressions using the LINEST function in Microsoft Excel.

## 3. Results

### 3.1. Effect of pH on lead, zinc, and copper sorption

Fig. 1 shows the sorption of lead, zinc, and copper to viable and dehydrated cells over time, at pH 2.5 and 45 °C. It can be seen that viable-cell systems equilibrated more slowly than dehydrated-cell systems. Using equilibrium concentrations, Lineweaver–Burk plots were used to calculate loading capacities and binding-site affinities. Fig. 2 shows the Lineweaver–Burk plot for the sorption of zinc to viable BC13 cells, at pH 2.5 and 45 °C. Results for all conditions tested for lead, zinc, and copper sorption are not shown, but showed similar trends and variances. $R^2$ values for these plots varied between 0.88 and 0.98 (data not shown).

Fig. 3 shows the effect of pH on the loading capacities and binding-site affinities values of lead, zinc, and copper to viable

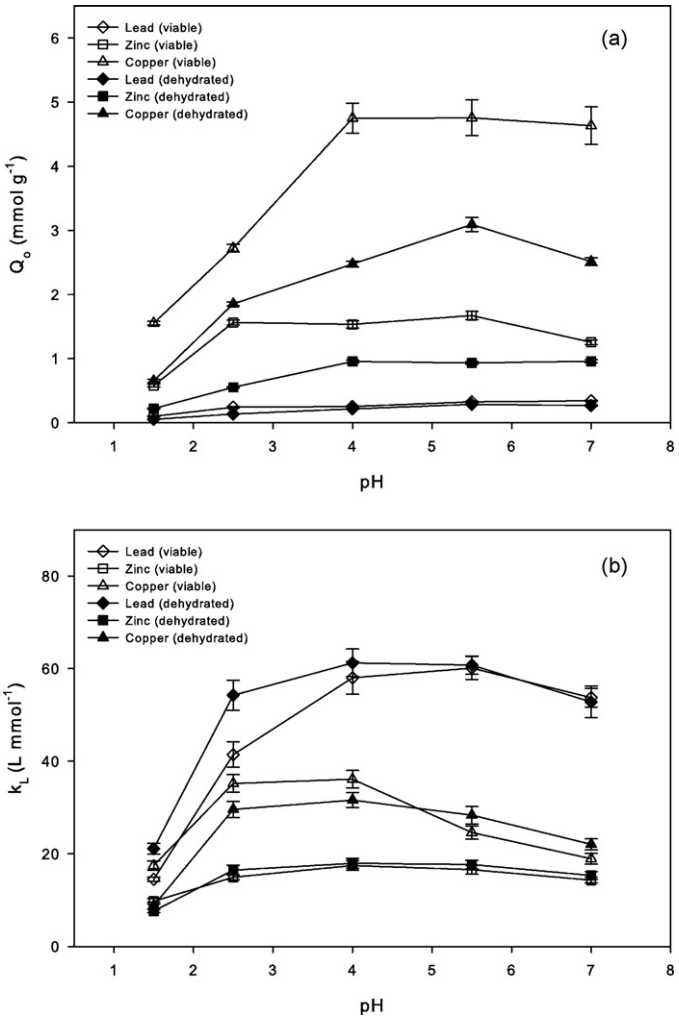


**Fig. 3.** The effect of pH on the (a) loading capacity ($Q_o$), and (b) binding-site affinity ($k_L$) for the sorption of lead, zinc, and copper to viable and dehydrated *Acidithiobacillus caldus* strain BC13 cells. Error bars represent 95% confidence intervals.

