



## Temperature dependence of Cd and Pb binding onto bacterial cells

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### ABSTRACT

We measured aqueous Cd and Pb adsorption onto bacterial surfaces at 5 °C, 50 °C, and 80 °C. We performed the adsorption experiments using one Gram-positive species, *Bacillus subtilis*, and one Gram-negative species, *Pseudomonas mendocina*. We use a surface complexation approach to determine the thermodynamic stability constants for the important metal–bacterial surface complexes for each temperature and each bacterial species.

Our results indicate that temperature affects metal adsorption onto the surfaces of Gram-positive and Gram-negative species differently. *B. subtilis* exhibits little difference in its metal-binding capacity over the temperature range of this study. At elevated temperatures,  $\text{Pb}(\text{OH})^+$  is the dominant form of Pb that binds to the surface while  $\text{Pb}^{2+}$  adsorption predominates at lower temperatures. However, the overall extent of Pb adsorption does not vary significantly with temperature. Conversely, the extent of both Cd and Pb adsorption onto *P. mendocina* is higher at 5 °C relative to the 50 °C and 80 °C data, but only under low pH conditions. The temperature dependence of metal adsorption onto bacteria depends upon both the metal and the bacterial species involved in the reaction. For both bacterial species studied here under circumneutral conditions, there is no significant temperature dependence to the extent of Cd adsorption or to the speciation of the important Cd–bacterial surface complexes. Therefore, a single set of averaged stability constant values can be used to account for Cd adsorption onto the cells as a function of temperature. Conversely, Pb more readily hydrolyzes in solution with increasing temperature than does Cd. Therefore, although the extent of total Pb adsorption does not change with temperature above pH 5, the speciation of the important Pb–bacterial surface complexes does vary with temperature. For metals such as Pb, the effect of temperature on the identity and stability constants of the bacterial surface complexes must be determined in order to accurately model metal speciation and transport as a function of temperature in bacteria-bearing geologic systems.

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### 1. Introduction

Adsorption of aqueous metal cations onto bacterial surfaces can affect metal speciation and mobility in a range of geologic systems. In order to predict metal adsorption onto bacterial surfaces, the concentration of binding sites as well as the stability constants for proton and metal adsorption reactions involving those binding sites must be determined. Determination of proton- and metal–bacterial surface stability constants has been limited almost exclusively to room temperature. However, bacteria are present in a range of geologic systems at temperatures from just above freezing in ocean-bottom environments and groundwater settings to temperatures just below boiling, such as in geothermal hot springs, mid-ocean ridge hydrothermal systems, and deep sedimentary basins (e.g., Walter et al., 1972; Jones and Renault, 1996; Konhauser et al., 2001). Furthermore, bacterial adsorption of cationic radionuclides may affect radionuclide mobility and speciation in geologic nuclear waste repositories where

temperature profiles can change dramatically with the evolution of the waste.

Although the effect of temperature has not been studied for bacterial metal adsorption reactions, temperature exerts a significant effect on the adsorption behavior of mineral surfaces. Most studies of the effects of temperature on adsorption have focused on protonation of mineral surface sites (see reviews by Blesa et al., 1990; Machesky, 1990). For example, Machesky et al. (1998) studied proton adsorption onto rutile surfaces from 25 °C to 250 °C, documenting a change in the  $\text{pH}_{\text{zpc}}$  of rutile from 5.4 to 4.3 over this temperature range. Machesky et al. (2001) used a refined MUSIC model to derive protonation constants and to account for the shift in the  $\text{pH}_{\text{zpc}}$  for rutile as a function of temperature. Sverjensky and Sahai (1998) used crystal chemical and Born solvation theories to model the variation in proton adsorption enthalpies for metal oxides, and to predict the proton adsorption enthalpies for silicate minerals. Johnson (1990) measured a significant change in the extent of Cd adsorption onto goethite between 10 °C and 70 °C, observing a shift in the pH adsorption edge (defined here by the pH at which 50% of the total Cd adsorbs onto the mineral surface) from 9.0 to 7.8 over this temperature range. Johnson (1990) attributed the increased adsorption at higher temperatures to a

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decrease in the effective size of the adsorbing cations due to progressively lower degrees of solvation with increasing temperature.

The effect of temperature on the adsorption of aqueous metal cations onto bacterial surfaces has not been studied as extensively as it has for mineral surface adsorption. Strandberg et al. (1981) performed adsorption kinetics experiments with uranium onto *Pseudomonas aeruginosa* and the yeast species *Saccharomyces cerevisiae* at 20 °C, 30 °C, 40 °C, and 50 °C. They found that higher temperatures increased the rate of uranium adsorption onto *S. cerevisiae*, but had a negligible impact on the rate of adsorption onto *P. aeruginosa*. Hu et al. (1996) performed uranium adsorption experiments onto *P. aeruginosa* at 4 °C and 31 °C. They observed only a slight decrease in uranium sorption at 31 °C, and concluded that uranium sorption onto *P. aeruginosa* was not significantly affected by temperature. Tsezos and Volesky (1981) performed uranium and thorium adsorption isotherm experiments onto the fungus *Rhizopus arrhizus* at 5 °C, 23 °C, and 40 °C. They concluded that temperature increase favored the equilibrium uptake of both thorium and uranium.

Wightman et al. (2001) conducted potentiometric titrations of suspensions containing *Bacillus subtilis* and TOR-39 bacterial cells from 25 °C to 75 °C, and used a discrete site surface complexation model approach to calculate protonation constants and concentrations of sites on the bacterial surface at each temperature. The calculated  $pK_a$  values decrease 0.6 to 1.5 log units over this temperature range, with a standard deviation for each value ranging from 0.6 to 0.8 log units. Thus, Wightman et al. (2001) concluded that any apparent temperature shift was smaller than the uncertainties associated with the measurement of these parameters, and that a single set of acidity constants and site concentrations could offer a reasonable approximation for proton adsorption over the experimental temperature range.

