Experimental study of iron and silica immobilization by bacteria in mixed Fe–Si systems: implications for microbial silicification in hot springs[1]

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Abstract: The immobilization of silica and iron by the bacteria *Bacillus subtilis* was monitored in controlled microcosms to elucidate the role iron may play in aiding bacterial silicification in hot springs. Silica and iron immobilization was monitored as a function of bacterial concentration, iron concentration, and silica concentration (both undersaturated and oversaturated with respect to amorphous silica). Results demonstrate that bacterial cells do immobilize more Fe than bacteria-free systems in solutions with iron concentrations ≤50 ppm Fe. However, as iron concentrations increase, the difference between Fe immobilization in bacterial and bacteria-free systems decreases as non-bacterially mediated precipitation processes dominate. Additionally, bacterial systems that had immobilized more Fe compared with bacteria-free systems did not immobilize more silica than bacteria-free systems. By comparing molar ratios of (silica in solution)/(bacterially bound Fe), it is evident that insufficient iron is bound to the bacterial surface to act as an effective salt bridge for silica sorption. This appears to be because much of the iron is immobilized by non-bacterially mediated precipitation of phases such as $Fe(OH)_3$ and poorly ordered hydrous iron silicates. It follows that in silica-enriched hot springs, silica and iron immobilization processes are significantly dominated by non-bacterially mediated precipitation. Any bacterially mediated processes are exceedingly small and outside the resolution of these experiments.

Résumé : L'immobilisation de la silice et du fer par la bactérie *Bacillus subtilis* a été suivie dans des microcosmes contrôlés afin d'élucider le rôle que pourrait jouer le fer dans la silicification des bactéries dans les sources thermales. L'immobilisation de la silice et du fer a été suivie en fonction de la concentration bactérienne, de la concentration en fer et de la concentration en silice (sous-saturée et sursaturée par rapport à la silice amorphe). Les résultats démontrent que les cellules bactériennes immobilisent en effet plus de Fe que les systèmes sans bactéries dans des solutions où les concentrations de fer ≤ 50 ppm Fe. Toutefois, à mesure que s'accroît la concentration de fer, la différence entre l'immobilisation du Fe dans les systèmes avec ou sans bactéries décroît alors que dominent les processus de précipitation non assistés par les bactéries. De plus, les systèmes bactériens qui avaient immobilisé des quantités supérieures de Fe comparativement aux systèmes sans bactéries n'ont pas immobilisé plus de silice que les systèmes sans bactéries. En comparant les rapports molaires (silice en solution)/(Fe lié par les bactéries), il est évident qu'il n'y a pas assez de fer lié à la surface bactérienne pour agir en tant que pont de sel pour la sorption de la silice. Cela serait dû au fait qu'une grande partie du fer est immobilisée par la précipitation, non assistée par les bactéries, de phases telles que $Fe(OH)_3$ et des silicates de fer hydraté mal organisés. Il s'en suit que, dans les sources thermales enrichies en silice, les procédés d'immobilisation du fer et de la silice sont dominés de façon significative par une précipitation non assistée par les bactéries. Tous les procédés assistés par les bactéries sont extrêmement petits et sont en dehors du degré de résolution de ces expériences.

[Traduit par la Rédaction]

Introduction

Silica immobilization by bacteria is found in abundance in a number of natural environments ranging from silica-enriched hot springs (Walter et al. 1972; Walter 1976; Ferris et al. 1986; Cady and Farmer 1996; Hinman and Lindstrom 1996; Schultze-Lam et al. 1995; Konhauser and Ferris 1996; Jones and Renaut 1997; Jones et al. 1998; Renaut et al. 1998) to epilithic biofilms in fresh water rivers (Konhauser et al. 1993, 1994; Konhauser and Urrutia 1999). A number of studies suggest the adsorption and precipitation of silica by bacteria can notably influence silica cycling on the Earth's

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surface, resulting in the formation of chemical sediments, such as silica stromatolites in hot springs (e.g., Jones et al. 1997; Jones et al. 1998; Konhauser et al. 2001), clay deposits (Konhauser et al. 1993, 1994; Konhauser and Urrutia 1999), and siliceous precipitates in acidic mine tailings (Fortin and Beveridge 1997). The bacterial sorption of silica may also result in the preservation of microfossils (Cloud 1965; Shultze-Lam et al. 1995). Furthermore, silica immobilization by bacteria may be advantageous, providing protection against high intensity light, ultraviolet (UV) radiation, and dehydration (Phoenix et al. 2000, 2001). Certainly, the immobilization of silica onto bacterial surfaces in the hot-spring environment is well documented (e.g., Ferris et al. 1986; Shultze-Lam et al. 1995; Renaut et al. 1998; Jones et al. 1998; Jones et al. 1999), yet the mechanisms of the microbe–silica interaction and whether the microbial surface enhances silica immobilization compared with inorganic precipitation processes in such an environment are questions still open to debate. Several experimental studies performed under laboratory conditions have attempted to resolve the processes involved in the silicification of organic material. Early studies suggest that silica is bound to organic surfaces through hydrogen bonding of hydroxyl groups in silicic acid to hydroxyl groups on the surface of the organic material (e.g., Leo and Barghoorn 1976; Westall et al. 1995). Other studies have proposed that silica may bind through electrostatic attraction of negatively charged silica ions (such as SiO_3^{2-}) to electropositive functionalities such as NH_4^+ on the bacterial surface (Urrutia and Beveridge 1993). More recent studies suggest, however, that such a mechanism is unlikely to be the dominant silica immobilization process due to the extremely low concentrations of anionic silica species (at pH < 9) and the repulsive force created by the overall negative charge of the bacterial surface (Fortin and Beveridge 1997; Fein et al. 2002). Because the bacterial surface is commonly negatively charged and silica species are neutrally charged, or negative if colloidal, silica has a very low affinity for the bacterial surface. Thus it has been proposed that a salt bridge is required to enable silica to bind to the bacterium (Urrutia and Beveridge 1993, 1994; Fortin and Beveridge 1997; Fein et al. 2002). The role of metals as a bridging complex has been studied and demonstrated in detail, either in mixed metal–Si systems (Urrutia and Beveridge 1993; Fortin and Beveridge 1997) or in systems where the bacteria are pretreated with a metal solution prior to reaction with dissolved silica (Urrutia and Beveridge 1994; Fein et al. 2002). These investigations suggest that increased silica retention by bacteria is due to a two step process whereby the metallic species first binds to the cell wall and then reacts with the dissolved silica phase (Urrutia and Beveridge 1993, 1994; Fein et al. 2002). However, these studies focused upon systems undersaturated with respect to amorphous silica. In this study, we utilize experimental methods to elucidate mechanisms of microbial–iron–silica interaction in the hot-spring environment and thus we investigate systems both under- and oversaturated with respect to amorphous silica. Furthermore, to more closely mimic a cooling geothermal fluid after expulsion from the vent, we use mixed Fe–Si solutions (as an alternative to treating cells with Fe prior to reaction with dissolved silica), and the experimental solutions are cooled from 90 to 25 °C.