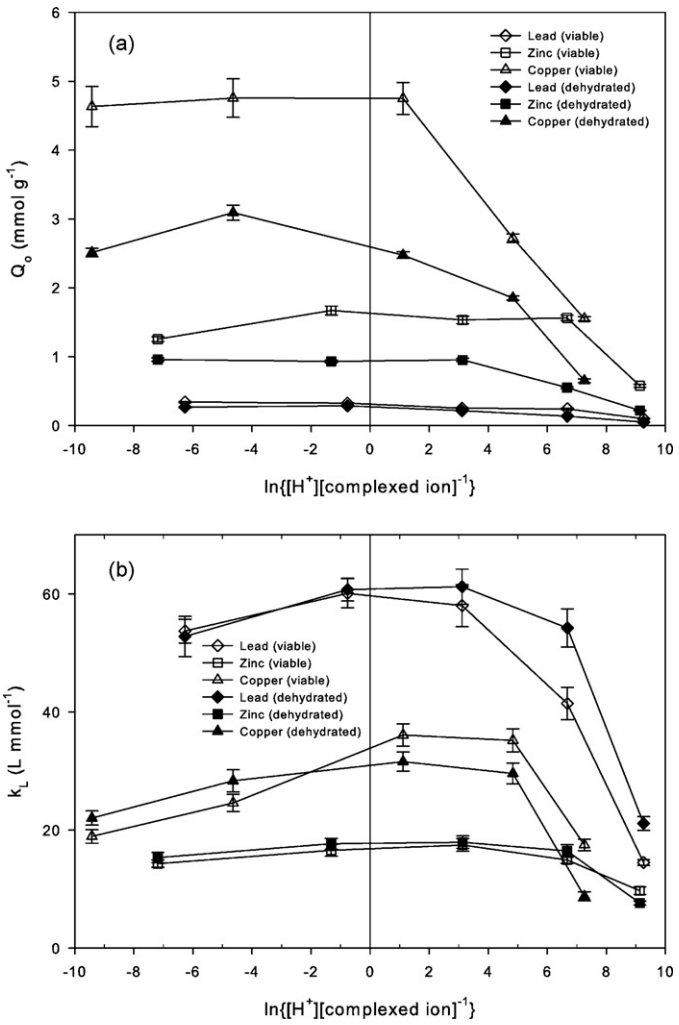


**Fig. 4.** The relationship between the free-proton and complexed lead, zinc, or copper concentrations on the (a) loading capacity ($Q_o$) and (b) binding-site affinity ($k_L$) for the sorption of lead, zinc, and copper to viable and dehydrated *Acidithiobacillus caldus* strain BC13 cells. Concentrations of 0.24, 0.92, and 1.57 mM for lead, zinc, and copper, respectively, were used in the speciation model. These were the highest aqueous concentrations used in this study, and represent conditions were each binding site could theoretically interact with a metal molecule. Error bars represent 95% confidence intervals.

and dehydrated cells at 45 °C. Viable cells were observed to have higher loading capacities, for zinc and copper, than dehydrated cells. However, in the case of lead, the loading capacities for viable and dehydrated cells were very close. Copper exhibited the highest loading capacities, $4.76 \pm 0.28$ and $3.09 \pm 0.11$ mmol g$^{-1}$ to viable and dehydrated cells, respectively, at pH 5.5 (Fig. 3a). Conversely, the highest binding-site affinities observed were for lead, $60.1 \pm 2.5$ L mmol$^{-1}$ at pH 5.5, and $61.2 \pm 3.0$ L mmol$^{-1}$ at pH 4.0, for viable and dehydrated cells, respectively (Fig. 3b).

The ratios of free-proton to complexed-metal concentrations are plotted on a natural logarithm scale against loading capacity (Fig. 4a) and binding-site affinity (Fig. 4b). A vertical line at $y = 0$ represents the pH where the free-proton concentration is equal to the complexed-metal concentration. The ratio at which the loading capacity was maximized varied among lead, zinc, and copper, and between viable and dehydrated cells. However, Fig. 4b shows that, for each metal and cell condition, the binding-site affinity was maximized when the free-proton concentration was higher than the complexed-metal concentration.

