

MICROBIALY-AIDED SiO₂ BIOMINERALIZATION

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By

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ABSTRACT

Microorganisms inhabiting silica solute-rich environments often show various degrees of SiO₂ mineralization as a consequence of exposure to SiO₂-saturated waters. As such, it has been thought that microorganisms exert a prominent role in the immobilization of amorphous silica phases. While this intimate spatial relationship of microorganisms and amorphous SiO₂ phases are almost always observed in hot springs, the exact mechanisms by which microbes affect SiO₂ secondary mineral precipitation is still poorly understood. Further, available laboratory investigations to date consistently showed that microbes do not significantly impact SiO₂ immobilization, suggesting that microbial silicification is a mere consequence of exposure to a largely abiogenically-driven SiO₂ precipitation.

This study demonstrates that discernible microbially-mediated silicification can occur under conditions where the potential for microbial opportunity to biomineralize is promoted. Identification of the key geochemical requirements for biosilicification to occur include thermodynamically favorable, but sluggish silica reaction kinetics associated with acidic conditions, and the necessity for colloidal silica rather than dissolved silicic acid species. This work provides the first results to bridge the apparent literature discrepancy between widespread, *in-situ* observations of microbial silicification, and the inability to demonstrate a detectable microbial effect in this process under well-constrained laboratory conditions.

Acid conditions promote microbial silicification by overriding the dominant repulsive forces arising from charge similarities between SiO₂ and cell surfaces, via neutralization of deprotonated surface silanol and carboxylic groups, respectively. Mechanistic consideration for SiO₂ coordination to cell surfaces suggests direct chemical bonding of silanol to carboxylic groups forming stable inner-sphere complexes largely insensitive to environmental perturbations. This result indicates that microbially immobilized SiO₂ are more tenaciously-coordinated on cell surfaces and not simply electrostatically-held.

Surface-dependent silicification showed higher SiO₂ mineralization propensities for unmineralized microbial cells compared to silica-encrusted cell matrices. Moreover, the extent and style of microbial SiO₂ mineralization is impacted by cellular level of metabolic activity. These results suggest that a biological overlay may be discernible in microbially induced biosilicification.

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PREFACE

This thesis encompasses three major stand-alone chapters, which collectively form the highlights of the author's research endeavor in partial fulfillment of Ph.D. degree. Chapter 2 is a slightly modified version of the manuscript published in *Chemical Geology*, 240: 298-312. For this published manuscript, the mat consortia sampled from two contrasting hot springs system in Yellowstone National Park, USA were chosen to represent the polarities in environmental conditions along a geochemical - biological driven SiO₂ precipitation continuum research framework detailed in Chapter 1. A total of 11 microbial mat consortia collected from four hot spring pools in Yellowstone were subjected to the same experiments in Chapter 2, but only three were included in the final manuscript. Of these 11 samples, only the microbial mat from the Yellowstone acidic pools showed significant silica immobilization under its *in-situ* conditions.

All aspects of research work in this thesis were done by the author: from the conceptualization and research framework formulation stage, field sampling and laboratory analyses, data processing and interpretation, to the final manuscript writing. Dr. Lesley Warren provided guidance, logistical support and editorial comments throughout the execution of this thesis, while Dr. Scott Smith shared his expertise on the FOCUS pK_a model. Dr. Kurt Konhauser gave valuable inputs on *in-situ* microbial silicification during the fieldwork phase of this work. Otherwise, the overall direction, interpretation of modeling and empirical results, and writing of this thesis were carried out by the author.

CHAPTER 1. MICROBIALLY-AIDED SiO₂ BIOMINERALIZATION

To place the discussions in the succeeding thesis Chapters in perspective, the following introductory sections are constructed with the objective of laying the groundwork of the fundamental concepts central to the development of this thesis. These key concepts are a collection of available literature information highlighting the more recent developments in microbially-aided silicification, with particular emphasis on induced (i.e., cell surface and indirect metabolic effects) silicification. Throughout this section, the introduction of these key concepts is linked to specific chapter/s from this work where salient empirical results and additional discussions can be found. In this work, the terms and notations: “amorphous silica” (SiO_{2(am)}) and “silica” (SiO₂) will be used interchangeably to mean the non-crystalline, low-temperature variety of silicon dioxide, unless otherwise specified (i.e., quartz, which is the symmetrical, high temperature SiO₂ polymorph: Zoltai and Stout, 1984). Chapter 1 is by no means an exhaustive compilation of available data on microbial silicification; nonetheless, the intent of this introduction is to acquaint the reader with the fundamental concepts and guiding framework used collectively in this thesis. Moreover, the key “missing pieces” from the current literature will be highlighted and addressed in the succeeding chapters of this work. Foremost of these are the apparent contradiction in laboratory observed silicification and the effects of “living” cells in mineralization, both detailed in Chapter 2 and Chapter 3, respectively. Specifically, Chapter 1 will focus on the nature of biomineralization (controlled vs. induced) and the associated key parameters initiating biosilicification. In particular, the lesser known non-enzymatic (i.e., induced) SiO₂

biomineralization; the potential linkages of system conditions (i.e., pH, cell level of activity, $[\text{SiO}_2]$ saturation and relative SiO_2 precipitation kinetics) in promoting biosilicification that is potentially distinct from abiotic counterparts; and the role of colloidal nano- SiO_2 particles as relevant silica species for perceptible biosilicification to occur will be examined.

1.1 Biomineralization

Biomineralization refers to the process by which organisms form minerals (Mann, 2001; Weiner and Dove, 2003; Konhauser, 2007). The controls exerted by the organisms typically distinguish biomineralization from a purely abiotic mineralization (Perry, 2003; de Vrind-de Jong and de Vrind, 1997). Over the last century, the discoveries among the intimate linkages of biology (organisms), chemistry (minerals and organic molecules) and geoscience (physical environment) collectively initiated the burgeoning field of biogeochemistry, whose main goal is to understand the past and future of the Earth's evolution, with no less than these very "biominerals" at the forefront of such works (Weiner and Dove, 2003). Perhaps one of the more recent spillovers of the field is in the application to biomimetrics in materials sciences, with the aim to discover novel hybrid materials typically aided by organismal activities (Mann, 1995; Lopez, et al., 2005). Biomineralization is expressed across all life forms, from the simplest, single-celled microorganisms (e.g., archaea, bacteria, algae) to the most complex "higher" life (e.g., plants, humans: Mann, 2001; Weiner and Dove, 2003). The following discussions will be limited to the role of single-celled microorganisms or colonies initiating the biomineralization processes.

A biomineral refers to the solid-phase inorganic component (i.e., mineral) that is produced by (micro)organisms. It encompasses mineralized composites of both organic and inorganic nature (Weiner and Dove, 2003; Belton, et al., 2004). Further, because these biominerals are affected (either directly or indirectly) by the presence of microorganisms, these mineral composites have properties unlike any other inorganically produced counterparts. Hence while these biominerals meet the criteria of true minerals, they are often distinguishable from abiotically produced equivalents (e.g., size, morphology, presence of organic macromolecules, etc.: Mann, 2001; Veis, 2003; Weiner and Dove, 2003).

1.1.1 Types of Biomineralization: Controlled vs. Induced

Biomineralization processes may be conveniently grouped into two broad categories: (1) Controlled and (2) Induced Mineralization (Mann, 2001; de Vrind-de Jong and de Vrind, 2003; Veis, 2003; Konhauser, 2007).

In biologically controlled mineralization (BCM), the organism uses cellular activities (i.e., enzymatic) to initiate the nucleation, growth, habit, morphology and subsequent location of the mineral that is deposited. This biomineralization scheme is synonymous to “direct catalysis” (Thompson and Ferris, 1990; Fortin, et al., 1997) or “active mineralization” (Ledin, 2000; Southam, 2000); however, both terms are usually more strictly reserved for microbial-metal transformations. Regardless of the degree of the control of the organism on the ensuing biomineral product (i.e., may vary across species), BCM processes largely occur in isolated environments (Weiner and Dove, 2003). That is, strictly speaking, the location of the formation of the biomineral in

controlled mineralization is distinct and specific, and may proceed either within (intra) or outside (extra) the cell, which distinguishes the formed biominerals by virtue of the site of mineralization relative to the cell (Towe, 1990; Weiner and Dove, 2003). Whether BCM proceeds intra- or extra-cellularly, the consistent theme for BCM processes is that microorganisms exert a significant influence with regard to the size, morphology, habit, degree of hydration, isotopic and trace element composition of biomineral products, hence these microbially controlled biominerals are truly distinct from their inorganically produced counterparts (Towe, 1990; Tebo, et al., 1997; Perry, 2003; Weiner and Dove, 2003).

1.1.2 Induced mineralization: microbes as reactive surfaces and living entities

In biologically induced mineralization (BIM), microbial cells often act as causative agents for the precipitation of secondary minerals resulting from the interaction between cellular surfaces and their activities with the immediate environment (De Yoreo and Vekilov, 2003; Frankel and Bazylinski, 2003; Weiner and Dove, 2003). Unlike BCM, microorganisms responsible for BIM have little or no control over the resulting biomineral composition, morphology and location of deposition (Southam, 2000; Veis, 2003). There are two main themes regarding the nature of BIM due to microorganismal presence from the current literature information: (1) microbes as living, actively metabolizing systems, and (2) microbial cells as reactive surfaces. Often, microbial metabolic activities mediate the surrounding cellular geochemical conditions, such as pH, $p\text{CO}_2$ and extraneous cellular secretions or metabolic by-products (Stone, 1997; Warren and Haack, 2001; Haack and Warren, 2003; Frankel and Bazylinski, 2003; Gilbert, et al.,

2005). Synonymous terms often encountered in the literature for this type of BIM is “indirect” (Fortin, et al., 1997; Ledin, 2000), “active BIM” (Southam, 2000) or “environmental biomineralization” (Towe, 1990). These geochemical changes typically occur on a local, micro-scale (Warren and Haack, 2001) surrounding the cell interfacial region, and are usually the driving forces favoring the “indirect” abiotic precipitation of certain types of minerals (Fortin, et al., 1997; Southam, 2000; Weiner and Dove, 2003; Konhauser, 2007).

Often, microbial cells need not be metabolizing (i.e., doing any sort of activity) to induct secondary mineral formation. Cell surfaces can act as templates for solid-phase biomineral precipitation from dissolved constituents. Cell surface reactivity to dissolved mineral constituents as a driving force for BIM is also otherwise known as “passive” microbial mineralization (Southam, 2000; Konhauser, 2007). In other words, the mere presence of organic surfaces such as microbial cells may be sufficient to induce the precipitation of secondary minerals. The reason for this phenomenon is straightforward: cell surfaces contain a myriad of organic functional groups, which are readily available for ionic interactions with the solution phase (Urrutia and Beveridge, 1993; Fortin, et al., 1997; Warren and Haack, 2001; see Chap. 4). The relative concentrations of these reactive groups may vary from species to species, or even within a given microbial strain dependent on its growth histories (Engl and Kunz, 1995; Ledin, 2000; Borrok et al., 2004); however, the identities of these organic groups are typically similar across all microbes (Smith and Ferris, 2003; Fein, et al., 2005). What governs the activation of these organic functional sites, and therefore their reactivity, is system pH. Depending on the pK_a of these organic functional groups, they deprotonate successively at increasing

pH values, and thus typically rendering cell surfaces net negatively charged particularly at circum-neutral pH where much of natural waters' pH values lie (Phoenix, et al., 2002; Konhauser, 2007). The effect of these net negatively charged cell surfaces (due to site deprotonation) is that they become ideal sites for metal coordination, an initial step in biomineralization process (Fortin, et al., 1997; Southam, 2000).

Perhaps the hallmark of microbially induced mineralization is the enormous compositional heterogeneity of the biominerals formed (Weiner and Dove, 2003). Because the role of microorganisms in BIM is to catalyze an inorganically-driven secondary mineral precipitation process, the “biological effect” typically distinguishing biominerals from BIM as compared to inorganically precipitated counterparts, may be obscured or completely obliterated.

1.2 Microbial silicification as BIM: proposed mechanisms

The biogeochemical cycling of SiO_2 is largely controlled by (micro)biotia (Dixit and Van Cappelen, 2002; Perry, 2003; Michalopoulos and Aller, 2004; Likhoshway, et al., 2005) via immobilization of amorphous silica precipitates. Much of our current understanding on microbially aided silicification is focused mainly on the more “well-known” silicifying microorganisms such as diatoms, although it has been known for quite some time that other microorganisms (e.g., bacteria: Bonny and Jones, 2003; Perry, 2003; Konhauser, 2007) were thought to participate in silica immobilization. With the exception of the unicellular eukaryotic diatoms and radiolarians that manufacture delicate lace-like tests and micro-skeletons, the current literature consensus is that microbial (i.e., bacterial and archaeal) silicification is initiated non-enzymatically, hence is an “induced”

mineralization (Ferris, et al., 1986; Konhauser, et al., 2004; Konhauser, 2007). In other words, these silicified microorganisms do not have a significant control over the size, habit, crystallinity, and organization of SiO_2 on or within the cell itself (Mann, 2000; Perry, 2003; Weiner and Dove, 2003). As with BIM, there are two general themes as to the nature of non-enzymatic microbial silicification: (1) cell surface effects, and (2) metabolic effects.

1.2.1 Cell-surface effects

In silica-saturated solutions, the formation of SiO_2 may be initiated by microbes via the enhancement of precipitation kinetics, analogous to heterogeneous nucleation mineral precipitation (Schultze-Lam, et al., 1995; Konhauser, 2001; Yee, et al., 2003). Foreign interfaces such as cell surfaces, enhance silica nucleation by reducing the activation energy barrier to solid formation (Fig. 1.1), and can be envisioned as a scaffold or template on which mineral precipitation occurs (Brock and Madigan, 1991; Fortin, et al., 1997). Mechanistically, foreign surfaces such as cell interfaces act as catalysts for mineral precipitation due to the lowered interfacial energy between the cell surface and the mineral as compared to the interfacial energy between the mineral and the solution phase (i.e., as in homogeneous nucleation, activation without catalyst; Fig. 1.1: Stumm, 1992; Brock, et al., 1994). Therefore, heterogeneous nucleation may be favored under conditions of slightly lower saturation ratio, and is thought to be the most important mechanism for a variety of environmental mineral formation (Stumm, 1992; Stumm and Morgan, 1996). The newly formed amorphous mineral phases are typically stable and less prone to dissolution/remobilization because the cell wall reduces the interfacial

tension between the nucleus and the bulk solution phase (Bratina, et al, 1998; Southam, 2000).

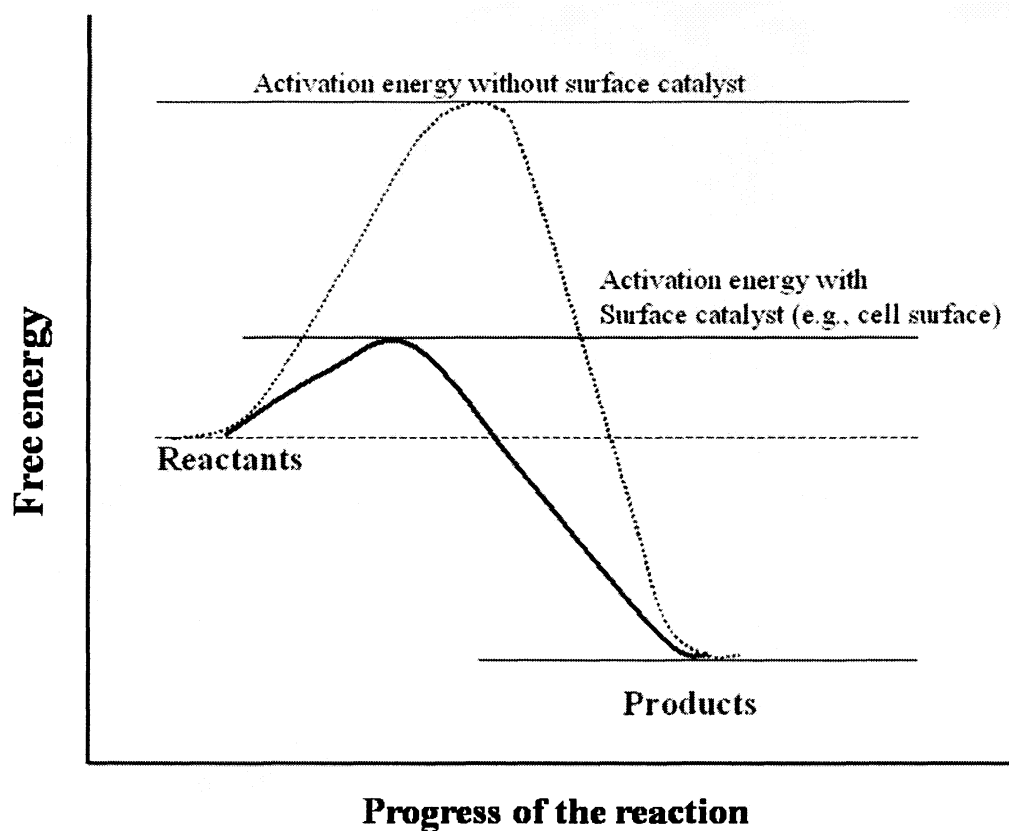


Figure 1.1 Progress of reaction in the presence of cell surface catalyst; the activation energy required for the formation of minerals (products) from constituent ions (reactants) is lowered in the presence of cell surface catalysts (bold curve), compared to the activation energy via homogeneous nucleation (without surface catalyst, dashed curve); figure modified from Brock, et al., 1994.

Conceivably, one apparent requisite to invoke a “cell-surface effected” heterogeneous SiO_2 precipitation is that the aqueous silica need to be at a certain degree of saturation for perceptible BIM to occur (Fortin, et al., 1997; de Vrind-de Jong and de Vrind, 1997; Southam, 2000). The degree of silica saturation appears to be an essential requirement because there has been no proof to date that silica (as dissolved H_4SiO_4 species) may be precipitated out of undersaturated SiO_2 solution by cell surface induced BIM process alone (Fein, et al., 2002; Yee, et al, 2003). Only BCM has been shown to selectively precipitate silica from undersaturated solution (e.g., diatoms in seawater; Mann, 2001; Perry, 2003); therefore, the level of saturation, for the most part determines whether heterogeneous SiO_2 BIM nucleation will ensue. It is therefore not surprising that microbially induced silicification via heterogeneous SiO_2 nucleation is the main mechanism invoked for the microbially-aided silica precipitation from various saturated to super-saturated silica pools, such as those silicified microbes encountered in many hydrothermal springs (Jones, et al., 1999; Bonny and Jones, 2003; Guidry and Chafetz, 2003). However, most hydrothermal springs are characteristically supersaturated with respect to amorphous silica (>300 ppm: Aramaki, et al, 2004; Gorbach, et al, 2006; see Chap. 2) hence are thermodynamically favored to spontaneously polymerize abiotically with or without the presence of these foreign (i.e., cell) surfaces (Mountain, et al., 2003; Konhauser, et al., 2004; Benning, et al., 2005). From recent laboratory works investigating the role of microbial surfaces in silica precipitation, it has been shown that microbial surfaces do not significantly enhance the rate (i.e., kinetics) or the amount of precipitated silica compared to abiogenic SiO_2 polymerization from supersaturated solution (Phoenix, et al., 2003; Benning, et al., 2004). Therefore, the role of microbes as

surface catalysts for SiO_2 precipitation in SiO_2 supersaturated systems such as hydrothermal springs has been put into question (Konhauser, et al., 2004).

One recurring commonality among previous investigations elucidating the likely mechanism of microbial silicification as cell surface influenced BIM is that these works centered on a silicic acid (H_4SiO_4) source (e.g., Yee, et al., 2003; see Chap. 2). This fundamental assumption is invoked, particularly because microbial cells become charged surfaces due to functional site protonation/deprotonation reactions, and may be ideal reaction sites for H_4SiO_4 species. Direct chemical interaction between H_4SiO_4 has been proposed on the cell surface functionalities via carboxylic (Fein, et al., 2002) or amine (Urrutia and Beveridge, 1993; Fortin and Beveridge, 1997) sites on the cell surface structure. While this cell silicification mechanism may seem plausible, available laboratory investigations have consistently shown otherwise: that is, direct silicic acid-cell functional site interactions were measured to be negligible (Fein, et al., 2002; Phoenix, et al., 2003; Konhauser, et al., 2004). Nonetheless, most research was performed under pH conditions where the supposed charge interactions were actually not apparent (i.e., near-neutral pH). At circum-neutral pH values, the majority of silicic acid species is still protonated ($\text{pK}_a \sim 9.5$; Iler, 1979), hence are mostly uncharged at pH 7 (Fig. 1.2). Cell surfaces on the other hand would have a net negative charge due to deprotonated acidic sites (Ledin, 2000; Yee, et al., 2004; see Chap. 3) under the same pH, thus the proposed occurrence of charge interaction mechanism may be weak. Where considerable ionization of H_3SiO_4^- occurs (slightly alkaline pH; Fig. 1.2) charge repulsion would have been the dominant interaction due to the like-charged (i.e., both negative) cells and monomeric silica species, thus preventing their effective interaction. Picking up

from the ionized nature of H_3SiO_4^- , other workers proposed the direct interaction of H_3SiO_4^- with positive cell surface sites (i.e., NH_3^+ : Urrutia and Beveridge, 1993; Schultze-Lam, et al., 1996; Fortin and Beveridge, 1997); however, this mechanism has yet to be proven (Konhauser, et al., 2004). It would appear that this cell-silicic acid mechanistic interaction via positive cell surface sites is unlikely because available macro- and molecular-scale investigations have shown that positive site moieties are less abundant relative to the total site concentration of cell surface sites, particularly for the more common silicifying microorganisms (e.g., cyanobacteria; Urrutia and Beveridge, 1993; Yee, et al., 2004; see Chap. 4).

Another mechanism proposed for cell-surface BIM is the role of cationic bridges, notably Fe and Al (Urrutia and Beveridge, 1993; Fein, et al., 2002; Phoenix, et al., 2003; Konhauser, et al., 2004;) to coordinate ionized silicic acid onto the dominantly negatively charged cell surfaces. Metal sorption to bacterial surfaces, particularly Fe, is a well-documented phenomenon (Warren and Ferris, 1998; Southam, 2000; Warren and Haack, 2001), and has been invoked to be an important process in silica(te) precipitation reaction (Urrutia and Beveridge, 1993; Fortin, et al., 1997). Metals act as bridge between the anionic constituents in the cell wall functional groups and the anionic silica, a process which has been shown for *Thiobacillus*' silicate sequestration in acidic media (pH ~ 2: Fortin and Beveridge, 1997). The rationale behind this cationic bridge mechanism is that cell surfaces are effective dissolved metal sorbents and could readily scavenge aqueous Fe or Al, eventually acquiring a net positive charge (i.e., charge reversal: Collins and Stotzky, 1992; Ahimou, et al., 2002). Positive charge development on cell surfaces from Fe and Al sorption would make it plausible for H_3SiO_4^- to coordinate indirectly with the

cell surfaces via these metal bridges, particularly at neutral to slightly alkaline pH when both cell surface functionalities and silicic acid species are ionized. One of the biggest obstacles as to the viability of this mechanism is that microbial cells would need to selectively accumulate metals on their surfaces prior to the coordination of silica. At circumneutral pH, metal ions particularly, Fe are susceptible to hydrolyze (Stumm, 1992; Stumm and Morgan, 1996; Warren and Ferris, 1998) and may be nucleating instead metal-oxyhydroxide solid precipitates on the cells, and not being scavenged as metal ions *per se*. Perhaps more importantly, Fe and Al have high affinity with solution H_4SiO_4 , and would promote the spontaneous, abiotically-driven polymerization of Fe/Al-silicate nucleates regardless whether or not microbes are present in these systems (Swendlund and Webster, 1999; Phoenix, et al., 2002; Yokohama, et al., 2004).

It is probably not an overstatement that the message of these collective literature data is that microbial surfaces are largely incompatible with dissolved silica. However, the effect of silica speciation and its role on cell surface interaction has not been thoroughly explored. Polymeric colloidal silica species is a significant component of the total aqueous silica pool (Dove and Rimstidt, 1994; Rao and Gelb, 2004) and may be the relevant form of SiO_2 that can effectively coordinate with cell surfaces (see Chap. 2; Chap. 3) and not dissolved silicic acid species as has been previously thought. This thesis has shown that direct silica-cell silicification is possible via the colloidal silica pathway (see Chap. 2). While there is no direct evidence that microbial surfaces induce/enhance the polymerization of silicic acid (see Chap. 2), this process is probably unimportant because pre-formed silica colloids (irrespective whether abiogenically

nucleated) were observed to be the relevant species that form chemically stable, inner-sphere complexes with cell surface groups (see Chap. 3).

1.2.2 Cell metabolic effects

For the cell surface effects for BIM of silica detailed above, microbial cells are assumed to be “static” or “inactive” surfaces composed of an organic framework interlaced with a suite of reactive functional sites (Beveridge, 1989; Schultze-Lam, et al., 1996). For most available laboratory investigations on biosilicification, the elucidation of microbially induced SiO_2 mineralization have been evaluated primarily by microorganismal presence as a reactive interface with only a few works assessing the effect of cell metabolic activity (Fortin, and Beveridge, 1997; Phoenix, et al. 2000). Throughout the following section, the term “metabolic effects” refers to any microorganismal activity except those cellular machineries specifically programmed to precipitate SiO_2 , as in the enzymatic (e.g., sillafin: Perry, 2003; Vrieling and Gieskes, 1999) BCM of siliceous tests of diatoms. Such “metabolic effects” referred to in this section may include: photosynthesis, respiration, proton pumping, locomotion, release of organic exudates and a myriad of other energy transforming activities such as the redox transformation of metals (Brock and Madigan, 1991; Little, et al., 1997; Nealson and Stahl, 1997; Ledin, 2000; Warren and Haack, 2001; Frankel and Bazylinski, 2003; Konhauser, 2007). In short, metabolic effects as they relate to BIM are the consequence of cell “doings” as a living entity and the collective effects of one or any combination of these metabolic processes is straightforward: they may initiate changes in the microgeochemical conditions at the cellular interfacial region. These microgeochemical

changes may in turn promote the precipitation of minerals that are otherwise not favored in the bulk aqueous phase. Unless otherwise specified, the term “metabolic effects” will be used interchangeably with *active BIM* to avoid confusion with “direct” and “active” terms which are also encountered in BCM (i.e., active/direct mineralization; Ledin, 2000).

There have been very few laboratory and field studies of the metabolic BIM effects of cells/microbial consortia in the formation of silica, probably because much of the silicified microorganisms observed in silica-rich systems (e.g., cyanobacteria) do not require silica for growth or normal functions. Phoenix, et al. (2000; 2001) has shown that cyanobacteria entombed in SiO_2 were able to photosynthesize and induce alkalization of the surrounding microgeochemical envelope, thereby promoting the (further) polymerization of silicic acid. Fortin and Beveridge (1997) showed from cell silicification experiments that both live and dead cells immobilized silica at acidic conditions; however, it is difficult to extrapolate the net effect of living cells because the experimental conditions used was a mixed Fe-Si system.

Whether the BIM mechanism invoked for cell silicification is cell surface-effected, metabolically induced or a combination of both, there is no denying that microbes succumb to silica mineralization. In fact any surface, whether organic or inorganic, will be susceptible to silica encrustation given enough solution silica saturation (Konhauser, et al., 2004). If then any surface can be enshrouded with SiO_2 , perhaps the more relevant question to ask is *if biologically induced silica mineralization by microbes can be promoted, and can be distinguished from an abiogenically driven SiO_2 precipitation?* Although this thesis may not have answered comprehensively this

inquiry; nonetheless this work laid down the necessary conditions (see Chap. 2) and possible mechanisms (Chap. 3) for which biologic mediation of silica formation can occur, both as surface-effected and metabolically-influenced process.

1.2.3 BIM of SiO₂: field vs. laboratory evidences

There is probably not a more dramatic illustration of microbial silicification than the extensive silicified microbial mats observed in many hydrothermal systems. In hot spring environments, microscopic investigations of silica sinter deposits clearly indicate microbial (specifically Bacteria and Archaea) cells encrusted with amorphous silica, both extracellularly (i.e., sheaths and cell walls; Ferris, et al., 1986; Kato et al., 2004) and intracellularly (i.e., within the cytoplasm; Konhauser et al., 2001; Phoenix et al., 2003; Bonny and Jones, 2003) as a consequence of exposure to silica supersaturated solutions. Naturally, a lot of work has been dedicated to unraveling the role of microorganisms in the biosilicification process using hydrothermal spring analogs. However, while collective evidences clearly show the intimate spatial relationships of nucleated silica masses and simultaneously, the silica encrustation of various types of microorganisms; they do not address definitively whether microbes mediate silica precipitation or biomineralize in some selective manner. Investigations of modern hydrothermal sinters almost always showed widespread and copious silicification of a wide variety of microbial species (Jones, et al., 2001; McKenzie, et al., 2001; Smyth, et al., 2003), and are interpreted to reflect that microbes are influential in the precipitation of biosilica (Ferris, et al., 1986; Ferris, et al., 1988; Phoenix, et al., 2001). However, available laboratory studies aimed at duplicating a mechanistic microbe-silica interaction from

these environments have failed to show significant silica biomineralization for a number of microbial strains (Fein, et al, 2002; Yee, et al., 2003; Phoenix et al, 2003). That is to say, current experimental data has produced a consensus that microbial silica encrustation observed in silica-rich waters is mechanistically similar to the entombment of any inert organic (wood, leaves, twigs) or inorganic (lithic/mineral, artificial) templates.

The key features of hydrothermal systems are that they are characteristically supersaturated with silica, metal laden and have the potential to condense solution silica by cooling or evaporative processes (Gaudry and Chafetz, 2002; Gorbach, et al., 2006). In other words, these hydrothermal waters are bound to precipitate silica abiotically, and thus there is no need for a microbial presence to initiate SiO_2 precipitation (Konhauser, et al., 2004; Konhauser, 2007). This statement is not meant to discredit hydrothermal environments as natural laboratories to investigate the intimate microbial-silica association; rather, it will be argued in this thesis that to be able to show perceptible silica biomineralization in the laboratory, conditions need to be such that the impending precipitation of SiO_2 is not almost exclusively abiotically driven as in the case of most hydrothermal systems. Laboratory experimentation from this work has shown that perceptible silicification of natural microbial consortia sampled from hydrothermal environment can be promoted under a specific set of geochemical conditions (see Chap. 2).

1.3 The framework to establish microbial BIM of SiO_2

In this thesis, an alternative view of investigating a likely microbial influence on silica precipitation is proposed that has not been systematically addressed before. The

argument posed in this research is that to be able to accurately investigate microbial mediation in any mineral formation, experimental conditions must be tailored such that two necessary conditions are satisfied: (1) a favorable thermodynamic reaction, and (2) slow kinetics.

One of the important requirements for microbial biomineralization is that a certain degree of saturation must be achieved, a direct consequence of the presence of an energy activation barrier that inhibits the spontaneous formation of insoluble minerals from solution (Stumm and Morgan, 1996; Fortin et al., 1997; Warren and Haack, 2001; Fig. 1.1). For amorphous silica precipitation to occur even in the presence of bacteria, conditions must be such that the ensuing reaction is thermodynamically favorable (Reysenbach, and Shock, 2002; Stumm, 1992; Nealson and Stahl, 1997), but not supersaturated such that the spontaneous SiO_2 precipitation dominates. The precipitation of $\text{SiO}_{2(\text{am})}$ is controlled by the solubility limits of H_4SiO_4 :



where, $K_{\text{sp}}\text{SiO}_{2(\text{am})}$ is the solubility product of amorphous silica (Langmuir, 1997). At 25°C, and at concentration below approximately 100-140 ppm (10^{-3} - $10^{-2.8}$ M), aqueous silica (H_4SiO_4 or simply $\text{SiO}_{2(\text{aq})}$) is in equilibrium with $\text{SiO}_{2(\text{am})}$ (Fig. 1.2; Iler, 1979; Benning, et al., 2005); therefore, H_4SiO_4 is stable in solution and no solid $\text{SiO}_{2(\text{am})}$ precipitates even in the presence of heterogeneous (e.g., bacteria) nucleation templates (Muñoz-Aguado, and Gregorkiewitz, 1997; Perry and Tucker, 2003). Not surprisingly, laboratory investigations on microbial biomineralization conducted under silica

undersaturated solutions ($[\text{SiO}_{2(\text{aq})}] \leq 60\text{ppm}$: Yee, et al., 2003; Fein, et al, 2002; Phoenix et al, 2003) did not show any significant biosilicification, reflecting the need for a thermodynamically-favored (close to) saturation state even for microbial SiO_2 mediation.