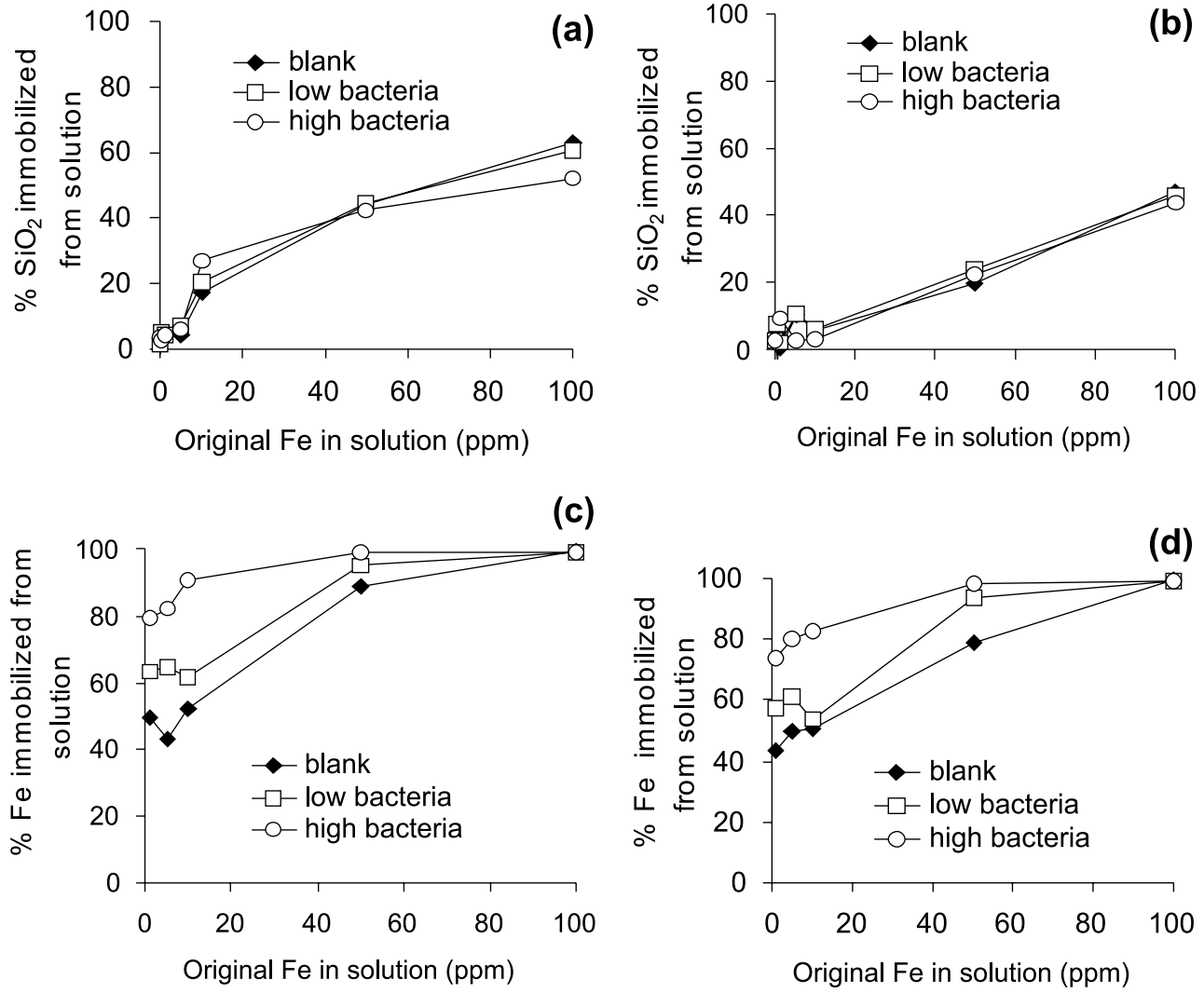
Methods

In the first study, the loss of Fe and Si from Fe–Si solutions containing both 60 ppm SiO_2 (undersaturated with respect to amorphous silica at 25 °C; saturation index (log Q/K) = –0.29) and a range of Fe concentrations (0, 0.2, 1, 5, 10, 50, and 100 ppm Fe) were monitored. Experiments were run in microcosms containing either high or low concentrations of bacteria and in blanks (without bacteria). In the second experiment, the same procedure was performed, but with a higher silica concentration of 400 ppm SiO_2 (supersaturated with respect to amorphous silica at 25 °C; log Q/K = 0.54). This range of silica and iron concentrations was chosen to cover a broad spectrum of typical hot-spring water chemistries.

The gram-positive bacteria *Bacillus subtilis* was used in this investigation as the surface reactivity and metal-binding capacity of this common microorganism are well documented (e.g., Fein et al. 1997; Warren and Ferris 1998; Cox et al. 1999; Fowle and Fein 1999; Fowle et al. 2000; Martinez and Ferris 2001; Fein et al. 2002). Furthermore, such studies have demonstrated that *Bacillus subtilis* has a strong metal-sorption capacity and thus is particularly suitable for studying Fe–Si immobilization by bacteria. Cultures were grown in tryptic soy broth on a rotary shaker (150 rpm) at 30 °C. Cells were harvested at late exponential growth phase (~18 h) and washed four times in deionized water by centrifugation (7000 × *g*). Twenty mL aliquots of washed bacterial suspension with an optical density of 1.9 at 525 nm were then added to 50 mL polypropylene centrifuge tubes (resultant biomass = 20 mg dry mass per tube). To a second set of tubes, a lower bacterial density was added by the addition of 5 mL aliquots of bacteria with an optical density of 1.6 at 525 nm (resultant biomass = 3 mg dry mass per tube). Both sets of tubes were then spun down to pellet the bacteria, and the eluent was then decanted.