Although bacterial surface protonation constants appear not to vary significantly with temperature from 25 to 75 °C, the effect of temperature on the stability constants of metal–bacterial surface complexes has not been determined. The temperature dependence of equilibrium constants is described by the van't Hoff equation, which relates the temperature derivative of the stability constant to the enthalpy of the reaction in question (e.g., Stumm and Morgan, 1996). Metal and proton adsorption enthalpies may differ significantly for a particular surface. Therefore, experimental evidence is still needed to determine whether metal adsorption onto bacterial surfaces is affected by temperature to any extent.

The objective of this study is to determine the effect of temperature on metal binding onto representative Gram-positive and Gram-negative bacterial surfaces. We performed bulk metal adsorption experiments at 5 °C, 50 °C, and 80 °C, and we modeled the results using a surface complexation approach in order to determine and compare metal-binding stability constants at those temperatures. We discuss the implications of the results for estimating the effect of temperature on metal–bacterial adsorption in real systems.

## 2. Experimental procedures

The bacterial species used in this study were *Bacillus subtilis*, a Gram-positive species, and *Pseudomonas mendocina*, a Gram-negative species. Both species are mesophilic and ideally grow in solutions with pH values between 6 and 8. Both types of bacteria were cultured under identical conditions. Bacterial cells were cultured initially in 3 mL of trypticase soy broth (TSB) with 0.5% yeast extract for 24 h at 32 °C, after which they were transferred to 2 L of TSB with 0.5% yeast extract and grown for another 24 h. These conditions were chosen to yield cells in early stationary phase growth (Daughney et al., 2001). Cells were removed from the growth medium by centrifugation for 10 min at 5800 g. *B. subtilis* cells were washed twice in 0.1 M NaClO<sub>4</sub>, soaked in 0.016 M HNO<sub>3</sub> (pH = 1.8) for 30 min, then rinsed in 0.1 M NaClO<sub>4</sub> five more times. *P. mendocina* was rinsed seven times in 0.1 M NaClO<sub>4</sub> without an acid wash step. This procedure removes competing cations from bacterial surfaces, while preserving the outer membrane of the

Gram-negative species. The bacteria were then transferred to two pre-weighed test tubes and centrifuged twice for 30 min at 6300 g, with the supernatant poured off after each centrifugation. The test tubes were weighed again to obtain a wet mass of bacteria used in each experiment, a value that is approximately 10 times the bacterial dry mass (Fein and Delea, 1999).

Solutions of Cd and Pb were prepared by diluting a 1000 ppm aqueous metal standard with 0.1 M NaClO<sub>4</sub> (the electrolyte used in these experiments). The initial concentrations for each experiment were 2 ppm ( $1.8 \times 10^{-5}$  M) Cd and 10 ppm ( $4.8 \times 10^{-5}$  M) Pb. The bacterial cells were suspended in the solutions to obtain 2 g (wet mass)/L bacterial cells in the Cd solution, and 1 g (wet mass)/L in the Pb solution. Each homogeneous bacteria–metal–electrolyte suspension was divided into multiple Teflon bottles. The pH of each suspension was adjusted by adding small aliquots of concentrated HNO<sub>3</sub> and/or NaOH to obtain initial suspension pH values from 2.0 to 10.0.

The bottles were then submerged for 2 h in a thermostated water bath set at 5°, 50°, or 80 °C. Fowle and Fein (2000) have shown that 2 h is sufficient time for the system to attain equilibrium and that adsorption reactions are reversible over this time period. We conducted kinetics experiments at 5 °C (results not shown) that demonstrated that 2 h was sufficient for equilibrium to be attained for systems containing either bacterial species. The pH value of each solution was measured after the 2 h equilibration period with the reaction vessel still immersed in the water bath. The pH meter was calibrated using buffer solutions that were placed in the temperature-controlled water bath and the pH of these solutions was known at the temperatures of interest. Samples taken from solutions were then filtered (0.45 μm) and the resulting solution was analyzed for dissolved metal content using inductively coupled plasma optical emission spectrometry (ICP-OES), with standards made in 0.1 M NaClO<sub>4</sub> in order to eliminate matrix effects on the analyses. The concentration of metal adsorbed onto the bacteria was determined by difference between the known initial metal concentration and the final measured concentration of metal remaining in solution. The ICP-OES yielded results with approximate uncertainties of ±3% for Cd and ±4% for Pb as determined by repeat analyses of external standards. All experiments were performed twice to insure reproducibility.

Controls experiments with metal, but no bacteria, were performed at both room temperature and 80 °C to determine whether evaporation (during pH measurement when the reaction bottles were open to the atmosphere) affected the metal concentration measurements. The results indicate that evaporation in the Cd solutions was negligible within analytical error. However, the Pb evaporation results indicate a 4.9% increase in Pb concentration for the 80 °C solutions, and therefore the measured Pb concentrations from the 80 °C experiments were corrected by decreasing the concentration of Pb remaining in solution after filtration by 4.9% for each solution. Loss due to evaporation for the 50 °C experiments was negligible.

We performed control experiments that were identical to the metal adsorption experiments described above, but without any metal in the solution, at 5 °C and 80 °C, and measured for dissolved organic carbon (DOC) using a Shimadzu 5000 DOC analyzer, and calibration standards were made by diluting a 1000 ppm C, potassium hydrogen phthalate, standard with the same electrolyte used in experiments. The DOC analyses were conducted in order to determine the stability of the bacteria under the experimental conditions. Cell lysis results in the release of organic material into solution, thereby increasing the DOC content. Before analysis, the samples were purged of inorganic carbon by acidification with 2 M ultrapure HCl. DOC results had analytical uncertainties of approximately ±3%.

