The effect of Fe on Si adsorption by *Bacillus subtilis* cell walls: insights into non-metabolic bacterial precipitation of silicate minerals

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Abstract

Si adsorption onto *Bacillus subtilis* and Fe and Al oxide coated cells of *B. subtilis* was measured both as a function of pH and of bacterial concentration in suspension in order to gain insight into the mechanism of association between silica and silicate precipitates and bacterial cell walls. All experiments were conducted in undersaturated solutions with respect to silicate mineral phases in order to isolate the important adsorption reactions from precipitation kinetics effects of bacterial surfaces. The experimental results indicate that there is little association between aqueous Si and the bacterial surface, even under low pH conditions where most of the organic acid functional groups that are present on the bacterial surface are fully protonated and neutrally charged. Conversely, Fe and Al oxide coated bacteria, and Fe oxide precipitates only, all bind significant concentrations of aqueous Si over a wide range of pH conditions. Our results are consistent with those of Konhauser et al. [Geology 21 (1993) 1103; Environ. Microbiol. 60 (1994) 49] and Konhauser and Urrutia [Chem. Geol. 161 (1999) 399] in that they suggest that the association between silicate minerals and bacterial surfaces is not caused by direct Si–bacteria interactions. Rather, the association is most likely caused by the adsorption of Si onto Fe and Al oxides which are electrostatically bound to the bacterial surface. Therefore, the role of bacteria in silica and silicate mineralization is to concentrate Fe and Al through adsorption and/or precipitation reactions. Bacteria serve as bases, or perhaps templates, for Fe and Al oxide precipitation, and it is these oxide mineral surfaces (and perhaps other metal oxide surfaces as well) that are reactive with aqueous Si, forming surface complexes that are the precursors to the formation of silica and silicate minerals. © 2002 Elsevier Science B.V. All rights reserved.

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1. Introduction

Both field and laboratory studies have documented close associations between bacteria and pre-cipitated silica, as well as Fe and Al silicates (Ferris et al., 1986, 1987; Konhauser et al., 1993, 1994; Urrutia and Beveridge, 1993, 1994; Schultze-Lam et al., 1995; Konhauser and Ferris, 1996; Fortin and Ferris, 1998; Konhauser et al., 1999). These precipitates are often amorphous when they first form (Urrutia and Beveridge, 1994; Fortin and Ferris, 1998), yet they can serve as precursors to clay minerals

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upon aging (Ferris et al., 1987; Konhauser et al., 1993, 1994). The formation of these minerals on bacterial cell walls can have implications not only for microfossil formation, but it can represent an important sink for a wide variety of elements in bacteria-bearing water–rock systems (Beveridge and Fyfe, 1985; Schultze-Lam et al., 1995; Konhauser and Ferris, 1996). Furthermore, the formation of these precipitates may represent a fundamental process in the weathering of primary rock to mature soil and in the formation of low temperature clay horizons (e.g., Konhauser et al., 1993, 1994). In order to better understand these geochemical processes, it is crucial to determine the mechanism of mineral formation and the role of bacteria in affecting the mineralization.

Organic acid functional groups on bacterial cell walls can adsorb and concentrate a wide range of metal cations (e.g., Beveridge and Murray, 1976, 1980; Beveridge and Koval, 1981; Crist et al., 1981; Harvey and Leckie, 1985; Gonçalves et al., 1987), and it has been proposed that the formation of the silicate precipitates is caused by cell wall adsorption and concentration processes (e.g., Ferris et al., 1987; Konhauser et al., 1993, 1994; Urrutia and Beveridge, 1993). In this scenario, Si, Fe, and/or Al atoms concentrate at the cell wall surface by adsorbing onto surface functional groups. One possible explanation for the association between bacteria and these Fe, Al silicates is that bacterial surface accumulation causes an elevation of the activities of the mineral-forming elements, creating local supersaturation and precipitation on the cell wall. If this process is responsible for mineralization, then the bacterial cell walls would cause precipitation in otherwise undersaturated solutions, thereby enhancing the extent of precipitation in the overall system. Conversely, the association between bacteria and these precipitates may be due to bacterial enhancement of precipitation kinetics from saturated solutions. In this study, we concentrate on the adsorption process only in order to provide constraints on the mechanism of mineralization.