The mixed Fe–Si experimental solutions were prepared using $Na_2SiO_3 \cdot 9H_2O$ and $FeCl_2 \cdot 4H_2O$ to the concentrations described earlier in the text. The solutions were then adjusted to pH 7.0 ± 0.2 with 2 M (1M = 2·mol/L) HCl. Four hundred mL of each solution was then added to 500 mL metal-free polypropylene bottles and placed in a constant temperature oven at 90 °C for 19 h to allow the solutions to equilibrate (in particular to allow the silica in the 400 ppm SiO_2 system (supersaturated at 25 °C) to depolymerize to monomeric silica. Almost all the 400 ppm SiO_2 at 90 °C should be in the monomeric state as the saturation index for amorphous silica at this temperature is ~0). Ten mL aliquots of the heated solutions were then added to the pre-prepared 50 mL centrifuge tubes, which were immediately capped and shaken to resuspend the bacteria. These were then placed in a water bath at 25 °C to cool the microcosms down to a constant and equal temperature. This was done to mimic cooling of a hydrothermal fluid upon expulsion from a hot spring vent. This cooling causes the microcosm solutions, which contain 400 ppm SiO_2, to become supersaturated with respect to amorphous silica (log Q/K = 0.54 at 25 °C); the 60 ppm SiO_2 solutions remained undersaturated with respect to amorphous silica at all temperatures throughout the experiment. After 4 h, the microcosms were centrifuged (4000 × *g*, 5 min) and the solutions filtered through 0.2 μm Supor® membrane filters,

Fig 1. Percent of SiO_2 or Fe immobilized (either precipitated homogeneously, or adsorbed or precipitated heterogeneously, onto the bacterial surface or microcosm wall) from a 60 ppm SiO_2 solution as a function of original Fe concentration. (*a*) Percent SiO_2 immobilized after 4 h. (*b*) Percent SiO_2 immobilized after 24 h. (*c*) Percent Fe immobilized after 4 h. (*d*) Percent Fe immobilized after 24 h. Key: blank, microcosms without bacteria; low bacteria, low bacterial density microcosms; high bacteria, high bacterial density microcosms. The data for the Fe immobilization in the 0.2 ppm Fe systems are not plotted as solutions had to be diluted to prevent silica buildup in the ICP–AES and thus analytical error is significant at these low iron concentrations.



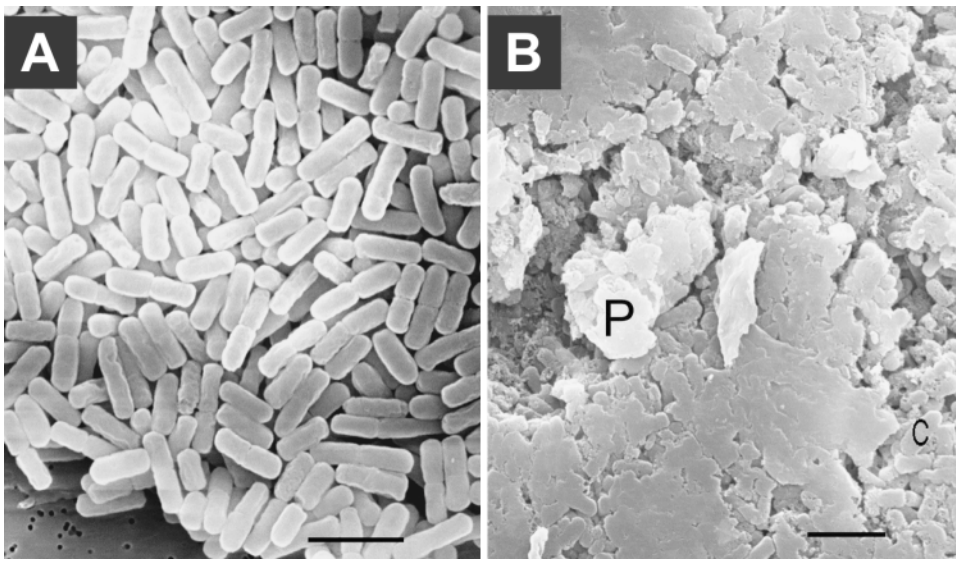
acidified in a matrix of 5% HCl, and analyzed by inductively coupled plasma atomic emission spectroscopy (ICP–AES) for total silica and iron concentrations (during analysis a standard was repeatedly analyzed to check for drift, and the results were then drift corrected). The same analysis was performed on a second set of identical microcosms after 24 h.

Samples were also collected during the experiments for SEM (scanning electron microscopy) analysis. After both the 4 and 24 h incubation periods, bacterial samples were collected, centrifuged at 10 000 rpm and the excess solution decanted. After fixing the samples in 2.5% glutaraldehyde, they were then processed for SEM by filtering onto 0.2 μm Nucleopor® track-etch filters. After being rinsed with deionized water the samples were dehydrated through a series of ethanol solutions (10 min in each 10%, 50%, 80%, and 100% ethanol solution), then dried overnight in a desiccation chamber. Dried samples were then placed on aluminum SEM stubs, sputter-coated with gold, and analyzed on a Joel 840 SEM operating at 15 kV. Qualitative precipitate chemistry was determined using a PGT EDS (energy dispersive spectroscopy) system.

Results

The results for the 60 ppm SiO_2 (undersaturated with respect to amorphous silica) experiment are discussed first. In the bacterial systems with only silica (i.e., no iron), there was no immobilization of silica after either 4 or 24 h (Figs. 1*a*, 1*b*), demonstrating the low affinity of silica for the bacterial surface. Increased levels of initial Fe in the system did appear to result in increased levels of silica immobilization at both 4 and 24 h (e.g., in the high-density bacterial system, ~0% SiO_2 was removed in the 0 ppm Fe system increasing up to ~45%

Fig 2. (A) SEM image of bacterial cells from 60 ppm SiO_2 – 0 ppm Fe system (low bacterial density) after 24 h. Note the lack of precipitates. Scale bar = 3 μm. (B) SEM image of bacterial cells and precipitates from 60 ppm SiO_2 – 100 ppm Fe (low bacterial density) system. Note the dominance of precipitate not associated with bacteria. P, non-bacterially associated precipitate; C, cells. Scale bar = 3 μm.



in the 100 ppm Fe system; Figs. 1*a*, 1*b*). However, there was no notable difference between the amounts of silica immobilized in the bacteria-containing systems compared to the blank, even after 24 h (Figs. 1*a*, 1*b*), again suggesting that the bacterial surface had failed to enhance immobilization of significant quantities of silica. Only solutions (both bacterial and blank) with starting Fe concentrations ≥50 ppm showed any notable silica immobilization after 24 h (Fig. 1*b*). Conspicuously, for these and the 10 ppm Fe solutions, the amount of silica immobilized actually decreased (10%–20%) between 4 and 24 h (for solutions with starting Fe concentrations <10 ppm, there was no significant change in silica immobilization over the 24-h period; compare Figs. 1*a* and 1*b*).