## 3. Results

The results of the metal adsorption experiments are depicted in Fig. 1. The extent of adsorption is minimal at low pH for each metal and

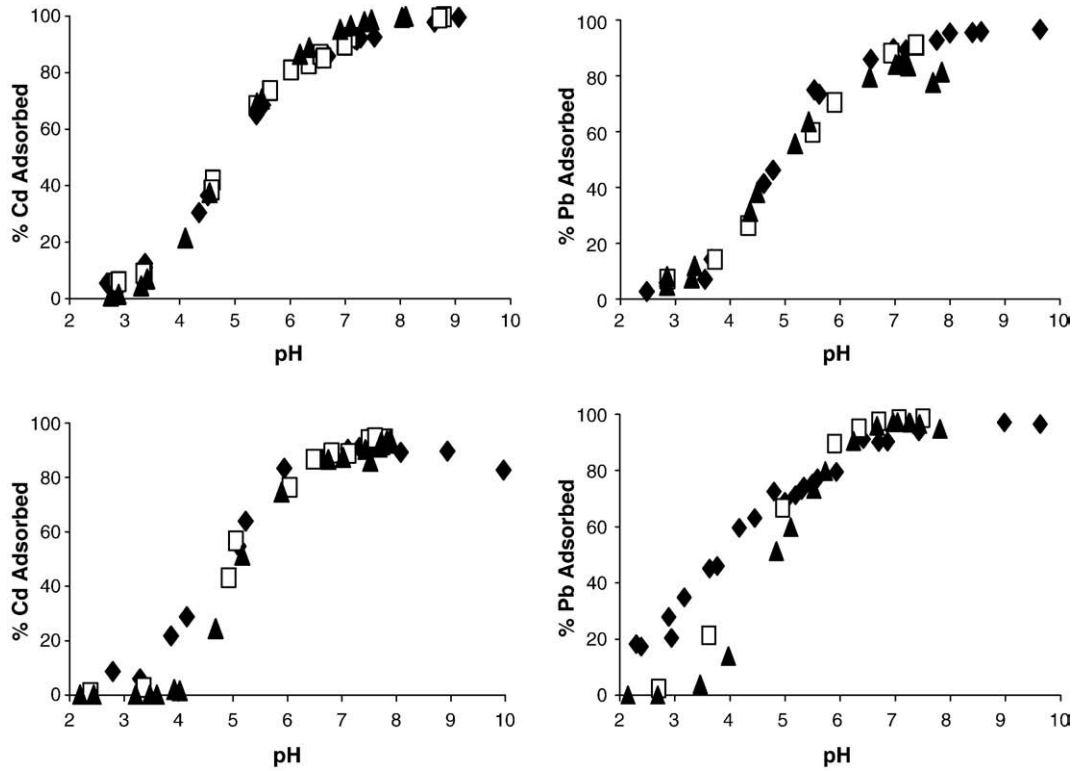


Fig. 1. Adsorption of Cd and Pb onto *B. subtilis* (top) and *P. mendocina* (bottom) at 5 °C (diamonds), 50 °C (squares), and 80 °C (triangles).

each bacterial species studied, with increasing adsorption with increasing pH over the pH range of approximately 2 to 8. Under the experimental conditions, the bacteria adsorbed >95% of the dissolved metal in each system when solution pH values were above approximately pH 8. This type of pH dependence is typical for adsorption of divalent cations onto bacterial surfaces, and has been observed repeatedly (e.g., Fein et al., 1997; Ngwenya et al., 2003; Haas et al., 2001). For the *B. subtilis* cells, the experiments indicate that there is no significant change in the extent of adsorption with temperature over the temperature range studied. The *P. mendocina* experiments also showed no discernable temperature effect on adsorption for pH>5. However, under lower pH conditions, the extent of metal adsorption observed in the 5 °C experiments was significantly greater than that observed at the higher temperatures. At pH=4, for instance, 20% of the total Cd in the experimental system was adsorbed onto *P. mendocina* at 5 °C, whereas only 2% of the total Cd was adsorbed at 80 °C. At pH=4, over 50% of the Pb was adsorbed at 5 °C, whereas only 18% was adsorbed at 80 °C.

The DOC values from the control experiments were lowest at near-neutral pH and increased both toward higher and lower pH values in all systems studied (Fig. 2). In general, the *B. subtilis* control experiments exhibited significantly lower DOC values than did the *P. mendocina* controls at both temperatures. The highest *B. subtilis* measurement was 11.7 ppm, and the highest *P. mendocina* measurement was 21.7 ppm. The *B. subtilis* controls also exhibited less variation with temperature, showing a maximum concentration difference of 7.5 ppm over the temperature range, whereas the *P. mendocina* controls showed a maximum concentration difference of 10.5 ppm. The higher DOC concentrations in the 80 °C samples relative to the 5 °C samples suggests that more cell lysis occurs in the higher temperature experiments. However, this difference is not large. Wightman et al. (2001) demonstrated that DOC from bacterial lysis and/or exudate material is largely proton-inactive. It is possible that a portion of this DOC material, although not proton-active, can bind aqueous Cd and/or Pb, but we assume that its presence at the

concentrations found in our experiments is unlikely to affect the speciation of the aqueous metals significantly.