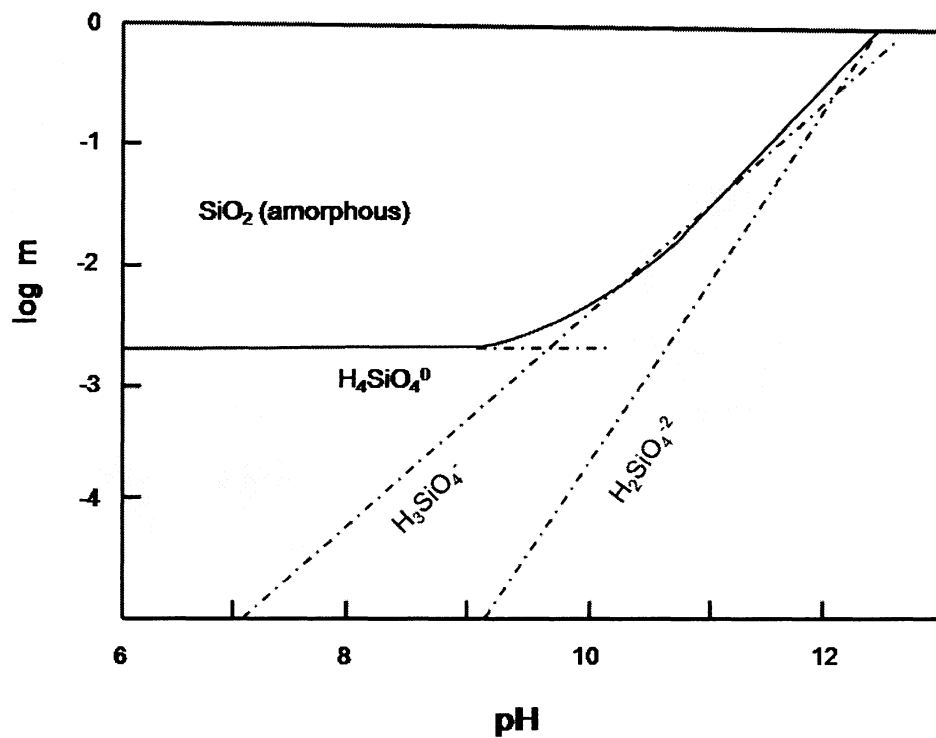


Figure 1.2 Solubility of $\text{SiO}_{2(\text{am})}$ where, m is the concentration of aqueous silica species, as a function of pH; broken lines indicate $[\text{H}_{(4-x)}\text{SiO}_4^{x-}]$ species; figure adopted from Dove and Rimstidt, 1994; Langmuir, 1997.

Other than thermodynamically favorable conditions, the slow kinetics of mineral formation is also an important parameter that needs to be considered in investigating microbial biomineralization. For microbial participation in any energetic chemical reactions, they must capitalize on the kinetic energy barriers of thermodynamically favorable reactions, consistent with what has been shown for the formation of other biominerals such as: Mn oxyhydroxides (Tebo, et al., 1997; Haack and Warren, 2003); carbonates (Thompson, et al., 1990; Little, et al., 1997); and silicates (Fortin and Beveridge, 1997; Frankel and Bazylinski, 2004). For $[\text{H}_4\text{SiO}_4]$ at or above saturation with respect to $\text{SiO}_{2(\text{am})}$, the kinetics of silica polymerization and aggregation is controlled primarily by system pH, and may be conceptualized as a variable kinetic gradient (Fig. 1.3, *section 1*). $\text{SiO}_{2(\text{am})}$ precipitation kinetics is minimum at $\text{pH} \sim 3$ to almost instantaneous in the neutral/mid-alkaline end ($\text{pH} \sim 7\text{-}9$). For instance, the $\text{SiO}_{2(\text{am})}$ polymerization rate is about 2 orders of magnitude slower at $\text{pH} 4$ as compared to $\text{pH} 6$ (Iler, 1971; Coradin and Livage, 2001). Maximum condensation (i.e., polymerization) reaction rates were measured between $\text{pH} 8$ and 9 within the measured pH range of $4\text{-}10$ (Coradin and Livage, 2001). In another study, a supersaturated silica solution ($[\text{SiO}_{2(\text{aq})}] = 0.45\text{M}$) underwent complete gelation (i.e., aggregation) in minutes at pH values between $6\text{-}9$, while SiO_2 gelling did not occur after at least one day at $\text{pH} 3$ (data estimated from Muñoz-Aguado and Gregorkiewitz, 1997). Therefore, in acidic milieus, the slow kinetics of silica condensation might provide a *biological window of opportunity* to investigate the potential for microbial biosilicification (BIO potential; Fig. 1.3, *section 1*); as they can possibly participate in these impending energetic reactions in contrast to the instantaneous, geochemically-controlled $\text{SiO}_{2(\text{am})}$ condensation (GEO control; Fig.

1.3, *section 1*) at higher pH regimes. Current laboratory studies on microbial silicification (Ferris, et al., 1988; Phoenix, et al., 2003; Yee, et al., 2003) justified investigation of microbial biomineralization at pH ~7 to be reflective of most hydrothermal systems as near neutral to slightly alkaline (Fig. 1.3, *section 1*). While this motivation is valid, it does not address nonetheless an important precondition that microbial biomineralization is favored under slow kinetics of reaction. Needless to say, a rapid and spontaneous $\text{SiO}_{2(\text{am})}$ precipitation kinetics such as those encountered in cooling, SiO_2 -supersaturated hydrothermal waters do not probably reflect a bacterially mediated mineralization mechanism (Konhauser, et al., 2004), where sufficiently sluggish reaction kinetics is warranted even if there is enormous potential for the reaction to proceed (Nealson and Stahl, 1997; Reysenbach, and Shock, 2002).

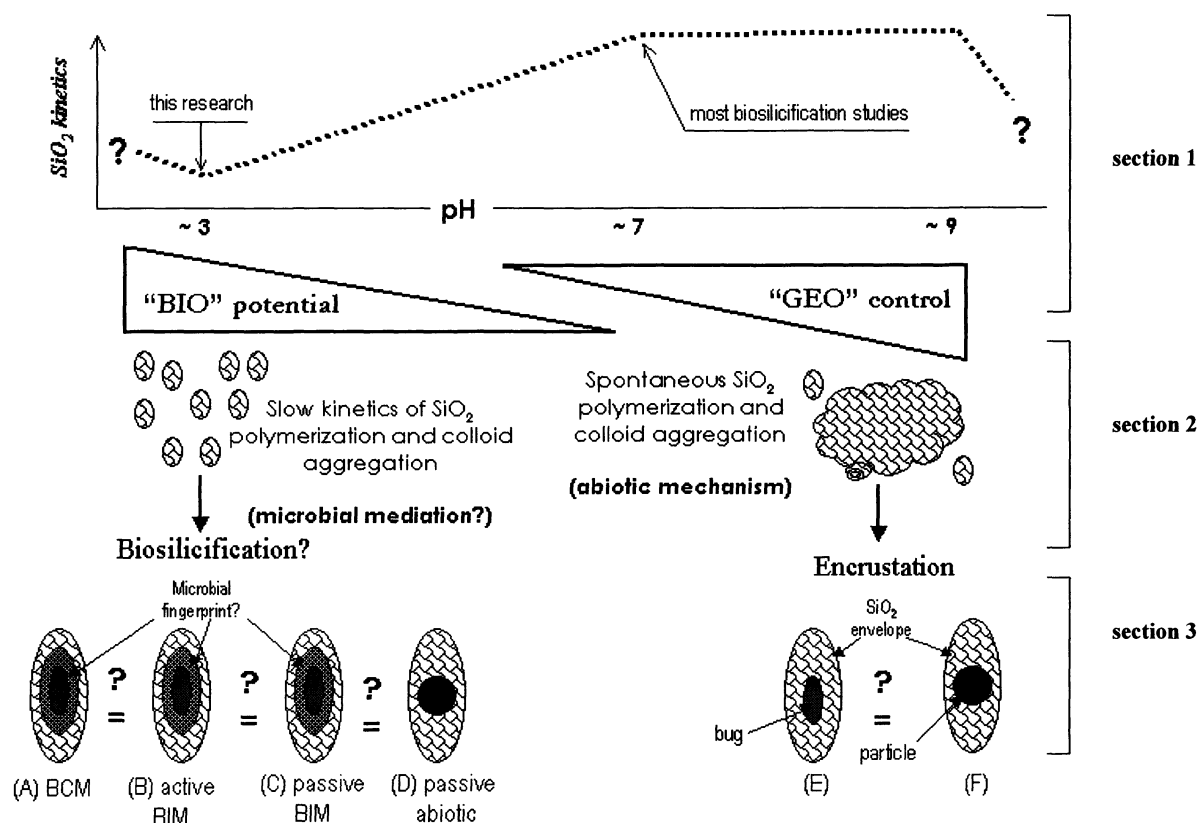


Figure 1.3 Conceptual framework for biological (BIO) mediation–geochemical (GEO) control continuum along a SiO_{2(am)} precipitation kinetic gradient defined roughly by system pH (dashed line, section 1); SiO₂ kinetics shown is relative and does not represent actual values; (section 2) colloidal SiO₂ forms across the whole pH range, however kinetics of aggregation is fastest at pH 7-9; (section 3) possible biosilicification mechanisms leading to biological overlay(?) on bacterial cells (A-C) and abiotic particle (D) at pH ~3, and rapid encrustation of cell (E) and particle (F) at pH 7-9; *see text for explanation*

Thus, if any microbial influence is to be observed in silicification processes, it is most optimal to investigate these phenomena in systems where $[\text{SiO}_{2(\text{aq})}]$ is close to the SiO_2 saturation values where microbes can take advantage on these impending energetic reactions. However, as mentioned, laboratory investigations of biosilicification conducted in unsaturated $[\text{SiO}_{2(\text{aq})}]$ did not show significant microbial-silica interactions. Where silicification was promoted onto bacterial surfaces, typical $[\text{SiO}_{2(\text{aq})}]$ used were beyond published $\text{SiO}_{2(\text{am})}$ saturation values (i.e., ≥ 300 ppm; see Chap. 2). That is to say, similar to the widespread microbial silica encrustation observed in many hydrothermal systems, the entombment of microorganisms observed in the laboratory conducted under $[\text{SiO}_{2(\text{aq})}]$ supersaturated solutions do not reflect a microbial mediation process because $\text{SiO}_{2(\text{am})}$ will spontaneously precipitate with or without the presence of these microorganisms.

The slow kinetics of imminent SiO_2 polymerization and gelation (i.e., aggregation) may play a critical role in promoting a likely microbial control in biosilica formation. The kinetic constraints of thermodynamically favorable silica precipitation reactions might provide a *window of opportunity* for microbes to capitalize on. While there appears to be no direct evidence for a metabolic (i.e., enzymatic; Fortin, et al., 1997) microbial catalysis for biosilica formation except those shown for eukarya (Vrieling and Gieskes, 1999; Perry, 2003), indirect bacterial influences may play important controls; such as metabolic-induced (a.k.a., metabolic effects, active BIM) changes of the enveloping solution locale, or as nucleation templates (a.k.a., surface effects; passive BIM).

Moreover, if indeed microbial biosilicification is promoted under acidic conditions, it is further hypothesized that potentially identifiable biological overlay, (i.e., fingerprint) are effected on the resulting siliceous envelopes which may be distinct from their abiotic counterparts (Fig. 1.3, *section 3*). Because natural hydrothermal analogs are composed of both organic (microbial biofilms) and inorganic (mineral) substrates, it is necessary to investigate silicification products from both types of templates if a biological effect is to be invoked (see Chap. 3; Chap. 4). Where the prospect for microbial silicification is maximum (BIO potential; Fig. 1.3), microbes can affect $\text{SiO}_{2(\text{am})}$ formation either as BCM (exclusive to eukarya: Perry, 2003) or BIM. As mentioned, BCM occurs when the cell exerts a significant degree of control over the nucleation, growth, composition, size, habit and intracellular location of the biominerals (Fig. 1.3, *section 3*, A), and were thought to be governed by microbial genetic and metabolic controls (Asada and Tazaki, 2001; Perry, 2003; Frankel and Bazylinski, 2004). BIM on the other hand, has been invoked to govern bacterial silicification processes (Schultz-Lam, et al., 1996; Fortin and Beveridge, 1997), and is broken down into *active* BIM (cell metabolic effects: Fig. 1.3, *section 3*, B); and (2) *passive* BIM (cell surface effects; Fig. 1.3, *section 3*, C). Because the mechanisms of microbial influence for biosilicification in all three biomineralization pathways (Fig. 1.3, *section 3*; A, B, C) are different (Southam, 2000; Frankel and Bazylinski, 2004), it is probable that distinct microbial fingerprints are found among the resulting biosiliceous solids. For this work, biological overlay or fingerprint pertains to any likely SiO_2 -cell association that would suggest microbial intervention (see Chap. 3).

Another factor to consider is the relevant species of aqueous SiO_2 inferred to occur in natural systems. Many laboratory silicification experiments employed a monomeric silica source (H_4SiO_4 or H_3SiO_4^-), presumably due to the general assumption that the biomineralization processes commence from the immobilization of dissolved constituents (Navrotsky, 2004). However, there is increasing evidence that clusters, nano-particles and other complex metastable phases in the solution phase play an important role in biomineralization processes (Banfield, et al., 2000; Perry and Keeling-Tucker, 2003; Navrotsky, 2004). In silica solutions in excess of the amorphous silica solubility, H_4SiO_4 polymerizes through a series of reactions involving linear, cyclic and three dimensional polymeric silica species (Iler, 1979; Ohsawa, et al., 2002). Nanometer-sized polymeric silica colloids have been shown to represent the dominant silica form (ca. $\geq 50\%$) equilibrated in the aqueous phase for a variety of silica-saturated artificial and engineered systems (Dove and Rimstidt, 1994; Muñoz-Aguado and Gregorkiewicz, 1997; Davis, et al., 2002; Ohsawa, et al., 2002; Rao and Gelb, 2004). While there have been suggestions that microbes are subjected to silicification via these colloidal silica phases (Phoenix, et al., 2000; Inagaki, et al., 2003; Ferris and Magalhães, 2004; Konhauser, et al., 2004; Benning, et al., 2005; Mocko, et al., 2005; see Chap. 2), there has been no systematic evaluation of the role of colloidal silica in microbial SiO_2 biomineralization.

1.4 Thesis Objectives

There are two fundamental questions that this research intends to explore:

- (1) do microbes induce silica immobilization in a manner that can be categorically surface and/or metabolic influenced BIM?; and,

- (2) what are the important controls and likely mechanisms underpinning microbially induced silicification?

To be able address the above research inquiries, specific objectives were formulated in each of the stand-alone chapters constituting this thesis using these general research questions as an overall guide. Chapter 1 presents an overview of the biomineralization and a synthesis of the current ideas on microbially-induced silica mineralizations. This chapter also highlighted the apparent irony between field-based observations of widespread intimate silica-microbial association in silica-rich systems (e.g., hydrothermal pools), and the inability to show a microbial impact in laboratory silicification efforts. Moreover, a working framework was constructed in such a way that the potential for biological mediation of SiO_2 mineral formation will be promoted.

Chapter 2 is a direct application of the formulated research framework to investigate a microbial influence to promote laboratory biosilicification of natural mat consortia sampled from two contrasting hydrothermal spring conditions. The specific objectives were to evaluate:

- (1) if discernible microbial biosilicification can be promoted when the SiO_2 precipitation “kinetic slider” is shifted towards greater biological opportunity; and,
- (2) whether colloidal SiO_2 is important in biologically-mediated silicification processes.

Chapter 3 examines the mechanisms involved in colloidal silica immobilization by microbes, and explores the effects of living cells versus compromised cells in this immobilization process. The specific objectives were to:

(1) investigate the likelihood of surface charge interactions as the mechanism for the microbially-aided silicification of microbial cells dependent on cell/silica surface proton-charge speciation; and,

(2) evaluate the effects of microbial cell viability (hence the potential for metabolic activity) on SiO_2 immobilization among live, dead and cell- SiO_2 mineral composite, through batch silica experimentation at pH 3 and pH 7.

Chapter 4 is a macroscopic investigation of the surface reactivity to protons of a variety of solids consisting of end-members of fully exposed unmineralized cells and abiotic silica surfaces, and an intermediate matrix of silica-mineralized cell composites. The variability in surface proton-charging behavior among living cells compared to inactivated, dead cells is also highlighted in this work. The specific objectives were to:

(1) investigate the proton-binding properties of surfaces representing compositional gradient from fully exposed cells to purely inorganic silica surfaces and an intermediate cell- SiO_2 composite; and,

(2) compare the surface proton-charging behavior of cells showing some putative level of cellular activity versus inactivated cells, by acid-base titration.

Chapter 5 is a summary of all the significant findings concluded from the three main chapters (Chapters 2-4), and an overall synthesis and broader implications reckoned from the major results of this thesis.

CHAPTER 2. IDENTIFYING WHEN MICROBES BIOSILICIFY: THE INTERCONNECTED REQUIREMENTS OF ACIDIC pH, COLLOIDAL SiO₂ AND EXPOSED MICROBIAL SURFACE

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Identifying when microbes biosilicify: the interconnected requirements of acidic pH, colloidal SiO₂ and exposed microbial surface. *Chemical Geology*, 240: 298-312.

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2.1 Introduction

Field observation of modern hydrothermal sinters has shown widespread and copious *in situ* silicification of a wide variety of microbial species (Jones, et al., 1999; McKenzie, et al., 2001; Guidry and Chafetz, 2003; Smyth, et al., 2003), both extracellularly (i.e., sheaths and cell walls: Ferris, et al., 1986; Kato et al., 2004) and intracellularly (i.e., within the cytoplasm: Konhauser et al., 2001; Phoenix et al., 2003). However, to date, cumulative laboratory investigations have failed to demonstrate a mechanistic role for microorganisms to explain the observed biosilicification process in natural systems. For example, laboratory investigations conducted at silica undersaturated conditions (Fein, et al., 2002; Phoenix et al., 2003; Yee, et al., 2003) found no quantifiable direct microbe-SiO₂ interaction. These results have been interpreted to indicate that the presence of microbes does not enhance the precipitation of solid-phase silica under dilute or undersaturated silica solutions (Inagaki et al., 1998; Yee, et al., 2003). Conversely, laboratory studies conducted under supersaturated silica solutions have shown that the presence of microbial cells affected neither the rate, nor the extent of spontaneous silica polymerization, as compared to abiotic controls (Phoenix, et al., 2003; Yee, et al., 2003). From these results, it has subsequently been hypothesized that abiotic, geochemically controlled silica precipitation (e.g., cooling and evaporation:

Guidry and Chafetz, 2002; Lowe and Braunstein, 2003; Konhauser, et al., 2004) of silica-supersaturated waters is the dominant silicification pathway in hydrothermal systems with very minimal to nil microbial intervention.

However, some of the discrepancies between widely observed *in situ* biosilicification and the inability to demonstrate this process under well-constrained laboratory conditions, may reflect two factors that have not been simultaneously considered in existing laboratory experimentation: (1) the pH-dependent kinetics of silica precipitation and the related, relative ability of microbes to impact the process to an observable extent; and (2) the differential reactivities of colloidal vs. dissolved silica forms in silicification occurring at microbial surfaces.

While thermodynamic equilibrium provides the realm of possible chemical reactions, kinetics largely control the arena of possible microbially catalyzed geobiological processes. For any energetically favorable reaction, microbial catalysis will be most evident when kinetic energy barriers impede the rate of an abiogenic process, as has been shown for the formation of a variety of biominerals such as Mn oxyhydroxides (Tebo, et al., 1997; Haack and Warren, 2003), Fe oxyhydroxides (Warren and Ferris, 1998), carbonates (Thompson, et al., 1990; Little, et al., 1997) and silicates (Fortin and Beveridge, 1997; Frankel and Bazylinski, 2004). The kinetics of aqueous silica ($\text{SiO}_{2(\text{aq})}$) polymerization and aggregation are controlled primarily by system pH, and are typically at a minimum close to pH ~3, but increase to an almost instantaneous rate at neutral/slightly-alkaline pH values (pH ~7-9: Iler, 1979; Bergna, 1994; Brinker, 1994). For instance, rates of $\text{SiO}_{2(\text{aq})}$ polymerization are nearly two orders of magnitude slower at pH 4 compared to pH 6 (Iler, 1979; Gorbach, et al., 2006) while maximum

silica polymerization rates have been measured to occur between pH values 8 and 9 over an examined pH range of 4-10 (Coradin and Livage, 2001 and refs. cited). Complete gelation (i.e. aggregation) of silica was observed to occur in minutes in a supersaturated solution ($[\text{SiO}_{2(\text{aq})}] = 0.45\text{M}$) at pH values between 6-9, while no $\text{SiO}_{2(\text{aq})}$ gelling was observed after 24 hours at pH 3 under the same $[\text{SiO}_{2(\text{aq})}]$ (Muñoz-Aguado and Gregorkiewitz, 1997). Thus, the relatively slower kinetics of acidic silica polymerization may represent a more biologically favorable window in which a microbially-driven process can be distinguished from abiotic contributions.

Further, to date, published laboratory studies have evaluated only dissolved, monomeric silica species (i.e., silicic acid, H_4SiO_4) in microbial biosilicification experiments. Silicic acid occurs dominantly as the neutral H_4SiO_4^0 species over the pH range of most environmental systems (i.e. $\text{pH} < 8$, Fein, et al., 2002; Lopez, et al., 2005 and refs. cited) making the coordination of neutral silicic acid species with the predominantly negatively-charged microbial surface challenging to invoke mechanistically. Where considerable ionization of silicic acids (H_3SiO_4^-) occurs under alkaline conditions ($\text{pH} > 9$; Iler, 1979; Coradin and Livage, 2001), cation bridges (e.g., Fe: Ferris, et al., 1986; Ferris, et al., 1988; Urrutia and Beveridge, 1993) and metal oxyhydroxide (co)precipitation mechanisms (e.g., Fe-OOH: Fortin, et al., 1997; Phoenix, et al., 2003; Benning, et al., 2004) have been considered as possible routes for the coordination of negative H_3SiO_4^- species to negatively charged bacterial surfaces in biosilicification processes. However, these investigations on the roles of both cation/salt bridges and metal-oxyhydroxide- SiO_2 (co)precipitates as potential mechanisms for $\text{SiO}_{2(\text{aq})}$ sequestration on bacterial surfaces have only limited success, weakening these

hypotheses as plausible mechanisms to explicitly show microbial influence in biosilicification.

Another consideration is that microbially-influenced silicification in nature may reflect a colloidal silica pathway. Biomineralization processes generally assume that the starting point for biological concentration and transformation of geochemical components commence from dissolved forms (Navrotsky, 2004). However, there is increasing evidence that clusters, nanoparticles, and other complex metastable constituents in the solution phase play an important role in biomineralization (Banfield, et al., 2000; Perry and Tucker, 2003; Navrotsky, 2004). Suggestions that microbes utilize polymeric SiO_2 (i.e., colloidal nanospheres) in biosilicification have been discussed or alluded to in the literature (Schultze-Lam, et al., 1995; Phoenix, et al., 2000; Konhauser, et al., 2001; Inagaki, et al., 2003; Benning, et al., 2004; Konhauser, et al., 2004), and three-dimensional, nm-sized silica colloids have been shown to represent the dominant silica form (ca. $\geq 50\%$) equilibrated in the aqueous phase for a variety of silica-saturated artificial and engineered systems (Dove and Rimstidt, 1994; Muñoz-Aguado and Gregorkiewitz, 1997; Davis, et al., 2002; Rao and Gelb, 2004). Most hydrothermal systems are characteristically saturated with respect to amorphous silica, and thus the presence of colloidal silica in these systems is plausible, but has not been directly evaluated to date in the literature. While colloidal (i.e., polysilicic) silica nanoparticles have specifically been suggested to play a role in both field (Konhauser and Ferris, 1996; Smyth, et al., 2003; Asada and Tazaki, 2001) and laboratory (Inagaki, et al., 1998; Benning, et al., 2004) studies of biosilicification, no systematic investigation has yet been carried out to evaluate the potential role of colloidal silica species; and specifically,

consider experimental conditions where biological mediation of silica would be most favorable relative to abiotic silica polymerization. Thus, the specific objectives of this study were to evaluate: (1) if discernible microbial biosilicification can be promoted when the “kinetic slider” is shifted towards greater biological opportunity (i.e., near $\text{SiO}_{2(\text{aq})}$ saturation and acid pH values where reactions are favorable but kinetically slow abiotically); and, (2) whether colloidal SiO_2 is implicated in biologically-mediated silicification processes.

2.2 Experimental methods

Batch silica uptake experiments were performed with three microbial mat systems collected from two shallow (<8cm), contrasting hot springs system of Yellowstone National Park (YNP), USA in July of 2005 (Table 2.1). Mat samples were sampled from two different hotsprings with system conditions determined prior to sampling (Havig, Jeff and Shock, Everett, 2005; pers. comm.) to reflect a continuum of potential possible biological mediation from most biologically favorable (acidic, lower temperature) to less biologically favorable (alkaline, lower temperature) to least favorable (alkaline, higher temperature: Iler, 1979; Rao and Gelb, 2004; Tossell, 2005). Both hotspring systems are characteristically saturated with respect to amorphous silica (total $[\text{SiO}_{2(\text{aq})}]$: Goldielocks Springs = 235-300 ppm; Bison Pool = 360-420 ppm). The acidic, mesophilic (AM) mat was collected from Goldielocks Springs, with ambient pH values ~ 3 , and moderate temperature ($\sim 35^\circ\text{C}$), located within the Sylvan Springs hydrothermal system ($44^\circ 42' 0.4''$ N Latitude, $110^\circ 45' 52.9''$ W Longitude). The other two mats were collected from Bison Pool ($44^\circ 34' 10.6''$ N Latitude, $110^\circ 51' 54.5''$ W Longitude) an alkaline (pH ~ 8) spring in

the Sentinel Meadows Mound Springs system. The alkaline, thermophilic mat (ALK-T; $T = 80^{\circ}\text{C}$) and the alkaline, mesophilic mat (ALK-M; $T = 35^{\circ}\text{C}$) represent the proximal and distal parts from the discharge of Bison Pool, respectively (Table 2.1). At the time of sampling for each mat, overlying water temperature and pH were measured at the site and water samples were taken for silica analyses (dissolved and colloidal). Samples for the three mats were collected using sterile spatula or tweezers, placed in sterile 50mL polypropylene tubes (PP; Falcon), hydrated with 25% (v/v) ambient mat-specific hotspring water, and stored on ice until return to the laboratory (3-5 days from collection) where they were stored at 4°C until experimentation. All laboratory glass and plastic ware for geochemical analyses were soaked overnight in 4% (v/v) HCl, and rinsed with ultrapure water (UPW: Milli-Q, $18.2\ \Omega$; Millipore). Materials used for microbial batch experiments were either manufacturer-certified sterile, or autoclaved at 121°C for 30 minutes (Castle[®], Conbraco Industries, Inc.).

Sample	Source	Relative distance from source (m)	In-situ T(°C±5)	^d In-situ [^c SiO ₂]	In-situ pH(±0.5)	^e [SiO _{2(aq)}] ⁰
^a AM _{mat}	Goldielocks Springs	Middle (18)	35	≤5%	3	150ppm
^b ALK-T _{mat}	Bison Pool	Proximal (1.5)	80	23%	8	350ppm
^c ALK-M _{mat}	Bison Pool	Distal (25)	35	50%	8	150ppm

Table 2.1 Microbial mat source and mat *in-situ* T°C and pH conditions. ^aAM_{mat}=acid, mesophilic mat; ^bALK-T_{mat}=alkaline, thermophilic mat, ^cALK-M_{mat}=alkaline, mesophilic mat; ^dpercent *in-situ* colloidal silica determined from overlying water column; ^e[SiO_{2(aq)}]⁰ near-saturated aqueous silica working concentration (dissolved or colloidal) used in phase 1 batch silicification experiments adjusted according to mat-specific, field *in-situ* temperature (Tarcn, 2001; Mountain, et al., 2003).

Laboratory batch silicification experiments were carried out in two experimental phases. In phase 1, individual experiments were performed for each mat at its respective *in situ* T°C and pH conditions, with both dissolved silica ($^D\text{SiO}_2$: $\text{Na}_2\text{SiO}_3 \cdot 5\text{H}_2\text{O}$, 27%; Waterglass, Sigma-Aldrich) and colloidal silica ($^C\text{SiO}_2$: Ludox, 30%, nominal dia. $\sim 14\text{nm}$; Sigma-Aldrich) forms (identical nominal starting $[\text{SiO}_{2(\text{aq})}]$) in separate batch systems. Thus, phase 1 experiments varied in pH, temperature and absolute $[\text{SiO}_{2(\text{aq})}]$ for each of the three mats to provide near-saturated silica conditions, and to ensure that each mat was experimentally maintained at its *in situ* temperature and pH. Nominal starting aqueous silica concentrations ($[\text{SiO}_{2(\text{aq})}]^0$; Table 2.1, column e) for each mat treatment were selected to maintain the saturation index for amorphous SiO_2 constant across all treatments (saturation index = $\log \{Q/K\} \sim 0$; Iler, 1979; Tarcán, 2001; Mountain, et al., 2003) under varying pH and temperature scenarios. Both abiotic particle (TiO_2 , 99.9+%; $<5\mu\text{m}$ dia.; Sigma-Aldrich) and solution ($\text{SiO}_{2(\text{aq})}^0$ only) controls were also evaluated at identical pH, temperature and $[\text{SiO}_{2(\text{aq})}]^0$ for each of the three microbial treatment systems. TiO_2 solids were chosen over SiO_2 as abiotic control surfaces for silicification to prevent the homogeneous polymerization of $\text{SiO}_{2(\text{aq})}$ associated with SiO_2 surfaces specifically, and because of the TiO_2 particles' similarity to microbial cell size. All five treatments (AM_{mat} , $\text{ALK-T}_{\text{mat}}$, $\text{ALK-M}_{\text{mat}}$, abiotic TiO_2 and $\text{SiO}_{2(\text{aq})}$ controls) were then evaluated for silica scavenging in the three experimental systems for both colloidal and dissolved SiO_2 batch experiments for 60 hours, with aliquots collected every 12 hours for determination of solution silica concentration.

In the second phase of experimentation, batch silicification experiments were repeated at the same pH (pH=3), temperature (T=35°C) and starting aqueous colloidal

silica ($[^{\text{C}}\text{SiO}_2]^0 = 150$ ppm) conditions as the AM mat system used in phase 1 experiment. All three mats, as well as associated TiO_2 particle and solution controls were evaluated under these conditions only for $^{\text{C}}\text{SiO}_2$ uptake. All five treatments in the phase 2 experiments were evaluated for $^{\text{C}}\text{SiO}_2$ scavenging for 120 hours with aliquots collected every 12 hours for determination of solution silica concentration.

For all experimental systems (phase 1 and 2), silica solutions were pH-adjusted (± 0.1) using sterile, high purity H_2SO_4 or NaOH and were set up as follows: 100 mg (nominal wet weight) mat sample was first acclimatized to room temperature from 4°C for approximately one hour, then rinsed with sterile UPW, placed in sterile 50 mL PP tubes containing 45 mL of appropriate starting experimental aqueous silica concentrations (dissolved or colloidal; Table 2.1, column e), and then heated in a water bath (Forma Scientific model 2568) to the desired temperature ($\pm 0.5^\circ\text{C}$) and shaken under low setting such that tubes were minimally agitated. Phase 1 silicification experiments were performed in single experimental tubes due to limited mat sample availability. In phase 2 silicification experiments, triplicate tubes were run for each treatment. Silica values varied less than 12% across triplicate tubes, thus silica values for triplicate tubes in each batch system were pooled to generate a mean and standard error ($X \pm \text{S.E.}$) for each treatment (AM_{mat} , $\text{ALK-T}_{\text{mat}}$, $\text{ALK-M}_{\text{mat}}$, and the abiotic and solution controls).