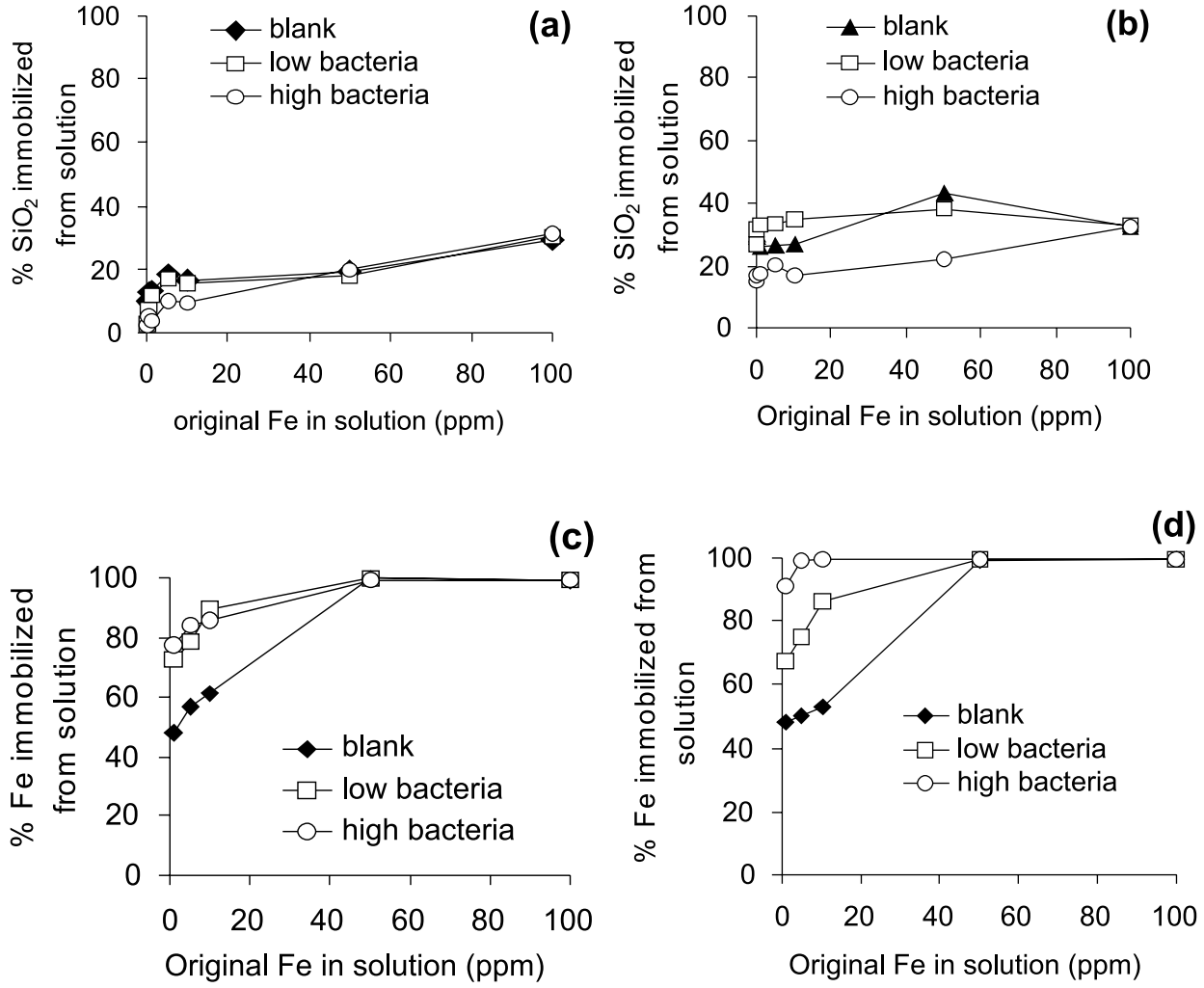
In contrast to the lack of difference in silica immobilization between blank and bacterial systems, immobilization data for Fe did demonstrate contrasts between these systems. For example, although the blank did show significant Fe immobilization (ranging from ~50% in the lower Fe solutions to 100% at 100 ppm original Fe concentration; Figs. 1*c*, 1*d*), the effect of the bacteria on Fe immobilization is clear. Examination of Figs. 1*c* and 1*d* demonstrates that increased densities of bacteria in the microcosm significantly increases the percentage of iron immobilized. This can be attributed to the sorptive properties of bacteria. The bacterial influence on iron immobilization, however, decreases with increasing original Fe concentration as inorganic (i.e., non-bacterially associated) precipitation processes, as highlighted in the blank, become more dominant at the higher original Fe levels (Figs. 1*c*, 1*d*). There is little difference in Fe immobilization between 4 and 24 h (compare Figs. 1*c* and 1*d*), indicating that the adsorption and precipitation processes involved in iron immobilization were rapid and had reached completion by 4 h.

These observations were corroborated by SEM and EDS analyses. For example, Fig. 2A shows cells from the 60 ppm SiO_2 – 0 ppm Fe system (low bacterial density) after 24 h. Clearly the cells are free from any notable precipitate, highlighting the low affinity of silica for the bacterial surface. This contrasts dramatically with Fig. 2B, which shows the 60 ppm SiO_2 – 100 ppm Fe – low density bacterial system after 24 h. The system is dominated by a considerable quantity of iron–silica precipitates not associated with bacteria (Fig. 2B) and thus highlights how increased levels of Fe in the original solution result in increased levels of inorganic precipitation. EDS analysis confirmed the Fe–Si composition of the precipitates.