4. Discussion

Although the adsorption data shown in Fig. 1 indicate that there are minimal changes in the extent of Cd adsorption onto *B. subtilis* and

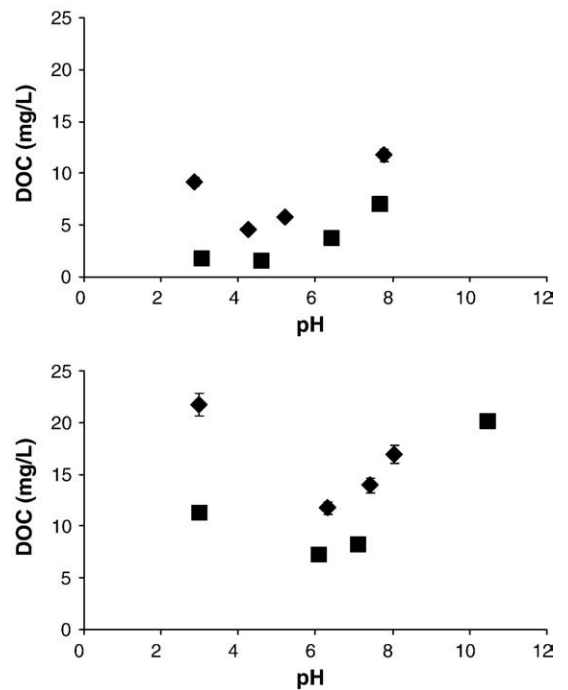


Fig. 2. Dissolved organic carbon concentrations from control experiments involving *B. subtilis* (top) and *P. mendocina* (bottom) at 5 °C (diamonds) and 80 °C (squares). Error bars indicate analytical uncertainties associated with the DOC measurements.

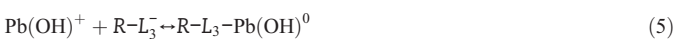
*P. mendocina* between 5 and 80 °C, we can not conclude from this evidence alone that there is no temperature dependence to the stability constants of the important metal–bacterial surface complexes. Changes in other thermodynamic parameters such as stability constants for aqueous metal–hydroxide complexes and the dissociation constant of water could offset changes in bacterial surface stability constants to yield no net change in the bulk extent of metal adsorbed by the bacteria. Therefore, in order to determine whether the bacterial surface stability constants change significantly with temperature, we explicitly calculate and compare stability constant values for the important metal–bacterial surface complexes in the experimental systems.

We calculate the best fitting stability constants for metal–bacterial surface complexes using FITEQL 2.0 (Westall, 1982), accounting for both surface and aqueous complexation reactions in the experimental system. Activity coefficients for aqueous species are calculated by FITEQL using the Davies equation (Westall, 1982), and the program calculates an output variance parameter,  $V(Y)$ , to constrain the goodness of fit between a model and the experimental data. In general, a  $V(Y)$  value less than 20 indicates a reasonable fit between a model and experimental data (Westall, 1982). We use the non-electrostatic 4-site surface protonation model proposed by Fein et al. (2005) for *B. subtilis*, and the non-electrostatic 4-site surface protonation model proposed by Borrok and Fein (2005) for *P. mendocina* to account for the protonation state of the cell wall functional groups on each species of bacteria. In these models, functional group speciation is described by the following reaction stoichiometry:



where  $L_i$  represents one of the four distinct site types active on the cell wall, and  $R$  represents the cell wall macromolecule to which each functional group is attached. The equilibrium constant for reaction (1) is the acidity constant for the site type in question. The pKa (negative logarithm of the acidity constant) values for the four sites on *B. subtilis* are 3.3, 4.8, 6.8, and 9.1, respectively, and the corresponding site concentrations are  $8.1 \times 10^{-5}$ ,  $1.1 \times 10^{-4}$ ,  $4.4 \times 10^{-5}$ , and  $7.4 \times 10^{-5}$  mol of sites/g wet mass bacteria, respectively. The pKa values for the four sites on *P. mendocina* are 3.4, 4.7, 6.5, and 9.3, respectively, and the corresponding site concentrations are  $9.6 \times 10^{-5}$ ,  $1.2 \times 10^{-4}$ ,  $5.1 \times 10^{-5}$ , and  $8.1 \times 10^{-5}$  mol of sites/g wet mass bacteria, respectively. We refer to these sites as Sites 1–4, respectively.

The Cd and Pb adsorption data provide constraints on the identity and thermodynamic stabilities of the important metal–bacterial surface complexes. We tested models that involved the interaction of the metal cations present in the experimental systems with the deprotonated form of Sites 1, 2, and 3 on the bacterial surface, according to the following reactions:



where  $M^{2+}$  represents either  $Cd^{+2}$  or  $Pb^{+2}$ . Reaction (5) is written only for the adsorption of  $Pb(OH)^+$  because the concentration of  $Cd(OH)^+$  is

never significant in the pH range of our experiments. However, with increasing temperature, Pb–hydroxide complexes increase in importance in the total aqueous Pb budget, especially at higher pH. In fact, at 80 °C,  $Pb(OH)^+$  is the dominant form of Pb in solution above pH 7. Therefore, the best-fitting models require adsorption of  $Pb(OH)^+$  onto one of the deprotonated bacterial sites. Above pH 7, Sites 1, 2, and 3 exist predominantly as deprotonated sites and, therefore, are the most likely to play a role in  $Pb(OH)^+$  adsorption. However, our data can not uniquely constrain the distribution of  $Pb(OH)^+$  between the sites on the bacterial cell wall. We arbitrarily ascribe all adsorption of  $Pb(OH)^+$  onto Site 3 of the bacterial cell wall and determine the temperature dependence of the stability constant for this complex. The results of this exercise should not be taken to represent the actual distribution of  $Pb(OH)^+$  between the different bacterial cell wall sites, but the modeling does serve to demonstrate the effect of temperature on the importance of  $Pb(OH)^+$  adsorption relative to that of  $Pb^{+2}$  adsorption on the cell wall.