Although studies have demonstrated a strong interaction between aqueous cationic Fe and Al and the functional groups on cell walls (e.g., Fein et al., 1997; Daughney et al., 1998; Warren and Ferris, 1998), the mechanism of Si interaction is uncertain. Si may be incorporated into the precipitates either due to direct interaction with the cell wall (e.g., Urrutia and Beveridge, 1993, 1994), or due to interaction with adsorbed or precipitated cations (e.g., Konhauser et al., 1993, 1994). Although field studies have clearly indicated that Fe, Al silicate precipitation occurs on bacterial surfaces, neither field studies nor laboratory investigations have constrained the precipitation process. Urrutia and Beveridge (1993, 1994) propose that silica anion adsorption onto amine surface functional groups may be responsible for the Si concentration by the bacteria, but enhanced Si uptake in the presence of aqueous cations such as Fe and Al suggests that cationic bridging may also play a role (Urrutia and Beveridge, 1993, 1994). Although these and other studies provide some constraints on Si adsorption by bacteria, because they were conducted with oversaturated systems, adsorption and precipitation reactions occurred simultaneously during the experiments, and precise determination of adsorption or precipitation mechanisms are not possible from the data.

Although bacterial cell walls can concentrate aqueous cations through adsorption, there is no direct evidence that such adsorption leads to precipitation in bulk solutions that are undersaturated. Warren and Ferris (1998) demonstrate that the formation of hydrous ferric oxides (HFO) is influenced by bacterial surfaces, and that there is a relationship between iron adsorption and HFO precipitation. However, their data support the interpretation that bacteria serve merely to enhance the precipitation kinetics of solids in supersaturated solutions. The cell wall functional groups act as nucleation sites, but the presence of the bacteria does not increase the extent of precipitation. Fowle and Fein (2001) conducted a study of aqueous Cu removal by non-metabolizing bacteria as a function of bulk solution saturation state and determined that bacteria do not enhance the extent of precipitation. The extent of precipitation of amorphous Cu hydroxide was identical in bacteria-bearing and abiotic control systems, and precipitation did not occur under undersaturated conditions.

One problem in identifying the precipitation mechanism of silicates arises because all experimental and field observations of bacterially associated precipitates have been made in supersaturated systems. Under these conditions, both surface area kinetic enhancements and adsorption/concentration

reactions occur. In this study, we isolate the adsorption reactions by only examining undersaturated solutions. The experimental results do not determine which precipitation scenario is correct. However, whether kinetic enhancements or adsorption/concentration cause bacterial associations with silicate precipitates, adsorption of Si, Fe, and Al constitutes a preliminary process to mineralization and, therefore, it is important to determine how the pertinent cations interact with bacterial cell walls. We examine sulfate adsorption onto bacterial cell walls in order to determine if anion binding to cationic surface sites is possible, and we also measure Si adsorption onto cell walls to determine if direct Si–bacteria interactions occur. We compare these results with measurements of Si adsorption onto Fe and Al oxide coated bacteria to determine the most likely controls on Si association with bacterial cell walls.

2. Experimental procedures

All experiments conducted in this study were batch adsorption experiments using a non-complexing 0.1 M $NaClO_4$ background electrolyte to buffer ionic strength. Five sets of experiments were conducted: (1) aqueous SO_4^{-2} adsorption onto a bacterial surface; (2) aqueous Si adsorption onto a bacterial surface; (3) aqueous Si adsorption onto a hydrous ferric oxide (HFO)-coated bacterial surface; (4) aqueous Si adsorption onto a HFO precipitate only; and (5) aqueous Si adsorption onto an aluminum oxide coated bacterial surface. All bacteria-bearing experimental systems used *Bacillus subtilis* as the bacterial species, following the growth and wash procedures previously reported by our research group (e.g., Fein et al., 1997), except the wash procedure consisted of washes only of a 1-h soak in pH 2 HNO_3, followed by five washes in the experimental electrolyte. *B. subtilis* is a Gram-positive species, the cell wall characteristics and charging properties of which are well-characterized (Beveridge and Murray, 1980; Fein et al., 1997). Gram-positive cell walls do not contain the outer membrane extracellular polysaccharides found on Gram-negative cell walls and, therefore, represent the most simple starting point for examining adsorp-