Results for the 400 ppm SiO_2 systems depict similar trends to those of the 60 ppm SiO_2 system. In bacterial systems without Fe, silica immobilization was ~2% after 4 h, again highlighting the low affinity of silica for the bacterial surface (Fig. 3*a*). Increased levels of initial Fe in the system did appear to result in increased levels of silica immobilization after 4 h (e.g., in the high-density bacterial system, ~2% SiO_2 was removed in the 0 ppm Fe system increasing up to ~30% in the 100 ppm Fe system; Fig. 3*a*). However, there was no notable difference between the amounts of silica immobilized in the bacteria-containing systems compared with the blank, again suggesting the bacterial surface had failed to enhance immobilization of significant quantities of silica. After 24 h, the amount of silica immobilized in both bacterial and blank systems had increased moderately (Fig. 3*b*), most notably in the lower Fe systems and generally to a lesser extent in the higher Fe systems (compare Figs. 3*a* and 3*b*). Although at lower original Fe concentrations (≤10 ppm) the low-density bacterial system immobilized slightly more SiO_2 than the blank, the high-density bacterial system immobilized the least silica. Thus, from SiO_2 immobilization data at both 4 and 24 h there is no strong evidence to suggest that the presence of Fe enhances silica immobilization onto the bacterial surface (certainly within the resolution of this experiment).

Trends for iron immobilization in the 400 ppm SiO_2 systems also show similarities to the 60 ppm SiO_2 systems. Although there is significant iron immobilization in the blank, for solutions with original Fe concentrations < 50 ppm the bacterial systems immobilized significantly more Fe (~25%) than the blanks after 4 h (Fig. 3*c*). The percentage of Fe immobilized appears, in general, to increase with increasing

Fig 3. Percentage of SiO_2 or Fe immobilized (either precipitated homogeneously, or adsorbed or precipitated heterogeneously onto the bacterial surface or microcosm wall) from a 400 ppm SiO_2 solution as a function of original Fe concentration. (*a*) Percent SiO_2 immobilized after 4 h. (*b*) Percent SiO_2 immobilized after 24 h. (*c*) Percent Fe immobilized after 4 h. (*d*) Percent Fe immobilized after 24 h. Key as in Fig.1.



original Fe content in both the blanks and bacterial systems (Figs. 3*c*, 3*d*), and at 50 and 100 ppm original Fe, 100% Fe immobilization in both bacterial and blank systems was observed. Again, this suggests that with increasing concentrations of Fe, non-bacterially associated precipitation processes become more dominant. Interestingly, after 24 h, there was no notable change in the amount of Fe immobilized by the low-density bacterial and blank systems and only the high-density bacterial system showed an increase in Fe immobilization (~100% immobilization at all original Fe concentrations) (Fig. 3*d*). This suggests that after 4 h the bacteria in the low-density system had reached their sorption potential, whereas in the higher density system Fe nucleation sites were still available.

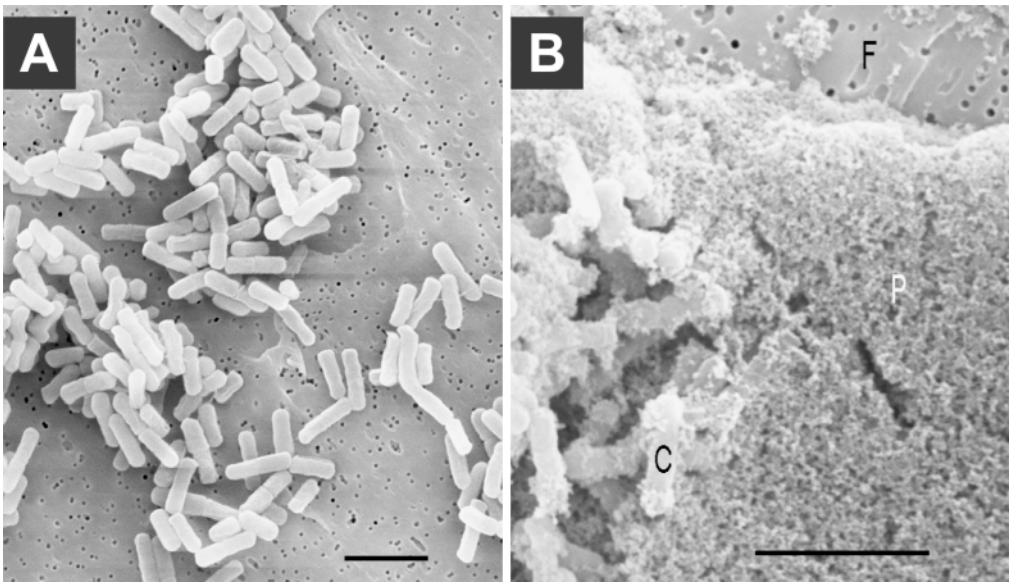
Visual analysis using SEM again highlights the low affinity of silica for the bacterial surface, as shown in Fig. 4A. At higher original Fe concentrations, significant non-bacterially mediated precipitates were observed (Fig. 4B). Bacterial cells in this system do have some precipitate upon their surfaces, but it is not possible to confirm from these SEM images whether the precipitates nucleated upon the cell surface or whether they precipitated homogeneously in solution (Fig. 4B). This observation and the dominance of precipitates not associated with bacteria again suggest that the bacterial surface may not be playing an important role in precipitate nucleation in these systems. EDS analysis confirmed the Fe–Si composition of the precipitates.

Discussion

In both the 400 and 60 ppm SiO_2 systems that contained no iron, there was no evidence of significant silica binding onto the bacterial surface when compared to the blank. This corroborates recent findings which demonstrate low bacterial–silica affinities in systems undersaturated with respect to amorphous silica (Fein et al. 2002). Furthermore, this current study demonstrates that bacterial–silica affinities are also low in systems supersaturated with respect to amorphous silica. Thus, silica immobilization either via adsorption (in undersaturated systems) or adsorption and precipitation (in supersaturated systems) does not appear to be enhanced upon bacterial surfaces.

In systems that contained Fe, commonly higher levels of Fe immobilized from solution resulted in higher levels of

Fig 4. (A) Scanning electron microscopy (SEM) image of bacterial cells from 400 ppm SiO_2 – 0 ppm Fe system (low bacteria) after 4 h. Note the lack of precipitates. Scale bar = 3 μm. (B) SEM image of bacterial cells and precipitates from 400 ppm SiO_2 – 100 ppm Fe (low bacteria) system. Note the dominance of precipitate not associated with bacteria. P, non-bacterially associated precipitate; C, cells; F, filter paper. Scale bar = 3 μm.