SiO_2 mass balance was performed for the phase 2 series of batch experiments to account for variable authigenic SiO_2 associated with the mats, i.e. formed *in situ* and collected with the mats. Pre- and post-experiment mat samples were first lightly washed in sterile UPW, digested with NaOH for an hour at 105°C (Iler, 1979; Clesceri et al., 1998), and then analyzed for silica to determine the initial pre- $^{\text{C}}\text{SiO}_2$ exposure (i.e.,

authigenic) and post- $^{13}\text{C}\text{SiO}_2$ (authigenic + experimentally scavenged) associated with the biotic solids. Microbial mat blank treatments (i.e., mat sample + pH 3_{UPW}; no $^{13}\text{C}\text{SiO}_{2(\text{aq})}$) were also evaluated to account for any potential authigenic silica dissolution under the $^{13}\text{C}\text{SiO}_2$ batch silicification experimental conditions.

Silica analysis was done via spectroscopy (Pharmacia Biotech, Ultraspec 3000, 410nm) using the molybdate-yellow technique (Clesceri, et al., 1998). NaOH was used to digest the unreactive $^{13}\text{C}\text{SiO}_2$ ($T^\circ\text{C} \sim 105$) in lieu of NaHCO_3 from Clesceri et al's (1998) procedure to prevent turbidity from CO_2 liberation upon acidification, prior to the distinctive yellow color development by molybdate addition. Silica absorbance values were calibrated against silica standards (HACH Co.) for both $^{29}\text{SiO}_2$ and $^{13}\text{C}\text{SiO}_2$ ($R^2 \geq 0.99$). Analytical uncertainty measured from this method is $< 2.5\%$. Silica uptake was determined as the difference between the nominal starting $[\text{SiO}_{2(\text{aq})}]^0$ (for both $^{29}\text{SiO}_2$ and $^{13}\text{C}\text{SiO}_2$) and measured silica values in the solution phase. Variability in solution controls ($^{29}[\text{SiO}_2]^0$ and $^{13}[\text{SiO}_2]^0$ only; $< 5\%$) were within published analytical uncertainty (5%; Coradin, et al., 2002), indicating that $[\text{SiO}_{2(\text{aq})}]^0$ loss greater than this value can be considered to reflect an interaction between $\text{SiO}_{2(\text{aq})}$ and the experimental solids used.

A hierarchal, nested multi-level structure classified by silica form, experimental conditions and solid treatment systems was used in the statistical analyses for both phase 1 and 2 experiments to identify any factor(s) or interaction(s) among experimental parameters important for the observed trends (Anderson, 1987). Differences in silica observations, as impacted by each level in the hierarchal design, were tested for significance (t-statistic) using MLwiN® Software version 2.02. A “rigid” multi-level data analysis was applied where the combined contributions from each hierarchy: silica

forms (dissolved vs. colloidal), experimental pH, temperature and $[\text{SiO}_{2(\text{aq})}]$ conditions (AM, ALK-T, ALK-M) and solid treatment systems (solution, abiotic, and biological mat) were factored in simultaneously. The acceptance criteria applied for significance level testing for all experiments was at $p < 0.05$ significance level, where d.f. = 107 and d.f. = 54 for phase 1 and 2, respectively.

Imaging and elemental analyses of the biological and abiotic particles' pre- and post-experimental silicification were performed using an environmental scanning electron microscope (ESEM, System 2020, ver. 3.53) equipped with energy dispersive x-ray spectroscopy (EDS, PGT IMIX system). Post-silicification microbial cell viability was evaluated using the live/dead cell assay (Baclight L7012, Molecular Probes) as per manufacturer protocols (Invitrogen Molecular Probes Product Information, 2004) and imaged under an epifluorescence light microscope (Leica DMR, Leica Microsystems) fitted with a digital camera (Hamamatsu Digital CCD Cam ver. 1.1), and associated imaging software (Openlab ver. 2.2.5). Accuracy of the live/dead staining procedure was evaluated with cultured freshwater cyanobacterial isolate (*Synechococcus cedrorum*; grown at 25°C, pH ~7.2; >95% live), and dead cyanobacterial cells (>95 % dead; boiled vigorously for 20 minutes in 10% v/v HCl and resuspended in 70% isopropyl alcohol for 1.5 hours; images not shown).

2.3 Results

2.3.1 Phase 1: $^{\text{D}}\text{SiO}_2$ silicification at individual mat-specific T(°C) and pH

No statistically significant silicification was observed for any of the microbial and abiotic particle treatment systems, at any of the three experimental pH and temperature conditions (data not shown, $p > 0.15$) exposed to near-saturated $^{\text{D}}\text{SiO}_2$ concentrations. In all batch systems, less than 7% of the starting $^{\text{D}}\text{SiO}_2$ was removed from the aqueous phase after 60 hours, indicating that none of the three microbial consortia nor the abiotic particle solids induced significant polymerization of $^{\text{D}}\text{SiO}_2$ over a range of acidic-moderate, to alkaline-moderate to high temperature conditions, despite favorable near-saturation $^{\text{D}}\text{SiO}_2$ concentrations.

2.3.2 Phase 1: $^{\text{C}}\text{SiO}_2$ silicification at individual mat-specific T(°C) and pH

In contrast to the above dissolved silica results, significant differences in colloidal silica uptake under the same set of pH, temperature and $[\text{SiO}_{2(\text{aq})}]^0$ conditions were observed after 60 hours, particularly for the acid, mesophilic conditions associated with the AM mat (Fig. 2.1-a; $p < 0.001$), and the AM abiotic particle treatment (Fig. 2.1-a; $p < 0.05$), as well as for the ALK-T abiotic control treatment (Fig. 2.1-b; $p < 0.002$). Both the AM mat and its corresponding abiotic particle control showed $^{\text{C}}\text{SiO}_2$ scavenging under acidic-mesophilic conditions (Fig. 2.1-a) relative to the solution control. However, the AM biological mat showed significantly higher $^{\text{C}}\text{SiO}_2$ uptake (~20%; $p < 0.001$) than its abiotic particle control (~10%; $p < 0.05$) counterpart. The ALK-T abiotic particle control treatment also showed observable $^{\text{C}}\text{SiO}_2$ uptake compared to ALK-T solution control (~10%; Fig. 2.1-b; $p < 0.001$), while no significant $^{\text{C}}\text{SiO}_2$ uptake was measured in

the ALK-T microbial mat system. Both the ALK-M microbial mat, and its abiotic particle counterpart system showed insignificant $^{29}\text{SiO}_2$ uptake compared to the ALK-M solution control treatment (Fig. 2.1-c; $p > 0.1$).

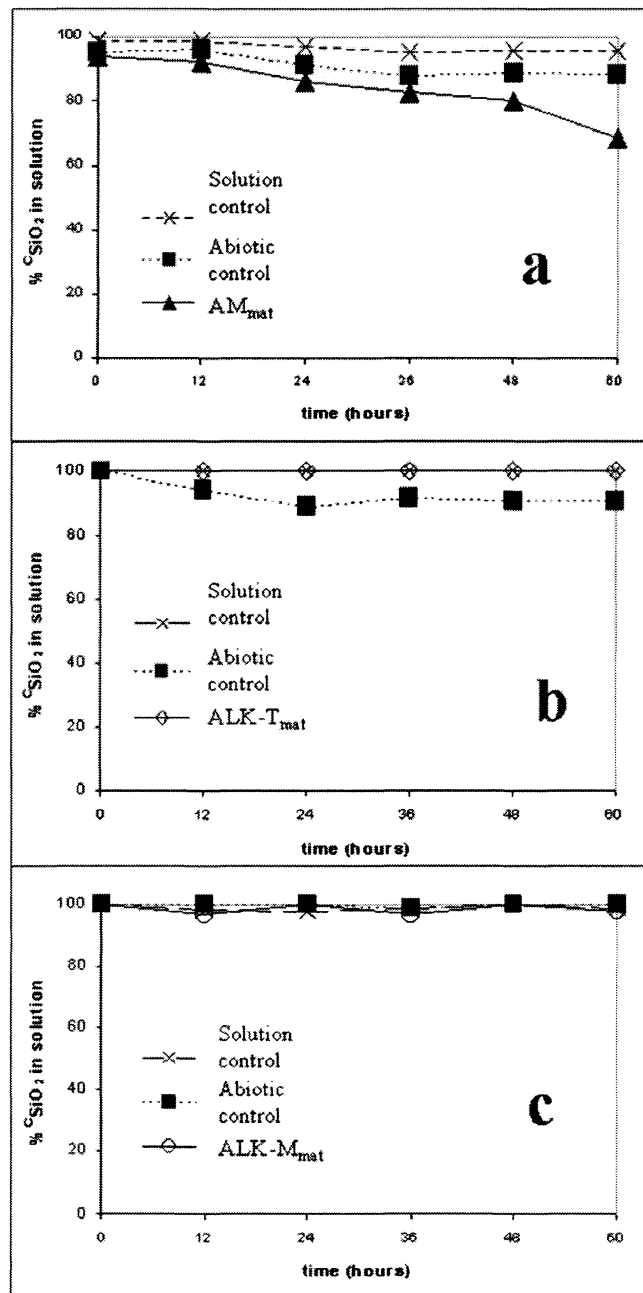


Figure 2.1 Percent colloidal silica ($^C\text{SiO}_2$) loss (as % $^C[\text{SiO}_2]^0$) as a function of time in experimental conditions: (a) AM, $^C[\text{SiO}_2]^0=150\text{ppm}$, pH 3, 35°C ; (b) ALK-T, $^C[\text{SiO}_2]^0=350\text{ppm}$, pH 8, 80°C ; (c) ALK-M, $^C[\text{SiO}_2]^0=150\text{ppm}$, pH 8, 35°C for 60 hours designed to mimic microbial *in-situ* temperature and pH conditions. All solution controls showed $<5\%$ $^C[\text{SiO}_2]^0$ variability.

2.3.3 Phase 2: $^{29}\text{SiO}_2$ uptake under acidic (pH=3) and mesophilic (T=35°C) conditions for all treatments

In these experiments, all three microbial mats were exposed to identical experimental $^{29}\text{SiO}_2$, and the acidic, moderate temperature conditions observed *in situ* for the AM mat system (pH = 3, T = 35°C). Results show that the two mat systems from the higher pH (pH = 8: both ALK-T and ALK-M) and higher temperature (T = 80°C; ALK-T), as well as the moderate temperature regimes (T = 35°C; ALK-M) scavenged greater $^{29}\text{SiO}_2$ compared to the solution control ($p < 0.04$; Fig. 2.2) under these lower pH and temperature conditions. This trend is in contrast to their results obtained in phase 1 experiments at the same silica saturation but at their *in situ* higher pH for both ALK-T and ALK-M mats and, in the case of the ALK-T mat, higher temperature conditions (Fig. 2.1-b; Fig. 2.1-c). These results indicate biosilicification is sensitive to pH under constant, favorable $[\text{SiO}_{2(\text{aq})}]$ saturation. The AM mat treatment, which had shown the highest $^{29}\text{SiO}_2$ uptake in phase 1 experiments conducted at mat-specific pH and temperature conditions (Fig. 2.1-a), again took up significantly more $^{29}\text{SiO}_2$ relative to both of the other two mat treatments ALK-T and ALK-M, as well as the abiotic particle system ($p < 0.001$; Fig. 2.2) under the same experimental conditions in phase 2, indicating differential scavenging occurred under identical geochemical conditions. After 120 hours, the AM mat had scavenged ~45% of the total colloidal silica added (total $^{29}\text{SiO}_2$ in batch systems = 112.5 μmol), compared to only ~14% observed in the ALK-M mat system, and ~7% for both the ALK-T mat and the abiotic TiO_2 control particles (Fig. 2.2).

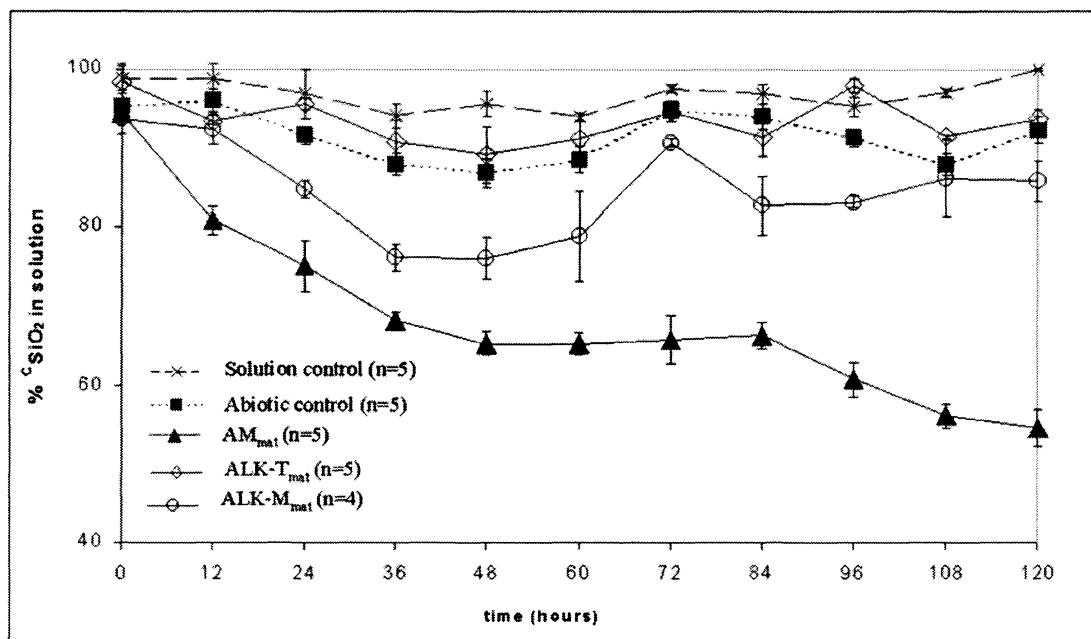


Figure 2.2 Percent colloidal silica ($^C\text{SiO}_2$) loss (as $\% ^C[\text{SiO}_2]^0$) as a function of time fixed at acidic, mesophilic AM conditions: $^C[\text{SiO}_2]^0 = 150\text{ppm}$, pH 3, 35°C containing mat treatments: AM_{mat} , $\text{ALK-T}_{\text{mat}}$, $\text{ALK-M}_{\text{mat}}$, and abiotic TiO_2 particles and solution controls over 120 hours (5 days); data shown are mean values ($\pm$ S.E.); variability in $^C\text{SiO}_2$ solution control is $<5\%$ (note y-axis scale).

Evaluation of $^{14}\text{C}\text{SiO}_2$ uptake normalized by mat dry weight ($\text{mM } ^{14}\text{C}\text{SiO}_2 \text{ mg}^{-1}$; Fig. 2.3), shows that the acidic, mesophilic AM mat took up $0.12 \text{ mM } ^{14}\text{C}\text{SiO}_2 \text{ mg}^{-1}$ of the initial $2.5 \text{ mM } ^{14}\text{C}\text{SiO}_2$ after 120 hours, at least 0.08 mM mg^{-1} higher than either the 0.04 mM mg^{-1} net $^{14}\text{C}\text{SiO}_2$ taken up by the alkaline, mesophilic ALK-M mat, the 0.02 mM mg^{-1} $^{14}\text{C}\text{SiO}_2$ taken up by the alkaline, thermophilic ALK-T mat or the 0.02 mM mg^{-1} associated with abiotic TiO_2 particle system. In all particle treatments, $^{14}\text{C}\text{SiO}_2$ uptake increased initially, then shows an observable plateau in further uptake followed by variable responses until the end of the experiment. However, results show that the most observable contrast in the pattern of observed $^{14}\text{C}\text{SiO}_2$ uptake occurs in the AM mat treatment, where a sustained increase in $^{14}\text{C}\text{SiO}_2$ uptake was observed compared to the other two mat systems and the abiotic particle control until the conclusion of the experiment (Fig. 2.3). Further, there was no statistically significant difference in $^{14}\text{C}\text{SiO}_2$ uptake between the ALK-T mat and the abiotic TiO_2 control treatment ($p > 0.45$; Fig. 2.2; Fig. 2.3). These results indicate that differences in $^{14}\text{C}\text{SiO}_2$ uptake among microbial mats can be as great as those observed between microbial and abiotic particle systems.

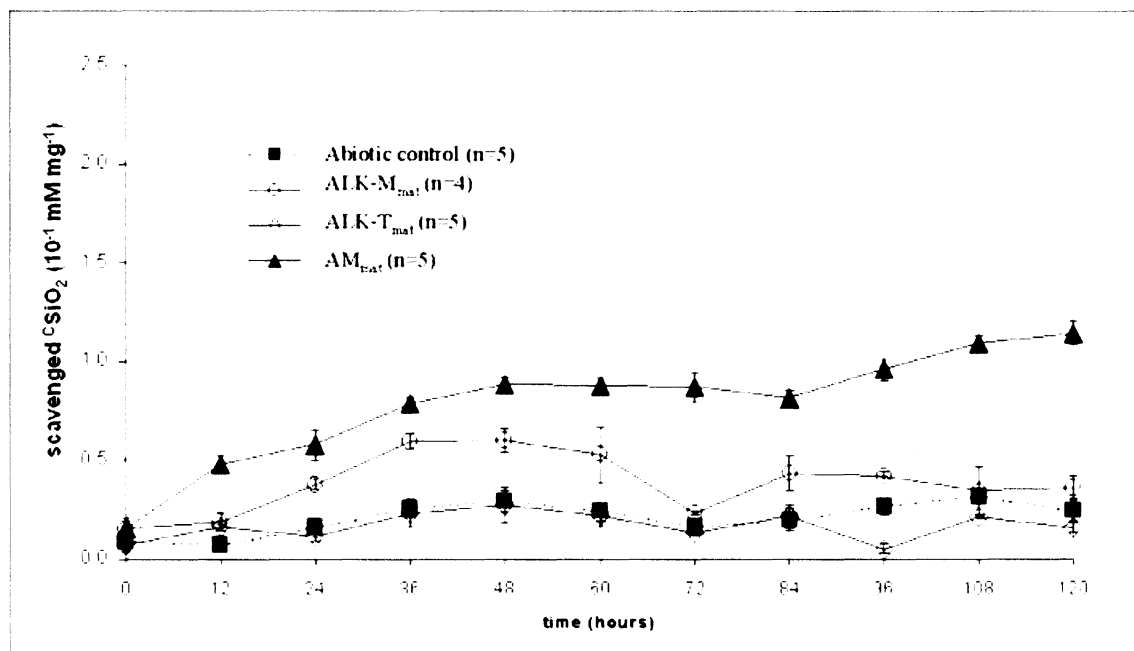


Figure 2.3 $^{2}\text{SiO}_2$ (mM) uptake as a function of time per mg (dry) experimental solid ($\text{mM } ^2\text{SiO}_2 \text{ mg}_{\text{dry}}^{-1}$) for mats: AM_{mat}, ALK-T_{mat}, ALK-M_{mat} and abiotic TiO_2 particle treatments (mean $\pm$ S.E.); $^{2}\text{SiO}_2^0 = 2.5 \text{ mM}$ in 45mL batch experimental solutions under AM conditions (i.e., pH=3, T=35°C).

2.3.4 Phase 2: SiO₂ mass balance and evaluation of authigenic SiO₂ dissolution

The authigenic SiO₂ concentrations, i.e., derived *in situ*, associated with the three mats varied indicating differential surface encrustation of the mats, where the mats taken from the alkaline Bison Pool hotspring system show greater native silicification compared to the mat taken from the acidic Goldielocks Springs (Fig. 2.4). Mass balance calculations from the measured difference between the “total” SiO₂ (i.e., post-experimental ^CSiO₂ = authigenic SiO₂+experimentally scavenged ^CSiO₂) and pre-experimental (i.e., pre-^CSiO₂ = authigenic SiO₂), for each mat system showed an experimentally associated increase in ^CSiO₂ concentrations for all mats that inversely reflects the degree of prior encrustation: AM_{mat} > ALK-M_{mat} > ALK-T_{mat} (Fig. 2.4). That is to say, the greater the native silica encrustation of the mat surface, the lower net ^CSiO₂ uptake observed in batch experiments run at identical pH of 3 and temperature of 35°C conditions (Fig. 2.2; Fig. 2.3). Extensive variability in the values obtained for pre-experimental associated silicification of each mat precluded an accurate mass balance determination of ^CSiO₂ uptake in these experiments. However, the mmol SiO₂ difference measured between pre- and post-experiment ^CSiO₂ was always positive, confirming that uptake had occurred during experimental exposure above and beyond any mat associated, authigenic SiO₂ derived *in situ*, but varied by as much as a factor of eight for the ALK-T mat compared to the actual measured ^CSiO₂ loss during batch experimentation (i.e., sorbed ^CSiO₂; Fig. 2.4) and by a factor of three for the ALK-M and AM mat systems. No detectable dissolved SiO₂ in solution (as molybdate-reactive silicic acid) was observed in the phase 2 experimental pH 3, 35°C mat blank systems (mat sample + pH 3_{UPW}) for all three biological solids after 120 hours (data not shown), indicating that dissolution of

mat-associated authigenic SiO_2 did not occur under the acidic moderate temperature conditions of the experiment and does not influence the observed pre- and post-silicification mass balance results.

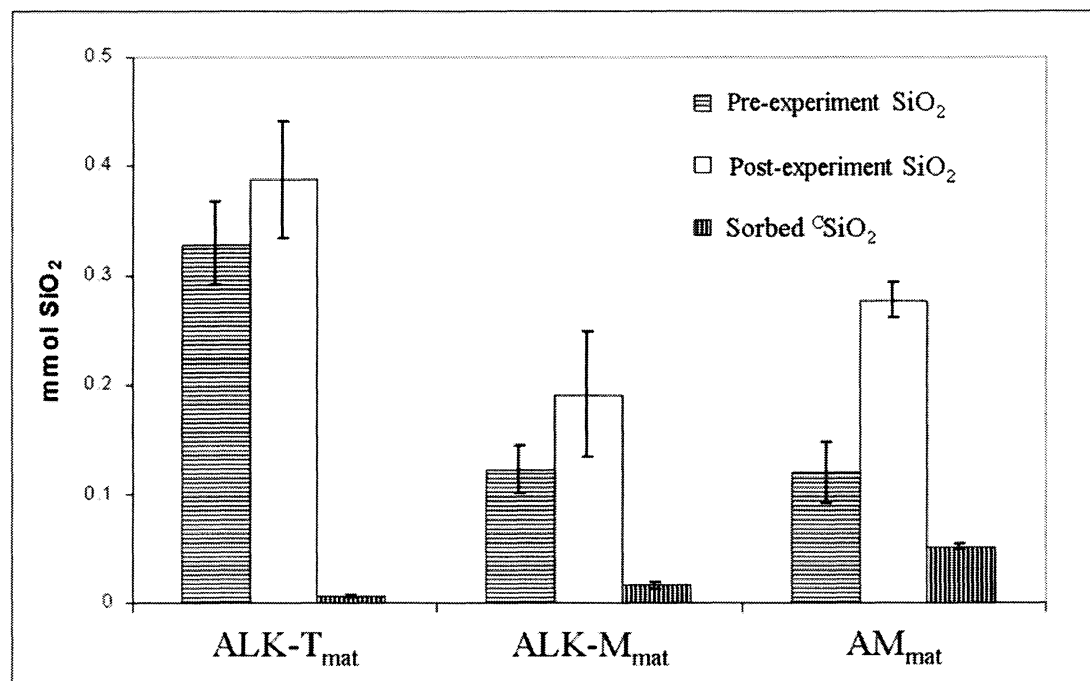


Figure 2.4 Microbial mat pre- and post- experiment [̑]SiO₂ silicification at pH 3 mass balance calculations (mmol). Difference between post-experiment SiO₂ (i.e., “total”) and pre-experiment (i.e., authigenic) SiO₂ shows net scavenged [̑]SiO₂ in the batch silicification experiments. Actual measured mmol [̑]SiO₂ loss in the presence of mat solids (i.e., sorbed [̑]SiO₂) varied by up to eight times compared to the difference between post- and pre- experiment silicification SiO₂ values (see text for explanation); total [̑]SiO₂ added in batch systems = 112.5 μ mol; mmol SiO₂ values shown are means ($\pm$ S.E.; n=3; sorbed [̑]SiO₂, n=4 or 5).

2.3.5 Phase 2: Microbial mat live/dead cell assay

Post-silicification cell viability tests for the biological AM and ALK-M mat systems were both greater than 95% (data not shown) indicating that cells were still viable after experimentation in the laboratory. However, no meaningful viable cell counts could be obtained for the ALK-T mat reflecting its heavy encrustation with authigenic SiO₂, which most likely prevented penetration of the molecular probes to the cells.

2.3.6 Phase 2: Experimental solids ESEM imaging and EDS

Visual examination by ESEM of the three mats indicated that authigenic SiO₂ associated with these mats was greatest for the ALK-T mat, followed by the ALK-M mat, and the least for the AM mat (Fig. 2.5-i; Fig. 2.5-e; Fig. 2.5-a, respectively), consistent with the bulk geochemical analysis of authigenic SiO₂ determined from the environmental mat samples presented above (i.e., pre-^CSiO₂; Fig. 2.4). *In situ* mat associated native SiO₂ particles showed variability in particle size from several microns to tens of microns-sized diameter, and extent of coverage both for a given mat as well as across mat samples. Native SiO₂ particles ranged from subhedral to anhedral grains attached on the cells (AM mat, Fig. 2.5-a; ALK-T mat; Fig. 2.5-i) and the extracellular polymeric substances within the mat structure (EPS: AM and ALK-M mats; Fig. 2.5-b; Fig. 2.5-e, respectively) for both the acidic and alkaline springs. Larger (~30µm diameter) authigenic SiO₂ particles also appear to be overgrown by microbial mat and incorporated in the biological matrix (ALK-M mat; Fig. 2.5-e), and are typically characterized by elemental peaks in Na, Al and K in addition to Si (EDS analyses, not

shown). Larger-sized native SiO₂ particles (> 10 µm) typically occurred on mats from the alkaline Bison Pool (ALK-M, Fig. 2.5-e; ALK-T, not shown), while smaller authigenic SiO₂ (few µm; Fig. 2.5-a; Fig. 2.5-b) was observed in the acid Goldielocks Spring.

The acidic, mesophilic AM mat morphologically appears to be composed of globular cells (~5µm coccoids) encased in EPS, forming strands (Fig. 2.5-a; Fig. 2.5-b, inset) that make up the gross streamer-like mat architecture anchored along the hydrothermal spring substrate. Pennate diatoms (Fig. 2.5-a) and filamentous megamicrobes (100's µm; not shown) comprise < 10% of the polymicrobial AM mat community. In contrast, the ALK-M mat appeared microscopically homogeneous in composition (i.e., unimicrobial; Fig. 2.5-e; Fig. 2.5-f), composed almost entirely of 5 to 10 µm-length rods and EPS material. ESEM photomicrographs of the ALK-T biological mat revealed heavy encrustation of micron-sized authigenic SiO₂ (Fig. 2.5-i), which precluded visualization of the underlying microbial morphologies.

Imaging and EDS analyses of the four particle systems' post-^CSiO₂ exposure were unable to resolve a distinct overlay of the experimental ^CSiO₂ particles used in the experiments, likely reflecting the small size of the experimental ^CSiO₂ nanoparticles (~14 nm dia.) and/or the relatively low [^CSiO₂]⁰ used (i.e., 150 ppm). However, qualitative comparison of the relative intensities of Si peaks from EDS spectra of pre- and post-^CSiO₂ experimentation for solid systems observed to scavenge ^CSiO₂ in the batch experiments, i.e. the AM (pre-^CSiO₂, Fig. 2.5-c) and ALK-M mats (pre-^CSiO₂, Fig. 2.5-g), and the abiotic TiO₂ surfaces (pre-^CSiO₂ not shown), showed higher Si signals after ^CSiO₂ exposure (analyses were specifically targeted at microscopically identified

unsilicified portions of the cell and EPS pre- and post-experimentation: Fig. 2.5-d, Fig. 2.5-h; Fig. 2.5-l, respectively), consistent with net colloidal silica uptake. In contrast, the ALK-T mat results showed invariant Si peaks from EDS analysis pre-and post- $^{13}\text{C}\text{SiO}_2$ experimental exposure (Fig. 2.5-k), consistent with the lack of significant observed experimental $^{13}\text{C}\text{SiO}_2$ uptake and the extensive authigenic SiO_2 initially present on the mat surface (Fig. 2.5-i).