**Table 1**
The heat of sorption ($\Delta H_{sorption}$) for lead, zinc, and copper to viable and dehydrated *Acidithiobacillus caldus* strain BC13 cells, calculated from an Arrhenius plot. The Arrhenius pre-exponential factor ($A$) and $R^2$ value (indicating goodness of fit) are also shown.

| | $\Delta H_{sorption}$ (J mmol$^{-1}$) | $A$ (L mmol$^{-1}$) | $R^2$ |
|---|---|---|---|
| Lead (viable) | −18.4 | $6.8 \times 10^{-2}$ | 0.978 |
| Zinc (viable) | −32.8 | $6.4 \times 10^{-5}$ | 0.989 |
| Copper (viable) | −12.1 | $3.6 \times 10^{-1}$ | 0.884 |
| Lead (dehydrated) | −26.0 | $2.9 \times 10^{-3}$ | 0.998 |
| Zinc (dehydrated) | −32.1 | $9.1 \times 10^{-5}$ | 0.988 |
| Copper (dehydrated) | −32.5 | $1.4 \times 10^{-4}$ | 0.992 |

### 3.2. Temperature effects

At pH 2.5, the loading capacities of lead and zinc onto viable and dehydrated cells were not significantly dependent on temperature; neither was the loading capacity of copper onto dehydrated cells. However, the loading capacity of copper onto viable cells decreased from $3.2 \pm 0.1$ mmol g$^{-1}$ at 35 °C, to $2.7 \pm 0.06$ mmol g$^{-1}$ at 45 °C (Fig. 6a). Conversely, binding-site affinities for lead, zinc, and copper sorption to viable and dehydrated cells consistently decreased with increasing temperature. The largest decrease was observed for the sorption of lead to dehydrated cells, where the binding-site affinities were $104.9 \pm 6.5$, $74.5 \pm 5.6$, and $54.2 \pm 3.2$ L mmol$^{-1}$ were observed at 25, 35, and 45 °C, respectively (Fig. 6b). From Eq. (6), heats of sorption were calculated and are shown in Table 1.

### 3.3. Desorption experiments

For each cell condition tested, at pH 2.5, 4.0, and 7.0, between 94.6% and 99.5% of lead and zinc desorbed from cellular surfaces in an NTA wash. However, at each pH, much less copper desorbed from viable cells than any of the other cell conditions tested. For example, at pH 2.5, only $78.4 \pm 2.8$% of the copper desorbed from viable cells, whereas $94.6 \pm 1.7$, $95.8 \pm 0.4$, and $95.6 \pm 2.4$ desorbed from dehydrated, lysed-viable, and lysed-dehydrated cells, respectively (Table 2).

### 3.4. Mixed-metal sorption

Fig. 7 shows that lead and zinc sorption decreased significantly when present in a mixture containing copper. In contrast, the presence of lead or zinc did not significantly reduce the sorption of copper. When metals were presented individually, at the high-

**Table 2**
Percent of lead, zinc, or copper that desorbed from *Acidithiobacillus caldus* strain BC13 cells in a 5-mM nitriloacetic acid (NTA) wash at equilibrium. ± values represent 95% confidence intervals.

| | Percent of sorbed metal removed in NTA wash | | |
|---|---|---|---|
| | Lead | Zinc | Copper |
| pH 1.5 | | | |
| Viable cells | 96.4 ± 3.1 | 94.2 ± 1.3 | 78.4 ± 2.8 |
| Dehydrated cells | 98.1 ± 1.7 | 98.1 ± 1.0 | 94.6 ± 1.7 |
| Lysed-viable cells | 96.3 ± 0.8 | 96.5 ± 0.9 | 95.8 ± 0.4 |
| Lysed-dehydrated cells | 96.8 ± 0.6 | 97.5 ± 1.2 | 95.6 ± 2.4 |
| pH 4.0 | | | |
| Viable cells | 95.4 ± 0.6 | 94.6 ± 1.5 | 83.1 ± 3.3 |
| Dehydrated cells | 97.6 ± 0.6 | 96.0 ± 2.8 | 97.4 ± 1.3 |
| Lysed-viable cells | 98.2 ± 2.0 | 97.5 ± 1.3 | 94.5 ± 2.4 |
| Lysed-dehydrated cells | 99.0 ± 1.2 | 97.4 ± 1.6 | 95.3 ± 1.3 |
| pH 7.0 | | | |
| Viable cells | 98.3 ± 1.0 | 98.0 ± 0.4 | 90.5 ± 0.8 |
| Dehydrated cells | 99.5 ± 0.5 | 98.4 ± 1.1 | 96.9 ± 0.4 |
| Lysed-viable cells | 97.0 ± 0.5 | 97.7 ± 2.2 | 94.4 ± 3.1 |
| Lysed-dehydrated cells | 97.4 ± 1.0 | 97.4 ± 1.2 | 96.2 ± 1.0 |