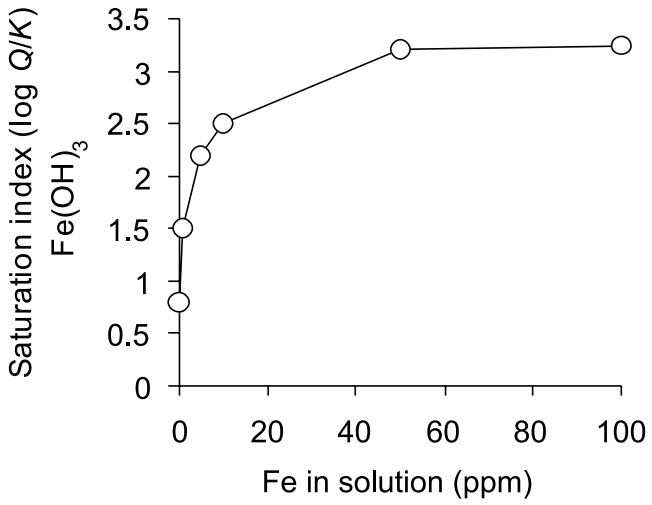


SiO_2 immobilization. This is to be expected considering the high reactivity of silica with iron (e.g., Manceau et al. 1995; Swedlund and Webster 1999; Davis et al. 2002; Fein et al. 2002). However, although higher levels of iron immobilization in general correlate with higher levels of SiO_2 immobilization, increased Fe immobilization in bacterial systems did not appear to enhance SiO_2 immobilization compared to the blank. Thus, iron(oxide–hydroxide) binding by bacteria does not appear to notably enhance SiO_2 binding onto the bacterial surface under these mixed Fe–SiO_2 conditions. For example, in the 60 ppm SiO_2 system, the bacterial systems clearly bound a greater percentage of Fe compared with the blank (from all solutions except the 100 ppm Fe solutions) (Figs. 1*c*, 1*d*). However, these higher levels of bacterially bound iron did not result in greater levels of SiO_2 immobilization in the bacterial systems compared to the blank (Figs. 1*a*, 1*b*). The same trend can be seen for the 400 ppm SiO_2 systems, where again higher levels of iron immobilization by the bacterial systems did not conclusively result in higher levels of SiO_2 precipitation (Fig. 3). It is thus evident that this trend appears applicable to systems both undersaturated and oversaturated with respect to amorphous silica.

To speculate further on the reasons for these trends, it is important to understand how Fe may be behaving in these systems. Iron may precipitate either as an amorphous $Fe(OH)_{3(s)}$ phase or as a poorly ordered hydrous iron-silicate. Certainly, iron and silica in geothermal solutions will readily polymerize to form poorly ordered hydrous iron-silicate precipitates similar to, for example, hisingerite ($(Fe,Mn)SiO_3,Fe_2^{3+}Si_2O_7{\cdot}2(H_2O)$) and minnesotaite ($(Fe,Mg)_3Si_4O_{10}(OH)_2$), as exemplified by a study of the Salton Sea geothermal system, California, U.S.A. (Manceau et al. 1995). Therefore, the significant proportion of iron immobilized in the blanks of both the 400 ppm SiO_2 and 60 ppm SiO_2 systems is likely due to the precipitation of such hydrous iron-silicate and $Fe(OH)_{3(s)}$ phases. Obviously, the non-bacterially nucleated precipitation of these phases will account for a significant quantity of iron removal in the bacterial systems also. However, bacterial systems do demonstrate higher levels of iron immobilization than the blanks (except at very high concentrations of iron in solution) (e.g., Fig. 1*c*). This imposes the question of what reactive iron phases are complexing with the bacterial surface to result in such increased levels of iron immobilization. Iron in the "aqueous" phase may be present as free aqueous species, such as $Fe(OH)_2^+$ or $Fe(OH)_{3(aq)}$, as suspended reactive iron hydroxide colloids, or as polymerized silica–iron colloids. A plot of the saturation index for $Fe(OH)_{3(s)}$ shows that at all levels of Fe used (i.e., 0.2, 1, 5, 10, 50, and 100 ppm), the system was supersaturated with respect to $Fe(OH)_{3(s)}$ (Fig. 5). Under these conditions most of the suspended iron will be present as reactive iron hydroxide colloids or as polymerized silica–iron colloids; only a very small percentage will be present as free aqueous iron species. In fact, only around 1.4×10^{-12} mol will be present as free aqueous Fe species (calculated using the Geochemist's Workbench software package) and are therefore unlikely to play a significant role in iron immobilization. Considering this, it seems apparent that the binding of suspended iron hydroxide colloids or silica–iron colloids are probable mechanisms for Fe immobilization onto the bacterial surface. The binding of iron hydroxide colloids is corroborated by a recent study by Glasauer et al. (2001), which demonstrated that nanometre scale ferrihydrite, goethite, and hematite readily adheres to the bacterial surface. Evidently, bacteria may immobilize Fe onto their surfaces by sequestering iron particles (Glasauer et al. 2001), as well as through the adsorption of aqueous Fe phases.

Despite the evidence here that the presence of bacteria increases iron immobilization, there is no direct evidence that the bacterial immobilization of iron increases levels of silica immobilization in these systems. At first, this may appear contradictory to studies by Urrutia and Beveridge (1993) and

Fig 5. Saturation index (log Q/K) for $Fe(OH)_{3(s)}$ at pH 7, 25 °C and under fully oxic conditions in solutions with different Fe concentrations (0.2, 1, 5, 10, 50, 100 ppm Fe). Plot generated using Geochemists Workbench.