tion reactions. During the experiments, the bacterial cells remain intact, but are non-metabolizing and, therefore, the results reflect cell wall processes only. We report bacterial concentrations as standardized wet weight values (after 1 h of centrifugation at 7500 rpm and removal of supernatant water), values that are 9.9 ± 1.1 times the dry weight of the biomass (Fein and Delea, 1999).

The sulfate experiments were conducted with 20 ppm SO_4^{-2} initially present in solution, added from a sulfuric acid volumetric standard. Sulfate adsorption was measured as a function of bacterial concentration in suspension at pH 2.3 and 3.2, with pH adjusted using small additions of concentrated HNO_3. The experimental systems were allowed to equilibrate for 4 h, and then were centrifuged and filtered through 0.45-μm membranes. Si adsorption was measured in a similar fashion, using 1.4 ppm Si in the initial solution, and adsorption was measured as a function of both pH and bacterial concentration.

Si adsorption onto HFO-coated bacteria was measured by conducting similar experiments to the Si adsorption measurements described above, using bacteria that were first exposed to a low pH 5×10^{-3} M ferric nitrate solution. This concentration was chosen because it was in slight excess of the calculated total site concentration of organic functional groups on the bacterial surface (as determined by the titrations of Fein et al., 1997). The pH of the bacteria–ferric nitrate system was incrementally increased by small additions of NaOH until the pH of the suspension reached approximately 6.0, causing substantial precipitation of hydrous ferric oxide onto the bacterial surfaces. X-ray diffraction analysis of the precipitate indicated that the hydrous ferric oxide precipitates were poorly ordered ferrihydrite, a result consistent that of Swedlund and Webster (1999) who formed ferrihydrite following a similar technique. The HFO-coated bacteria were removed from the precipitating solution via centrifugation and rinsed two times in 0.1 M $NaClO_4$ prior to use in the Si adsorption experiments. Si uptake was measured as a function of pH at two bacterial concentrations (75 and 2.5 g bacteria/l) and as a function of bacterial concentration at pH 5.2. Si adsorption onto HFO alone was also measured to determine if the bacteria affected uptake. In these experiments, HFO was precipitated following the same procedure used to

coat the bacteria, only precipitation was done in a bacteria-free solution. Si adsorption was measured as a function of pH, using a HFO concentration of 2.22 g/l. Si adsorption onto Al oxide coated bacteria was measured in experiments similar to the ones involving HFO-coated bacteria, except Al oxide was precipitated onto the bacterial surfaces by first exposing the bacteria to a low pH 5×10^{-3} M Al solution, and incrementally adjusting pH to 7.3, allowing the precipitating suspension to equilibrate overnight. After two washes in 0.1 M $NaClO_4$, Si adsorption was measured as a function of solid concentration at pH 6.0. Sulfate (measured as total S) and Si concentrations in the samples were measured by ICP–AES, with an analytical uncertainty of $\pm 6\%$ and $\pm 2\%$, respectively. All element standards were made using the same electrolyte as was used in the experiments in order to avoid potential matrix effects.