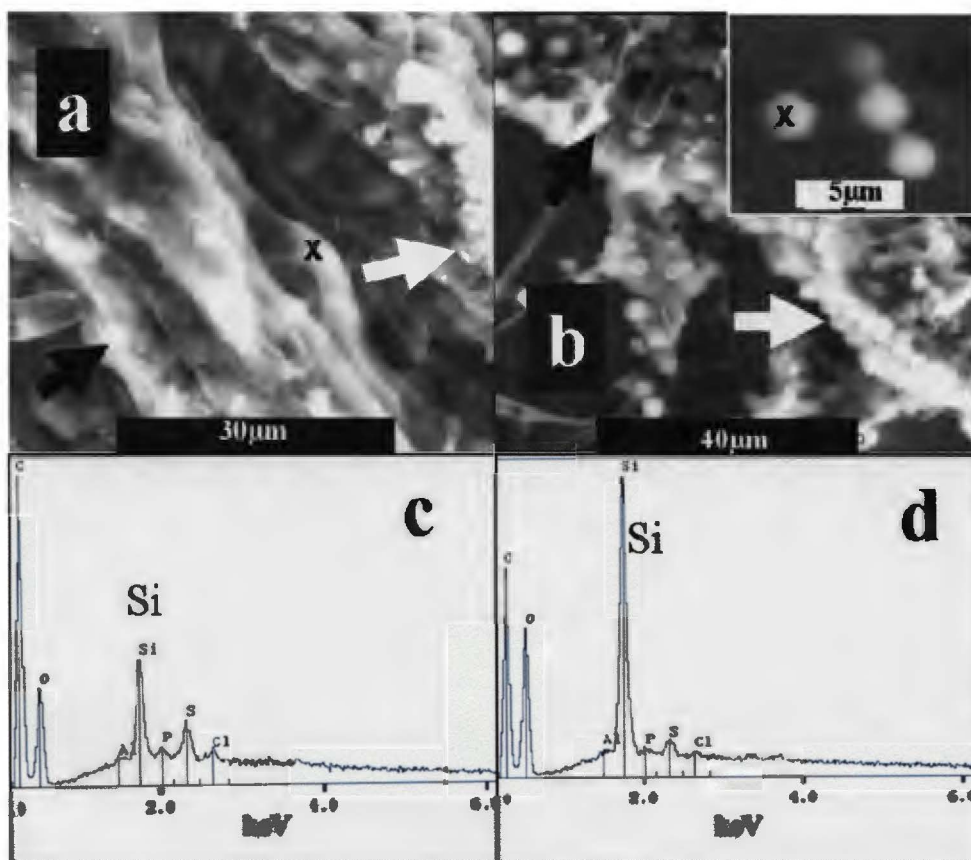
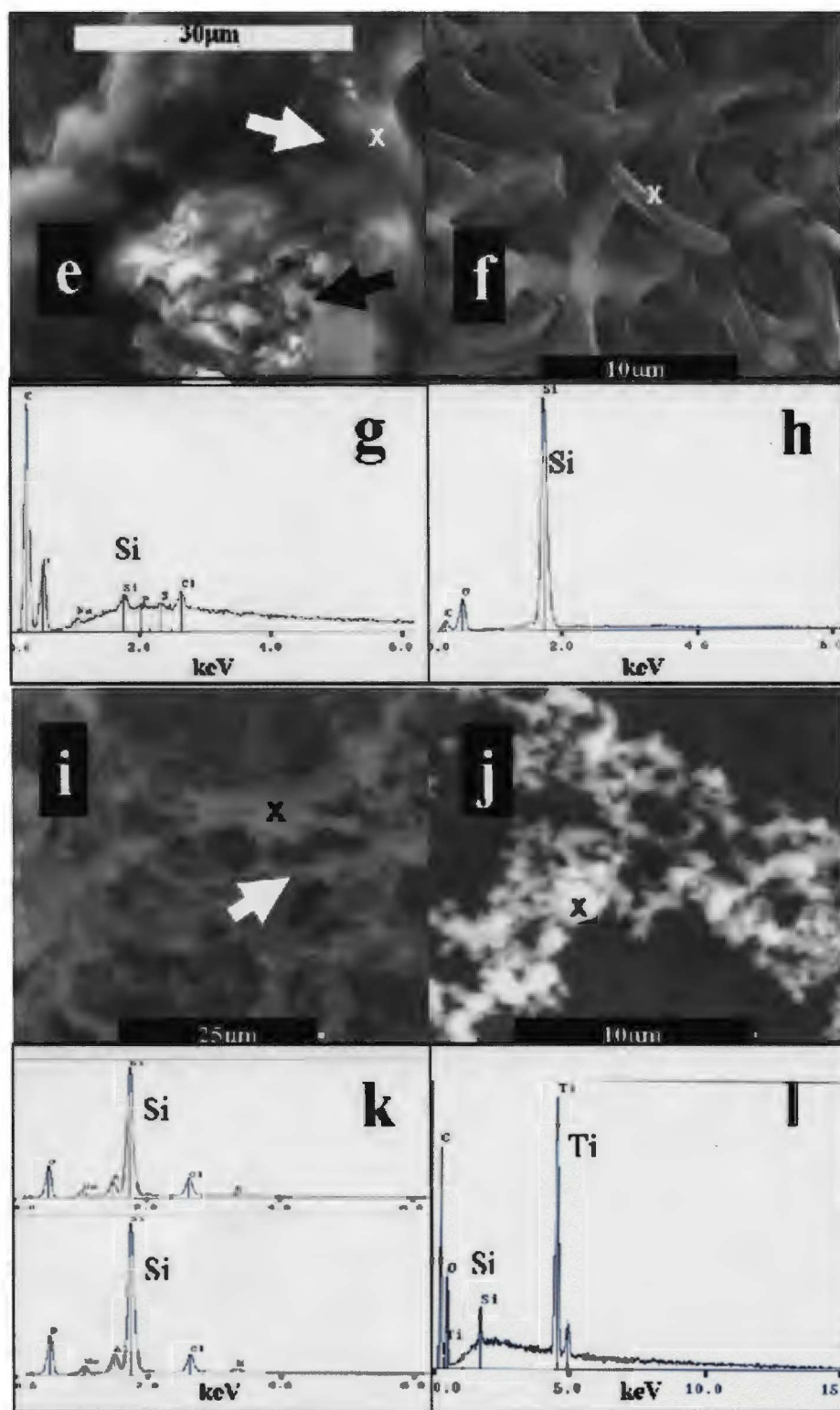


Figure 2.5 ESEM photomicrographs and EDS elemental scans of pre- and post- $^{13}\text{C}\text{-SiO}_2$ -exposed experimental solids at acid, mesophilic system conditions: (a) pre- $^{13}\text{C}\text{-SiO}_2$ AM mat showing globular cells encased in EPS (left arrow) and $<10\ \mu\text{m}$ authigenic SiO_2 encrustation of cells (right arrow), diatom frustules on top of left arrow; “x” denotes EDS target beam for all samples; (b) post- $^{13}\text{C}\text{-SiO}_2$ AM mat showing authigenic SiO_2 on EPS (top arrow) and intact globular cells in EPS strands (bottom arrow), isolated AM cell globules (*inset*); (c, d) respective pre- and post- $^{13}\text{C}\text{-SiO}_2$ EDS analysis of AM mat showing increased Si peaks in (d); (e) pre- $^{13}\text{C}\text{-SiO}_2$ ALK-M mat showing authigenic SiO_2 (bottom arrow) encased in cell and EPS matrix (top arrow); (f) disaggregated post- $^{13}\text{C}\text{-SiO}_2$ ALK-M cells and EPS materials; (g, h) respective pre- and post- $^{13}\text{C}\text{-SiO}_2$ EDS elemental scan of ALK-M cells showing increased Si peak intensity in (h); (i) pre- $^{13}\text{C}\text{-SiO}_2$ ALK-T mat heavily encrusted in authigenic SiO_2 (post- $^{13}\text{C}\text{-SiO}_2$ image not shown); (j) abiotic TiO_2 particles after $^{13}\text{C}\text{-SiO}_2$ exposure (pre- $^{13}\text{C}\text{-SiO}_2$ image not shown); (k) invariant Si peak intensity of ALK-T mat at pre-(top) and post-(bottom, area scan) $^{13}\text{C}\text{-SiO}_2$ exposure; (l) post- $^{13}\text{C}\text{-SiO}_2$ abiotic TiO_2 particles EDS scan (pre- $^{13}\text{C}\text{-SiO}_2$ scan not shown, *note keV scale*).

**Figure 2.5 (continued).**

2.4 Discussion

Results of this work counter laboratory based arguments that microbes do not exert a distinguishable impact on silicification beyond providing a surface for silica accumulation (Benning, et al., 2004; Phoenix, et al., 2003; Konhauser, et al., 2004 and refs. cited), thus reopening the door to re-evaluate biosilicification processes so widely observed in hotspring systems. The results shown here identify the requirements for thermodynamically favorable, slow silica polymerization kinetics, and colloidal SiO_2 for biosilicification to occur in a detectable manner. Our results show that no net uptake of silica occurred under near saturated $\text{SiO}_{2(\text{aq})}$ conditions when silica was present in dissolved (i.e., H_4SiO_4 ; $p > 0.15$, data not shown) form, for either the natural microbial mats or the TiO_2 mineral solids under any of the three series of experimental conditions, consistent with published literature to date (Fein, et al., 2002; Phoenix, et al., 2003; Yee, et al., 2003). The observed absence of dissolved silica uptake by all three microbial mats and their counterpart abiotic particles at mat-specific hotspring pH and temperature, parallel results from similar microbial silicification experiments using undersaturated aqueous $^{\text{D}}\text{SiO}_2$ (Inagaki, et al., 1998; Phoenix, et al., 2003; Yee, et al., 2003). In undersaturated $^{\text{D}}\text{SiO}_2$ solutions, where silicic acid “molecules” remain monomeric, direct chemical interaction between H_4SiO_4 and microbial surfaces has not been observed, most likely due to the dominantly uncharged H_4SiO_4^0 over most working pH conditions investigated ($\text{pH} < 8.2$: Fein, et al., 2002). Unless the solution pH is close to the $\text{pK}_{\text{a}1}$ of H_4SiO_4 ($\text{pK}_{\text{a}} \sim 9.5$; Iler, 1979; Brinker, 1994), silicic acid “molecules” are predominantly neutral. At the pH conditions used in this study (i.e., pH 3 and 8), the majority of the dissolved silica species thus remain uncharged, with negligible electrostatic interaction

occurring between neutral H_4SiO_4^0 species and the surfaces of any of the biotic and abiotic particles.

However, the dissolved silica results are contrasted by significant observable microbial and abiotic mineral colloidal silica uptake at similar silica saturation level (phase 1: Fig. 2.1-a; Fig. 2.1-b; $p < 0.05$) and same nominal aqueous silica concentration (phase 2: Fig. 2.2; $p < 0.04$), indicating that the previous interpretation that no silicification via a silicic acid pathway is evidence that microbes do not appreciably affect biosilicification in natural systems, have not considered the relevant form of silica involved in microbial silicification pathways, i.e., colloidal SiO_2 .

The highest colloidal silica uptake was observed for the acidophilic, mesophilic AM mat in both experimental phases demonstrating that $^{\text{C}}\text{SiO}_2$ scavenging is not simply a function of system pH and temperature at constant silica saturation. Rather, mat associated surface characteristics must also play an important role. In phase 1 experiments, the AM mat exhibited 20% greater $^{\text{C}}\text{SiO}_2$ scavenging than either of the other two mats as well as the abiotic TiO_2 particles (Fig. 2.1-a; $p < 0.005$). The abiotic TiO_2 particle treatment in the alkaline, thermophilic ALK-T conditions also showed significant (10%; Fig. 2.1-b; $p < 0.05$), although lower net $^{\text{C}}\text{SiO}_2$ uptake compared to AM mat. These results indicate two important characteristics of biosilicification which have not been elucidated before: first, the distribution of silica, either dissolved or colloidal, in the solution phase is a key factor even when total $[\text{SiO}_{2(\text{aq})}]$ may be at saturation or even oversaturated; and second, colloidal SiO_2 scavenging is surface dependent, signifying that fingerprints may be discernible in the process, as mat and abiotic particle surfaces showed differing reactivities for colloidal silica uptake.

The surprising result of significantly higher observed $^{13}\text{C}\text{SiO}_2$ uptake in the abiotic TiO_2 particle treatment compared to the biological mat under ALK-T conditions (Fig. 2.1-b; $p < 0.001$), may reflect two independent solid-associated factors: colloidal silica dynamics and the highest native surface SiO_2 coverage of the ALK-T mat compared to the other two mats. It has been shown that the rates of amorphous silica precipitation at 80°C increase as pH increases from 4 to 8 (Carroll, et al., 1998), and the presence of seeding surfaces such as TiO_2 in the current experiment may have initiated partial $^{13}\text{C}\text{SiO}_2$ scavenging from solution at alkaline, thermophilic conditions that for some reason was not activated for the corresponding ALK-T mat encrusted with native SiO_2 under the same conditions. The latter result will be discussed in more detail subsequently. It is apparent that colloidal silica nanoparticles are more reactive with biological interfaces than dissolved silica, preferentially associating with organic surfaces particularly under acidic, mesophilic conditions (i.e., AM regimes). In phase 2 experiments, when all three biological and abiotic solids were exposed to identical pH 3, moderate temperature (35°C) and $[^{13}\text{C}\text{SiO}_2]$ ($[^{13}\text{C}\text{SiO}_{2(\text{aq})}]^0 = 150 \text{ ppm}$) conditions, the AM mat treatment again showed consistently higher $^{13}\text{C}\text{SiO}_2$ scavenging than either of the two mats as well as the TiO_2 mineral particle systems, indicating the importance of both system conditions and mat surface characteristics in observed biosilicification (Fig. 2.2; Fig. 2.3; $p < 0.001$).

The importance of microbially favorable reaction windows in biosilicification is borne out by the results for the two alkaline mats. In phase 1 experiments, results show no discernible microbial colloidal silica scavenging under their indigenous habitat conditions at alkaline, high to moderate temperature conditions (i.e., ALK-T and ALK-M; Fig. 2.1-b; Fig. 2.1-c, respectively) despite near saturated $^{13}\text{C}\text{SiO}_2$ conditions. However,

when these same mats are exposed again at near-saturated $^{\text{C}}\text{SiO}_2$ conditions, but at lower pH, and in the case of the ALK-T mat, also lower temperature conditions, both mats showed 10-20 % more $^{\text{C}}\text{SiO}_2$ scavenging (Figs. 4 & 5; $t=60$ h). These results are consistent with our hypothesis that a potentially favorable biological window of opportunity for microbes to biosilicify occurs under acid conditions specifically with the colloidal silica form because there is a greater possibility to attach the colloids to the bacterial surfaces.

It is clear that the presence of microbial surfaces initiated significant measurable $^{\text{C}}\text{SiO}_2$ uptake at AM system conditions, and greater than those associated with abiotic TiO_2 particles (Fig. 2.1-a; Fig. 2.2; Fig. 2.3; $p < 0.04$). We interpret the enhanced colloidal silica scavenging of the ALK-T mat, ALK-M mat, and the consistently highest observed $^{\text{C}}\text{SiO}_2$ uptake by the AM mat under acidic, mesophilic conditions, to be the result of the charge interactions between the biological surfaces and $^{\text{C}}\text{SiO}_2$ nanoparticles. It may also reflect community microbial metabolic activity, which can create interfacial conditions conducive for colloidal silica uptake. The surface charge behavior of the three microbial mat samples was not determined in this work, but it has been shown that under acidic conditions ($\text{pH} < 5$), a net positive charge occurs for a variety of microbial surfaces (van der Wal, et al., 1997; Smith and Ferris, 2003; Châtellier and Fortin, 2004; Yee, et al., 2004). Silica particles on the other hand, have been shown to exhibit increasing negative surface charge at pH values greater than ~ 2 (Sonnenfeld, et al., 1995; Laven and Stein, 2001; Dove and Craven, 2005). Thus at pH values of 3, the right balance between net bacterial surface positive charge functionalities and $^{\text{C}}\text{SiO}_2$ particle net negative charge promotes charge interactions, while at the higher pH values indigenous to ALK-T and

ALK-M mats (pH = 8), both bacterial surfaces and $^{29}\text{SiO}_2$ particles would become increasingly negatively charged causing greater repulsion between them. Phase 2 experimental results show greater surface scavenging abilities for the biological mat surfaces compared to the abiotic TiO_2 mineral. Moreover, differing $^{29}\text{SiO}_2$ reactivities were observed among the three mats and abiotic particles investigated ($\text{AM}_{\text{mat}} > \text{ALK-M}_{\text{mat}} > \text{ALK-T}_{\text{mat}} \sim \text{abiotic TiO}_2$; Fig. 2.2; Fig. 2.3; $p < 0.04$). These results showing differential mat sequestration at identical system conditions may reflect an influence of previous mat biomineralization on subsequent $^{29}\text{SiO}_2$ uptake. The extent of *in situ* derived authigenic SiO_2 mineral surface coverage was least for the AM mat, i.e. it had the greatest extent of un-mineralized biological surface (Fig. 2.4; Fig. 2.5-a), and the ALK-T mat had the highest silica-mineralized surface coverage (Fig. 2.4; Fig. 2.5-i). The observed, weight normalized $^{29}\text{SiO}_2$ sequestration among the three biological mats in phase 2 experiments ($\text{mM } ^{29}\text{SiO}_2 \text{ mg}^{-1} \text{ hr}^{-1}$; Fig. 2.3), is negatively correlated with the extent of surface exposed cells/biological matrix (i.e., least amount of associated authigenic SiO_2 : $\text{AM}_{\text{mat}} \leq \text{ALK-M}_{\text{mat}} < \text{ALK-T}_{\text{mat}}$; Fig. 2.4; Fig. 2.5), suggesting that either the biological mat surface must possess a greater number of reactive sites, or these sites have a higher relative affinity for $^{29}\text{SiO}_2$ compared to either TiO_2 surface sites, or mat-associated surface authigenic SiO_2 mineral phases (i.e., ALK-T_{mat}). Thus, the extent of exposed biological cells/matrix surface may play a role in mat reactivity to colloidal silica and it appears from the results presented in this work that the greater the prior silicification of that surface, the less observable subsequent $^{29}\text{SiO}_2$ scavenging even under favorable biological conditions. In addition, results also support the notion that biosilicification is not merely a function of the mineralized-to-nonmineralized ratio of

microbial surfaces. The amount of observed authigenic SiO_2 associated with both AM and ALK-M mats surfaces is similar, both qualitatively by microscopic examination (Fig. 2.5-a; Fig. 2.5-e) as well as quantitatively by chemical analysis (Fig. 2.4). Thus, they should show similar $^{29}\text{SiO}_2$ scavenging if system conditions and the degree of surface mineralization were the only influencing factors to biosilicify. However, significant difference in these two mats' relative abilities to silicify occurred ($\sim 30\%$; $p < 0.001$; Fig. 2.2; Fig. 2.3), indicating that other factors in addition to, or in lieu of, the extent of biological exposed surface may be involved. Such characteristics may include differential microbial cell surface charge properties (e.g., types and densities of surface charge functionalities reflecting different microbial strains and/or nutritional histories; van der Wal, et al., 1997), and/or the establishment of metabolically influenced cell surface geochemical interfacial conditions in metabolically active cell systems (Warren and Haack, 2001 and refs. cited). Both the AM and ALK-M mat systems showed greater than 95% viability at the end of the experiments, which does not definitively indicate active metabolism but does indicate similar viability of cells. Analogous *in vitro* silicification experiments have shown that microbial cells were not only viable after silica exposure with subsequent encrustation, but were still able to metabolize (e.g., *Calothrix*, Phoenix, et al., 2000; Phoenix, et al., 2001) despite a profuse silica envelope. The highest net $^{29}\text{SiO}_2$ uptake by the AM mat may reflect its adaptation to thrive under acidic, moderate temperature conditions, i.e., metabolic rates may have differed among the AM and ALK-M mats, and led subsequently to their differential $^{29}\text{SiO}_2$ uptake. SiO_2 does not act as either electron donor or acceptor (Inagaki et al., 2003; except for enzymatic uptake by diatoms: Kröger, et al., 1999; Poulsen, et al., 2003), thus it is not likely that the AM

mat has some requirement for SiO₂ that is reflected in its greater relative biosilicification compared to the ALK-T and ALK-M mats. However, microbial metabolic activity is well documented to control the geochemical conditions in its surrounding interfacial region, dependent on the type of metabolic pathway(s) involved. For instance, photosynthetic mats have been shown to have markedly different geochemical conditions at their interface with the aqueous phase, compared to the bulk water column (Warren and Haack, 2001; Haack and Warren, 2003; Pope, et al., 2003). Increases in dissolved O₂ saturation and pH, associated with biofilm activity specifically in hot spring systems have been shown to influence the microbial-aided formation of an assortment of biominerals such as Fe-oxides (Pierson and Parenteau, 2000), carbonates (Bonny and Jones, 2003) and potentially siliceous (sinter) deposits (Pope, et al., 2003). While we are not able to distinguish the mechanisms responsible for the observed levels of biosilicification in these current laboratory results, they do provide the first evidence demonstrating that microbial silicification can be an active process, and that microbially associated mat-dependent surface characteristics and/or types and/or levels of metabolic activity appear to be implicated in biosilicification.

The above laboratory results indicate that the tendency for microbial surfaces to biosilicify is favored when a colloidal silica pathway is present under acidic conditions, contradictory to the *in situ* propensities of these three mats to silicify inferred from the observed surface extent of authigenic silicification associated with the collected mat samples. The mat that showed the greatest silicification in the laboratory, the AM mat (Fig. 2.1-a; Fig. 2.2; Fig. 2.3), had the lowest amount of authigenic silica mineralization (Fig. 2.4; Fig. 2.5-a), similar to that observed for the ALK-M mat which showed

intermediate silicification in the laboratory. In contrast, the mat that showed no detectable to nominal colloidal silica uptake in the laboratory, the ALK-T mat (Fig. 2.1-b; Fig. 2.2; Fig. 2.3) had the highest native authigenic SiO_2 mineralization loads of the three mats (Fig. 2.4; Fig. 2.5-i). However, examination of the field results in fact provides direct evidence to support our contention that a number of factors play a role in biosilicification, and the absence of one will impede discernible biosilicification. While all three mats came from hotspring systems that were supersaturated with respect to amorphous silica, the AM Goldielocks Springs system had very little indigenous colloidal silica ($\leq 5\%$), while the ALK Bison Pool system had a detectable and substantive (23-50%) colloidal SiO_2 component in its total aqueous silica pool (Table 2.1, column d). Thus, even when the AM hotspring system was silica-saturated ($[\text{SiO}_{2(\text{aq})}] = 235\text{-}300$ ppm), the lack of colloidal silica, i.e. the form we argue is required for biosilicification, prevented active biosilicification of this mat *in situ*. In contrast, the alkaline Bison Pool system, while also saturated with respect to amorphous silica ($[\text{SiO}_{2(\text{aq})}] = 380\text{-}420$ ppm), contained colloidal silica accounting for 23% at ALK-T site and 50% at the mesophilic ALK-M site (Table 2.1, column d). Thus, these mats experienced *in situ* silicification potentially by both abiotic precipitation (more favored at higher pH values) as well as through biologically mediated colloidal SiO_2 uptake when the mat surface was not as encrusted with authigenic silica. However, while the highest proportion (50%) of colloidal silica was observed at the ALK-M *in situ* site, laboratory based $^{\text{C}}\text{SiO}_2$ silicification of this mat was insignificant under its ambient pH and temperature conditions (Fig. 2.1-c), but increased under more acidic conditions (Fig. 2.2; Fig. 2.3), providing further support that both (1) colloidal silica and, (2) acid conditions which

favor biological opportunities, are important parameters in microbial biosilicification. The extensive silica encrustation of the ALK-T mat from the higher temperature, proximal site at Bison pool, is most consistent with abiotic, silica polymerization kinetics due to rapid cooling of supersaturated hydrothermal waters (>400 ppm; Jones, et al., 2001a; Jones, et al., 2001b; Guidry and Chafetz, 2003) and subsequent entombment of the mat.

The results of this study provide the first laboratory evidence to substantiate field-based arguments that different microbial communities interact uniquely with silica in the solution phase (Konhauser, et al., 2004; Konhauser, et al., 2001; Jones, et al., 1999). To date, field studies have coupled observations of differential formation of mega- and micro-textural fabrics of hotspring sinter deposits (Jones, et al., 2001a; Konhauser, et al., 2001) and determination of associated microbial communities through molecular-based approaches (Walker, et al., 2005; Blank, et al., 2002; Smyth, et al., 2003), as indirect evidence that microbial community-dependent fingerprints on sinter formation occur, but have lacked laboratory substantiations as well as evaluation of the pathways and necessary conditions involved for biosilicification to occur. However, it still remains to be evaluated across a wide spectrum of pH, temperature and colloidal SiO₂ concentrations conditions *in situ* to establish the boundaries on discernible biosilicification.

2.5 Conclusion

The combined results of the laboratory based batch ²⁹SiO₂ experiments, ESEM imaging and EDS elemental analyses, and silica mass balance evaluation demonstrate

that microbial mats can exert a significant influence in biosilicification under specific conditions. Namely this process reflects a number of interconnected factors including acid pH and temperature conditions that provide thermodynamically favorable but slower kinetics with respect to abiogenic silica polymerization, as well as the requirement for colloidal SiO_2 , rather than dissolved silicic acid species. Further, the differential microbial $^{29}\text{SiO}_2$ scavenging observed across natural mats under the same temperature, pH and colloidal silica concentration conditions, indicates variable susceptibility to silicify that is likely determined by any or all of a number of microbially-dependent factors such as the nature of the exposed organic microbial surface and extent of prior biomineralization, interfacial micro-geochemical conditions, and level of metabolic activity. This study provides the first direct laboratory support for field observations suggesting a biological role in the formation of hotspring sinters, highlighting the need to consider a biogeochemical framework in microbe-sinter interaction and the need to evaluate these factors simultaneously in field investigations to further our understanding of how extensive discernible biosilicification may be in hotsprings.

CHAPTER 3: EVALUATING CELL SURFACE CHARGE STATUS EFFECTS AND THE IMPACT OF LIVING MICROBES IN BIOMINERALIZATION

3.1 Introduction

Microorganisms inhabiting solute-rich environments typically biomineralize as a consequence of their ability to bind metal ions from solution, thereby serving as templates for the attendant nucleation of minerals (Beveridge, 1980; Ehrlich; 1996; Southam, 2000). This ability of microbes as impetus that drives mineral precipitation lies mostly in their inherent cell surface characteristics, whereby exposed reactive groups are readily available for chemical interactions with the surrounding solutes (Stumm, 1992; Fortin et al., 1997; Nealson and Stahl, 1997). Amorphous silica is one of the more commonly thought biologically mediated minerals whose widespread occurrence is linked closely to microorganismal presence (Mann, 2001; Perry, 2003; Weiner and Dove, 2003). This intimate spatial association of microbes and silica is apparent in many field investigations of modern and ancient silica-rich systems (e.g., hydrothermal springs; acid mine tailings: Konhauser and Ferris, 1996; Fortin and Beveridge, 1997; Konhauser, et al., 2001); however the controls underpinning microbial silicification are still poorly understood (Fortin and Beveridge, 1997; Konhauser, et al., 2004). Processes such as direct monomeric silica (H_4SiO_4) sorption to cell surface ligands (Urrutia and Beveridge, 1993; Schultze-Lam, et al., 1996), cation bridging (Fein, et al., 2002), heterogenous nucleation (Benning, et al., 2004) and Fe/Al-metals (co)precipitation (Phoenix, et al., 2003) mechanisms have been proposed to describe these cell-silica associations (see Chap. 1). However, despite these myriad of proposed microbially-aided silicification

scenarios, the definitive impact of microbial presence on biosilicification (i.e., as induced SiO_2 biomineralization; see Chap. 1) has been elusive.

Results from Chapter 2 have successfully demonstrated in a controlled laboratory setting, the silica-scavenging abilities of environmental microbial mat sampled from hydrothermal springs; and have identified the requisite colloidal silica forms, acid pH and extent of exposed cell surfaces among microbial mats encrusted with native SiO_2 , as major controls initiating perceptible biosilicification. Besides the enzymatically controlled biosilicification machinery resolved in many higher organisms (e.g., diatoms: Martin-Jézéquel, et al., 2000), conflicting interpretations arise about non-enzymatic microbial silicification mechanisms (i.e., as cell surface or metabolic induced; see Chap. 1), particularly for cyanobacterial species whose close association with widespread silica deposits have been widely observed (e.g., *Calothrix*: Konhauser, et al., 2001; Jones, et al., 2001).

Colloidal silica ($^{\text{C}}\text{SiO}_2$) is a significant species in the total aqueous silica pool of many natural and engineered systems (Findlay, et al., 1996; Davis, et al., 2002; Rao and Gelb, 2004), and has been shown to be the relevant form of silica responsible for the observed biosilicified cells (see Chap. 2), as has also been inferred from other colloidal mineral – cell biomineralization processes (Fortin and Beveridge, 1997; Banfield and Hamers, 1997). Results from Chapter 2 showed that insignificant microbial silica immobilization occurred when dissolved H_4SiO_4 species were used for biosilicification, presumably due to the uncharged nature of silicic acids (H_4SiO_4^0) at most environmental pH ($\text{pK}_a \sim 9.5$; Iler, 1979; Fig. 1.2) consistent with other published works (Fein, et al., 2002; Phoenix, et al., 2003; Yee, et al., 2003) to date. However, both silica colloids and

cell interfaces bear charged surfaces over a wide pH range of environmental interest (pH > 4; see Chap. 4), hence there is a potential for electrostatic (Stumm and Morgan, 1996; Yamanaka, et al., 1997) interaction as the mechanism responsible for the nature of cell silica mineralization. Electrostatic interactions resulting from charged interfaces play a significant role in many separation processes, such as deposition and aggregation in both natural and artificial systems (Mendez, et al., 2003; Taboada-Serrano, et al., 2005). Several works have investigated bacteria-silica interactions resulting from the electrostatic attraction on both organic and mineral surfaces; however, these works were conducted mainly in the context of bacterial adhesion to silica substrates (as in biofilm formation: Lawrence, 1995; Yee, et al., 2000; Parent and Velegol, 2004). For example, bacterial adsorption to SiO₂ mineral surfaces has been shown to be a product of a combination of both hydrophobic and electrostatic interactions between the cells and the mineral surfaces arising from surface attractive/repulsive forces (Yee, et al., 2000). Hence charge interactions due to ionized surface ligands on bacteria and colloidal silica may be responsible for silica coordination to microbial surfaces, akin to the same controls governing bacterial attachment to SiO₂ substrates during biofilm formation. Further, because the charge determining moieties on any given surface, whether organic or inorganic are governed by the speciation of these reactive groups, system pH would naturally exert a significant influence in this process (Smith and Ferris, 2001; Smith and Ferris, 2003). For instance, at neutral pH conditions, both cell and colloidal silica interfaces are net negative due to deprotonation of surface acidic groups (e.g., carboxylic; phosphoric: Cox, et al., 1999; Warren and Haack, 2001; Konhauser, 2007) and silanol functionalities (Nawrocki, 1991; Bergna, 1994; Nawrocki, 1997), respectively and would

expectedly result to charge repulsion. If surface charge interactions and/or hydrophobicity were responsible for bacterial colloidal silica scavenging mechanisms, it is therefore imperative to investigate microbial silicification phenomenon at pH windows where changes in comparative cell and/or colloidal silica surface speciation will be apparent.

(Cyano)bacterial silica mineralization is unlikely a consequence of silica utilization as energy requirement for growth and normal functions (i.e., enzymatic: Fortin and Beveridge, 1997; Konhauser, et al., 2004; Benning, et al., 2005). Such is that in many live bacterial laboratory investigations, cells were assumed to be viable but “inactivated” which although construed as non-metabolizing due to the absence of essential nutrients, were typically alive and capable of inducing cell-related activities in response to environmental perturbations (e.g., pH, temperature, salinity: Urrutia, et al., 1992; Claessens, et al., 2004; Taboada-Serrano, 2005). This level of microbial cell activity can translate to cell membrane functionality, and may be key for the cell propensity to biomineralize (Fortin, et al., 1997). It is uncertain whether the overall impact of living/metabolizing cells promotes or hinders biomineralization. For example, reported alkalization of interfacial cell-solution geochemistry of live, photosynthesizing cells were shown to enhance CaCO_3 precipitation around the cell envelope, which were otherwise unfavorable in the bulk solution phase (Merz, 1992). In another study, metal uptake by living bacteria was significantly reduced (*Bacillus subtilis*: Urrutia et al., 1992), hence was less prone to mineralization due to H^+ competition for the available anionic binding sites on the cell wall fabric from actively proton-pumping cells (Schultze-Lam, et al., 1996). Whether the net effect of live cells is to augment or hinder

bacterial susceptibility to mineralize is still subject to debate; nonetheless, it is clear that live bacterial cells showing putative level of metabolic activity will affect the cells' overall biomineralization potential. If such were the case, then a biological overlay may be discernible among mineralized “living” cells as opposed to mere organic interfaces provided by non-viable (i.e., dead) microbial cells.

It was shown from the results in Chapter 2 that natural microbial consortia with prior silica encrustation scavenge less silica compared to unmineralized cells with fully exposed organic surfaces. Therefore it is necessary to probe the effect of fully exposed organic cell surfaces compared to highly silicified cell envelopes, and the latter's combined outcome on (further) silica sequestration. The more practical rationale for probing differences in the silicification behavior, if any, as impacted by prior cell mineralization and level of activity is that in most naturally occurring environments where biomineralization (silicification) is occurring, the mineralizing substrates realistically contain a matrix of both naked live and dead biomass, and previously mineralized cells of varying degrees (Jones, et al., 2001b; Bonny and Jones. 2003). If any form of surface charge interaction is invoked as a plausible mechanism to explain microbial silicification, it is necessary to isolate and evaluate the potential impacts of important parameters mentioned that influence microbial cell and/or silica surface charge status such as pH, cell viability and the influence of prior SiO₂ mineral envelope.

To test the hypothesis whether bacterial colloidal silica scavenging is surface charge initiated, cell ^CSiO₂ sequestration is investigated at neutral and acid pH windows where the net charge functionalities on both cell and SiO₂ surfaces are highly net negative and neutral/less negative, respectively from surface deprotonation/protonation reactions

(see Chap. 4). The effect of putative cell activity (live vs. dead) and the consequence of prior cell-SiO₂ mineral encrustation in induced biosilicification will also be assessed. If indeed the mechanism of microbial silica sequestration is surface charge controlled, a qualitative comparison of cell-induced SiO₂ immobilization behavior will be made relative to two inorganic mineral substrates with known and contrasting surface protonation status and pH zero point of charge values (pH_{zpc}): iron oxyhydroxide (FeOOH; pH_{zpc} = 8.0: Dzombak and Morel, 1990; Smith and Ferris, 2003) and potassium feldspar (KFELD; pH_{zpc} = 2.0: Vidyadhar and Rao, 2007). At pH 7, FeOOH surfaces are positively charged, while KFELD minerals are highly net negative. Under acidic pH 3 conditions, FeOOH surfaces become “more” positive while KFELD surfaces are “less” negative. The objectives therefore of this paper were to (1) investigate the likelihood of surface charge interactions as the mechanism responsible for the microbially-aided silicification dependent on cell/SiO₂ surface proton-charge speciation; and, (2) evaluate the effects of microbial cell viability (hence the potential for metabolic activity) on SiO₂ immobilization among live, dead and cell-SiO₂ mineral composite, through batch silica experimentation at pH 3 and pH 7.

3.2 Materials and methods

3.2.1 Solutions preparation

All laboratory glass and plastic ware used for geochemical analyses were soaked overnight in 4% (v/v) trace metal grade HCl (Sigma Aldrich), and rinsed with ultrapure water (UPW: Milli-Q, 18.2 Ω; Millipore). All solutions were prepared analytically and were carefully prepared to be metal-free and sterile. To avoid silica contamination, all

vessels used for batch silicification experiments were either polyethelyne (PE) or polypropylene (PP) make. Materials used for microbial batch experiments were manufacturer-certified sterile, or autoclaved at 121°C for 30 minutes (Castle[®], Conbraco Industries, Inc.).