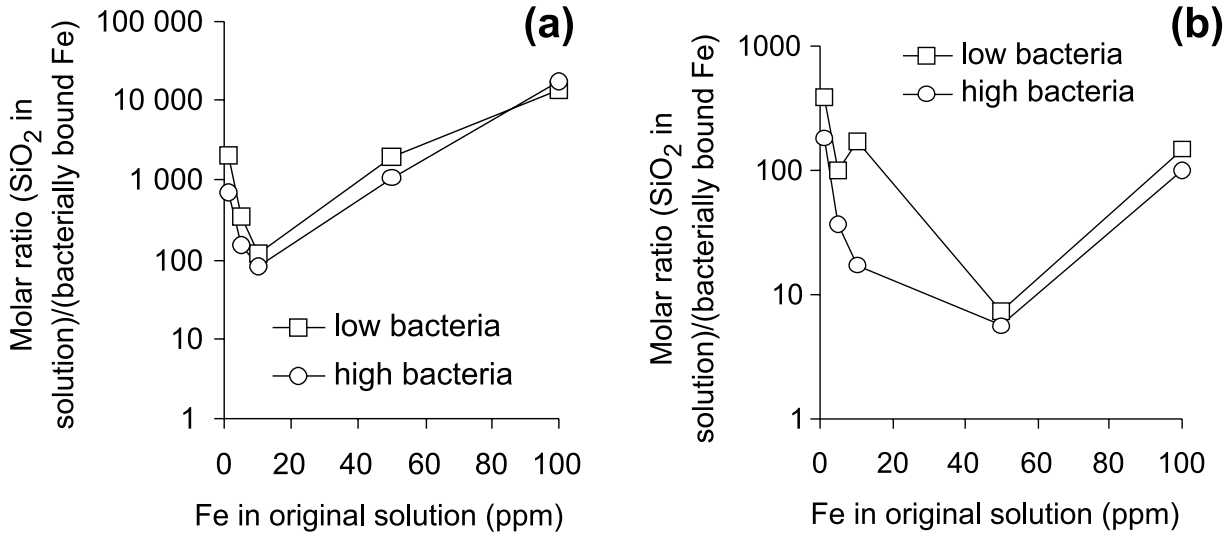


Fein et al. (2001); however, further consideration reveals this is not necessarily the case. Firstly it is important to consider the ratio of silica in solution to iron bound to the bacterial surface. For example, Fig. 6*a* shows the molar ratio of (silica in solution)/(bacterially bound Fe) for the 400 ppm SiO_2 system after 24 h (the amount of bacterially bound iron was determined as bacterially bound Fe = [Fe immobilized$_{Bacteria}$] – [Fe immobilized$_{Blank}$]). Inspection of Fig. 6*a* demonstrates that the molar ratio of (silica in solution)/(bacterially bound Fe) is always very high. Considering there is between 100 and 10 000 times less iron bound onto the bacterial surface compared with SiO_2 in solution, it is unsurprising that the bacterially bound Fe has no notable influence over silica immobilization. If one assumes, for purposes of discussion, that there is a 1:1 stochiometric relationship between bacterially bound iron and the molar concentration of silica it can potentially sorb, then, at the very best, the bacterially bound iron could only immobilize 1% of the silica in this example. In contrast to this study, the study by Fein et al. (2002) used (silica in solution)/(bacterial bound Fe) ratios that were very small, such that bacterially bound iron concentrations were several orders of magnitude greater than the silica concentrations in solution. Hence, Fein et al. (2002) demonstrated significant silica immobilization onto bacterially bound Fe. However, the current study is interested in bacterial Fe–Si immobilization in silica-enriched hot-spring systems, and therefore the concentrations of Fe and silica used in this study were chosen to be representative of typical hot-spring chemistry.

In the 60 ppm SiO_2 systems, the (silica in solution)/(bacterial bound Fe) ratios are approximately an order of magnitude lower than for the 400 ppm systems, as exemplified in Fig. 6*b*. At intermediate original Fe concentrations, where the (silica in solution)/(bacterial bound Fe) ratios approach 10 (Fig. 6*b*), one might expect some influence of silica immobilization by bacteria. However, no increased levels of bacterial SiO_2 immobilization compared to the blank were observed (Figs. 1*a*, 1*b*). Again this may be because the (silica in solution)/(bacterial bound Fe) ratios are still too high. This hypothesis appears to be supported by a recent study of SiO_2 sorption onto $Fe(OH)_{3(s)}$ by Davis et al. (2002). Their study reported that in solutions of 60 ppm SiO_2 at circum-neutral pH, the molar ratio of $Fe(OH)_3$ precipitate to sorbed SiO_2 was ~0.5. In the current study, maximum SiO_2 sorption by bacteria would be expected where (silica in solution)/(bacterial bound Fe) ratios where lowest, such as at intermediate original Fe concentrations that exhibit (silica in solution)/(bacterial bound Fe) ratios ~ 6–7 (Fig. 6*b*). Using the sorption capacity of $Fe(OH)_{3(s)}$ (~0.5) from Davis et al. (2002), under these conditions, one can calculate an expected maximum of 8% more SiO_2 immobilized in bacterial systems compared with blanks. Considering this is the maximum predicted value, it is not surprising then that bacterial systems do not show notable evidence of SiO_2 immobilization compared to the blank. Clearly, bacterial SiO_2 immobilization in mixed Fe–Si systems is negligible compared to inorganic SiO_2 immobilization processes. This is because only small quantities of iron hydroxides are able to bind to the bacterial surface due to considerable iron immobilization in non-bacterially mediated (inorganically precipitated) phases, such as poorly ordered hydrous iron silicates. Furthermore, when bacterial systems do immobilize notably more Fe than the blanks, the amount of bacterially bound iron is insufficient to sorb significant quantities of SiO_2. It follows that levels of SiO_2 immobilization by bacterially bound $Fe(OH)_{3(s)}$ are insignificant. In addition to this, it is also important to consider that bacterially bound iron hydroxides may have less sorptive capacity than their non-bacterially bound counterparts. Recent studies on the sorptive capacities of bacteria–iron hydroxide composites have suggested that when iron hydroxides are bound to the bacterial surface, masking effects occur (Small et al. 1999). During this process, reactive polymers on the bacterial surface interact with reactive functionalities on the iron hydroxide surface, essentially neutralizing the sorptive capacities of these reactive sites (Small et al. 1999). Such masking effects may further decrease the ability of bacterially bound iron compounds to sorb silica onto the bacterial surface.

The ratios of (silica in solution)/(bacterial bound Fe) may also explain why the possible binding of aqueous iron–silica colloids to the bacterial surface would appear to only increase bacterial iron immobilization whilst not notably effecting silica immobilization. For example, after 4 h in the 400 ppm SiO_2 – 10 ppm Fe system, the bacterial microcosms had immobilized 25% (~2.5 ppm) more Fe than the blanks (Fig. 3*c*). If this was due to the sorption of an iron–silica complex with a silica:iron stochiometry of 1:1, then the bacterial system would only immobilize ~2.5 ppm more SiO_2 than the blank. In a system containing 400 ppm SiO_2, such a small change in SiO_2 concentration would not be resolvable. Again one might expect to see evidence of this process in the 60 ppm SiO_2 system, where small changes in silica concentration are more resolvable. However, there is no evidence of increased silica immobilization by bacteria in this system, indicating such a mechanism does not occur significantly in systems undersaturated with respect to amorphous silica (this may also be true of the supersaturated system). What is clear is that the binding of iron–silica complexes to the bacterial surface does not occur significantly enough to notably enhance

Fig. 6. Plot of molar ratios of (SiO_2 in solution)/(bacterially bound Fe) after 24 h for (*a*) 400 ppm SiO_2 system and (*b*) 60 ppm SiO_2 system. Key as in Fig. 1.



silica immobilization in bacterial systems compared to bacteria free systems.