Solving for the stability constants ( $K$  values) for the important bacterial surface complexes (Reactions (2)–(5)) requires a knowledge

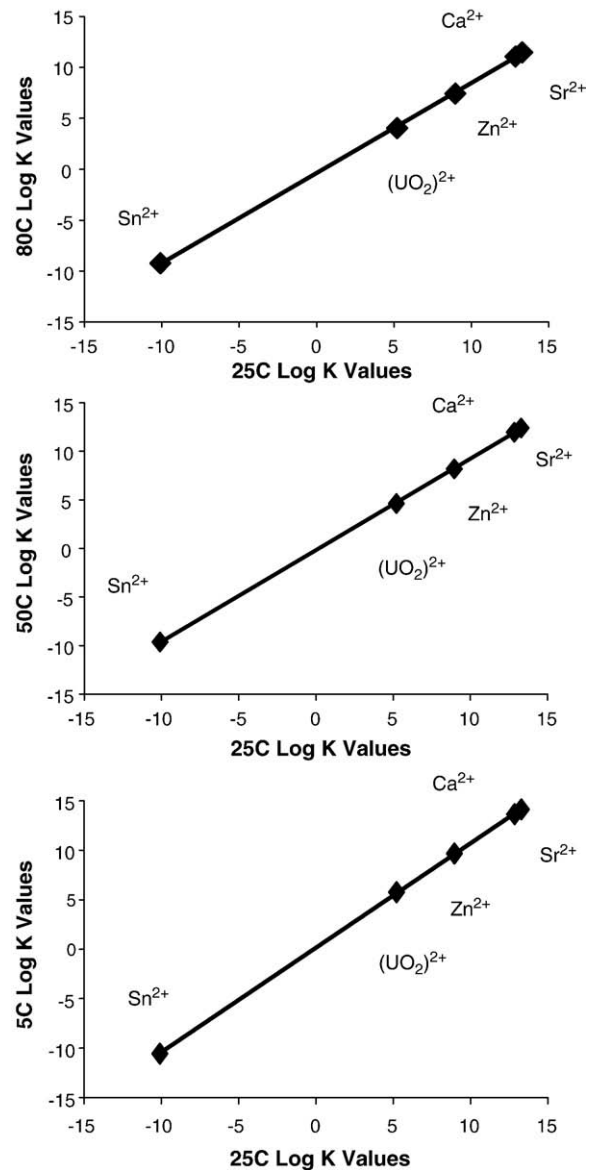


Fig. 3. Relationships between the 25 °C pK values for the first hydrolysis reaction (Eq. (6) in text) and the corresponding values for a range of metal cations at 80 °C (top); 50 °C (middle); and 5 °C (bottom).

Table 1

Species	Metal	Temperature	Log K (2) <sup>a</sup>	Log K (3) <sup>b</sup>	Log K (4) <sup>c</sup>	Log K (5) <sup>d</sup>	V(Y)
<i>B. subtilis</i>	Cd	80 °C	N/A	4.1±0.1	6.1±0.5	N/A	0.1
		50 °C	N/A	4.3±0.1	5.8±0.5	N/A	0.3
		5 °C	N/A	4.3±0.1	5.7±0.5	N/A	0.3
	Pb	80 °C	N/A	4.7±0.1	5.7±0.7	8.0±0.6	1.7
		50 °C	N/A	4.6±0.1	5.6±0.7	6.9±0.6	1.7
		5 °C	N/A	4.5±0.1	6.5±0.7	5.9±0.6	1.1
<i>P. mendocina</i>	Cd	80 °C	N/A	3.1±0.1	5.6±0.5	N/A	0.3
		50 °C	N/A	4.0±0.1	5.6±0.5	N/A	0.2
		5 °C	N/A	4.3±0.1	5.4±0.5	N/A	0.4
	Pb	80 °C	N/A	N/A	6.6±0.5	7.5±0.6	0.3
		50 °C	N/A	4.8±0.1	6.3±0.5	6.6±0.6	1.8
		5 °C	4.6±0.1	4.4±0.1	6.1±0.5	N/A	2.5

<sup>a</sup> Log K for the reaction  $M^{2+} + R - L_1 \leftrightarrow R - L_1 - M^+$ .  
<sup>b</sup> Log K for the reaction  $M^{2+} + R - L_2 \leftrightarrow R - L_2 - M^+$ .  
<sup>c</sup> Log K for the reaction  $M^{2+} + R - L_3 \leftrightarrow R - L_3 - M^+$ .  
<sup>d</sup> Log K for the reaction  $M(OH)^+ + R - L_3 \leftrightarrow R - L_3 - M(OH)^0$ .

of the thermodynamic stabilities of the Cd- and Pb-hydroxide species at the temperatures of interest. These values are known for 25 °C (Martell and Smith, 1977), but the temperature dependence is not well-established. We apply the van't Hoff equation to the EQ3 (Wolery, 1991) database of 0 to 300 °C stability constants for a range of 1:1 aqueous metal-hydroxide complexes involving divalent cations ( $Sr^{+2}$ ,  $Zn^{+2}$ ,  $Ca^{+2}$ ,  $Sn^{+2}$ , and  $UO_2^{+2}$ ) to constrain the linear relationship between log K values