3.2.2 Cell culture growth and preparation

Synechococcus-type cyanobacterial cultures were generously donated by Maria Dittrich (from C. Calleri collection) isolated from the water column of a stratified lake (Plöner See, Germany). This *Synechococcus*-type strain was previously described as “green picocyanobacteria” (Dittrich and Sibling, 2005), and was used throughout the experiments presented in this work. Silicified oxygenic photoautotrophic cyanobacterial communities were typically found in the rock record, and were assumed to have played a major role in the precipitation of silica deposits in ancient (Likhoshway, et al., 2005) and modern (Konhauser et al., 2001) silica-rich environments. Cell cultures were grown in 2 L flasks supplemented with a modified BG-11 media (Schlösser, 1994) at room temperature and ambient light conditions (~10 Watts/m²; Li Cor, L1-1400) under approximately 10h/14h light/dark cycle. Cyanobacterial cells were harvested during their early to mid-stationary growth phase (approximately 6 weeks) for all microbial treatments. Calibration curves of bacterial suspensions were generated from absorbance values at 600 nm optical density (OD_{600nm}) of known bacterial aliquots to transpose absorbance readings into cell concentration (mg/L). Bacterial dry weight was obtained by oven drying (Fisher Scientific Isotemp Oven, Model 650G) filtered cell suspensions (Whatman[®] Microfibre Filters) at 50°C to a constant weight. For the batch silicification

experiment at pH 3, a separate flask of cyanobacterial isolate (initial pH ~ 7.2) was first “conditioned” in a two-step pH reduction scheme (i.e., ~ pH 5 and 4) using filter-sterile H₂SO₄ for about one week at each step, before harvesting. Cells were prepared for batch silicification by centrifugation at 7000 rpm for 5 minutes (Sorvall RC5C PLUS). The growth media was decanted and the pelleted cells were washed with 1 mM EDTA (Fisher) and rinsed with sterile UPW. Cleaned cells at this stage were used for batch silicification without additional treatment, and were designated as naked LIVE cyanobacteria.

All killed cells were sampled from a single batch of cell suspension. Previously cleaned LIVE cyanobacterial cells were resuspended in sterile UPW to a final concentration of about ~80 mg/L. The cell suspension was stirred (Fisher Scientific) and placed in a secondary flask containing UPW and heated to 74°C for 30 seconds (Lewin, et al., 2003). The cell suspension turned light green, and was transferred immediately to a cool water bath and continuously stirred to room temperature. The killed cells were then pelleted as described and used for silicification experiments designated as DEAD cells.

The procedure for coating cyanobacterial cells with silica to produce a silica-coated cell composite (COMP) for batch silicification at pH 3 and pH 7 was done in a single batch. Cell suspension of known OD_{600nm} was cleaned as described, and combined with concentrated silica solution (Ludox LS, 30% w/w SiO₂; Sigma-Aldrich) to a final concentration of 1 M SiO₂. The cell-silica suspension was then pH-adjusted to pH ~ 7 (initial pH ~ 10) using 50% v/v filter-sterile HCl with moderate stirring. The suspension was left stirred for five days; thereafter, the silica-cell composites were pelleted at 17000

rpm for 10 minutes and the excess silica solution was discarded. The COMP solids were washed successively with 1mM EDTA and UPW prior to batch silicification. No measurable silica (either dissolved or colloidal) was detected from the decanted final UPW rinse. COMP dry weight (mg cell + mg SiO₂ coating) was estimated from oven-dried composites of known dry cell mass. Visual inspection and elemental scanning for Si verified the method described above effectively coated the cyanobacterial cells with μm -thick silica crusts (Fig. 3.7; Fig. 3.8-c).

3.2.3 Batch silicification experiments

Batch silicification experiments were performed under pH 3 and pH 7 conditions using a constant, near-saturated concentration of starting aqueous colloidal silica ($^{\text{C}}[\text{SiO}_2]^0 = 120 \text{ ppm}$; *saturation index* = $\log \{Q/K\} \sim 0$; Iler, 1979; Tarcn, 2001; Mountain, et al., 2003) at 25°C. For both experimental conditions (i.e., pH 3 and pH 7), $^{\text{C}}[\text{SiO}_2]^0$ was pH-adjusted ($\pm 0.1 \text{ pH}$) using filter-sterile H₂SO₄ or NaOH and were placed in sterile triplicate 50 mL PP tubes (Falcon) containing the appropriate experimental solids and capped with sterile, foam plugs (S|P diSPO®). Colloidal silica scavenging was evaluated for the following triplicate treatments: solution control ($^{\text{C}}\text{SiO}_2$ only); live naked cells (LIVE), killed naked cells (DEAD), silica-coated cyanobacterial cell composite (COMP) and inorganic silica beads (SILB) at pH 3 and pH 7.

All experimental tubes were immersed in water bath (Forma Scientific model 2568) set to $25^\circ\text{C} \pm 0.5^\circ\text{C}$ under continuous white light ($\sim 10 \text{ Watt/m}^2$) and slow shaking (end-to-end cycle $\sim 20/\text{min}$) conditions. Colloidal silica loss in the solution phase was evaluated at 0, 1, 4, 6 hours and every 12 hours thereafter for a total of 120 hours (5

days). The batch solution was initially turbid, hence about 1mL of aliquot was pipetted and micro-centrifuged at 10000 rpm for 1 minute (IEC Micromax) to separate the experimental solid-sorbents and associated fractionated $^{29}\text{SiO}_2$ from the “free” silica in the solution phase. This micro-centrifugation step for all silica analyses does not affect the distribution of “free” silica colloids in the solution phase, which were reported to be pelleted in excess of 100,000 rpm for an hour (Juhos, 1966). This contention was further verified by the observed identical silica values measured for both centrifuged and un-centrifuged $^{29}\text{SiO}_2$ controls in pH 3 and pH 7 systems. For the $\text{COMP}_{\text{pH3/pH7}}$ and $\text{SILB}_{\text{pH3/pH7}}$ treatments, blank controls (respective solids + $\text{UPW}_{\text{pH3/pH7}}$ only) showed no detectable silica partitioned back in the aqueous phase after 120 hours. Silica analysis was done via spectroscopy (Pharmacia Biotech, Ultraspec 3000) using a modified molybdate-yellow technique as discussed in Chapter 2. Variability in silica loss in $^{29}\text{SiO}_{2\text{pH3/pH7}}$ controls were less than 5%, hence any value larger than this benchmark was assumed to be the result of experimental sorbent- $^{29}\text{SiO}_2$ interaction. Experimentally scavenged silica ($^{29}\text{SiO}_{2\text{scav}}$) in all particle treatments was defined as the difference between the nominal starting colloidal silica ($[^{29}\text{SiO}_2]^0 = 120\text{ppm}$) and silica left in the solution phase. $^{29}\text{SiO}_{2\text{scav}}$ across triplicate treatments were pooled to generate a mean and standard error ($X \pm \text{S.E.}$). After 5 days of silica exposure, sub-samples of LIVE, DEAD and COMP cyanobacterial cell treatments from both pH 3 and pH 7 experimental systems were re-cultured in BG-11 nutrient media.

A hierarchal, nested multi-level structure classified by experimental conditions (i.e., pH 3 and pH7) and solid treatment effects was used in the batch silicification statistical analyses (Anderson, 1987). Differences in observed scavenged silica, as

impacted by each level in the hierarchal design were tested for significance (t-statistic) using MLwiN® Software version 2.02. A “rigid” multi-level data analysis was applied where the combined contributions from each hierarchy were factored in simultaneously. The acceptance criteria applied for significance level testing for all experiments was at $p < 0.05$ significance level, where $d.f. = 419$.

3.2.4 “Extractability” of cell-scavenged silica

To evaluate the relative strength of cyanobacteria-colloidal silica interaction, previously batch-silicified cells (i.e., exposed for 120 hours) LIVE_{pH3} cyanobacterial sub-samples with associated $^C\text{SiO}_{2\text{scav}}$ were washed lightly with UPW_{pH3}, pelleted and resuspended in three operationally-defined extractants: UPW_{pH7}; 0.1 M NaCl (Sigma Aldrich) and 1 mM amino acid (L-arginine: Sigma Aldrich) solutions. Operationally, UPW_{pH7} was used to assess the “reversibility” of cyanobacterial- $^C\text{SiO}_2$ interaction at pH 3, while NaCl and amino acid extractants were utilized to evaluate the “exchangeability” of bacterially scavenged silica. NaCl was chosen over CaCl_2 to prevent silica coagulation induced by divalent salts (Lee and Moon, 2003), and the [NaCl] used was such that the molarity of the “exchanging” ions is about 100 times higher than the maximum associated $^C\text{SiO}_{2\text{scav}}$. 0.1 M NaCl is also below the critical coagulation concentration reported for colloidal silica induced by monovalent salts (i.e., 0.45 M NaCl: Lee and Moon, 2003). L-arginine was used due to the presence of positive amine moieties (NH_3^+ ; Saoudi, et al., 1997) on its molecular structure over a wide range of environmental and physiological pH ($\text{pK}_a \sim 12.5$: Boudko, 2007) and thus can potentially coordinate with (negatively) charged silica (Mendez, et al., 2003; Belton, et al., 2004) particles and

“exchanged” from the cyanobacterial surfaces. The molarity of NH_3^+ (1 mM) was chosen such that it is about the same concentration as the $[\text{}^{\text{C}}\text{SiO}_{2\text{scav}}]$ on $\text{LIVE}_{\text{pH}3}$ cells. Triplicate tubes of post-silicified, $\text{LIVE}_{\text{pH}3}$ sub-samples and associated $^{\text{C}}\text{SiO}_{2\text{scav}}$ were dispersed separately in each of the three extractants (as opposed to a sequential extraction; Haack and Warren, 2003) and placed in an orbital shaker set at 100 rpm (Forma Scientific Model 430) for 24 hours under ambient conditions. Silica partitioned from the cyanobacterial surface to the solution extractants: $\text{Si}_{\text{UPWpH}7}$, Si_{NaCl} and $\text{Si}_{\text{NH}3+}$, respectively was evaluated following the silica spectroscopic analysis described in Chapter 2. Matrix-matched $^{\text{C}}\text{SiO}_2$ blanks (i.e., $^{\text{C}}\text{SiO}_2 + \{\text{UPW}_{\text{pH}7}; [\text{NaCl}]; [\text{NH}_3^+]\}$) analyses showed comparable $[\text{}^{\text{C}}\text{SiO}_2]$ as the standard ($^{\text{C}}\text{SiO}_2$ only; $\pm 5\%$) in the presence of the three extractants. After extraction, the spent $\text{LIVE}_{\text{pH}3}$ solids with associated residual “bound” silica ($^{\text{C}}\text{SiO}_{2\text{bound}}$; post-extracted $^{\text{C}}\text{SiO}_2$) were completely digested in NaOH and analyzed for SiO_2 , after which $^{\text{C}}\text{SiO}_2$ fractionation calculations were performed. Only the post-silicified $\text{LIVE}_{\text{pH}3}$ cyanobacterial suspensions were analyzed for “reversibility” and “exchangeability” of cell surface associated $^{\text{C}}\text{SiO}_{2\text{scav}}$, and silica mass balance analyses.

3.2.5 Cu^{2+} and SO_4^{-2} sorption of naked cells

To assess the “active” net surface charges (i.e., + / -) of live, naked cyanobacteria at pH 3 and pH 7, cyanobacterial scavenging of cation (Cu^{2+}) and anion (SO_4^{-2}) was performed. Microbial scavenging studies of relatively stable ions have been particularly useful in determining the surface electrochemical status of microbial surfaces conditional to pH (Fein, et al., 1997; Fowle and Fein, 2000), and are typically done in conjunction

with other macro and molecular scale techniques (e.g., potentiometry, spectroscopy: Borrok, et al., 2004; Yee, et al., 2004). About 100 mg/L suspensions of cleaned, live cyanobacteria were prepared in separate sterile PP tubes to a final concentration of 78.7 μM Cu^{2+} (Plasmachem Associates, Inc.) and 104.2 μM SO_4^{2-} (HACH Co.). Cu^{2+} and SO_4^{2-} amended cyanobacterial suspensions were then pH-adjusted using filter-sterile HCl or NaOH. Net scavenged cation and anion was determined from the Cu^{2+} and SO_4^{2-} lost from the original $[\text{Cu}^{2+}]$ and $[\text{SO}_4^{2-}]$ at 0, 1 and 12-hour intervals for a total of 48 hours. Cu^{2+} and SO_4^{2-} analyses were done respectively using factory-calibrated spectrophotometer (HACH DR/2010) and user-defined standard curve spectroscopy ($R^2 > 0.99$). The actual pH for the Cu^{2+} cyanobacterial sequestration analysis at neutral pH was adjusted to pH ~ 6.2 to prevent Cu^{2+} hydrolysis (HACH Co. Handbook).

3.2.6 Cell visualization, viability assay and Si x-ray spectroscopy

Imaging and elemental analyses for Si on the four treatment solids' post-experimental pH 3 batch silicification were performed using a combined field emission-scanning electron microscopy (FESEM JEOL JSM 7000F) with an x-ray detector (Oxford Instruments, Model 7558), and an environmental scanning electron microscope (ESEM, System 2020, ver. 3.53) equipped with energy dispersive x-ray spectroscopy (EDS, PGT IMIX system). Unmineralized (live and dead) and composite solids from both pH 3 and pH 7 were analyzed by ESEM-EDS, while high resolution FESEM work was done only for the LIVE_{pH3} and DEAD_{pH3} sub-samples.

Microbial cell viability was estimated using the live/dead cell viability assay (Baclight L7012, Molecular Probes) as per manufacturer protocols (Invitrogen Molecular

Probes Product Information, 2004) and imaged under an epifluorescence light microscope (Leica DMR A/RXA, Leica Microsystems) fitted with a digital camera (Hamamatsu Digital CCD Cam ver. 1.1), and imaging software (Openlab ver. 2.2.5). Accuracy of the live/dead staining procedure was evaluated prior using the same cyanobacterial isolate (see Chap. 2)

3.2.7 Mineral surface-induced ^{14}C SiO₂ scavenging

Synthetic FeOOH mineral was prepared from the controlled neutralization of 198 mM FeNO₃·9H₂O (Fisher) salt solution with 0.1 M NaOH standard (Sigma Aldrich) using a digital titrator (Mettler Toledo, DL70 ES) under positive N₂ atmosphere (Alphagaz, 99.99%) as described in Smith and Ferris (2001). Natural KFELD specimen was obtained from the McMaster-School of Geography and Earth Sciences mineral collection, and was crushed and pulverized to a texture of fine granulated sugar (approx. ~ 0.1mm). Both mineral sorbents were washed in 1mM EDTA and UPW, with an additional prior 4% HCl wash for the KFELD mineral. Both minerals were used for batch silicification under the same conditions, and using the same procedures as the cyanobacterial cell treatments described above. Colloidal silica scavenging of the two mineral surfaces at pH 3 and pH 7 was evaluated at 0, 1 and every 12 hours for a total of 48 hours.

3.3 Results

3.3.1 Batch silicification

Results showed statistically insignificant $^{\text{C}}\text{SiO}_2$ scavenging ($p > 0.07$) for all experimental treatments at pH 7 (Fig. 3.1). After 120 hours, less than 10% of the original $^{\text{C}}\text{SiO}_2$ ($^{\text{C}}\text{SiO}_2^0 = 120 \text{ ppm}$) was scavenged from the solution phase, indicating that none of the experimental solid treatments used induced significant silica sequestration at neutral pH. In contrast to the insignificant results obtained for batch silicification at pH 7 (Fig. 3-1), silicification of the same experimental solids at pH 3 showed substantial and significant colloidal silica sequestration particularly among the organic solid treatments: LIVE_{pH3} , DEAD_{pH3} and COMP_{pH3} ($p < 0.05$; Fig. 3.2). After 120 hours, 25-60 % of the initial $^{\text{C}}\text{SiO}_2$ was sequestered from the aqueous phase, indicating a more favorable experimental solids-colloidal silica surface interaction occurred in acid conditions compared to the same batch experiments conducted at pH 7. Moreover, as with the neutral pH system, no statistically significant colloidal silica scavenging occurred for the inorganic SILB_{pH3} sorbent (9%, $p > 0.10$; Fig. 3.2), and behaves similarly as the $^{\text{C}}\text{SiO}_2$ control ($^{\text{C}}\text{SiO}_2$ only; Fig. 3.2). These results are consistent with what was shown from Chapter 2 that perceptible colloidal silica sequestration of natural microbial mats occurs at acid pH (pH ~ 3), irrespective of the native ecological pH conditions where these mats were taken.

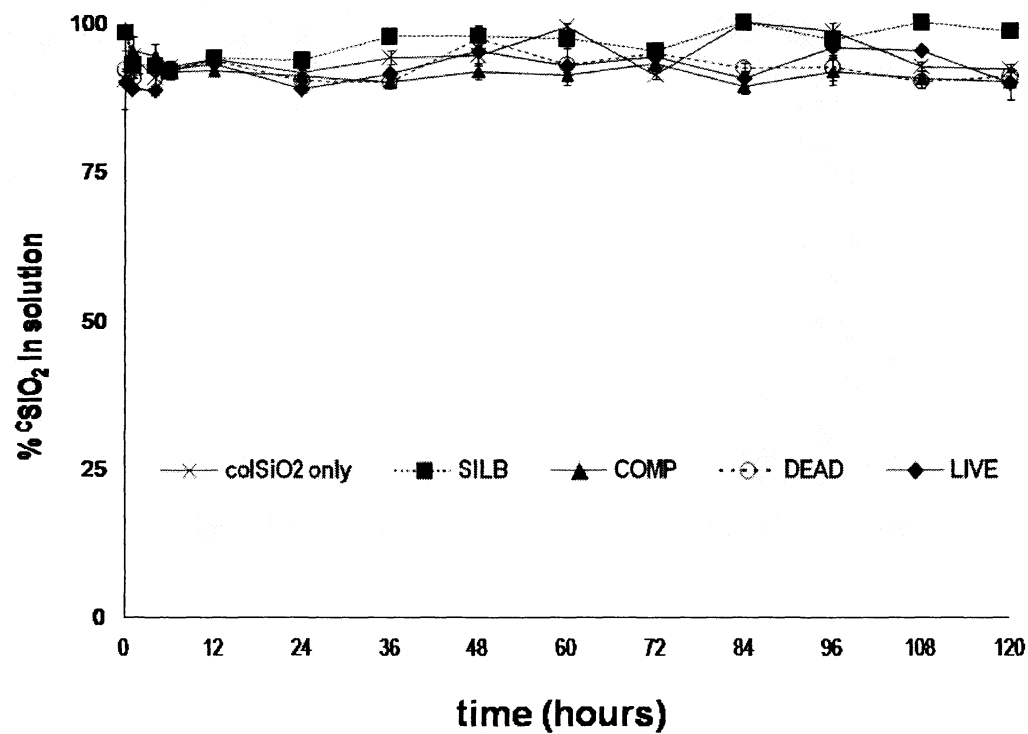


Figure 3.1 Percent $^{13}\text{CSiO}_2$ loss ($^{13}\text{CSiO}_2^0 = 120$ ppm) as a function of time at pH 7 using experimental solids: solution control ($^{13}\text{CSiO}_2$ only), inorganic silica beads (SILB), cell-silica composites (COMP), naked dead (DEAD) and live (LIVE) cyanobacteria; mean triplicate values shown $\pm$ S.E. bars; all treatments showed $<10\%$ $^{13}\text{CSiO}_2$ loss at neutral pH.

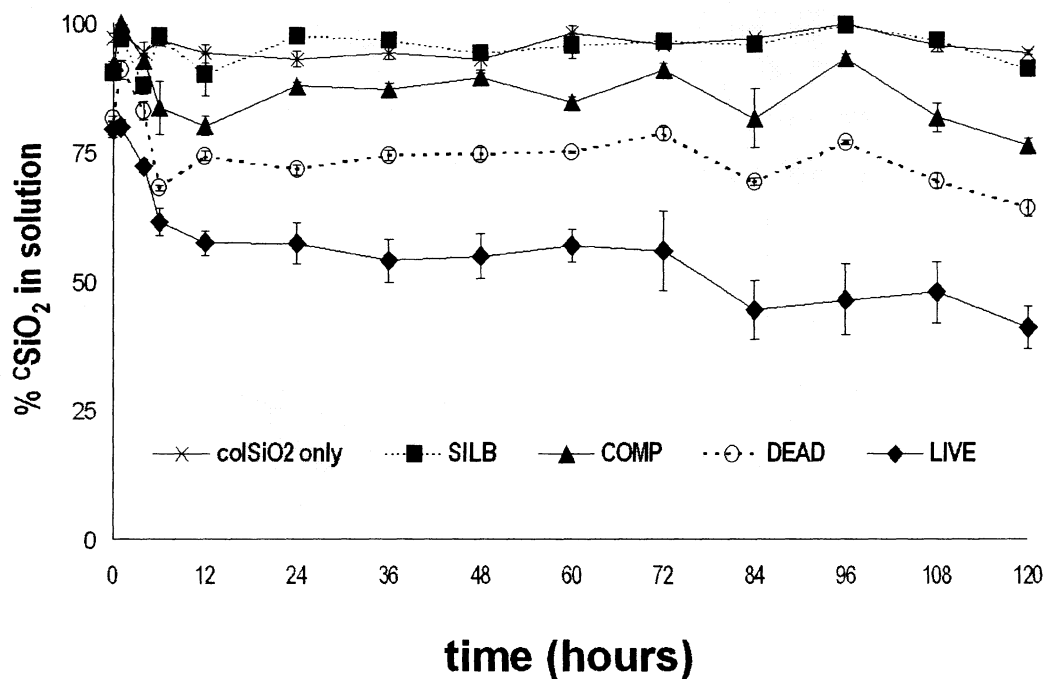


Figure 3.2 Percent $^{29}\text{SiO}_2$ loss ($^{29}\text{SiO}_2^0 = 120$ ppm) as a function of time at pH 3 using experimental solids: inorganic silica beads (SILB), cell-silica composites (COMP), naked dead (DEAD) and live (LIVE) cyanobacteria; mean triplicate values shown $\pm$ S.E. bars; solution controls ($^{29}\text{SiO}_2$ only) showed $<5\%$ variability.

Weight-normalized $^{13}\text{C}\text{SiO}_2$ scavenging per mg dry weight of experimental solids ($\text{mM } ^{13}\text{C}\text{SiO}_2 \text{ mg}^{-1}$) at pH 3 further showed a treatment-dependent rate for colloidal silica sequestration (Fig. 3.3). The highest net silica scavenging occurred in the $\text{LIVE}_{\text{pH}3}$ naked cyanobacteria ($1.2 \times 10^{-1} \text{ mM } ^{13}\text{C}\text{SiO}_2 \text{ mg}^{-1}$; $p < 0.01$), followed by the naked DEAD ($0.67 \times 10^{-1} \text{ mM } ^{13}\text{C}\text{SiO}_2 \text{ mg}^{-1}$; $p < 0.01$) and the bacteria- SiO_2 composite ($0.65 \times 10^{-1} \text{ mM } ^{13}\text{C}\text{SiO}_2 \text{ mg}^{-1}$; $p = 0.05$). The $\text{mM } ^{13}\text{C}\text{SiO}_2 \text{ mg}^{-1}$ values obtained for the silicification of these cyanobacterial isolates at pH 3 are comparable to those observed from batch-silicified natural mat consortia under identical conditions (see Chap. 2), suggesting that similar mechanisms govern the microbial silica scavenging of both environmental mats, and the pure isolates used in this current work.

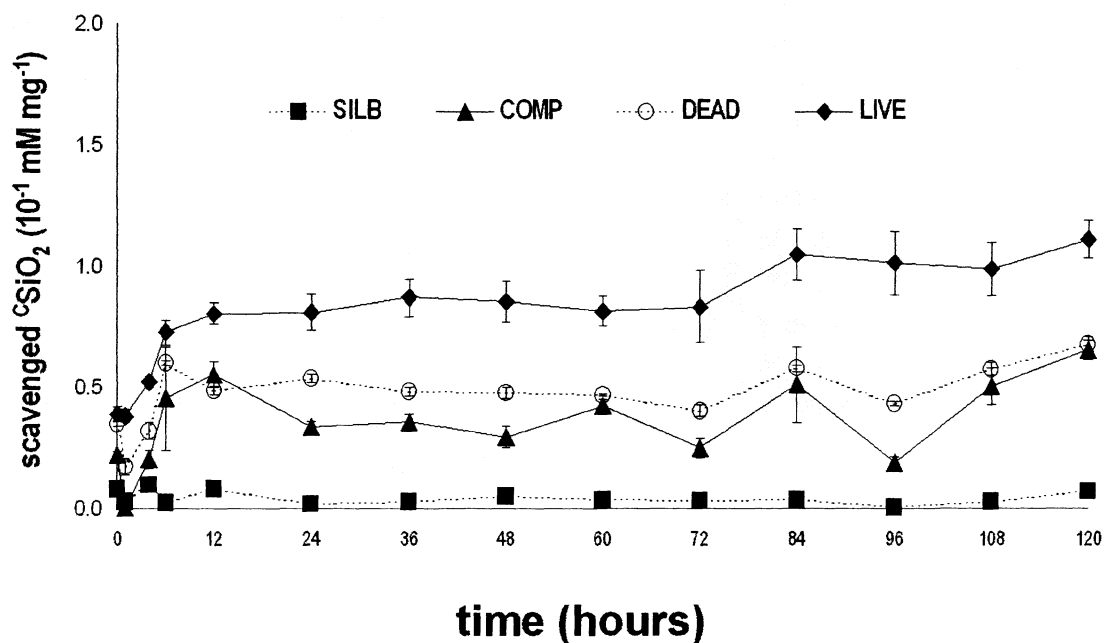


Figure 3.3 Immobilized $^{13}\text{C}\text{SiO}_2$ (mM) as a function of time per mg (dry) experimental solids ($\text{mM } ^{13}\text{C}\text{SiO}_2 \text{ mg}^{-1}_{\text{dry}}$) at pH 3: inorganic silica beads (SILB), cell-silica composites (COMP), naked dead (DEAD) and live (LIVE) cyanobacteria; mean triplicate values shown $\pm$ S.E. bars.

3.3.2 “Extractable” $^{\text{C}}\text{SiO}_2$ and mass balance calculations

“Extractable” silica as operationally defined in this work may be “reversible” (partitioned $\text{Si}_{\text{UPW}_{\text{pH}7}}$) or “exchangeable” (partitioned Si_{NaCl} or $\text{Si}_{\text{NH}_3^+}$) relative to the $^{\text{C}}\text{SiO}_{2\text{scav}}$ associated with the $\text{LIVE}_{\text{pH}3}$ cyanobacterial sorbent, exposed separately to these extractants. “Bound” silica ($^{\text{C}}\text{SiO}_{2\text{bound}}$) refers to silica digested from the spent, post-extracted cells, while total silica ($^{\text{C}}\text{SiO}_{2\text{tot}}$) is the sum of “extractable $^{\text{C}}\text{SiO}_2$ ” (for each of the 3 extractants) and their respective $^{\text{C}}\text{SiO}_{2\text{bound}}$. The following percentage $^{\text{C}}\text{SiO}_2$ mass balance calculations were performed only for the $\text{LIVE}_{\text{pH}3}$ samples. Recalculation of percentage extractable silica using $\text{UPW}_{\text{pH}7}$, NaCl and NH_3^+ extractants, and $^{\text{C}}\text{SiO}_{2\text{bound}}$ with respect to the initial amount of silica in solution ($[^{\text{C}}\text{SiO}_2]^0 = 120 \text{ ppm}$), showed a general agreement of $^{\text{C}}\text{SiO}_{2\text{tot}}$ (i.e., $^{\text{C}}\text{SiO}_{2\text{tot}} = \text{“extracted Si”} + ^{\text{C}}\text{SiO}_{2\text{bound}}$; Fig. 3.4) with the experimentally-derived scavenged silica ($^{\text{C}}\text{SiO}_{2\text{scav}} \sim 60 \%$; Fig. 3.4, dashed horizontal line). At the most, $^{\text{C}}\text{SiO}_{2\text{tot}}$ varied within 10 % relative to the batch silicification-derived $^{\text{C}}\text{SiO}_{2\text{scav}}$ for all the three extractants used. $^{\text{C}}\text{SiO}_2$ mass balance calculations showed that only less than 10% of the initial $[^{\text{C}}\text{SiO}_2]^0$ in the batch experiments can be mobilized from the cyanobacterial surfaces using either NH_3^+ or $\text{UPW}_{\text{pH}7}$, while no detectable silica was “exchanged” by Na^+ or Cl^- ions. In other words, almost all of the experimentally scavenged $^{\text{C}}\text{SiO}_2$ (ca. 90 % of $^{\text{C}}\text{SiO}_{2\text{scav}}$) was actually “bound” on the cyanobacterial surfaces. These results strongly suggest that the scavenged silica on the live, naked cyanobacterial surfaces at pH 3 is not simply electrostatically held but chemically-bonded, hence are more tenacious and do not readily partition back in the solution phase from environmental changes such as variations in pH, salinities or presence of organic complexants.

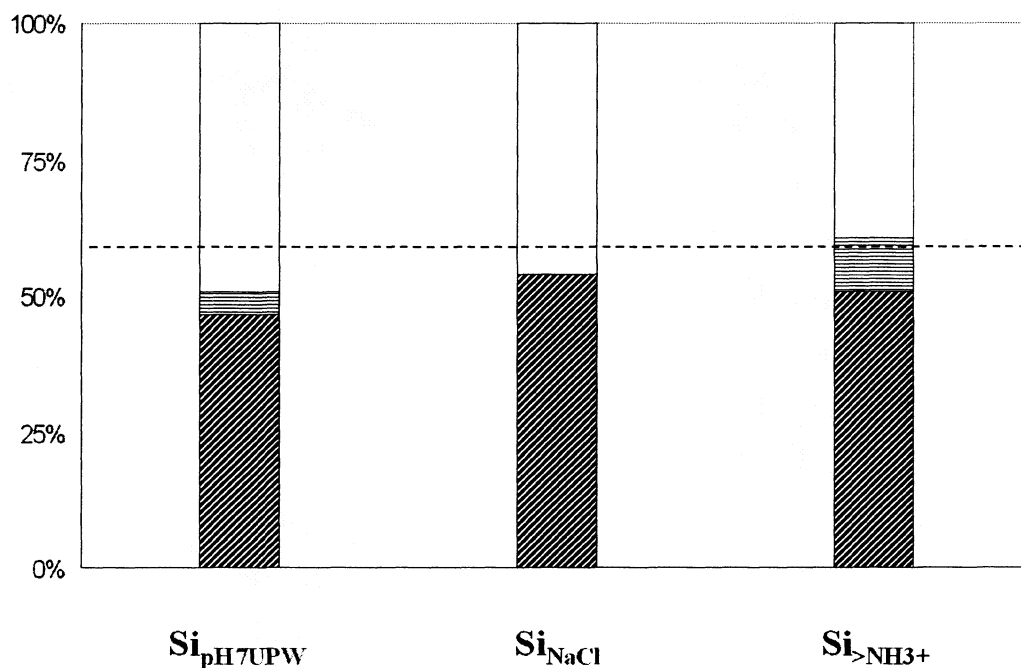


Figure 3.4 Partitioned $^{13}\text{C}\text{SiO}_2$ (horizontal fill) using extractants: UPW_{pH7} ($\text{Si}_{\text{pH7UPW}}$), NaCl (Si_{NaCl}) and amino acid ($\text{Si}_{>\text{NH3+}}$) from LIVE_{pH3} samples expressed as percentage of original $^{13}\text{C}\text{SiO}_2^0$; diagonal fill represent “bound” silica ($^{13}\text{C}\text{SiO}_{2\text{bound}}$) from the spent, post-extracted cells; dashed horizontal line is the experimentally derived scavenged silica from batch silicification ($^{13}\text{C}\text{SiO}_{2\text{scav}} \sim 60\%$); values shown are mean ($n=3$); *see text for explanation*.