A conspicuous trend is the slight decrease in SiO_2 immobilization (~15%) between 4 and 24 h in the 60 ppm SiO_2 system at original Fe concentrations ≥10 ppm (Figs. 1*a*, 1*b*). It is tentatively suggested here that this change is due to Ostwald ripening of $Fe(OH)_3$ precipitates. As the precipitates merge, their cumulative surface area decreases and thus releases surface-adsorbed silica into solution. Considering that the decrease in SiO_2 immobilization occurs equally in both bacterial and blank systems, this process must be a result of Ostwald ripening of $Fe(OH)_3$ precipitates not associated with bacteria (as non-bacterially associated precipitates are common to both systems). It is suggested that silica release through Ostwald ripening is not observable at original Fe concentrations <10 ppm (Figs. 1*a*, 1*b*) as the molar concentrations of $Fe(OH)_3$ precipitate is small and thus will have adsorbed minimal SiO_2. Furthermore, it is speculated that only the ripening of non-bacterial $Fe(OH)_3$ causes observable silica release because (*i*) there is more non-bacterial than bacterial nucleated $Fe(OH)_3$ precipitate and (*ii*) masking effects may inhibit silica adsorption onto bacterially bound $Fe(OH)_3$ (thus bacterially bound $Fe(OH)_3$ will have less SiO_2 to release during ripening). If Ostwald ripening of non-bacterial $Fe(OH)_3$ releases SiO_2 in the 400 ppm SiO_2 system, it is not observable (Figs. 3*a*, 3*b*). This may be because the system is supersaturated with respect to amorphous silica, which may inhibit desorption of SiO_2, or because the amount of SiO_2 released may not be within the resolution of the experiment considering the larger quantity of SiO_2 in solution (e.g., a ~15% drop in SiO_2 immobilization in the 60 ppm SiO_2 system would equate to a potentially unobservable ~2% drop in SiO_2 in the 400 ppm SiO_2 system if the same amount of SiO_2 was desorped).

Masking effects may also help explain why in the 400 ppm SiO_2 system after 24 h there was more silica immobilization in the low bacterial density systems than the high bacterial density system (at low original Fe concentrations; Fig. 3*b*). It is conceivable that sufficient iron hydroxide had been bound to the bacterial surface in the low-density bacterial system to significantly neutralize its electronegative surface charge. However, in the high bacterial density system, it is possible that insufficient iron binding had occurred to effectively neutralize the greater number of electronegative surface sites, thus leaving the bacterial surface with a strong negative surface charge, which may have repelled anionic silica colloids, which may be important in bacterial silicification in such supersaturated (with respect to amorphous silica) conditions (Phoenix et al. 2001). This hypothesis is corroborated by the observation that between 4 and 24 h there was no further iron immobilization in the low-density bacterial system (Figs. 3*c*, 3*d*), indicating the sorption capacity of the bacterial surface had been reached. However, in the high-density bacterial system, iron sorption continued over the 24-h time period (Figs. 3*c*, 3*d*), finally reaching 100% immobilization, indicating the bacteria's sorption capacity had not been reached, thus leaving the bacterial surface with a net negative surface charge. This tentative hypothesis does then suggest that at certain bacteria: silica:iron ratios some enhanced silica immobilization by iron-complexed bacteria is observable (i.e., the low-bacterial density system demonstrates higher silica immobilization than the blank at low original Fe concentrations; Fig. 3*b*). However, this is the only example and the difference in silica immobilization between the low bacterial system and the blank at these original Fe concentrations is still very small (<10%).

In this study, only one species of bacteria was used. Whether the results would vary markedly with the use of other bacteria can only be determined through further study. Consideration of this is pertinent as in the natural environment a single taxon community (*B. subtilis* or otherwise) is very unlikely. However, it is suggested that other bacteria would not significantly enhance silica immobilization. Firstly, considering the exceedingly low affinity of silica for *B. subtilis* (in iron-free systems), it is unlikely that other bacteria may exhibit a high affinity for silica. We suggest that unrealistic changes in cell wall chemistry would be required for this to occur. Secondly, *B. subtilis* was used in this study as it possesses a highly reactive cell surface capable of adsorbing significant quantities of metals, including iron (Daughney et al. 2001). Despite this,

in iron-bearing systems, silica immobilization was not notably greater in the bacterial systems compared to the blanks. This was because the bacteria failed to bind sufficient iron to act as a salt bridge for silica and (or) failed to bind significant aqueous iron–ilica complexes. Instead, non-bacterially mediated (inorganic) precipitation processes dominated. Although surface reactivity and metal-binding capacity does vary somewhat between different bacteria, there is a degree of similarity in the metal-binding character of different species (Yee and Fein 2001). Following this, it is suggested that the differences in iron-adsorbing capacity of different bacterial species are not great enough to cause notable silica immobilization through enhanced iron binding. This is corroborated by the observation that both high- and low-density bacterial systems immobilized the same levels of SiO_2, even when greater levels of Fe immobilization occurred in the high-density bacterial system.

Summary

It is evident that in hot-spring systems, both under and oversaturated with respect to amorphous silica, bacteria can enhance iron immobilization. However, at high concentrations of Fe in solution (≥50 ppm Fe) iron immobilization by bacteria becomes insignificant as non-bacterially mediated precipitation processes dominate. Additionally, it is evident that neither salt bridging by iron hydroxides onto bacterial surfaces, nor bacterial binding of aqueous silica–iron colloids can significantly enhance bacterial silica immobilization compared to the dominant non-bacterially associated precipitation processes. Moreover, when bacterial systems do bind significantly more $Fe(OH)_3$ than bacteria-free systems, the amount of bacterially bound Fe is insufficient to adsorb significant quantities of silica onto the bacterial surface.

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