3. Results

Control experiments conducted without either a bacterial or mineral sorbent demonstrated that sulfate and Si adsorption onto the experimental apparatus is negligible. Therefore, we assume that the difference between the starting elemental (either S or Si) concentrations and the measured final concentration in each sample is due entirely to adsorption, and we represent the data in terms of percentage of the
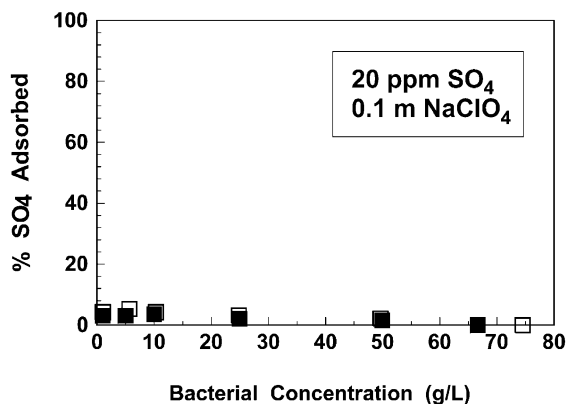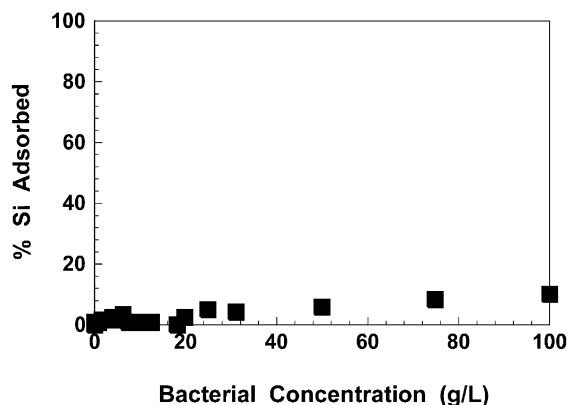
Fig. 2. Si adsorption onto *B. subtilis* at pH 5.2. Initial Si concentration was 1.4 ppm Si. The electrolyte used was 0.1 m $NaClO_4$.

original concentration that is adsorbed. Fig. 1 depicts the results from the sulfate adsorption experiments, and at both pH values studied, there is negligible adsorption of sulfate onto the bacterial surface, even to extremely high concentrations of bacteria (75 g bacteria/l).

Si adsorption onto the surface of *B. subtilis*, like that of SO_4^{-2}, is negligible under low to moderate bacterial concentrations (< 20 g bacteria/l) at pH 5.2 (Fig. 2). Under higher bacterial concentrations, adsorption becomes significant, with a slightly increasing extent of adsorption with increasing bacterial concentration. At 100 g bacteria/l, 8.3% of the initial aqueous Si adsorbed onto the *B. subtilis* surface. In contrast to this weak adsorption behavior, Fig. 3 depicts virtually complete removal of Si from solution when the HFO-coated bacteria are used as the experimental sorbent at a concentration of 75 g/l, and removal is pH independent over the pH interval studied (pH 3.6–7.3). The dependence of Si adsorption on the concentration of HFO-coated bacteria in pH 5.2 suspensions is shown in Fig. 4. We observed significant adsorption of Si even at the lowest concentrations of HFO-coated bacteria studied: at 0.1 and 0.6 g bacteria/l, 6.5% and 21.0% of the initial aqueous Si was removed from solution, respectively. Increasing the concentration of coated bacteria in the system increased the extent of adsorption over the entire concentration range studied.

Fig. 1. Sulfate adsorption onto *B. subtilis* at pH 2.3 (open squares) and 3.2 (filled squares).
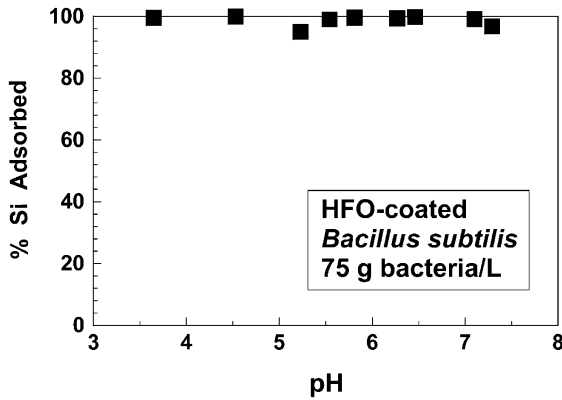
Fig. 3. Si adsorption onto HFO-coated *B. subtilis* cells as a function of pH with 75 g (wet weight) bacteria/l. Initial Si concentration was 1.4 ppm Si.
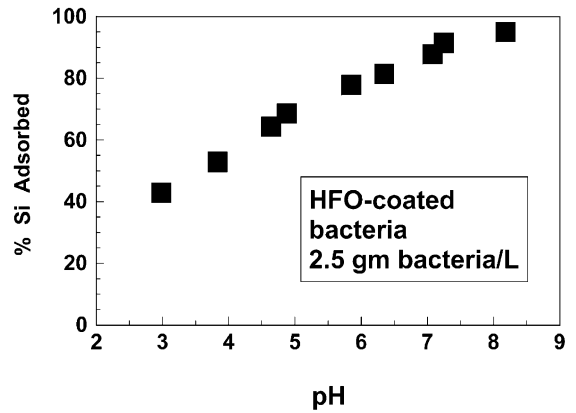