and reciprocal absolute temperature for each metal. Using these linear relationships to interpolate, we calculate 80 °C, 50 °C, and 5 °C stability constant values for the range of aqueous metal-hydroxide complexes considered, and plot them against the 25 °C stability constant for each complex (Fig. 3). We find that there are especially strong relationships ( $R^2=0.9997$ ,  $0.9999$ , and  $0.9999$ , respectively) between the interpolated 80 °C, 50 °C, and 5 °C stability constants and the known 25 °C values, with the relationships defined by the equations  $y=0.8833x-0.3703$  (for the 80 °C vs. 25 °C relationship),  $y=0.9416x-0.1810$  (for the 50 °C vs. 25 °C relationship), and  $y=1.0562x+0.1462$  (for the 5 °C vs. 25 °C relationship). In the above equations, y represents the log of the 80 °C, 50 °C, or 5 °C stability constant value, and x represents the log of the 25 °C stability constant value. In each case, the reaction stoichiometry corresponds to the following:



where M represents the divalent cation of interest. We use these relationships to estimate the unknown 80 °C, 50 °C, and 5 °C stability constant values for the aqueous Cd- and Pb-hydroxide complexes based on the known 25 °C stability constants for these complexes. These calculations yield 80 °C, 50 °C, and 5 °C log stability constant values of -8.5, -9.3, and -10.8 for the Cd-hydroxide complex, and -6.4, -7.1, -8.3 for the Pb-hydroxide complex, respectively.

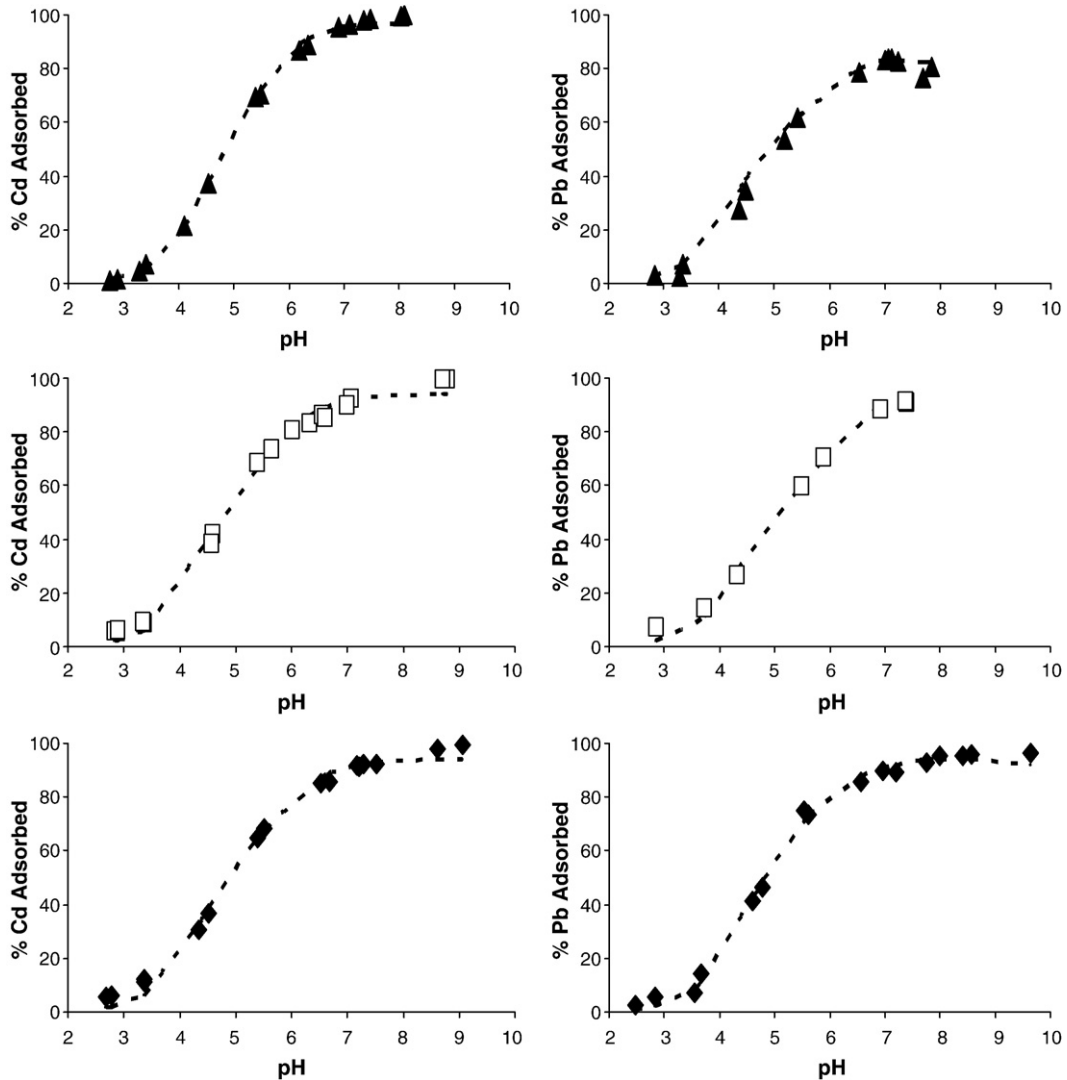


Fig. 4. Best-fitting models (dashed curves) of the Cd (left) and Pb (right) adsorption measurements involving *B. subtilis* at 80 °C (top), 50 °C (middle), and 5 °C (bottom).

The Cd and Pb adsorption data provide constraints on the thermodynamic stabilities of the important metal–bacterial surface complexes. In our approach, we determine the goodness-of-fit of models that involve Cd or Pb adsorption onto between one and four of the proton active sites on the bacterial cell walls. We used FITEQL's variance parameter,  $V(Y)$ , to compare quantitatively the relative fits of each model to the experimental data. In each case, we modeled the data with as few binding sites as were required in order to yield a reasonable fit to the data, and we attempted to fit the data with all possible combinations of binding sites. Because each site type is proton-active under different pH conditions from the other site types, the pH dependence of adsorption could be used to constrain the important binding mechanisms.