3.3.3 Naked cyanobacterial Cu^{2+} and SO_4^{-2} scavenging

At either pH 3 or pH 7 systems, no significant microbial SO_4^{-2} scavenging was detected within 48 hours (<5%; Fig. 3.5), signifying that these naked cyanobacterial cells do not appreciably possess (proton-active) positive sites at either acidic or neutral pH scales (see Chap. 4). This result is consistent with what has been shown for a number of bacterial sequestration studies that even when positive amine groups ($>\text{NH}_2 + \text{H}^+ \leftrightarrow >\text{NH}_3^+$) are present on bacterial cell surfaces that could theoretically impart positive electrochemical charges, such occurrences are very small and typically beyond the resolution of most macroscopic experimental works (Beveridge and Murray, 1980; Ledin, et al., 1995; Fein, et al., 2002; see Chap. 4). Molecular (spectroscopic) investigation also verified that basic amine sites represent only a small fraction of the total site density of cyanobacterial cell surfaces (Yee, et al., 2004), hence they do not considerably affect the overall cyanobacterial net negative surface charge status originating from higher density acidic (i.e., carboxylic; see Chap. 4) organic functional groups.

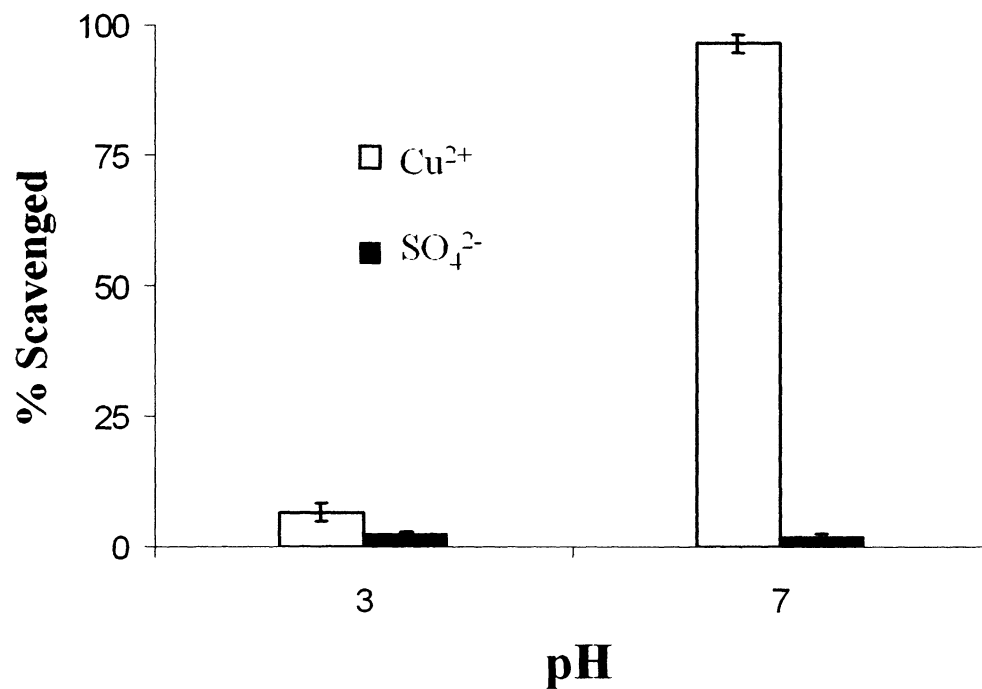


Figure 3.5 Percent scavenged Cu^{2+} and SO_4^{2-} by naked cyanobacterial cells at pH 3 and pH 7 (actual pH for Cu^{2+} - cyanobacteria system ~ 6.2; see 3.2.5); values shown are averages ($n = 10$) from pooled duplicate analyses per 12-hour time interval; error bars indicate standard deviation.

In contrast with SO_4^{-2} scavenging results, cyanobacterial Cu^{2+} sorption experiment showed almost complete (96%; Fig. 3.5) scavenging at circum-neutral pH for the first hour until the end of the 48-hour period. This result is consistent with adsorption of positively charged copper species onto highly negative-charged bacterial surfaces at circum-neutral pH values (Parker, et al., 1998; Ledin, 2000). At pH 3 however, only ~7% of Cu^{2+} was sequestered by the naked cyanobacteria (Fig. 3.5), indicating competition from solution H^+ , which has a higher affinity for cell surface organic ligands than do metals (Yee, et al., 2004). Therefore, results of Cu^{2+} and SO_4^{-2} sequestration experiments of naked cells showed that cyanobacterial surfaces are highly net negative at circum-neutral pH values and are important scavengers for counter-charged solutes (e.g., dissolved metals: Ehrlich, 1996; Warren and Haack, 2001; Rawlings, 2002); further, these organic surfaces do not appreciably possess positive (i.e., amine; see Chap. 4) site functionalities at either neutral or even acid conditions, hence are more likely to possess net neutral (or slightly negative) surfaces at pH 3.

3.3.4 FeOOH- and KFELD- $^{\text{C}}\text{SiO}_2$ sequestration

Silica scavenging of synthetic FeOOH ($\text{pH}_{\text{zpc}} = 8.0$) and natural KFELD ($\text{pH}_{\text{zpc}} = 2.0$) minerals showed a strong surface charge status dependency at pH 7 among these two mineral sorbents and the $^{\text{C}}\text{SiO}_2$ sorbate, dependent on their pH_{zpc} values. After 48 hours and at neutral pH, all of the colloidal silica was scavenged on the FeOOH mineral surfaces while no measurable $^{\text{C}}\text{SiO}_2$ sequestration occurred on the KFELD surfaces under the same pH conditions (Fig. 3.6). Surface FeOOH- and KFELD- $^{\text{C}}\text{SiO}_2$ interaction at both pH 3 and pH 7 was immediate (~1st hour) and invariant throughout the 48-hour

period. The results of the two mineral substrates' $^{13}\text{C}\text{SiO}_2$ sequestration behavior at pH 7 reflect the effects of surface charge interactions between the negatively charged $^{13}\text{C}\text{SiO}_2$ and oppositely charged FeOOH (attractive), while the reverse surface charge interaction (repulsive) dominate the KFELD- $^{13}\text{C}\text{SiO}_2$ system which are both net negative at pH 7. However, this surface charge interaction mechanism governing the silica scavenging behavior of these two mineral sorbents at pH 3 is less apparent. At pH 3, where FeOOH surface is “more” positive, about half (~60%; Fig. 3.6) of the colloidal silica was scavenged, whereas nominal (<10%; Fig. 3.6) silica immobilization occurred on KFELD surface under the same acidic conditions.

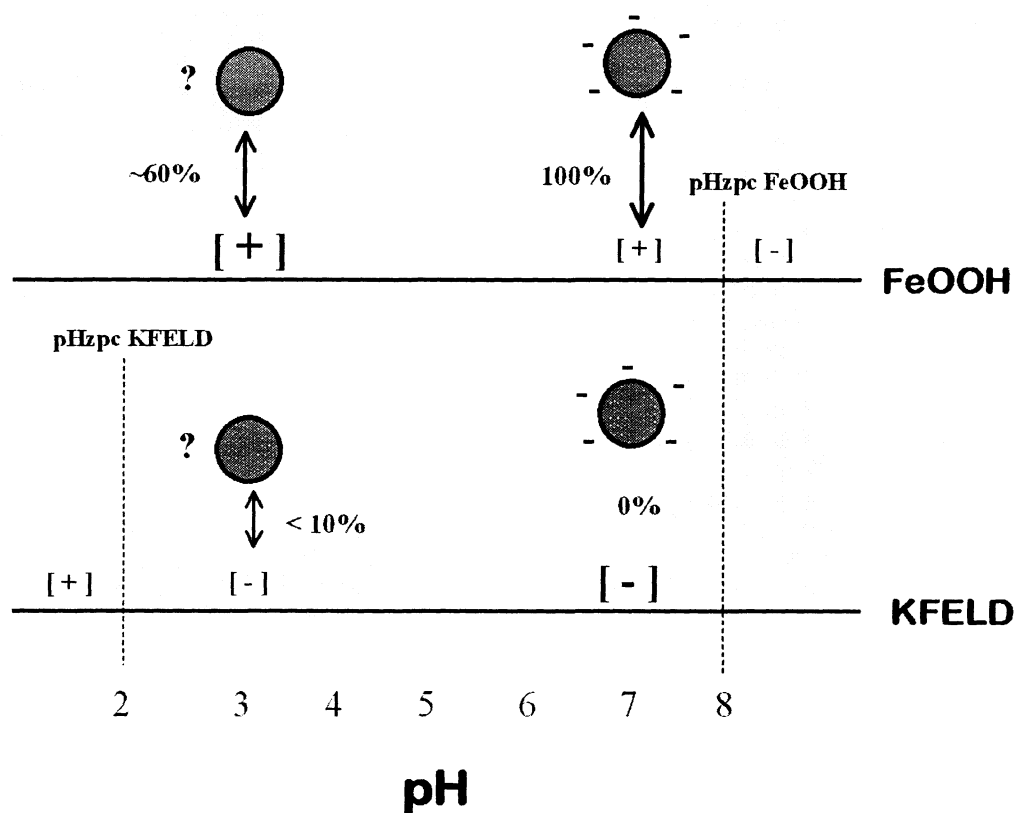


Figure 3.6 Schematic diagram showing the percentage $^{\text{C}}\text{SiO}_2$ immobilization ($^{\text{C}}[\text{SiO}_2]^0 = 120$ ppm) of FeOOH ($\text{pH}_{\text{zpc}} = 8.0$; dashed vertical line) and KFELD ($\text{pH}_{\text{zpc}} = 2.0$) minerals at pH 3 and pH 7; solid horizontal lines represent mineral surfaces and their relative net surface charges []; circles represent colloidal SiO_2 with inferred net surface charge status at pH 3 and pH 7 (*see text for explanation*).

3.3.5 Cell viability assay, visualization and Si elemental scanning

Live/dead staining procedure indicated >90% cell viability for the LIVE naked cyanobacterial cells in both pH 3 and pH 7 batch systems. Further, post-silicified re-cultured subsamples of LIVE cyanobacterial cells from both acid and neutral systems showed that the cells were not only viable after the 5-day experimentation, but were able to metabolize and grow when transferred to BG-11 growth media (3-5 days). Cell viability assay of the DEAD_{pH3/pH7} naked cyanobacterial cells indicated >85% non-viable cells before batch silicification; further, DEAD_{pH3/pH7} cells have become completely bleached from yellowish-green color after 3-4 days of re-culturing in nutrient medium indicating completely compromised cells. No meaningful cell viability assay was obtained for the COMP_{pH3/pH7} cyanobacterial treatment due to the heavy silica veneer on the cells preventing probe hybridization (see Fig. 3.7; Fig. 3.8-c); however, re-culturing of the batch-silicified COMP_{pH3/pH7} showed the cells were able to metabolize (i.e., O₂ evolution) and outgrow their silica envelope. Harvested cell-SiO₂ composites after one month in 1 M SiO₂-cyanobacterial suspension showed more vigorous growths when transferred to nutrient broth as compared to the live, naked cells (Fig. 3.7).

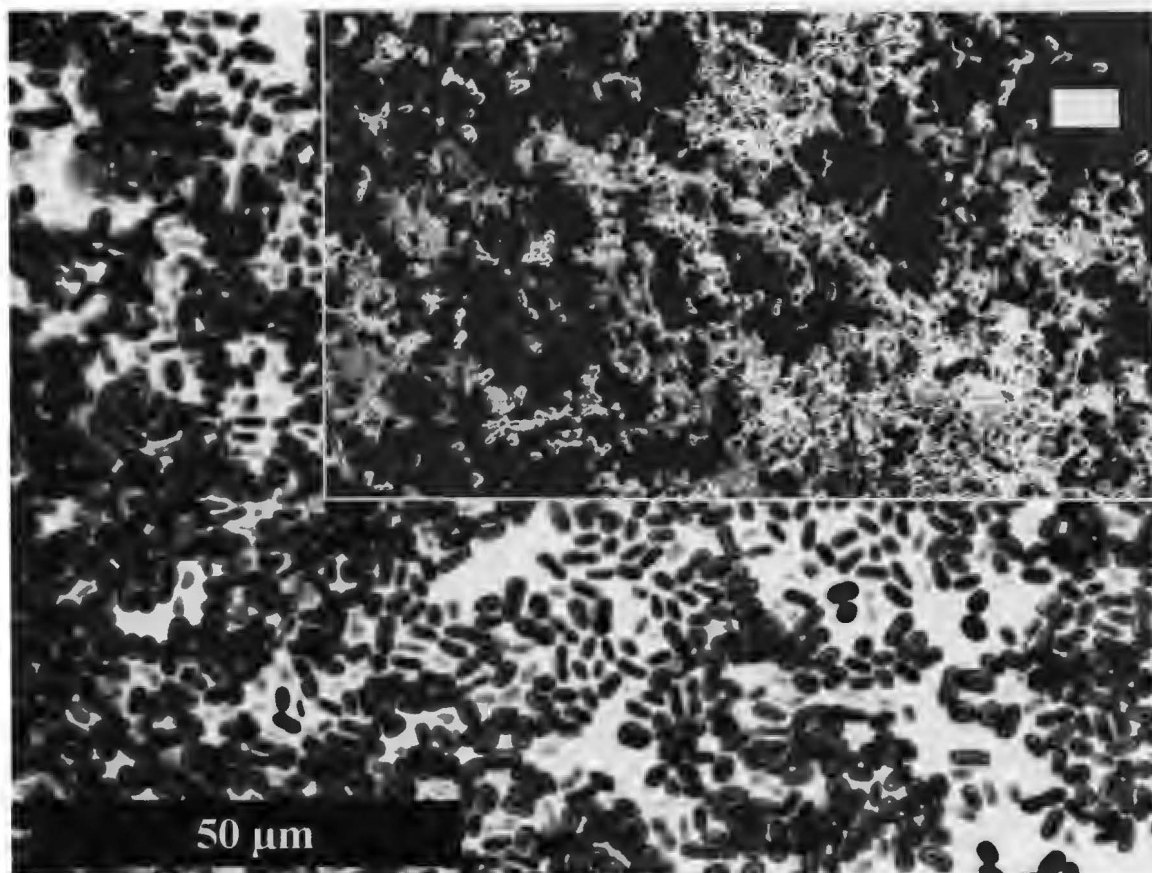


Figure 3.7 Photomicrograph of COMP solids under bright field optical microscope (foreground) showing SiO₂ halos on cyanobacterial cells; (inset) FESEM photomicrograph of COMP solids upon transferring to BG-11 nutrient medium after one month of preparation; note the outgrowths of cyanobacteria from the SiO₂ veneers (bright areas around the cell, scale bar = 10 μm); both COMP solids shown were not subjected to batch silicification.

Qualitative comparison of ESEM-EDS Si peaks on the surfaces of post-silicified naked cyanobacteria (live and dead) from both pH 3 and pH 7 systems showed minimal Si peaks detected in the pH 7 solids compared to the former, reflecting the pH 7 solids' insignificant ($\sim 10\%$; Fig. 3.1) associated $^{13}\text{C}\text{SiO}_{2\text{scav}}$ compared to pH 3 organic solids (for example, LIVE_{pH7} and EDS scan in Fig. 3.8-a and Fig. 3.8-b, respectively). The COMP Si elemental point scans showed variable Si peaks post- $^{13}\text{C}\text{SiO}_2$ exposure, probably indicating the variable thicknesses of the silica halos (for example, see Fig. 3.7). However, micron-sized area scans were generally invariant among the COMP_{pH3} pre-and post-silicification reflecting the more dominant silica encrustations on the cell surfaces relative to its experimentally scavenged $^{13}\text{C}\text{SiO}_{2\text{scav}}$ (Fig. 3.8-c; Fig. 3.8-d). Both LIVE_{pH3} (Fig. 3.9-a; Fig. 3.9-b) and DEAD_{pH3} (Fig. 3.9-c; Fig. 3.9-d) naked cells showed detectable Si peaks; however, the LIVE_{pH3} solids showed consistently higher relative Si peaks compared to the DEAD_{pH3} naked cells, verifying the highest $^{13}\text{C}\text{SiO}_2$ scavenging among the live treatments in the pH 3 batch treatments (Fig. 3.2; Fig. 3.3).

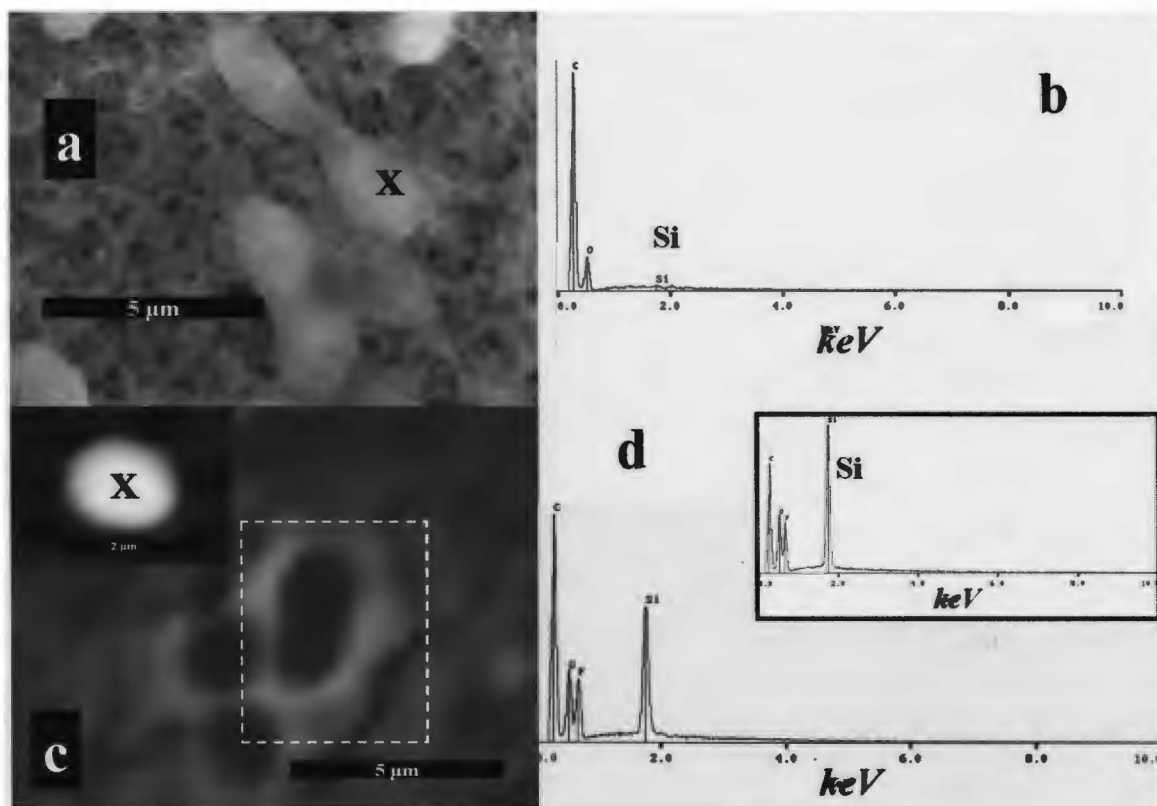


Figure 3.8 Representative ESEM photomicrographs of post-silicified LIVE_{pH7} cells (a; only LIVE_{pH7} solid shown for pH 7 batch experiments) and respective EDS Si elemental scan (b); COMP_{pH3} solids (c) and respective EDS scan (d); boxed spectra in d represent EDS scan of single COMP_{pH3} (inset in c); lower EDS spectra in d is an area scan of boxed region in c; “X” denotes EDS target beam.

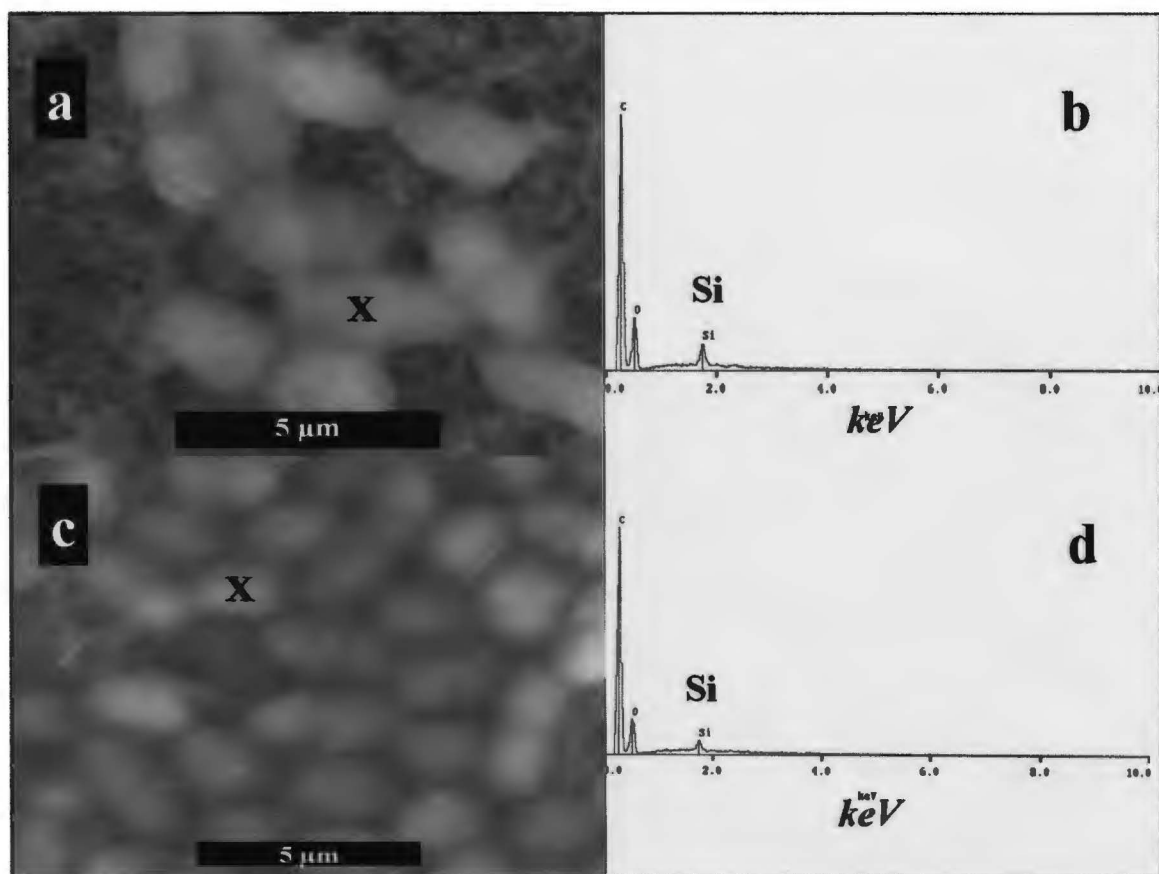


Figure 3.9 ESEM photomicrographs and associated EDS Si elemental scan for post-silicified LIVE_{pH3} (a, b respectively) and for DEAD_{pH3} (c, d respectively) solids; “X” denotes EDS target beam.

While ESEM-EDS Si elemental scans qualitatively verified a solid-dependent silica scavenging at pH 3, higher resolution FESEM analysis unequivocally showed a visual overlay of the silica nanoparticles (10's nm) on the LIVE_{pH3} and DEAD_{pH3} samples (Fig. 3.10; Fig. 3.11). There appears to be a spatial pattern of ^CSiO₂ distribution among the batch-silicified (i.e., 5 days) LIVE_{pH3} samples where SiO₂ nanoparticles were distributed at the “polar ends” of the cyanobacterial cells (Fig. 3.10-a; Fig. 3.10-c). Such trend may indicate a site-specific propensity for the initial ^CSiO₂ attachment on the cells, suggesting spatial surface heterogeneity on cell surfaces. For instance, relative Si peak comparison on the central part of LIVE_{pH3} cyanobacterium (Fig. 3.10-c; Fig. 3.10-d) qualitatively showed less Si peak compared to the polar end (Fig. 3.10-e; Fig. 3.10-f). Sheath-protrusions were also observed among the LIVE_{pH3} silicified samples (Fig. 3.10-c, white arrow), a typical cyanobacterial response mechanism to stressors such as high salinities and metal loads (Borbely, et al., 1990; Gardea-Torresdey, 1996). In contrast to the LIVE_{pH3} silicified cyanobacteria, the DEAD_{pH3} silicified cells do not show an apparent distribution of scavenged ^CSiO₂ colloids over the cell surface (Fig. 3.11). Moreover, unlike the LIVE_{pH3}, there were no evident sheath protrusions on the DEAD_{pH3} cells.

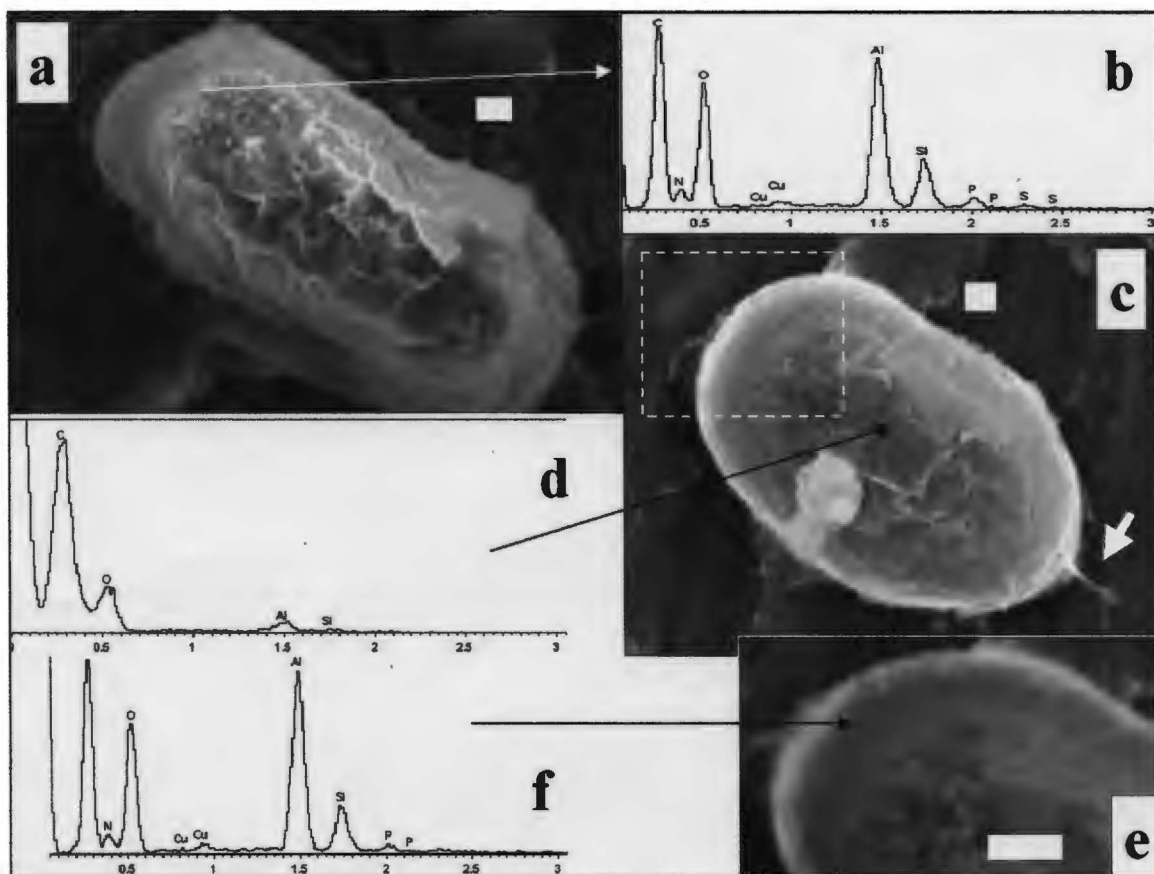


Figure 3.10 Representative FESEM photomicrographs and EDS elemental scans of post-silicified LIVE_{PH3} cells; thin arrows represent location of target beam on cell surface; plate (e) is higher magnification view from boxed area in (c); white bold arrow in (c) shows web-like cellular protrusion (*see text for explanation*); Al peaks from EDS originate from the aluminum stub used as platform; scale bar = 100 nm.

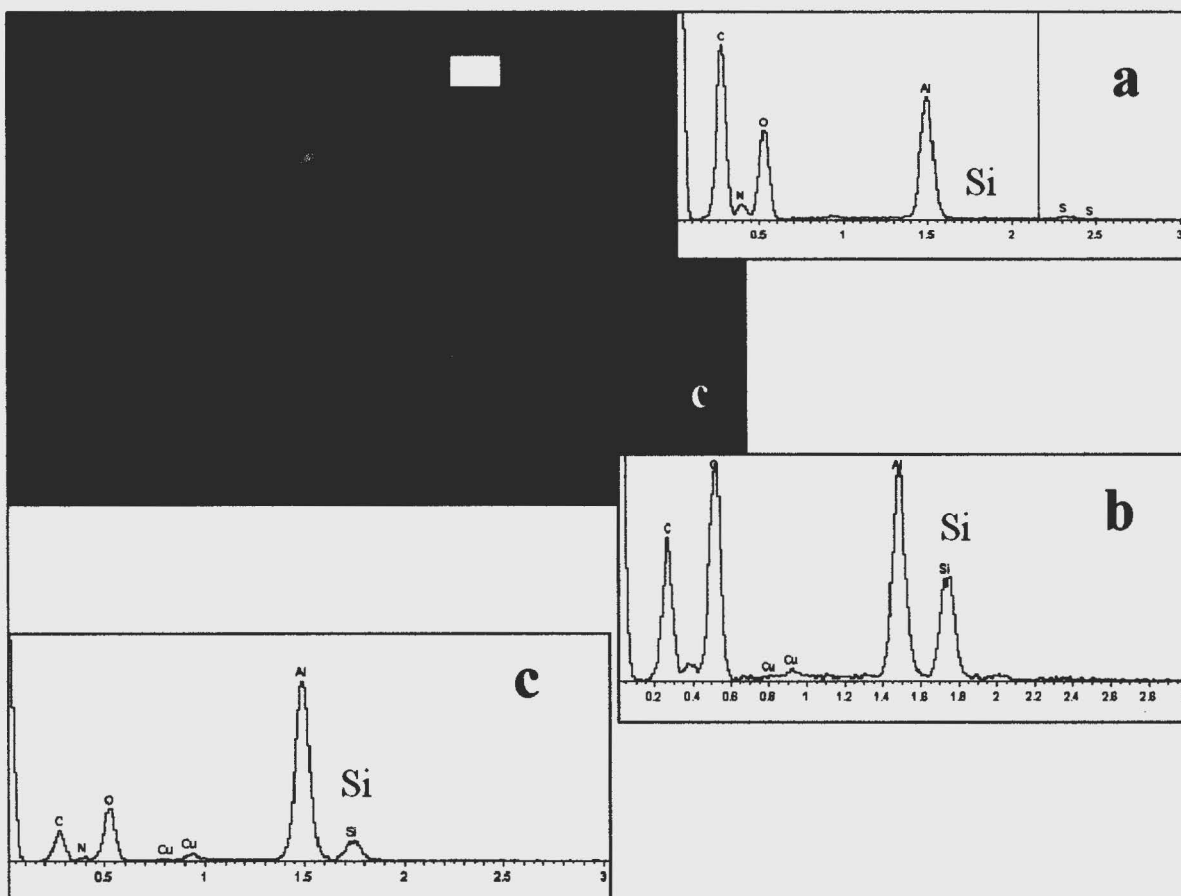


Figure 3.11 Representative FESEM photomicrograph and EDS elemental scans of post-silicified DEAD_{pH3} cyanobacterial cell; EDS spectra (a), (b) and (c) correspond to respective labeled areas on dead cell (*see text for explanation*); Al signal comes from the stub used; scale bar = 100 nm.