Fig. 5. Si adsorption onto HFO-coated *B. subtilis* cells as a function of pH with 2.5 g (wet weight) bacteria/l. Initial Si concentration was 1.4 ppm Si.

The pH dependence of Si adsorption onto 2.5 g/l of the HFO-coated *B. subtilis* cells is shown in Fig. 5. Adsorption is significant over the entire pH range studied (3.0–8.2), with the extent of adsorption increasing with increasing pH. A similar pH trend is depicted in Fig. 6, which shows the extent of Si adsorption onto the iron oxide precipitate only. The exact amount of iron oxide precipitate surface area present on the bacteria used in the coated bacteria experiments was not determined and, therefore, direct comparison of the extents of adsorption can not

be made between the HFO-coated bacteria experiments and those involving the precipitate only. However, the amount of iron oxide precipitate used yielded similar extents of adsorption to the HFO-coated bacteria experiments, and the pH trends in the two sets of experiments were very similar. Furthermore, the adsorption behavior of aqueous Si onto Al oxide coated bacteria at pH 6.0 (Fig. 7) is similar to that observed for the HFO-coated bacteria. Significant adsorption occurred even in the experimental systems containing 0.05 g bacteria/l, and adsorption
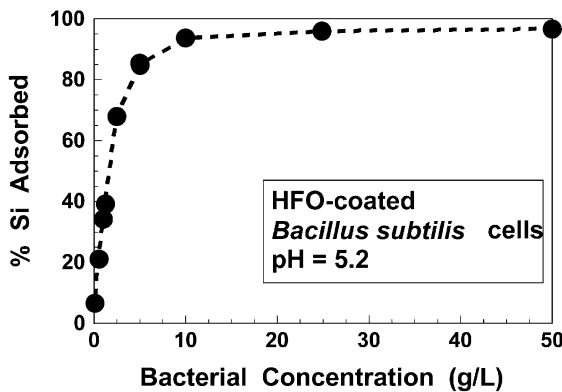

Fig. 4. Si adsorption onto HFO-coated *B. subtilis* cells as a function of bacterial concentration in suspension (values given in wet weight) at pH 5.2. Initial Si concentration was 1.4 ppm Si.
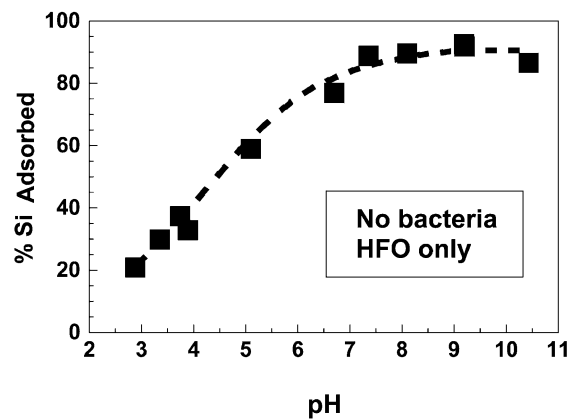