For the Cd systems for both bacterial species, models that involve adsorption of aqueous  $\text{Cd}^{+2}$  onto the deprotonated forms of Sites 2 and 3 yielded, on average, the best-fits to the experimental data; for these systems, models involving adsorption of Cd onto only one site, or onto other combinations of two or more sites, yielded significantly worse fits or failed to converge, indicating overconstraint by the model. For the Pb systems for both bacterial species, except for the 80 °C Pb–*P. mendocina* data, models involving  $\text{Pb}^{+2}$  binding onto the deprotonated forms of Sites 2 and 3 also provide the best fits to the experimental data. Because the extent of Pb adsorption at 80 °C onto *P. mendocina* is so low below pH 4, binding of  $\text{Pb}^{+2}$  onto Site 2 was not needed to account for the observed

adsorption behavior. However, the increased Pb adsorption observed at 5 °C relative to the other temperatures under low pH conditions required a model that invokes  $\text{Pb}^{+2}$  adsorption onto Site 1 (the only site that is proton-active under low pH conditions). In all other cases that we studied, adsorption under these low pH conditions is minimal, so metal binding onto Site 1 was not required to account for the observed extents of adsorption. With increasing temperature,  $\text{Pb}(\text{OH})^+$  becomes the dominant aqueous Pb species, and this was reflected in the modeling results. Each Pb system, except for the 5 °C Pb–*P. mendocina* experiments, required the inclusion of  $\text{Pb}(\text{OH})^+$  adsorption onto the deprotonated form of Site 3 to account for the observed Pb adsorption behaviors.

The thermodynamic modeling results are compiled in Table 1 for both bacterial species, and the model fits are depicted in Fig. 4 for *B. subtilis* and in Fig. 5 for *P. mendocina*. In each case, the adsorption model provides an excellent fit to the experimental data over the entire pH range studied. In Table 1, we compile the log  $K$  values for the important adsorption reactions in each model, and we also list the  $V(Y)$  values associated with each model. Each experiment was performed twice, and we modeled each data set separately. The uncertainties in  $K$  values were determined by finding the range of  $K$  values that fit both data sets within the certainties of our measurements. In these calculations, we used our estimated stability constants for the Cd- and Pb-hydroxide aqueous complexes. We used the 25 °C cell wall

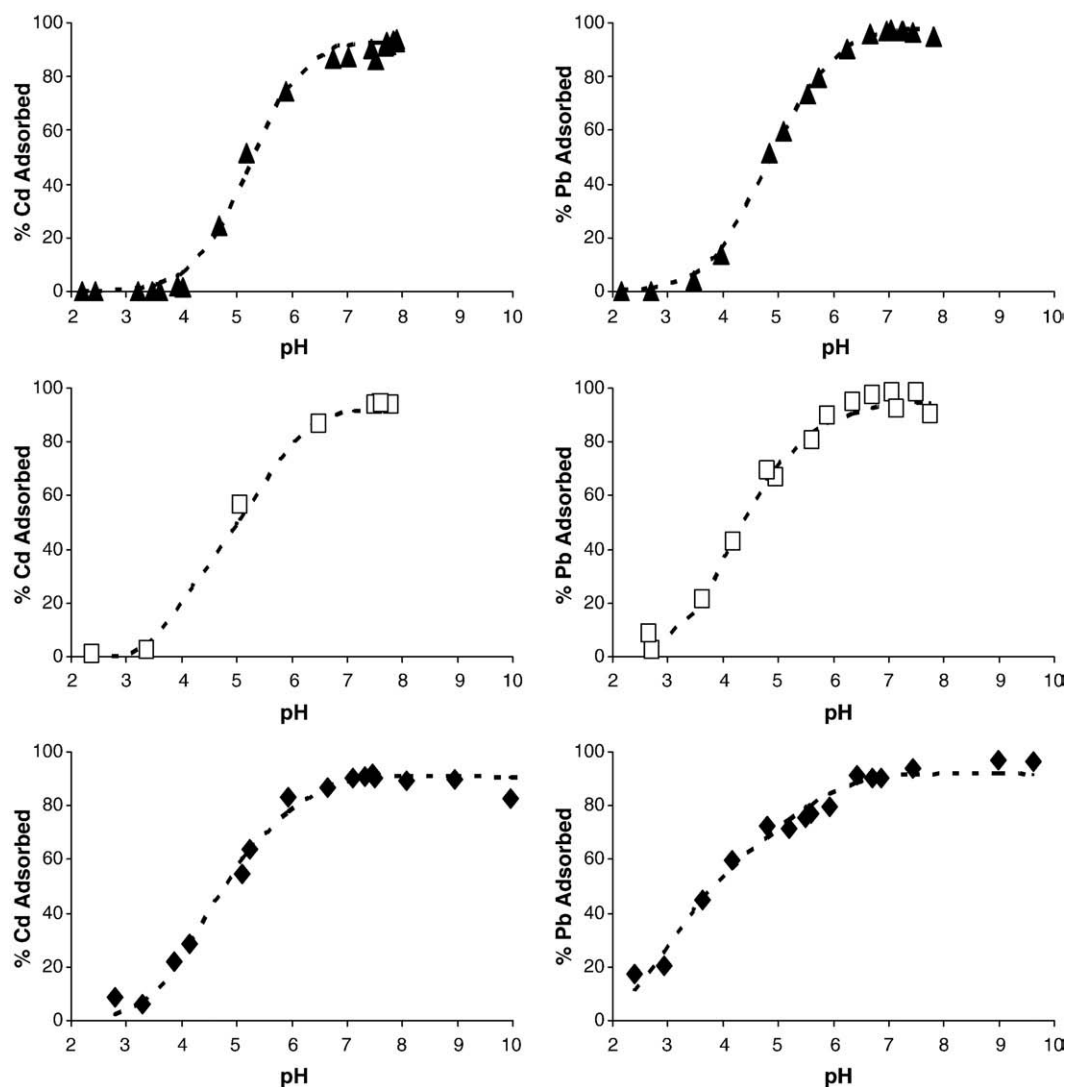


Fig. 5. Best-fitting models (dashed curves) of the Cd (left) and Pb (right) adsorption measurements involving *P. mendocina* at 80 °C (top), 50 °C (middle), and 5 °C (bottom).

acidity constants for each species for each temperature of interest because the stability constants for the protonation of the cell wall surface do not change significantly over the temperature range studied (Wightman et al., 2001).