est concentrations used in the previously described experiments (Section 2.1), 95.3 ± 2.3%, 92.7 ± 5.6%, and 94.8 ± 2.6% of the lead, zinc, and copper sorbed, respectively. However, when presented jointly with copper, only 46.5 ± 2.8% and 33.8 ± 5.5% of lead and zinc sorbed to BC13. Conversely, 96.0 ± 2.7% and 97.0 ± 2.9% of the copper sorbed to BC13 cells when presented jointly with lead or zinc, respectively. When lead and zinc were mixed there was not a significant effect, as 93.6 ± 3.3% and 95.6 ± 3.2% of the lead and zinc sorbed, respectively. When all three metals were mixed, 40.2 ± 4.3%, 28.7 ± 3.8%, and 91.3 ± 3.0% of the lead, zinc, and copper sorbed, respectively.

## 4. Discussion

### 4.1. Sorption of lead, zinc, and copper to BC13

Although various authors have suggested different metal properties that may affect binding-site affinity, the literature suggests that the relative affinities of metals are specific to the organism used. In the present study, the order of binding-site affinity was lead > copper > zinc. This matches the order of binding-site affinity for these metals to *Pseudomonas putida* as observed by Pardo et al. [37].

Ferris and Beveridge [38] suggested that a higher metal charge density contributes to greater affinity. However, the ionic radii of lead, copper and zinc are 133, 87, and 74 pm, respectively, so in the present case, it appears that other factors are greater contributors to binding-site affinity than charge density. It has also been suggested that metal acidity is an important factor [39]. The metal acidities of lead, copper, and zinc as represented by the stability constant of the first hydroxyl-metal complex, were calculated to be 6.2, 6.0, and 4.4 at pH 4.0, respectively. This corresponds with the order of binding-site affinities observed here, suggesting that metal acidity may be important in the affinity for metal sorption to BC13. Unlike the binding-site affinity, the sorption capacity for lead was the lowest of the three metals tested, as BC13 had a sorption capacity for zinc and copper over an order of magnitude greater than it did for lead (Fig. 3).

### 4.2. Effects of cell condition on metal sorption

Several studies have reported significant differences in metal sorption to viable versus non-viable biota (*i.e.* [40–43]). In the case of zinc and copper, binding-site affinity was not significantly affected by cell condition, however, a significantly higher loading capacity was observed for viable cells as compared to dehydrated cells (Fig. 3). In addition, Fig. 1 suggests that the sorption kinetics also differ between viable and dehydrated cells. Only 48% of the copper sorption to viable cells occurred before the first time point, compared to 93% of the sorption to dehydrated cells. These calculations were made for lead (61% versus 86%) and zinc (59% versus 80%). This suggests that changes to zinc and copper binding sites during the dehydrating process significantly affected zinc and copper sorption.

### 4.3. Dependence of metal sorption on pH

For viable and dehydrated cells, the loading capacities and binding-site affinities were dependent on pH (Fig. 3). In general, both increased to a plateau with increasing pH, and then decreased slightly. A review by Febrianto et al. [43] reported many studies that observed significant increases in the loading capacity with increasing pH, and in many cases, a subsequent decrease in the loading capacity with further increases in pH. Specific to bacteria, this observation has been reported by several researchers (*i.e.* [27,44–46]).