Fig. 6. Si adsorption onto HFO alone, with no bacterial cells present, as a function of pH with 2.22 g HFO/l. Initial Si concentration was 1.4 ppm Si.
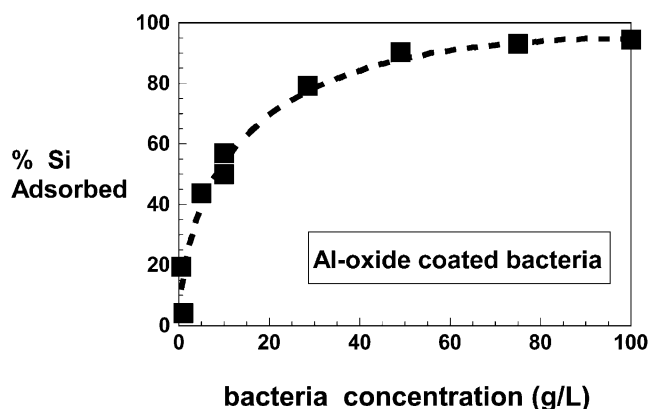
Fig. 7. Si adsorption onto Al-oxide coated *B. subtilis* cells as a function of bacterial concentration in suspension (values given in wet weight) at pH 6.0. Initial Si concentration was 1.4 ppm Si.

increased with increasing concentration of the bacteria–mineral sorbent.

4. Discussion

The lack of sulfate adsorption even at extremely high bacterial concentrations suggests that the amine functional groups on the surface of *B. subtilis*, if present, do not actively participate in anion adsorption. Beveridge and Murray (1980) showed that amine groups within the cell wall of *B. subtilis* do not contribute significantly to metal uptake by the bacteria. Furthermore, these sites also do not appear to contribute significantly to the overall surface charge of the cell wall of *B. subtilis*, which never becomes positively charged, even under extremely low pH conditions where all anionic sites are fully protonated and neutrally charged (Harden and Harris, 1953).

Si anions, as described by Urrutia and Beveridge (1993, 1994), exist in solution in significant quantities (> 1% of the total aqueous Si) only at pH values higher than approximately pH 8, and they only dominate the aqueous Si budget at pH values above approximately 9.5 (Iler, 1979). Under these conditions, the bacterial surface is highly negatively charged and the electrostatic repulsion between aqueous $HSiO_3^-$ and the cell wall is likely to prevent Si adsorption directly to the cell wall, even if positive amine groups exist within it under these condi-

tions. Likewise, organic acid anions do not adsorb onto bacterial surfaces under pH conditions that favor their deprotonation, while they do adsorb when protonated and neutrally charged (Daughney and Fein, 1998; Fein et al., 1999; Fein and Delea, 1999; Fein, 2000).

The lack of positively charged adsorption sites for anions under low pH conditions coupled with the electrostatic repulsion between anionic $HSiO_3^-$ and the bacterial cell wall suggests that the mechanism for Si uptake by bacterial cell walls during mineralization is not due to electrostatically controlled processes. Our measurements of aqueous Si adsorption onto the cell wall of *B. subtilis* suggest that the hydrophobic adsorption that causes neutrally charged organic acids to adsorb onto neutrally charged bacterial surfaces does not influence the adsorption of Si under most bacterial concentration conditions. We only observed significant Si adsorption under the most extreme bacterial concentrations. Under realistic concentrations, there is negligible direct interaction between neutrally charged $SiO_{2(aq)}^0$ and the cell wall. Therefore, it is unlikely that direct adsorption of aqueous Si onto the bacterial cell wall contributes to silicate biomineralization.

The only significant removal of Si from solution in our experiments occurred in either iron-or aluminum oxide bearing systems. This removal is most likely due to adsorption onto the mineral sorbents, with the extent increasing with increasing cell (and hence mineral coating) concentration. The pH trend