3.4 Discussion

Akin to any other sorbent-sorbate systems where charged surfaces interact with oppositely-charged particulate or dissolved species (i.e., aqueous metals, (nano)particulate solutes: Stumm, 1992; Morel and Hering, 1993; Warren and Haack, 2001), results from this work highlight the importance of charged interfaces for mineralization, particularly silicification whether these surfaces are of organic or inorganic in composition. The highly significant net scavenged SiO_2 of particle treatments at pH 3 (Fig. 3.2; Fig. 3.3) as compared to pH 7 (Fig. 3.1), principally for the naked cyanobacteria (both live and dead), and the SiO_2 -cell composites, attests to a strong pH-dependent solid interfacial effect on silica sequestration. These results were consistent with what has been shown in Chapter 2 that detectable silicification of natural microbial mats in near-saturated (colloidal) silica solution is intimately linked with acid pH conditions. The insignificant scavenging of aqueous silica at neutral pH among all four experimental solids: $\text{LIVE}_{\text{pH}7}$, $\text{DEAD}_{\text{pH}7}$, $\text{COMP}_{\text{pH}7}$, $\text{SILB}_{\text{pH}7}$ (Fig. 3.1) may be explained by surface charge repulsion effects of silica colloids and these respective interfaces. The proton-binding properties of both colloidal silica and the cyanobacterial surfaces used in this work have been characterized and are detailed in Chapter 4; however, results from acid-base characterization (i.e., proton binding; Chap. 4) will be referred to when necessary to illustrate the arguments in this chapter. At pH 7, $^{\text{c}}\text{SiO}_2$ particles were shown to have strong net negative surface charges (Janusz, et al., 1991; Sonnefeld, 1996; see Chap. 4). Similarly, microbial surfaces are largely net negative as well, due to deprotonation of acidic surface functional sites (for this cyanobacterial strain mainly carboxylic, >90%; see Chap. 4) as has also been shown for a variety of

cyanobacterial species (e.g., *Calothrix*, *Synechococcus*: Pitta and Berg, 1995; Phoenix, et al., 2002). An overall “active” negative surface charge for this *Synechococcus*-type cyanobacteria at pH 7 is further inferred from the complete sequestration of positively charged metal (Cu^{2+}) ions on the cell surfaces, while anionic (SO_4^{-2}) scavenging did not occur under the same pH conditions (Fig. 3.5). These results indicate that under conditions where both cyanobacterial surfaces and SiO_2 particles are strongly net negatively charged (i.e., circumneutral pH), electrostatic repulsion from both interfaces is more dominant hence no considerable cell- SiO_2 interaction occurred (Fig. 3.1; Fig. 3.8-a; Fig. 3.8-b). This conclusion is consistent with what has been mechanistically shown thus far from similar bacterial-silica mineral substrate adsorption investigations (Yee, et al., 2000). That is to say that, for any interaction to occur (either specific adsorption leading to chemical bonds and/or non-specific, ionic charge interactions) colloidal silica need to be close enough and overcome these repulsive forces to “stick” to sorbent surfaces (Gregory, 2006), regardless if these interfaces are organic, inorganic or a matrix of both.

It is clear that the propensity for a surface to scavenge silica and become silica mineralized is limited by surface charge repulsion effects, and is independent of the nature of the silicifying surface. While the fully or partly exposed organic surfaces (i.e., naked and composite solids, respectively) did not show silica sequestration at pH 7 due to the dominance of repulsive forces (both highly negative; Fig. 3.1), FeOOH mineral showed complete colloidal silica scavenging at the same pH (Fig. 3.6). This result is not surprising considering the FeOOH surfaces are positively charged at pH 7 ($\text{pH}_{\text{zpc}} = 8.0$) and would be ideal surfaces for interaction with oppositely charged SiO_2 , as has been shown by other similar works (Davis, et al., 2002; Luxton, et al., 2006). On the other

hand, KFELD mineral surfaces are strongly net negative at pH 7 ($\text{pH}_{\text{zpc}} = 2.0$), thus expectedly no interaction occurred with SiO_2 particles, again due to the dominance of charge repulsion mechanisms (Fig. 3.6). It is difficult however to establish the SiO_2 sequestration propensities shown by both mineral sorbents at pH 3. FeOOH partially sequestered more than half of SiO_2 at pH 3 (~60%; Fig. 3.6) when the surfaces of FeOOH are “more” positive compared to pH 7 (Fein, et al., 2002). It is suspected that this behavior has to do with the surface speciation and reactivity of $^{\text{c}}\text{SiO}_2$, reflecting likely the combined effects of residual negative surface charges at pH 3 and/or the inherent affinity of silica to Fe-oxide mineral surfaces (Swendlund and Webster, 1999; Davis, et al., 2001; Luxton, et al., 2006). KFELD and SiO_2 both have similar pH_{zpc} values ($\text{pH}_{\text{zpc}} \sim 2\text{-}3$; Goyne, et al., 2002) and would expectedly have similar proton-charging behavior, hence it is no surprise that SiO_2 scavenging did not occur at pH 7 due to charge repulsion from both negative interfaces. However, under acidic conditions, KFELD showed nominal SiO_2 scavenging (~10%; Fig. 3.6), suggesting a reduction of charge repulsive effects from protonation/neutralization of either surface reactive groups, and thus making KFELD and/or SiO_2 interfaces “less” negative or electrochemically neutral, respectively compared to pH 7 (see Chap. 4). As with FeOOH, aluminosilicate feldspar surfaces have been shown to have affinity for silica (Smith, 1998), but their coordinative propensities appear to be restricted by surface charge repulsion effects, which needed to be overcome first under pH conditions close to their pH_{zpc} ($\text{pH } 3 \sim \text{pH}_{\text{zpc}}$ for both KFELD and SiO_2). Obviously, this work does not attempt to describe the SiO_2 scavenging behavior of FeOOH and KFELD minerals in terms of surface charge interactions alone. It is argued however that when silica surfaces are highly ionized (i.e., at pH 7; Fig. 3.6; see Chap. 4),

electrostatic interactions (attractive or repulsive) exert a major control relative to the coordinating mineral surfaces. This contention is evident from the complete SiO_2 sequestration by net positive FeOOH at pH 7, whereas no detectable SiO_2 sequestration of KFELD occurred at the same pH, due to the overriding repulsive forces induced by both negative surfaces in the latter case. Therefore, parallel mechanism of silica non-sequestration between inorganic minerals and organic bacterial substrates, as dictated largely by their respective proton-charging behavior (i.e., pH_{zpc}), is clearly demonstrated in this work. These results evidently showed that electrostatic repulsion arising from charge similarities between the surface sorbent (either mineral or cell) and the silica sorbate restricts their effective interaction, and thus needs to be overcome prior, for perceptible silica immobilization to occur. While the significance of this electrostatic repulsion effect has not been fully explored within the context of microbial (non)mineralization, its importance has been widely recognized in a variety of subsurface colloid transport systems (Davis, et al., 2001; Gregory, 2005).

In marked contrast to pH 7 silicification results, acid conditions clearly favor the sequestration of colloidal silica by naked, both live and dead cyanobacterial cells as well as the silica-coated cyanobacteria cell composites (Fig. 3.2; Fig. 3.3), consistent with what was hypothesized to occur. This enhanced colloidal silica scavenging of both organic and silica-organic composites is interpreted to be the result primarily from the reduction of interfacial repulsive forces eventually leading to specific $^{\text{C}}\text{SiO}_2$ -cell surface group chemical reactions. From pH 7 down to pH 3, the net surface proton-charging condition on the unmineralized cyanobacterial cells changed from dominantly negative to neutral or “less negative” status as inferred from the reduced Cu^{2+} sequestration (i.e., ~

80% reduction from pH 7 to pH 3; Fig. 3.5), due to protonation/neutralization of cyanobacterial surface carboxylic groups ($pK_{a1 > \text{COOH}} \sim 3$; see Chap. 4). While these naked cyanobacterial cells possess surface neutral (or slightly negative) functionalities at pH 3, there was no evidence of an “active” positive charge occurrence inferred from anionic non-sequestration (SO_4^{2-} , Fig. 3.5; see Chap.4). The surface charge status of $^{\text{c}}\text{SiO}_2$ at pH 3 is more difficult to ascertain. Amorphous colloidal silica is a complex system and often, predicting its proton charging behavior may prove difficult due to discrepancies between theoretical and empirical observations (Nawrocki, 1997; Taboada-Serrano, 2005). Much of these translocations arise due to its complex structure (i.e., heterogeneity and/or random arrangement of surface silanols of varying types and proportions: Bergna, 1994; Mendez, et al., 2003), presence of impurities (e.g., alkali metals: Carroll, et al., 2002), microporosities and surface roughness (Goyne, et al., 2002). The emergence of surface charges on SiO_2 at the window pH 3 and pH 7 arise from protonation/deprotonation reactions of the surface silanol groups ($\equiv\text{SiOH}$, where “ $\equiv$ ” is the underlying lattice; see Chap. 4). At pH 7, the surface charge status of silica is net electronegative from deprotonated $\equiv\text{SiO}^-$ (Dove and Rimstidt; 1994; Perry, 2003); however, owing to silanol’s wide range of reported pK_a values (1.8 – 10; Nawrocki, 1991; Nawrocki, 1997), this divergence can drastically affect the surface charge development of SiO_2 . There is a general consensus that silica has an acidic pH_{zpc} between 2 and 4.5 (Vigil et al., 1994; Dove and Rimstidt; 1994; Carroll, et al. 2002). While this range of pH_{zpc} literature values does not constrain the overall surface proton charge state of silica used in this work at pH 3, there appears to be an agreement that positively charged silanols ($\equiv\text{SiOH}_2^+$) are negligible (Nawrocki, 1997; Laven and Stein,

2001; Carroll, et al., 2002; see Chap. 4) and can only occur at extreme acidities ($\sim 30\%$ at $\text{pH} \sim 0$; Pokrovsky, et al., 2006). From these collective literature data, it is reasonable to assume that the surface charge status of $^{\text{C}}\text{SiO}_2$ at $\text{pH} 3$ may be overall neutral or “slightly” negative.

The surface charging status of the cyanobacterial cells and the silica colloids are similar at both pH values investigated, i.e., both are strongly negative at $\text{pH} 7$ and neutral or “less” negative at $\text{pH} 3$. Intuitively, this scenario implies that charge repulsion arising from like-charging is lessened and overcome at $\text{pH} 3$ due to surface protonation compared to $\text{pH} 7$ (Fig. 3.12-a; Fig. 3.12-b, respectively). This result is consistent with the mineral KFELD- SiO_2 sequestration results (Fig. 3.6) showing that for surfaces with similar proton charging behavior (i.e., $\text{pH}_{\text{zpc cell}} \sim \text{pH}_{\text{zpc SiO}_2}$), charge repulsion may be overcome at pH conditions close to their pH_{zpc} values. Such is that, there is a greater tendency for the silica colloids to get in contact with the cyanobacterial cell surface functionalities and form specific chemical bonds.

Most ($>90\%$ of $^{\text{C}}\text{SiO}_{2\text{scav}}$) of the experimentally scavenged silica was chemically “bound” on the cyanobacterial surfaces (Fig 3.4) and is largely insensitive to perturbations in pH , salinity and organic complexants. These results show that microbially scavenged silica is chemically stable, and is not simply electrostatically coordinated (i.e., non-specific, electrostatic adsorption) which has been shown to be a reversible reaction (Ledin, et al., 1995; Yee, et al., 2000; Parent and Velegol, 2004). The surface functional sites of this cyanobacterial strain are composed largely of carboxylic $>\text{COOH}$ groups (see Chap. 4), hence it is reasonable to assume that direct silica chemical coordination occurred via these exposed reactive groups, as has also been suggested in

the literature (Konhauser, et al., 2004; Yokohama, et al., 2004; Benning, et al., 2005). Results from this work indicate that at acid conditions close to the interfacial solids' pH_{zpc} , a majority of the cell $>\text{COOH}^0$ and silica $\equiv\text{SiOH}^0$ surface groups are neutralized (Fig. 3.12-b), hence repulsive interactions are overcome and the potential for direct, specific chemical coordination is promoted. It is proposed that direct chemical reaction between the hydroxylated silica groups and the protonated carboxylic sites on the cell surfaces occurred via hydrogen bonding (Fig. 3.12; Eqn. 3.1):



No significant pH changes were measured post-silicification at pH 3 in all experimental treatments ($\text{pH} \pm 0.05$), thus it is suspected that Eqn. [3.1] would be sufficient to describe the specific chemical coordination inferred for cell SiO_2 sequestration. However, because the surface proton-charge status of both $^c\text{SiO}_2$ and cyanobacteria can only be deduced at pH 3 relative to pH 7, it may also be plausible that either $>\text{COO}^-$ or $\equiv\text{SiO}^-$ group is deprotonated, and coordinate with each other to liberate an hydroxyl (OH^-) ion instead of water molecule (Perry and Keeling-Tucker, 2003). Although it is clear from the results of batch silicification at pH 7 (Fig. 3.1) that when both surface groups are strongly deprotonated (i.e., $>\text{COO}^- \leftrightarrow ^-\text{OSi}\equiv$) and therefore net negative, electrostatic repulsion dominates and no observable chemical interaction occurred (Fig. 3.12-a). This proposition for a direct chemical bonding by silica onto cyanobacterial surfaces (Eqn. 3.1, via hydrogen bond) is consistent with what has been inferred from published works on cyanobacterial silicification (Phoenix, et al., 2000;

Konhauser, et al. 2004; Bening, et al., 2004a; Benning, et al., 2005). These works also conclude that cyanobacterial cells do not necessarily enhance the precipitation of monomeric H_4SiO_4 species, rather it was suggested that subsequently-condensed (abiogenic) silica nano-precipitates interact directly with the cyanobacterial surfaces (Phoenix, et al. 2000; Benning, et al., 2004a; Benning, et al., 2004b), consistent with the results from Chapter 2 that pre-polymerized SiO_2 colloids are responsible for biosilicification. The pH 3 silicification results (Fig. 3.1; Fig. 3.2) also parallel recent findings that surface charge neutralization (hence, surface hydrophobicity; Yee, et al., 2000; de Mesquita, et al., 2003; Parent and Velegol, 2006) under acidic conditions promotes bacterial attachment to mineral substrates, and that cell silicification is akin to the same process governing hydrophobic cell attachment to mineral substrates during biofilm formation. However, this hydrophobic mechanism cannot be fully invoked to explain the cyanobacterial silica sequestration observed in this work because the cell-scavenged SiO_2 is chemically bonded to the cell matrix (Fig. 3.12-d) and is neither reversible nor exchangeable (Fig. 3.4) unlike hydrophobic interactions (Yee, et al., 2000). Also, while cyanobacterial charge neutralization at pH 3 leading to cell hydrophobicity was apparent in the experiment from observed cell aggregation (not shown), it cannot be ascertained whether the silica particles were hydrophobic as well because all colloidal silica solutions (i.e., $^{\text{C}}\text{SiO}_2$ only) remained stable in solution with no evidence for colloid aggregation or separation from the aqueous phase at either pH 3 or pH 7 (Fig. 3.1, $^{\text{C}}\text{SiO}_{2\text{pH}7}$ only; Fig. 3.2 $^{\text{C}}\text{SiO}_{2\text{pH}3}$ only). Hence it is proposed in this work that for microbial silicification to occur, it is important that particle electrostatic repulsion effects has to be overcome (via charge neutralization of cell and/or silica surfaces), followed by

direct chemical coordination of SiO_2 silanol to cell carboxylic surface groups as described in Eqn. 3.1.

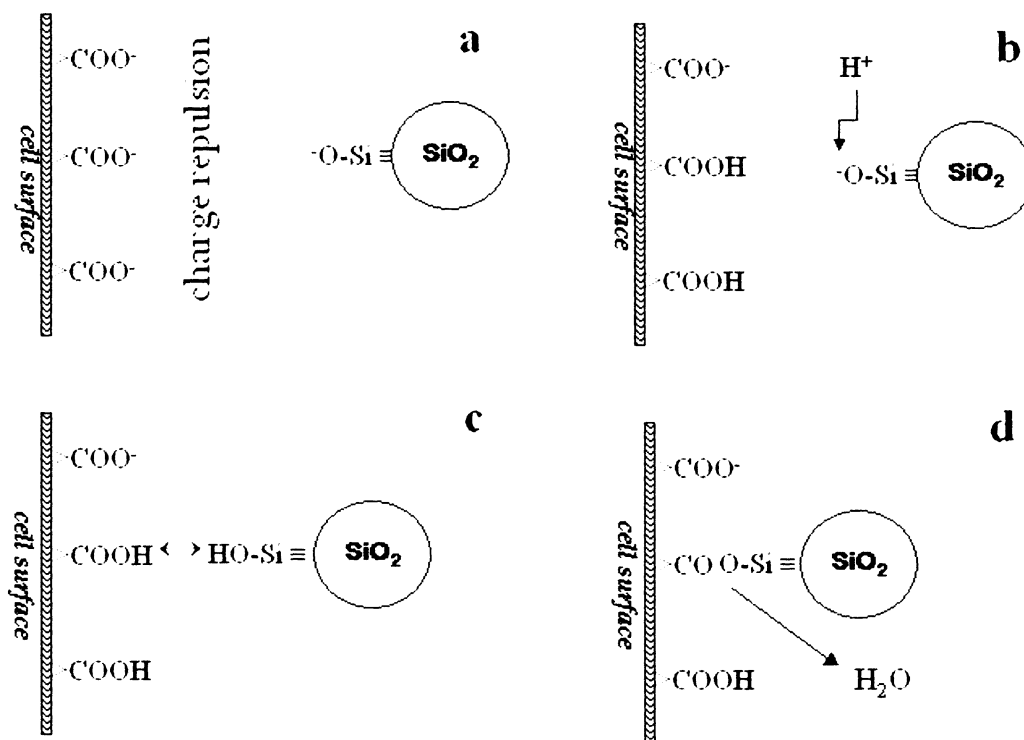


Figure 3.12 Schematic diagram illustrating proposed model for colloidal silica attachment to cyanobacterial surface; (a) deprotonated COO^- and SiO_2^- functionalities on cell and SiO_2 surfaces, respectively at pH 7 leads to charge repulsion; (b) protonation of cell and SiO_2 surface functional sites via acidification at pH 3; (c) reduction of surface (negative) charges at pH 3 on both interfaces initiates COOH and SiOH interaction via H-bonds; (d) bond formation of carboxylic and silanol groups with subsequent liberation of water molecule (*see text for explanation*).

Acid pH is identified as a “master variable” that promotes substantial silicification of organic cell surfaces via the formation of chemical bond of surface carboxylic groups with SiO₂ silanols (Eqn. 3.1); moreover, the extent of silica sequestration showed a strong treatment-dependence among the experimental solids used, particularly on the level of cell integrity, and the extent of exposed organic cell surfaces (Fig. 3.2; Fig. 3.3). Both the naked live and dead results will be discussed subsequently in more detail. The results however of the lower cell-SiO₂ composite silicification at pH 3 relative to the naked cells (either live or dead) shown in this work (Fig. 3.2; Fig. 3.3) parallel what was previously observed that the cell’s prior silica encrustation inhibits the cell surfaces’ effective silica mineralizing propensity (see Chap. 2). The silica scavenging behavior of the cell-SiO₂ composite is fairly close to that of naked dead cell solids rather than the abiotic silica beads (mM·mg⁻¹; Fig. 3.3), hence it is suspected that while the composites appeared to be visually encrusted with silica envelopes (Fig. 7; Fig. 3.8-c), there may be some residual organic functional sites available for aqueous SiO₂ interaction, as has also been shown for other cell-mineral composite investigations (Smith and Ferris, 2003; see Chap. 4). There was no measurable acid-induced silica dissolution of the SiO₂ veneer among the composite solids during batch silicification, therefore it is likely that the SiO₂ envelope is not homogeneously distributed over the entire cell surfaces such that exposed remnant organic functional sites may be available for the coordination of solution ^cSiO₂. The same SiO₂ and composite solids interaction via direct chemical reaction of the solution silica ≡SiOH with the residual >COOH (Eqn. 3.1) “sticking out” of the cell composite surfaces is invoked in this process. Silicification of the inorganic silica beads did not induce colloidal silica immobilization from the aqueous phase at either pH 3 (Fig. 3.2) or

pH 7 (Fig. 3.1); therefore, the role of the silica veneer on the SiO_2 coated cell mineral composite is to limit the available binding sites for colloidal silica coordination, consistent with what has been shown previously (see Chap. 2). It is interesting to note that the silica scavenging behavior of this cell-mineral composite is intermediate between those observed for pure inorganic SiO_2 and the unmineralized live cyanobacterial cells (Fig. 3.3), and is typically reflective of the surface site functionalities available for the mixed mineral-organic composite matrix (Smith and Ferris, 2003; see Chap. 4). The cell composites, despite their enshrouding silica, are still viable and are able to grow after experimentation (Fig. 3.7), thus showcasing silica scavenging properties intermediate between those of abiotic and viable fully exposed organic surfaces (Fig. 3.3).

Perhaps the most surprising result of this work is the observed significantly higher mineralization propensities of unmineralized live vs. dead cyanobacterial cells (Fig. 3.2; Fig. 3.3). From a microbial surface charge development perspective, this result contrasts with what has been typically shown for live (and potentially metabolizing) bacterial systems. Metal sequestration experiments involving live bacteria showed lower metal scavenging abilities due to solute (e.g., metal) competition for available cell surface binding sites from $[\text{H}^+]$ liberated via cell proton pumping (Urrutia, et al., 1992). Moreover, previous studies on the silicification of dead cyanobacterial isolate induced mineralization on both the cell wall and cytoplasm, while silicification among live cells tend to be restricted to the outer cell sheaths (Konhauser and Ferris, 1996; Phoenix, et al., 2000), implying more ideal sites for silica to form on dead cells (i.e., on both cell walls and cytoplasmic sites), compared to live cyanobacteria (sheaths only). While the mode of killing the cells in this current work might introduce cell surface architectural

reorganization induced by thermal denaturation (e.g., cell protein unfolding: Brock, et al., 1994); this scenario is unlikely because the nature of cell surface arrays destabilization will tend to expose previously inaccessible functional sites to the bulk solution phase and should, on the contrary be more favorable for microbial mineralization (Claessens, et al., 2004). One distinguishing feature observed from the silicification of naked live cells is the apparent heterogeneous spatial propensity of a living cell to biomineralize (Fig. 3.10). The onset of biomineralization on cell surfaces is not homogeneously distributed over the entire surface. In the earlier stages of cell silicification (i.e., 5 days), scavenged SiO₂ was typically observed to be distributed on the “polar ends” of the cyanobacterial cell (Fig. 3.10). This nano-scale heterogeneous susceptibility of cell surfaces to biomineralize has been observed in our laboratory from the microbially-mediated oxidation of manganese oxyhydroxides in acid mine drainage environments (Drs. Lesley Warren and Elizabeth Haack, pers. comm.), however its significance is yet to be fully understood.

It is interpreted that this SiO₂ nano-scale distribution on biosilicifying cells is an expression of the heterogeneous charge orientation of organic functional sites (Ledin et al., 1995; Vadilo-Rodriguez and Logan, 2006). The distribution of sequestered silica on the live cyanobacterial cells (i.e., distributed mostly on the cell “poles”; Fig. 3.10) may be explained by cell surface charge heterogeneity and charge polarity, which has also been observed in adhesion studies of bacteria (*E. Coli*) to silica surfaces (Parent and Velegol, 2004). However, this charge polarity/heterogeneous distribution of functional sites among the naked live cells cannot be fully invoked because the dead cells did not show the same spatial distribution of scavenged SiO₂ at the polar ends of the cell (Fig. 3.11). In this work, it is proposed that the observed significantly higher ^cSiO₂ sequestration and

apparent “polar” distribution of SiO_2 on the live cell surfaces compared to the compromised cells could be the result of any or the combination of the following: heterogeneity of surface cell structural array; cell interfacial modification induced by active cell metabolism; and/or cell surface architecture alteration in response to perturbations such as experimental solution silica or low pH.

There have been reports of heterogeneous functional site distribution over the entire cell surfaces (Cox, et al., 1999; Sokolov, et al., 2001; Vadillo-Rodriguez and Logan 2006), but this possibility cannot be invoked solely because there should be no difference between the silicification styles of the live and dead cells if this mechanism alone controls the variability shown by the two experimental systems (Fig. 3.10; Fig. 3.11). In addition, the total site densities of COOH groups reactive to SiO_2 determined from live cell surfaces in this work is comparable to or even lower, than those observed for the dead cells (see Chap. 4). Therefore if variability in surface reactive site alone governs the silicification of cells, it is expected that dead cells should coordinate more silica than do living cells, which was not observed for this current work.

On the other hand, even if the viable cyanobacterial cells are nutrient-depleted, they still have access to atmospheric $\text{CO}_{2(g)}$ and light, hence are capable of photosynthesizing. The ability of this particular cyanobacterial strain to recycle nutrients, or even grow under nutrient absent conditions has been observed before (i.e., from $\text{CO}_{2(g)}$ diffusion alone in distilled water: Amores, unpub. data). Photosynthetic (oxygenic) activity generates hydroxyl ions and the OH^- liberated can diffuse into the cell interfacial region, hence alkalinizing the local cell surface micro-environment (Visscher, et al., 1998; Phoenix, et al., 2000). Concomitant pH increase at the interface of live,

photosynthesizing cells favors the further (poly)condensation of solution silicas, particularly on the surfaces of initially reacted silica-carboxylic sites.

Another viable alternative to explain the increased silica scavenging of the live cells is their propensity to grow extracellular components in response to environmental stressors, in this case, from elevated silica in solution or acid pH. The acid-silicified live cyanobacteria were observed to grow “web-like” filaments on their surfaces, mostly at the “polar ends” of the cyanobacterium (Fig. 3.10-c, white arrow). High metal loads (Gardea-Torresdey, et al., 1996) and elevated silica (Phoenix, et al., 2001; Benning, et al., 2004 a/b) have also been shown to induce cell surface alterations. Similar ultrastructural projections were also observed from *E.coli* on the one side of the cell that makes them “stickier” possibly as a means of adhesion to substrates (Jones, et al., 2003). The microbial physiological function of these appendages emanating from the cyanobacterial cell wall is not clear; however, it is likely that these ultrastructures form as a type of defense barrier from possible toxicities brought about by solution SiO_2 or $[\text{H}^+]$. More importantly, the environmental consequence of these cell ultrastructural projections is that they may either offer additional favorable sites for silica coordination or may potentially “guide” the $^{\text{C}}\text{SiO}_2$ sequestration along the polar ends of the cyanobacterium (Fig. 3.10).

There are important microbial ecological consequences for silicifying under acid conditions. The waters liberated from silica coordination with cell surface carboxylic functional sites (Eqn.4.1) were thought to be important in regulating external cell pH and the subsequent microbial tolerance to acidity (Asada and Tazaki, 2001). The veneer of silica may also function as a microbial mode of protection from the harsh effects of

excessive $[H^+]$ in the external milieu, in addition to its “traditional” role as defense barrier from viral attack (Weiner and Dove, 2003), predation (Mann, 2001) and ultraviolet radiation (Phoenix, et al., 2001) as have also been proposed for many mineralizing microorganisms. While the cyanobacterial strain used in this work is not strictly acidophilic, the abovementioned microbial adaptations may lead us to further refine the exact mechanisms why “living” cells have higher preponderance, either cell-regulated, surface (charge) expressed or an ecological consequence, for perceptible microbial silicification to be more apparent in acid conditions. Clearly, there is some level of “metabolic factor” that shows consistently higher silica scavenging among fully-exposed live cells compared to dead/nonviable cells under these same acidic conditions.

3.5 Conclusion

Results from this work showed that perceptible microbial induced biosilicification is regulated primarily by surface charge effect. Just like any other sorbent-sorbate phases, charge interactions require that repulsive forces are overcome prior to effective silica sorbate interaction with sorbents, irrespective whether these coordinating surfaces are of organic (i.e., cell) or inorganic (i.e., mineral) constitution. Charge attractive/repulsive interactions are primarily dictated by the speciation of active functional sites on both cell and mineral surfaces, and are dependent on system pH. For silica biomineralization specifically, the propensity for SiO_2 coordination to cell surfaces is promoted at pH conditions close to SiO_2 sorbate and cell sorbent phases' respective pH_{zpc} values (i.e., in this case, $pH \sim 3$). Finer details of microbial silicification process indicate surface-dependent silica immobilization propensities wherein fully exposed cells

are more susceptible to silicify than do previously SiO_2 -mineralized cell composites consistent with what has been shown previously (Chap. 2). Further, these microbially coordinated SiO_2 phases are more tenaciously held on surface cellular matrices, indicating that cell immobilized SiO_2 is directly (i.e., chemically) bound to cell surfaces and not simply electrostatically held, hence are largely insensitive to environmental perturbations. Putative evidence from live, metabolizing cells showed intensified biosilicification propensities than do inactivated cells. This trend may be the result of any or combination of several possibilities such as heterogeneity of surface cell structural array; cell interfacial modification induced by active metabolism and cell surface architecture alteration in response to physico-chemical perturbations. Clearly, more work needs to be done to elucidate the mechanisms and the consequences of actively metabolizing cells leading to the formation of SiO_2 biominerals; nonetheless, this work has opened the doors for a more sensitive evaluation to distinguish a biological signature in microbially-induced silicification process.

CHAPTER 4: SURFACE PROPERTIES OF UNMINERALIZED (LIVE AND DEAD) CELLS AND SiO_2 END-MEMBERS AND THEIR COMPOSITE MATRIX: IMPLICATIONS FOR MINERALIZATION

4.1 Introduction

Microbes are ubiquitous in environmental systems, and have been implicated in the formation of a myriad of solid-phase secondary minerals in aqueous systems (Ehrlich, 1996; Nealson and Stahl, 1997; Tebo, et al., 1997; Warren and Haack, 2001; Classens, et al., 2004). Microbial mediation in mineral formation has been extensively investigated for a variety of biological minerals (i.e., biominerals) such as metal-oxyhydroxides (Tebo, et al., 1997; Warren and Haack, 2001; Haack and Warren, 2003), carbonates (de Vrind-de Jong, 1997; Visscher, et al., 1998) and silica/silicates (Fortin and Beveridge, 1997; Dixit and Van Cappellen, 2002). Microbial surfaces are highly reactive interfaces due to an abundance of surface binding sites where strong chemical interactions from dissolved ions can occur (Stumm, 1992; Fortin et al., 1997; Martinez, et al., 2003). Depending on the pK_a values of these structural moieties, they deprotonate successively at increasing system pH values, thus typically imparting a net negative surface charge to the microorganismal surfaces in natural aqueous environments (i.e., typical microbial surface $\text{pH}_{\text{zpc}} < 6$: Beveridge and Murray, 1980; Fein, et al., 1997; Cox, et al., 1999; Phoenix, et al., 2002; Konhauser, 2007). Similarly, mineral surfaces such as amorphous silica ($\text{SiO}_{2(\text{am})}$) acquire negative surface charges in aquatic systems, and are equally reactive to dissolved ionic constituents ($\text{pH}_{\text{zpc}} \text{SiO}_{2(\text{am})} < 4.5$: Carroll, et al., 2002; Cardenas, 2005). However, while both microbial and mineral surfaces acquire surface charges in natural waters, the former typically possess higher concentration and types of coordinating functional moieties compared to mineral surfaces, such as SiO_2 (Dixit and

Van Cappellen, 2002; Claessens, 2004). Amorphous SiO_2 is a common secondary mineral found in almost all environments: from soils and sediments (Fortin and Beveridge, 1997; Clarke, 2003); freshwater (Konhauser, et al., 1992; Inagaki, et al., 2003); and marine (Fortin, et al., 1998; Perry, 2003) systems. Moreover, it is not uncommon to observe matrices of SiO_2 mineralized microbial cells/colonies in various stages of cell integrities (i.e., mineralized “living” cells and dead cellular components) in many silica-bearing waters such as hydrothermal springs, acid mine drainage and groundwaters (Fortin, et al., 1997; Asada and Tazaki, 2001; Konhauser, et al., 2002; Inagaki, et al., 2003; Clarke, 2003).