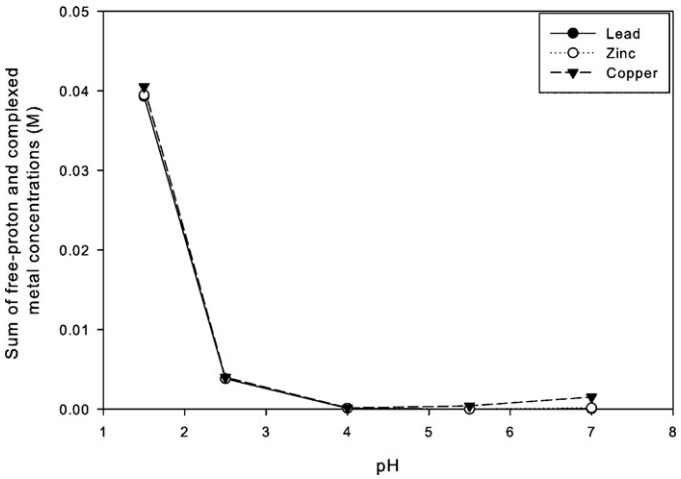


**Fig. 5.** Sum of the free-proton and complexed-metal concentrations, representing two sources of competition for metal sorption onto a cellular binding site. Concentrations of 0.24, 0.92, and 1.57 mM for lead, zinc, and copper, respectively, were used in the speciation model. These were the highest aqueous concentrations used in this study, and represent conditions where each binding site could theoretically interact with a metal molecule.

In many systems, there are two competing factors that affect metal sorption. First, the metal must compete with proton sorption at the binding site [47], especially at low pH. Secondly, metal speciation increases with pH, possibly reducing the biological availability of the metal [48]. Fig. 4 shows how these factors affect the loading capacity and binding-site affinity. The binding-site affinities were maximized between pH 4.0 and 5.5, where, interestingly, the sum of the free-protons and complexed ions was near a minimum for solutions containing lead, zinc, and copper, respectively (Fig. 5).

### 4.4. Dependence of metal sorption on temperature

Many published studies have reported an increase in loading capacities of metals with increasing temperature [16,23,49,50]. However, in the present report, temperature did not significantly affect the loading capacity of lead, zinc, or copper to viable or dehydrated cells. Conversely, the loading capacity of copper on viable cells did decrease slightly between 35 and 45 °C (Fig. 6). Similar observations were made of zinc sorption to several species of *Pseudomonas* [51].

Unlike loading capacities, binding-site affinities decreased significantly with increasing temperature suggesting, by LeChatelier's principle, that the sorption of these metals is an exothermic reaction and is dominated by reversible (physical) mechanisms [47]. Copper was the least exothermic of the three metals tested (Table 1), indicating some irreversible aspect to its binding mechanism.

### 4.5. Mechanistic inferences

Lead and zinc shared shared similar sorption behaviors. Specifically, Lineweaver–Burk analyses of sorption experiments carried out with lead and zinc resulted in $R^2$ values between 0.97 and 0.99 (Table 1), indicating excellent fit to the Langmuir model. Key assumptions in the derivation of the Langmuir equation is monolayer sorption onto a finite number of sites with similar affinities for the sorbent. The excellent fit of the data to a Langmuir model would suggest that the sorption mechanism of lead and zinc follows these assumptions. Surface adsorption to different functional groups that do not change