of the Si adsorption, which is identical whether the iron oxide is present on bacterial surfaces or not, also supports our conclusion that anionic aqueous Si is not responsible for the adsorption behavior. Fig. 8 illustrates the speciation of the iron oxide surface. Note that we have not conducted potentiometric titrations of the iron oxide precipitates formed in our experiments, so Fig. 8 should be viewed as a qualitative guide to the mineral surface speciation only. The dominant aqueous Si species in the pH range of the experiments is neutrally charged $SiO_{2(aq)}^0$, and the concentration of this species remains unchanged as a function of pH. Therefore, it is unlikely that the speciation of the aqueous Si controls the pH dependence of the adsorption behavior, and rather that the adsorption behavior is controlled by the speciation of the mineral surface. In this pH range, the iron oxide surface can be characterized using two surface species (e.g., Dzombak and Morel, 1990): $> FeOH_2^+$ and $> FeOH^0$. The fully deprotonated anionic surface site, $> FeO^-$, does not become significant under these pH conditions. Of these two surface species, only the neutrally charged site, $> FeOH^0$, exhibits an increase in concentration with increasing pH. Furthermore, the increase in $> FeOH^0$ site concentration mimics the increase in adsorbed Si concentration as a function of pH. Therefore, our data suggest that the aqueous Si adsorption is best accounted for by an interaction between $SiO_{2(aq)}^0$ and $> FeOH^0$.
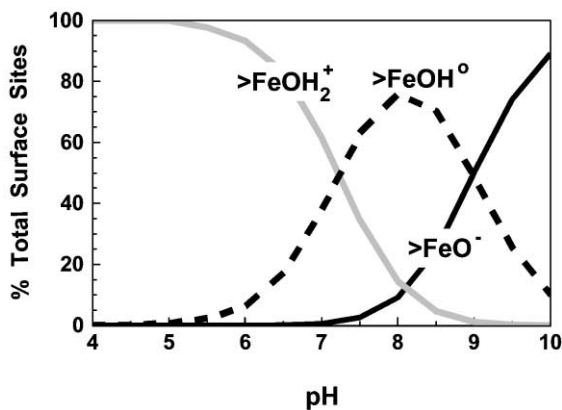

Fig. 8. Qualitative speciation diagram for the HFO surface in water, shown in terms of relative percentages of the three dominant surface site species as a function of pH. Calculations were conducted using the HFO surface acidity constant values given in Dzombak and Morel (1990).

Silicic acid adsorption onto ferrihydrite has been measured previously (Bruun Hansen et al., 1994; Swedlund and Webster, 1999). Between pH 3 and 5, Bruun Hansen et al. (1994) identified the dominant adsorption reaction as:

$$> FeOH^0 + SiO_{2(aq)}^0 \leftrightarrow > FeSiO_3H^0. \tag{1}$$
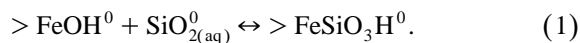
Swedlund and Webster (1999) also model Si adsorption onto ferrihydrite using reaction (1), but their data cover a wider pH range, and the following reactions become important with increasing pH:

$$> FeO^- + SiO_{2(aq)}^0 \leftrightarrow > FeSiO_3^{-1}, \tag{2}$$

$$> FeO^- + HSiO_3^{-1} \leftrightarrow > FeHSiO_4^{-2}. \tag{3}$$

Note that $SiO_{2(aq)}^0$ is thermodynamically equivalent to $H_4SiO_4^0$, without providing potentially misleading information concerning the number of solvating water molecules around the Si cation. Because we did not determine the amount, or spatial distribution/morphology, of the ferrihydrite on the bacterial surfaces, it is impossible to predict the amount of Si adsorption that would be expected using the stability constants for the adsorbed species proposed by Swedlund and Webster (1999). However, the pH trends of the two datasets are consistent. The increase in adsorption with increasing pH from pH 3 to 8 (Figs. 5 and 6) mimics the increase in $> FeOH^0$ site concentration on the ferrihydrite. Furthermore, the lack of decrease in Si adsorption above pH 8 (Fig. 6), where $> FeOH^0$ concentrations begin to decrease with increasing pH, indicates that an additional Fe–Si surface species becomes important under these pH conditions, and the stoichiometry proposed by Swedlund and Webster (1999) is consistent with our results.