For Cd adsorption onto *B. subtilis*, the log *K* values for each reaction do not change significantly as a function of temperature. The log *K* values for Pb adsorption onto Site 2 on *B. subtilis* also do not change significantly with temperature. Similarly, the temperature dependence for the log *K* (4) values for Pb adsorption onto Site 3 on *B. subtilis* remains constant within experimental uncertainty with increasing temperature. Conversely, *K*(5) values increase with increasing temperature, reflecting the fact that Pb(OH)<sup>+</sup> adsorption becomes more important relative to Pb<sup>+2</sup> adsorption as temperature increases. For Cd adsorption onto Site 3 on *P. mendocina*, the calculated *K* (4) values are not sensitive to temperature, but *K* (3) decreases with increasing temperature. As was the case with Pb adsorption onto *B. subtilis*, the calculated *K* (5) values for Pb(OH)<sup>+</sup> adsorption onto Site 3 on *P. mendocina* increase with increasing temperature, indicating that Pb(OH)<sup>+</sup> adsorption onto the bacterial surface becomes more important as temperature increases.

The slight temperature dependence that we observed in the extent of Cd and Pb adsorption onto *P. mendocina* under low pH conditions may be caused by the effect of acid on a feature present exclusively in the Gram-negative bacterial cell wall. *P. mendocina* does not exhibit metal-binding temperature dependence above pH 5.2, and the temperature trend is also not exhibited in the experiments conducted using the Gram-positive *B. subtilis* species. The only difference between the *P. mendocina* and *B. subtilis* experiments was the species of bacteria present in each system. Therefore, the difference in the low pH temperature dependences between *P. mendocina* and *B. subtilis* is most likely due to differences between the two bacterial cell wall types. Gram-positive bacteria have a thick cell wall primarily consisting of peptidoglycan. Gram-negative bacteria have a relatively thin cell wall surrounded by a lipopolysaccharide (LPS) outer membrane. The most likely difference between the behavior of *B. subtilis* and *P. mendocina* is due to response of the outer membrane to changes in temperature.

The fluidity of both the inner and outer membranes decreases with decreasing temperature (Kumar et al., 2002; Janoff et al., 1979; Nakayama et al., 1980). Bacteria typically respond to changes in membrane fluidity by altering the length of the fatty acid chains in their LPS, by changing the number of unsaturated carbons in the fatty acids, and by changing the number of branches in their fatty acid chains (Kumar et al., 2002). At a critical temperature, the outer membrane undergoes a phase transition from a liquid crystalline material to a solid gel (Janoff et al., 1979; Nakayama et al., 1980). Brandenburg and Seydel (1990) used Fourier transform infrared spectroscopy to determine that the LPS extracted from several bacterial species, including one *Pseudomonas* species, had transition temperatures ranging from 30 °C–40 °C, and that the transition temperature strongly depends on pH, with higher phase transition temperatures occurring under lower pH conditions. Brandenburg and Seydel (1990) demonstrated that the LPS exhibits increased rigidity at low pH as well.

Fluidity changes and phase transitions may be responsible for the adsorptive behavior exhibited by *P. mendocina*. The increase in adsorption at low temperature for pH < 5 may arise because the outer membrane undergoes a phase transition which alters its reactive properties by changing the binding energies of metal cations to membrane sites and/or by altering the site concentrations on the membrane. The effects of these fluidity changes may be minimal in natural environments with actively metabolizing bacteria because metabolizing bacteria can alter their membranes in order to reduce these effects (Russell, 1997).

## 5. Conclusions

This study examines Cd and Pb adsorption onto *B. subtilis* and *P. mendocina* cells at 5 °C, 50 °C, and 80 °C. At these temperatures, both species of bacteria exhibit nearly identical extents of metal binding as

a function of temperature under most of the conditions of this study. However, at 5 °C and at pH < 5, we observed enhanced Cd and Pb adsorption onto *P. mendocina* cells relative to that observed at the higher temperatures. This increase in adsorption is small and would not have an impact on contaminant transport models. Under higher pH conditions, the effect of temperature on adsorption depends both on the metal and on the bacterial species of interest. For Cd, a metal that does not hydrolyze to a significant extent over the pH range of this study, we observed no significant temperature dependence to the extent of adsorption or to the speciation or thermodynamic stability of the important Cd surface complexes. Therefore, we conclude that a single set of averaged *K* values can be used to account for Cd adsorption onto bacterial cells as a function of temperature over the temperature range studied here. Conversely, Pb more readily hydrolyzes in solution than does Cd and its hydrolysis products become more stable with increasing temperature. Therefore, although the extent of total Pb adsorption does not change with temperature above pH 5, the speciation of the important Pb-bacterial surface complexes do depend on temperature. For metals such as Pb, the effect of temperature on the stability constants of the bacterial surface complexes must be determined in order to accurately model metal speciation and transport as a function of temperature in bacteria-bearing geologic systems.

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