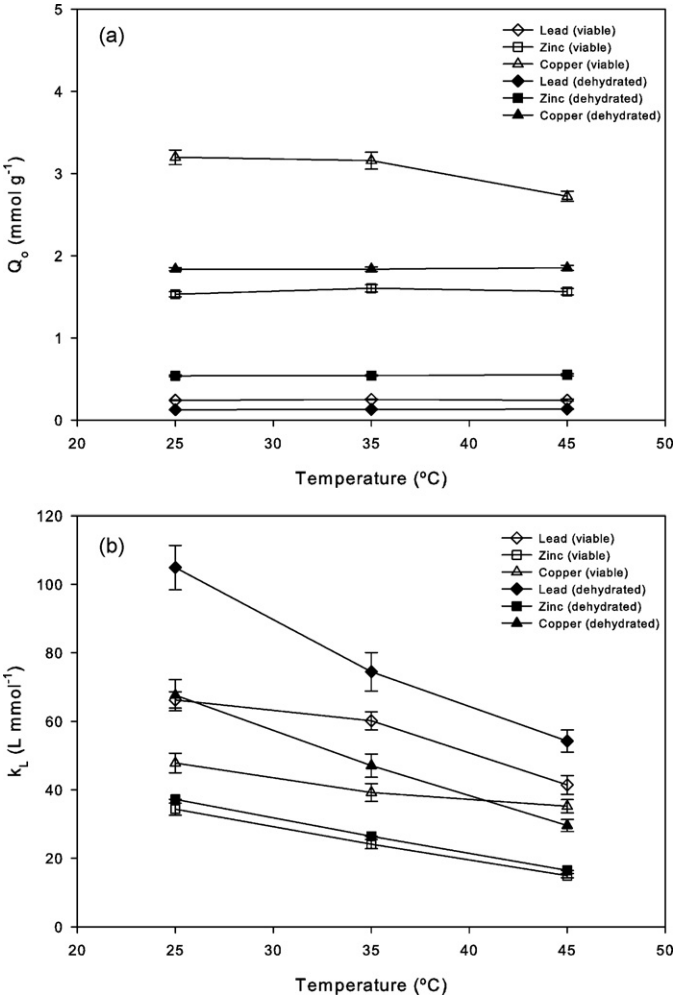


**Fig. 6.** The effect of temperature on the (a) loading capacity ($Q_o$) and (b) binding-site affinity ($k_L$) for the sorption of lead, zinc, and copper to viable and dehydrated *Acidithiobacillus caldus* strain BC13 cells. Error bars represent 95% confidence intervals.

significantly in composition or affinity would provide such a mechanism.

Conversely, Lineweaver–Burk analyses of copper sorption to viable cells resulted in average $R^2$ values of 0.884 (Table 1). This would suggest that an additional mechanism(s) played a role, one that is not well described by the Langmuir assumptions. Interestingly, the data in Table 2 shows that far less copper is recovered in an NTA wash of viable cells than lead and zinc. However, recoveries of copper from dehydrated, lysed-viable, and lysed-dehydrated cells were all similar to recovery of lead and zinc from cells subjected to different treatments. This may indicate that more copper had absorbed into the viable cells, rather than adsorbing to the cellular surface. This mechanism is not described by the Langmuir assumptions, so it would also explain the poorer fits from the Lineweaver–Burk analyses. Finally, the heat of sorption for copper was calculated to be less negative than those of lead and zinc, suggesting a less reversible sorption mechanism. Absorption, into the cell would also represent a less reversible process.

Alvarez and Jerez [52] suggested that the closely related *At. ferrooxidans* possesses a copper efflux system that requires polyphosphate kinase activity. If BC13 possesses a similar mechanism, electron-donor starved cells would likely lack the energy to pump phosphate–copper complexes out of the cell following absorption. Such a mechanism would explain the difference between copper sorption kinetics to viable versus dehydrated cells, the lower amount of copper that desorbed from viable cells in the NTA wash, and the significantly less negative heats of sorption calculated for copper sorption.

The desorption experiments and the calculated heats of sorption for lead and zinc suggest a mechanism(s) consisting primarily of surface adsorption. Conversely, the desorption experiments and calculated heat of sorption for copper suggests a mechanism(s) that contains a less reversible aspect, such as absorption. To better elucidate the mechanisms of sorption for BC13, Fourier transform infrared spectroscopy could be used to compare functional group coverage before and after the sorption of lead, zinc, and copper. This approach has been used by previous researchers [53,54], and may differentiate the binding of metals to the various functional groups of the cellular envelope (*i.e.* carboxylic, phenolic, hydroxyl, etc.). If absorption is indeed a significant mechanism for copper sorption by BC13, a difference in functional group coverage should be observed between lead and zinc versus copper sorption.

### 4.6. Comparisons to previous work with acidithiobacilli and other microorganisms

Previous metal sorption studies using *At. thiooxidans* and *At. ferrooxidans* have reported loading capacities for zinc and copper. To facilitate a comparison with the results presented here, the units for loading capacity in previous reports were converted from mg L$^{-1}$ to mmol g$^{-1}$.