5. Conclusions

The experimental results from this study demonstrate that, in undersaturated systems, aqueous Si does not adsorb significantly onto the surface of *B. subtilis*, except under the most extreme conditions of high bacterial concentrations where only slight amounts of adsorption were observed. Conversely, HFO-coated bacteria and iron oxides alone exhibit similar and strong affinities for $SiO_{2(aq)}^0$. The ob-

served adsorption of aqueous Si onto ferrihydrite (either on bacterial surfaces or alone) is consistent with an adsorption reaction involving $SiO_{2(aq)}^0$ and the neutrally charged HFO-coated surface site $> FeOH^0$. Adsorption of $SiO_{2(aq)}^0$ onto ferrihydrite above pH 8, where the concentration of $> FeOH^0$ sites decreases, is consistent with the Si adsorption model of Swedlund and Webster (1999) involving additional Fe–Si surface species under these higher pH conditions. We observed aqueous Si adsorption onto Al oxide coated bacteria, the extent of which was similar to that observed involving the ferrihydrite. Therefore, we hypothesize that the adsorption is caused by an analogous reaction to reaction (1):

$$> AlOH^0 + SiO_{2(aq)}^0 \leftrightarrow > AlSiO_3H^0. \qquad (4)$$
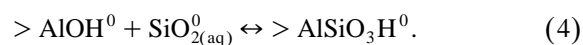
Because we did not measure the concentration of surface Al sites in the Al oxide coated bacteria experiments, it is impossible to use our data to constrain a value for the equilibrium constant for reaction (4).

Our experiments suggest that silica and silicate biomineralization does not result from direct specific Si interaction with the bacterial cell wall. The concentration of anionic aqueous $HSiO_3^-$ relative to neutrally charged $SiO_{2(aq)}^0$ at pH values below approximately 9 is vanishingly small, so adsorption of $HSiO_3^-$ onto positively charged amine-type bacterial surface sites can not explain the high degrees of association observed in natural and laboratory conditions. Furthermore, at least for *B. subtilis* and similar bacterial species, the concentration and/or reactivity of these amine-type sites is minimal, as shown by the lack of sulfate adsorption in our experiments over a wide range of bacterial concentrations under low pH conditions (where electrostatic repulsion between the sulfate and the anionic organic acid functional groups on the bacterial cell wall should be negligibly small).

The experiments in this study are consistent with the conclusions drawn from field observations by Konhauser et al. (1993, 1994) and summarized by Konhauser and Urrutia (1999), in that they suggest that the role of bacteria in silica and silicate mineralization is to concentrate Fe and Al through adsorption and/or precipitation reactions. Bacteria serve as bases, or perhaps templates, for Fe and Al oxide precipitation. It is these oxide mineral surfaces (and

perhaps other metal oxide surfaces as well) that are reactive with aqueous Si, with a significant amount of adsorption occurring in even undersaturated conditions over a wide range of pH conditions. The same type of adsorption reactions should operate in oversaturated bacteria–water–rock systems, and the precursors to the formation of silica and silicate mineral precipitates are likely to be the adsorbed Si species on the oxide mineral surfaces. Fe and Al oxides have extremely low solubilities and positive surface charges under near-neutral pH conditions, and would likely accumulate, or potentially even nucleate, on bacterial surfaces which exhibit a significant negative charge under these conditions (e.g., Yee et al., 2000). Therefore, the role of the bacteria in this form of 'biomineralization' is indirect. Silica and silicates tend to be associated with bacterial surfaces not due to direct Si interactions with the bacteria, but rather because Si is highly reactive with the Fe and Al oxides which commonly precipitate and become associated with the bacterial surfaces themselves. This work does not quantify the potential role bacterial surfaces play in enhancing the kinetics of precipitation in oversaturated solutions, and that role may be responsible for at least some of the associations between silicate minerals and bacterial surfaces. However, this study demonstrates that non-kinetic effects can give rise to a concentration of Si, Fe, and Al at the bacterial surface in undersaturated systems. This concentration of silicate-forming cations may not cause precipitation under undersaturated conditions, but it is likely to explain why silicates are associated with bacterial cell walls in oversaturated systems.

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