Liu et al. [28] used a Langmuir model to describe the sorption of zinc (at pH 2.0, 4.0, and 6.0) and copper (at pH 4.0 and 5.0) to viable *At. thiooxidans* cells at 25 °C. In contrast to the sorption of zinc to BC13 observed here, Liu et al. did not observe any sorption of zinc to viable cells below pH 6.0. At pH 6.0 a loading capacity of $0.66 \pm 0.01$ mmol g$^{-1}$ was reported, compared to $1.67 \pm 0.07$ mmol g$^{-1}$, in the present study at pH 5.5 at 45 °C. However, when Liu et al. pre-treated the cells with sodium hydroxide, rendering them non-viable, sorption occurred at pH 2.0 ($0.58 \pm 0.02$ mmol g$^{-1}$), pH 4.0 ($0.83 \pm 0.03$ mmol g$^{-1}$), and pH 6.0 ($1.46 \pm 0.06$ mmol g$^{-1}$). These values are over three-fold higher than those reported here for dehydrated BC13 cells at similar pH. The same study reported loading capacities for copper for both viable and non-viable cells that are significantly lower than those reported here, for BC13. This comparison suggests that viable BC13 cells may be capable of sorbing greater amounts of zinc and copper than *At. thiooxidans*, especially below pH 4.0.

In another study, Ruiz-Manriquez et al. [29] reported a loading capacity of 3.14 mmol g$^{-1}$ for copper sorption to *At. ferrooxidans* cells treated with sodium hydroxide at pH 6.0 and 37 °C, this is very close to the 3.09 mmol g$^{-1}$ reported for dehydrated BC13 cells, at pH 5.5 and 45 °C, in the present study. However, when viable cells were used, Ruiz-Manriquez et al. observed loading capacities of copper lower than those observed for viable BC13 cells in the present study. The ability of viable BC13 to sorb relatively high amounts of lead, zinc, and copper below pH 4.0 may suggest that it could play a significant role in the fate and mobility of these metals, especially in low pH environments such as acid-mine drainages. In addition, *At. caldus* may play an especially important role in the immobilization of copper at low pH as copper sorption did not decrease significantly when present in a mixture containing lead and zinc.

In addition to comparing the sorption of heavy metals by BC13 to other acidophilic prokaryotes, it is worth mentioning that the sorption capacities observed here for zinc and copper were significantly greater than those observed by other researchers using various strains of eukaryotic organisms and non-biological materials. For example, Gupta et al. observed a sorption capacity of 0.15 mmol g$^{-1}$ for chromium(VI) to a carbon slurry obtained from fertilizer waste [34], between 0.10 and 0.20 mmol g$^{-1}$ copper to the *Spirogyra* algal species [35], and up to 1.5 mmol g$^{-1}$ cadmium(II) to

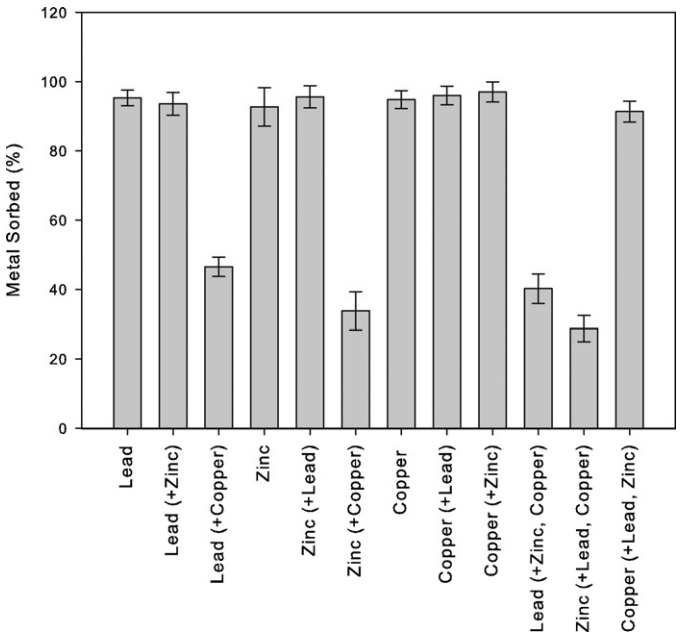


**Fig. 7.** Effect of lead, zinc, and copper mixtures on metal sorption by *Acidithiobacillus caldus* strain BC13. The initial concentration of each metal was 0.24, 0.92, and 1.57 mM for lead, zinc, and copper, respectively. These were the highest aqueous concentrations used in this study, and represent conditions where each binding site could theoretically interact with a metal molecule. *X*-axis labels indicate the metals present. The values are reported for the un-parenthesized label. Error bars represent 95% confidence intervals.

the *Oedogonium* algal species [36]. These values are comparable to those observed here for lead (up to 0.2 mmol $g^{-1}$), but significantly less than those observed for zinc and copper (between 1.0 and 5.0 mmol $g^{-1}$, depending on pH).

For further comparison, Sari and Tuzen determined the sorption capacities of cadmium(II) to *Ceramium virgatum* (0.02 mmol $g^{-1}$) [31], and a fungus, *Amanita rubescens* (0.38 mmol $g^{-1}$). In addition, Sari and Tuzen observed a sorption capacity of lead to *A. rubescens* of approximately 0.2 mmol $g^{-1}$ [33]. Similar work by Anayurt et al. reported sorption capacities of lead and cadmium(II) to a fungus, *Lactarius scrobiculatus*, of approximately 0.25 and 1.0 mmol $g^{-1}$, respectively [32]. Again these values are comparable for those observed here for lead, but in general, BC13 can efficiently sorb heavy metals.

It is also worth noting that although the observed sorption capacities were significantly different between BC13 and the various eukaryotic organisms discussed here, the optimal pH for sorption capacity in these studies was typically near 5.0, similar to that observed here, suggesting that the concentrations of free-protons and complexed-metal ions plays an important role in metal sorption in a variety of systems.

### 4.7. Mixed-metal sorption and application implications

The results reported here suggest that BC13 may be a candidate for the remediation of other acidic, metal-contaminated systems. Previous work has shown that biosorption can play an important role in the remediation of many metal-contaminated sites [10–22]. To characterize the ability of BC13 to sorb lead, zinc, and copper under conditions that better represent *in situ* conditions, experiments were carried out using metal mixtures. Fig. 7 shows that copper appeared to out-compete lead and zinc for cellular binding sites in mixed-metal environments containing high concentrations of copper. Interestingly, even a relatively high concentration of zinc did not significantly affect the sorption of either lead or copper. This is important as many acid-mine drainages and metal contaminated sites contain very high zinc concentrations [3,55,56]. These results are especially significant in the context of an applied system, where mixtures of metals may be present.

The relatively high sorption capacities observed here in both single and mixed-metal experiments suggest that BC13 is a viable candidate for the remediation of heavy-metal contaminated streams between pH 1.5 and 7.0. In addition, the relatively efficient removal of these metals from BC13 using a desorption wash suggests that cells could be recycled in a substrate-starved environment, without the introduction of a potentially harmful chelating agent into a waste stream.

### 4.8. Conclusions

This is the first report on the effects of cell condition, pH, or temperature on lead, zinc, or copper sorption to *At. caldus*. This is also the first study of these effects on the sorption of lead to any organism of the acidithiobacilli genus. Lead, zinc, and copper sorption was observed to be highly dependent on pH, with the greatest sorption occurring between pH 4.0 and 5.5. Temperature did not affect the loading capacities of lead, zinc, and copper significantly; however, the binding-site affinities decreased significantly with increasing temperature, suggesting that metal sorption to BC13 is an exothermic reaction.

Because experiments were not carried out identically, it is difficult to make an exact comparison with previous work; however it appears that viable BC13 cells are excellent sorbents of zinc and copper at low pH compared to viable *At. thiooxidans* and *At. ferrooxidans* cells [28,29], as well as eukaryotic microorganisms [31–33,35,36]. In addition, mixed sorption studies showed the presence of lead and zinc did not significantly decrease copper sorption from metal mixtures. This suggests that *At. caldus* may be especially important to the fate and transport of copper in acid-mine drainages, and may suggest a role in heavy-metal remediation, and that future work studying heavy-metal sorption by BC13 in applied systems would be of value.

This comprehensive study of lead, zinc, and copper sorption to BC13 cells contributes to understanding the role, and possible applications, of this microorganism in the fate and transport of heavy metals in acid-mine environments. In addition, its relatively high accumulation of zinc and copper identify *At. caldus* as a potential sorbent for these metals in other acidic systems.

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