The environmental significance of these interfaces of both organic (microbes) and mineral (silica), together with their combined cell-mineral matrix, is coupled to important physico-chemical processes in aqueous environments such as sorption, dissolution, precipitation and ion exchange reactions (Sjöberg and Lövgren, 1993; Stumm and Morgan, 1996) explored typically in the context of (bio)remediation of toxic metals (Subramaniam, et al., 2003; Vidyadhar and Rao, 2007) and other organic contaminants (e.g., PAH: Strnadova, et al., 1995; Foght and McFarlane, 1999). Knowledge therefore of the characteristics and reactivities of both microorganismal and SiO_2 mineral surfaces will not only aid us to better understand their function in pollutant immobilization, but can also give insights on the nature of cell surface-induced biomineralization, i.e., biosilicification in general. Results from Chapter 2 and Chapter 3 have shown that the silica mineralization propensity of natural microbial mat consortia is promoted by the amount of exposed organic cell surfaces; hence it is likely that the reactivity of these organic surfaces is reduced by the presence of these SiO_2 mineral veneers. While the

reality of the occurrence of microbial community-silica mineral assemblage in contact with the aqueous phase is that of a composite (Ferris, et al., 1986; Jones and Renault, 2003; Kato, et al., 2004); meaning, a matrix of mineralized cells (Bratina, et al., 1998; Haack and Warren, 2001), most available works probing the surface properties of microbes and silica were done separately as discrete entities. However, these cell-mineral composite sorbent phases do not necessarily behave additively if the individual components interact chemically with each other (Small, et al., 2000; Smith and Ferris, 2003). Comparative elucidation of functional site identities and densities of cell and mineral SiO₂ sorbent end-members, and an intermediate matrix of silicified cell phases is necessary to be able to evaluate their relative reactivities; hence their propensities to interact with ionic components in the aqueous phase. The relevance of investigating mineral and microbial surface charge behavior over a spectrum of pH values lies in their ability to coordinate counter-charged dissolved ionic constituents, an initial step in mineral precipitation, and in the case of microbial surfaces, biomineralization (Fortin, et al., 1997; Southam, 2000). Unlike their abiotic mineral sorbent counterparts, microbes often show variable surface reactivities even for the same strain (Urrutia, et al., 1992; Fein, et al., 2005), a direct consequence of microbes as “living” entities whose internal cell activities may translate to cell surface heterogeneity (Urrutia, et al., 1992; see Chap. 3). Cell surfaces typically host a standard suite of organic binding sites such as carboxylic, phosphoryl, amino and hydroxyl groups across species (Schultze-Lam, et al., 1996; Phoenix, et al., 2002; Martinez, et al., 2003; Scott and Ferris, 2003), but their relative proportions can significantly differ (Cox, et al., 1999; Sokolov, et al., 2001). Cell surface heterogeneity may be the result of any or a combination of the following factors:

age of analyzed cells in the course of their life cycle; their metabolic state; and, solution media chemistry (Beveridge, 1989; Daughney, et al., 2001; Dostalek, et al., 2004; Hong and Brown, 2006; Guine, et al., 2007). Therefore, it is often difficult to ascertain the overall microbial surface charging behavior across a pH spectrum even for the same microbial strain due to these intrinsic variabilities. For instance, growth media composition can lead to differential functional groups on the cell surface due to “insertion” of these sites on cell surfaces (e.g., ammonium and phosphate ligands from NO_3^- and PO_4^- salts: Engl and Kunz, 1995; Ledin, 2000). Repeated regeneration of a single microbial strain using high nutrition growth broth has also been shown to increase microbial surface functional group density compared to a newly isolated microbial consortia (Borrok et al., 2004).

Perhaps one of the most fundamental differences between the surfaces of minerals and microbial cells is that the latter are truly composed of three-dimensional macromolecular structures rather than two-dimensional arrays of functional groups such as those of mineral interfaces (Brown, et al., 1999; Claessens, et al., 2004). As living organisms, microbial cell wall components are designed to obtain nutrients from the environment, and protect the organism from harmful perturbations surrounding the immediate cell vicinity (Beveridge, 1989; Brock and Madison, 1991). Consequently, surfaces of living cells cannot be simply treated as “inert” interfaces under experimental conditions during which harmful physical and chemical changes are induced in the surrounding media such as high metal loads, high salinity and extreme pH (e.g., acid-base titrations) investigations (Claessens, et al., 2004). The function of cell walls of living cells may change via counter-metabolic responses to dampen the harmful effects of these

chemical or physical perturbations. For instance, in metal uptake studies of living bacteria (Urrutia et al., 1992), competition for the available anionic binding sites on the cell wall fabric from actively metabolizing cells occurred via proton pumping. As such, protons compete for binding sites around cell wall sites, thus reducing the number of metal ions which can bind to the cell wall surface (Urrutia, et al., 1992; Schultze-Lam, et al., 1996). It appears that the net effect of living (and presumably metabolizing) cells in metal sequestration studies is that they bind less metals than do dead cells, hence are less likely to be mineralized (Urrutia, et al., 1992; Guine, et al., 2007). Contrastingly, results from this current work have shown otherwise (see Chap. 3); that live cells showing some putative metabolic activity are more susceptible to mineralization (i.e., biosilicification) than dead cells. Therefore, the potential impact on cell interfacial region induced by factors such as level of microbial activity and/or growth histories is that microbial cells may show a wider range of possible surface charge forming moieties and concentrations, compared to inorganic mineral surfaces. Further, because microbes are living entities capable of inducing cell-related activities, (Fortin, et al., 1997; Claessens, 2004), the interfacial reactivities of live cells may differ from those of “inactivated” (i.e., dead) cells.

Acid-base titration is a relatively simple yet effective method to probe the surface structure and reactivity of various natural and artificial sorbents with respect to protons (Smith and Ferris, 2001; Smith and Ferris, 2003). Proton binding and charge characteristics of both microbial (e.g., cyanobacteria: Phoenix, et al., 2002; Fein, et al., 2005) and mineral surfaces (Sjöberg and Lövgren, 1993; Kraepiel, et al., 1998; Gaboriaud and Ehrhardt, 2003) have been investigated in detail using acid-base titrations

to determine the chemical nature and abundances of such ligands. These quantitative potentiometric investigations have been successfully applied to studies of both microbial and mineral solids with heterogeneous proton-binding sites (Cox, et al., 1999; Smith and Ferris, 2003; Lalonde, et al., 2007), and that the resolution for the determined site binding constants can be comparable with those of more sensitive molecular-scale techniques (e.g., spectroscopy: Yee, et al., 2004; Benning, et al., 2004).

The occurrence of bacteria-mineral assemblage in nature is typically mixed; hence it is unrealistic to envisage discrete bacteria and SiO₂ phases occurring separately in the natural environment. Moreover, these environmental sorbents are typically composed of a matrix of live cells, dead biomass and mineralized organic composites, each potentially possessing differing, surface-dependent reactivities (i.e., proton binding behavior). In this work, acid-base titrations were employed to investigate the proton-binding properties of four solids representing compositional gradient, and putative level of cellular activity, specifically: SiO₂, cell-SiO₂ mineralized composite, unmineralized live cells and dead cell biomass.

4.2 Materials and methods

4.2.1 Solutions preparation and cell culture growth conditions

All solutions were prepared analytically and were carefully made to be metal-free, sterile and degassed of O₂ and CO₂. Laboratory glass and plastic ware for geochemical analyses were metal-leached overnight in 4% (v/v) trace metal grade HCl (Sigma Aldrich) and rinsed with ultrapure water (UPW: Milli-Q, 18.2 Ω; Millipore). A known stock solution of KNO₃ electrolyte (Fisher) was prepared in an anaerobic chamber

(Forma Scientific, Inc. Model 1025/1029) using N₂-purged (Alphagaz, 99.99%) degassed sterile UPW. All N₂ purged salt solutions, UPW diluent and reagents for acid-base titrations were kept in the anaerobic chamber when not in use. To prevent silica contamination from glasswares, all vessels used for titration experiments were either polypropylene (PP) or polyethelyne (PE) make.

Synechococcus-type cyanobacterial isolate was obtained from Maria Dittrich (from C. Calleri collection) and was used in all experiments presented in this work. Cyanobacteria (e.g., *Synechococcus*) are oxygenic photoautotrophic microorganisms commonly encountered in silica-rich systems (e.g., hot springs: Konhauser, 1996; Lalonde, et al., 2007) and typically showing various stages of silicification (Ferris, et al., 1986; Konhauser, 2001; Bonny and Jones, 2003; Likhoshway, et al., 2005). Cells were grown in 2 L culture flasks supplemented with a modified BG-11 media (Schlösser, 1994) at room temperature and ambient light conditions (~ 10 Watts/m²) under 10h : 14h light:dark cycle. The cells were harvested during their early to mid-stationary growth phase at approximately 6 weeks. Calibration curves of bacterial suspension were generated from their absorbance values at 600 nm optical density (OD_{600nm}) of known stocks in both 0.1 M KNO₃ electrolyte and UPW suspensions, to transpose OD_{600nm} absorbance values into dry cell concentration (mg/L). Bacterial dry weights were obtained by oven drying (50°C; Fisher Scientific Isotemp Oven, Model 650G) filtered cell suspensions (Whatman® Microfibre Filters) to a constant weight.

Cyanobacteria are living entities, and the potential for varied cell surface characteristics dependent on their growth circumstances may occur for suspensions of the same strain; therefore affecting the overall cell surface acid-base properties. To minimize

cell culturing artifacts in all replicate titrations for each cyanobacterial treatment, replicate suspensions for each treatment were sampled from either of two prepared 2-L cyanobacterial cell isolates of identical age, growth conditions and cultured from the same “mother” cell transfer.

4.2.2 Experimental solids preparation

Colloidal silica

Commercially available colloidal silica ($^{\text{C}}\text{SiO}_2$; Ludox LS, 30% w/w SiO_2 ; Sigma-Aldrich) was characterized for acid-base properties without further pre-treatment. The $^{\text{C}}\text{SiO}_2$ solution was made up to a final concentration of 1000 mg/L in 0.1 M KNO_3 . Na^+ is a typical “contaminant” alkali metal from silica synthesis to stabilize the $^{\text{C}}\text{SiO}_2$ suspension (Iler, 1979; Vansant, et al., 1995). The $[\text{Na}^+]$ used in the original $^{\text{C}}\text{SiO}_2$ solution was determined by Atomic Emission Spectrophotometry (AES) and was accounted for in the charge excess expression calculations (*b-value*; see 4.2.4).

Live and dead cells

Cells were collected for acid-base titration via centrifugation at 7000 rpm for 5 minutes (Sorvall RC5C PLUS), followed by successive rinsing in 1 mM EDTA (Fisher), sterile UPW and the working electrolyte solution (0.1 M KNO_3). Cleaned cells from this stage were resuspended in background 0.1 M KNO_3 electrolyte for acid-base titration and designated as LIVE cells.

All killed cyanobacterial cells were prepared from a single pool of cell suspension. Previously cleaned LIVE cells were resuspended in sterile UPW to a final concentration of about ~80 mg/L. The cell suspension was placed in a secondary holding flask with sterile UPW and heated to 74°C for 30 seconds (Fisher Scientific) under constant stirring (Lewin, et al., 2003). The cell suspension turned light green, and was transferred immediately to a cool water bath and continuously stirred to room temperature. The killed cells were then pelleted, washed and resuspended in the working electrolyte as described for acid-base titration, and designated as DEAD cells.

Silica-coated cells

Silica-coated cell composites (COMP) were prepared from a single flask of cyanobacterial culture. Cleaned live cells of known OD_{600nm} were combined with concentrated silica solution (Ludox LS, 30% w/w SiO₂; Sigma-Aldrich) to a final concentration of 1 M. The cell-silica suspension was then pH-adjusted to pH ~ 7 (initial pH ~ 10) using 50% v/v filter-sterile HCl under constant stirring. The suspension was left stirred for five days, then the SiO₂-coated cells were pelleted at 17000 rpm for 10 minutes and the excess silica solution was discarded. The silica-cell composites were further washed successively with UPW, 1mM EDTA and 0.1 M KNO₃. There was no detectable silica measured from the decanted final washing of the COMP solids. Visual inspection and elemental scanning for Si verified the presence of silica veneer on the cells (for example, see Fig. 3.7).

4.2.3 Potentiometric titration and titrator settings

All titrations were carried out under ambient conditions ($\sim 22^{\circ}\text{C}$) using a digital titrator (Mettler-Toledo DL70 ES) interfaced by titration software (DLWin V3.0, MT) connected to a personal computer. The titrator unit was programmed in a *dynamic titration mode*, where variable amount of titrant was dispensed from the acid or base burette implement to maintain approximately equal pH changes ($\Delta\text{pH} \sim 0.2$). The titrant was automatically dispensed only when the potential is changing by less than 0.5 mV/min; thus ensuring system equilibrium before the next titrant addition. Silica-free 0.1 N NaOH standard (Sigma-Aldrich) and 0.1 N triple-distilled, ultra-pure HNO_3 were prepared and transferred to ultra-clean high-density PP amber bottles (Nalgene) in an anaerobic chamber. PP bottles were used as titrant receptacles to prevent silica contamination from conventional glass bottles, i.e., silica leaching verified in the base glass vessel in excess of 80 mg/L silica. NaOH titrant was standardized by potassium phthalate method (KHP; Smith and Ferris, 2003), which agreed well within manufacturer-certified concentration (0.1010 N). The HNO_3 titrant was standardized against the NaOH standard using the gran function (Smith and Ferris, 2003). The glass electrode probe used (Ag/AgCl; Mettler-Toledo, DG-1114SC) has a built-in movable polymer sleeve frit for easy cleaning, and was three-point calibrated in standard buffer solutions (pH 4.0, 7.0, 10.0; VWR International). Electrode calibration using standard buffer solutions (i.e., external) was done to determine the electrode's response time (~ 6 sec); however, “internal” calibration generated from data points at both acidic and basic extremes of the blank KNO_3 titrations was used in the actual mV/pH expression for all data transformations. Internal calibration was preferred over external calibration (i.e.,

standard buffers) to take into account specific solution characteristics (e.g., ionic strength: Dr. Scott Smith, pers. comm.) and to correct for any potential fouling of the glass electrode. In all calibrations (both internal and external), the slope of the mV-pH curve is consistently >98% of the ideal Nernstian value.

Colloidal silica solution ($^c\text{SiO}_2$), silica-coated cyanobacterial composites (COMP), unmineralized live (LIVE) and dead cyanobacterial cells (DEAD), and the background electrolyte blanks (0.1 M KNO_3) were titrated using the same titrator settings and employing the same protocols. A known KNO_3 stock was pipetted to the experimental solids in ultra-clean plastic titration cups and made up to volume using degassed, sterile UPW (actual $[\text{KNO}_3] = 0.097 \text{ M}$). All titrations were done in an “upward” direction, where a known volume of HNO_3 titrant was dispensed prior to acidify the suspension. This “upward titration” method was employed throughout the experiments because the cyanobacterial strain used, while not accustomed to either extreme of pH values, appears to be more tolerant of acid pH conditions. Also silica dissolution was reported to occur in basic conditions (Reymond and Kolenda, 1999; Sonnefeld, et al., 1995), which can potentially affect the titration results. Lastly, acidifying all suspensions and equilibration for one hour under constant stirring with continuous N_2 purging would get rid of extraneous $\text{CO}_{2(\text{g})}$.

All titration replicates for the DEAD and COMP treatments were prepared from a single respective bulk batch (subsequently divided into replicates); however, the LIVE cell titrations were harvested per replicate batch to assure cell integrity before each titration run (hours to no more than 1 day apart for consecutive replicates).

4.2.4 Modeling acid-base titration data

Experimental acid-base titration data were fit to smooth continuous pK_a spectra using the Fully Optimized ContinUous (FOCUS) pK_a spectrum method. This method optimizes for both goodness-of-fit and smoothness describing the titration data set (Smith and Ferris, 2001; Smith and Ferris, 2003), and has been successfully used in a variety of mineral (e.g., Mn/FeOOH; Smith and Ferris, 2001; Kennedy, et al., 2004) and organic solids (e.g., bacteria; Smith and Ferris, 2003; Martinez, et al., 2003) surface proton-binding behavior. Using the FOCUS model, the proton binding characteristics of polyelectrolytic solids such as bacteria and minerals can be represented by a continuous pK_a spectrum, which is thought to better represent the inherent surface heterogeneity of both natural and engineered solids with multiple functional group types (Sokolov, et al., 2001; Martinez, et al., 2003; Kennedy, et al., 2004). Moreover, continuous pK_a spectra would allow comparison among treated or mixed samples, such as individual end member spectra, i.e., cell and SiO_2 , of the cell- SiO_2 mineral composites presented in this work (Smith and Ferris, 2001; Martinez, et al., 2003). Experimental acid-base titration data were fitted assuming system equilibrium conditions, and the mixture of heterogeneous binding sites on the experimental solid surfaces is treated as the sum of m monoprotic sites. First, raw titration data points were transposed into a charge excess expression b :

$$b_{i,meas} = \frac{Cb_i - Ca_i + [H^+]_i - [OH^-]_i}{C_s}; b_{i,calc} = \sum_{j=1}^m \frac{K_j L_{Tj}}{K_j + [H^+]} + S_0 \quad [\text{Eqn. 4.1}]$$

where the charge excess from the i^{th} addition of titrant is shown on the left term ($b_{i,meas}$; Eqn. 4.1) and the terms on the right ($b_{i,calc}$) are fitting parameters; $b_{i,meas}$ is the charge excess in $\mu\text{mol/mg}$ of dry solid, Cb_i and Ca_i are the concentrations of base and acid titrants added, respectively, and the last two terms in the numerator on the left term are the proton ($[H^+]$) and hydroxyl ($[OH^-]$) concentrations in solution, determined experimentally via the glass electrode. Finally, C_s is the dry solids concentration in mg/L .

A pK_a spectrum represents the solid surface's overall proton binding defined by a unique pK_a value (K_j in Eq. 4.1) and a corresponding site concentration (L_{Tj}). A constant S_0 term is analogous to the acid neutralizing capacity (ANC) or the initial protonation state of the surface (Smith and Ferris, 2001; Martinez, et al., 2002), and is introduced to account for positive charge on the solid surface because an inherent artifact of representing the titration curve as the sum of monoprotic sites can only describe negative charges (Sokolov et al., 2001). Intuitively, S_0 can be envisaged as the difference between the proton binding sites that are always saturated and those that are always empty over the experimental titration pH range (Smith and Ferris, 2003). The influence of these sites is included in the constant offset parameter S_0 , to account for how much the titration curve has to be shifted so that the changes in shape can be represented as a sum of monoprotic negatively charged proton binding sites (Brassard et al., 1990).

Equation 4.1 can be solved by fixing the pK_a values in a constant sequence from some minimum value to a maximum value at constant intervals. In this work, the minimum and maximum pK_a values correspond to the minimum and maximum measured experimental pH, respectively, and the incremental pK_a steps are fixed at 0.2 units. This

way, only the L_{Tj} value associated with each K_j and the S_0 terms remain to be solved, using the matrix version of Eqn 4.1. Detailed discussions of the FOCUS model are given in Smith and Ferris (2001). It must be noted that the pK_a spectrum derived using the FOCUS method is an apparent spectrum, because electrostatic effects and site-site interactions are not considered (Smith and Ferris, 2003). The assignment of the peaks in the pK_a spectra is conditional on ionic strength used (for this work, 0.1M KNO_3) but nonetheless does give some idea of the types of the surface groups likely present on the microbial and inorganic solid surfaces (Sokolov et al., 2001). Overall, the effect of higher ionic strength on proton binding occurs prominently in the basic ends of the titration curve since surface ligands of (cyano)bacteria and silica predominantly acquire negative charges at higher pH values (Cox, et al., 1999). Parameter fitting was performed using MatlabTM (The MathWorks Inc.) Optimization Toolbox. The final pK_a spectrum for each of the experimental solids is expressed as a mean spectrum, and the associated standard deviation was obtained by fitting individual replicate spectrum for each of the four solid replicates.

4.2.5 Microscopy and cell viability assay

Microbial cell viability was estimated using the live/dead cell viability assay (Baclight L7012, Molecular Probes) following manufacturer protocols (Invitrogen Molecular Probes Product Information, 2004) and imaged under an epifluorescence light microscope (Leica DMR A/AXA Leica Microsystems) fitted with a digital camera (Hamamatsu Digital CCD Cam ver. 1.1), and imaging software (Openlab ver. 2.2.5). Accuracy of the live/dead staining procedure was evaluated prior using the same

cyanobacterial isolate (see Chap. 2). In addition to cell viability staining, sub-samples of the naked live and SiO₂-cell composites were collected after titration and were re-cultured in BG-11 nutrient media. The silica coating on COMP solids were visualized and verified for the presence of elemental Si via environmental scanning electron microscope (ESEM, System 2020, ver. 3.53) equipped with energy dispersive x-ray spectroscopy (EDS, PGT IMIX system; for example, see Chap. 3, Fig. 3.8).

4.3 Results

4.3.1 Cell viability and microscopy

Representative live cells (5 sub-samples spread over the entire titration replicates) were viable pre- and post-titration (> 87 %). The killed cyanobacterial cells were also verified as being compromised (> 90 %) using the same viability assay. Post-titrated LIVE sub-samples re-cultured in BG-11 media showed signs of growth after ~5 days, whereas killed cells were completely bleached after 3-5 days of resuspension in nutrient broth. These results validate the integrity (or lack of) cells before, during and after titration upon designation as live or dead cells. There was no gross morphological difference observed between the live and killed cells (e.g., see Chap. 3; Fig. 3.9), suggesting that despite the killed cells' machineries were compromised and non-functioning, the cell surfaces were largely intact.

No meaningful cell viability result can be obtained from the COMP sub-samples due to the presence of silica encrustation on the cells which likely prevented probe hybridization. However, re-cultured COMP sub-samples in the same nutrient media showed intense growths of post-titrated cells (see Chap. 3; Fig. 3.7). The COMP solids

were verified for the presence of SiO₂ envelope, and were compositionally determined to be elemental Si-bearing (SiO₂) via x-ray spectroscopy (shown in Chap. 3; Fig. 3.8-c and Fig. 3.8-d, respectively).

4.3.2 Surface charge excess (*b*)

Acid-base titration data transformed as surface charge excess expression ($b_{i\text{ meas}}$, hereforth referred to as “*b*”; left-hand expression of Eqn. 4.1) with respect to pH for all four experimental solids: ^cSiO₂, COMP, LIVE and DEAD are shown in Fig. 4.1, where the charging behavior of each titrated solid can be seen. Each sub-plot represents a minimum of 3 replicate titrations (Table 4.1), and the solid line for each of the sub-plots are best-fit *b* values (i.e., average) with corresponding confidence intervals about the mean defined by the standard deviation of the replicate titrations ($\pm 1 \sigma$; Fig. 4.1, dashed line). The best-fit model and $\pm 1 \sigma$ is calculated from the average FOCUS pK_a spectrum (see 4.3.3) from individual replicate spectrum for each of the four solids. It must be noted that the nature of the charge excess *b* ($\mu\text{mol mg}^{-1}$ dry solid) refers to the surplus H⁺ released in solution (i.e., from surface deprotonation: Fein, et al., 2005), hence *b* is envisaged as the “negative value” of the charge accumulation on the solid surfaces, that is, increasing positive *b*-value in Fig. 4.1 is synonymous to increasing deprotonation (i.e., negative) state of the respective surfaces. With the exception of the LIVE titrations (Fig. 4.1-c, e, f), the best-fit model describes all titration replicates well, with the residuals clustering randomly about the best fit (i.e., average) solid lines, and are within $\pm 1 \sigma$. Generally, the relative error values are higher in either acidic (< pH 4) or basic (> pH 8) ends of the titration pH range, consistent with the error function shape showing greater

titration errors at both or either ends of the titration curve (Smith et al., 1999; Smith and Ferris, 2001). For the complete LIVE data set (Fig. 4.1-c; LIVE_{ALL}), replicate titrations were pooled into two groups: “high” (LIVE_{HIGH}; Fig. 4.1-e) and “low” (LIVE_{LOW}; Fig. 4.1-f) each exhibiting approximately similar behavior with respect to surface charge excess accumulation. These “high” and “low” groupings are set arbitrarily from the complete set of LIVE_{ALL} pK_a spectra (see section 4.3.3), nonetheless delimited by comparable standard deviation values (2σ).

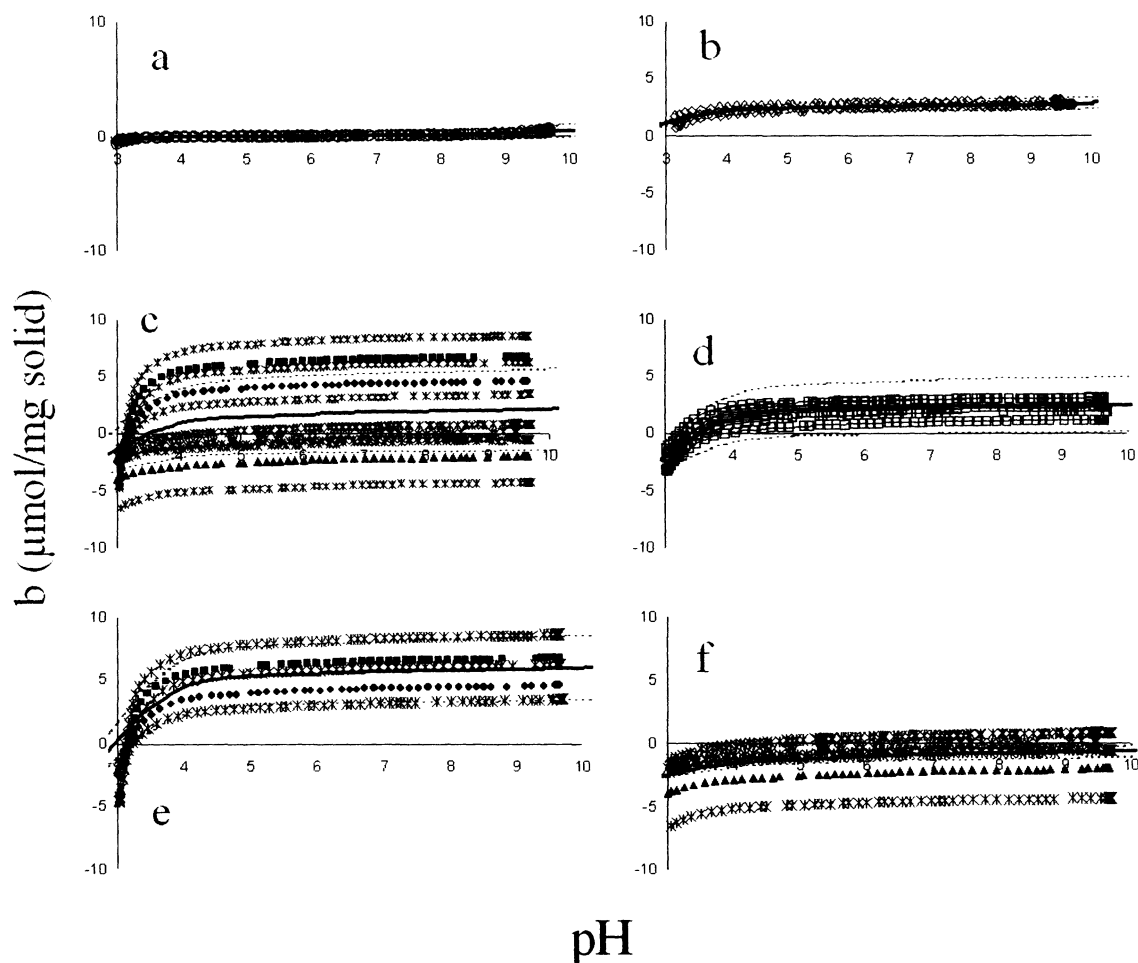


Figure 4.1. Charge excess expression (b , $\mu\text{mol mg}^{-1}$ dry solid) for replicate titration of $^{\text{C}}\text{SiO}_2$ (a), $^{\text{C}}\text{SiO}_2$ -cell composite (b), unmineralized LIVE_{ALL} (c), DEAD (d), $\text{LIVE}_{\text{HIGH}}$ (e), and LIVE_{LOW} (f) as a function of pH; solid lines show mean best-fit b value $\pm 1\sigma$ (dashed lines); solid circle, triangle and square, respectively show titration chronology for 3 replicates (shown in sub-plots c, e and f for LIVE_{ALL} , $\text{LIVE}_{\text{HIGH}}$ and LIVE_{LOW} , respectively).

Comparison of the charge accumulation on all four solids showed varying, surface-dependent proton-charging behavior with respect to pH (Fig. 4.1). In this work, it is only possible to estimate the apparent zero point of charge ($^{\text{app}}\text{pH}_{\text{zpc}}$) because all titrations were conducted at only one ionic strength (0.1 M KNO_3). “True” or “absolute” pH_{zpc} values can only be obtained if the background salt concentrations were varied, and the zero point of salt effect was determined (Smith and Ferris, 2003). Nonetheless, for comparative charging behavior purposes, it is sufficient to resolve the $^{\text{app}}\text{pH}_{\text{zpc}}$ conditional to the ionic strength used (Smith and Ferris, 2003). The pooled LIVE titration results will be discussed subsequently, but the general averaged $^{\text{app}}\text{pH}_{\text{zpc}}$ for the solids evidently showed acidic surfaces: $^{\text{C}}\text{SiO}_2$ ($^{\text{app}}\text{pH}_{\text{zpc}} = 4.3$); LIVE_{ALL} (3.5) DEAD (3.1) and COMP (2.0). These $^{\text{app}}\text{pH}_{\text{zpc}}$ values mean that at pH values above their respective derived $^{\text{app}}\text{pH}_{\text{zpc}}$ ($\text{pH} > 4.5$), these surfaces will acquire net negative surface charges. However, it must be noted that the COMP surfaces’ very acidic $^{\text{app}}\text{pH}_{\text{zpc}}$ (Fig. 4.1-b) should be taken with caution because the extrapolated $^{\text{app}}\text{pH}_{\text{zpc}}$ is way below the titratable range and may be subject to error (i.e., $< \text{pH } 3$; Smith and Ferris, 2001; Smith and Ferris, 2003). Overall, the significance of these acidic $^{\text{app}}\text{pH}_{\text{zpc}}$ values for all these surfaces investigated is that they accumulate negative charges from surface deprotonation at higher pH milieus. These would make these interfaces ideal coordination sites for counter ions (e.g., dissolved metals: Ledin, et al., 1995; Ehrlich, 1996; Warren and Ferris, 1998; Southam, 2000) over a wide range of pH values ($> \text{pH } 4.5$). These results are consistent with what has been shown thus far for silica mineral surfaces and bacterial surfaces acquiring net negative charges in natural waters of circumneutral pH values (Beveridge, 1980; Cox, et al., 1999; Dixit and Van Cappellen, 2002; Perry, 2003; Dove and Craven, 2005).

One of the most evident differences shown by the charge accumulation of live cyanobacterial cell surfaces is the marked variability exhibited by the LIVE_{ALL} titrations (Fig. 4.1-c) wherein 50% of the data set lies outside $\pm 1 \sigma$. While the average $^{app}pH_{zpc}$ of the LIVE_{HIGH} data set (Fig. 4.1-e) is comparable to that of the LIVE_{ALL}; the average extrapolated $^{app}pH_{zpc}$ of the LIVE_{LOW} (Fig. 4.1-f) is very basic and lies outside the titratable range ($> pH\ 11$). Similar to the COMP $^{app}pH_{zpc}$, this result showing extreme surface basicity should be interpreted with caution. Conspicuously, the results for the LIVE titrations strongly indicate that the surface charging behavior of live cells are highly variable even for the same strain investigated under identical conditions. Overall, there is no observable trend of surface charge accumulation for the LIVE_{ALL} systems with respect to either the replicate titration chronology (i.e., sequence of titration) or from the population source where the cells were harvested. For instance, the surface charge accumulation of LIVE replicate titrations 8, 9, 10 (Fig. 4.1-c: solid circle, triangle and square, respectively) were partitioned in both the LIVE_{HIGH} (Fig. 4.1-e) and LIVE_{LOW} (Fig. 4.1-f) data set, while these replicate cell suspensions were harvested from the same population flask in a single batch, and sequentially titrated continuously (i.e., minutes apart from the end of one titration to the start of next replicate run).

4.3.3 FOCUS pK_a spectra

It is often difficult to make meaningful comparisons among the proton-charge behavior of surfaces based solely on raw titration data. Assessment of the chemical identities and site densities of functional groups on $^C\text{SiO}_2$, naked live and dead cyanobacteria and the silica-cyanobacterial composites is possible from their respective

FOCUS pK_a spectra (Fig. 4.2). The solid lines represent the mean pK_a for each solid treatment, and the dashed lines are $\pm 1 \sigma$. It can be seen that acid pK_a 's (around pH ~ 3) are the dominant charge-forming groups characterizing the surfaces of all four solids, as expected from the surface deprotonation behavior shown in Fig. 4.1. A three-site pK_a model describes the protonation behavior for all surfaces, and the derived pK_a values for each of the three sites and their respective concentrations (L_T ; $\mu\text{mol/mg}$ solid) are summarized in Table 4.1.

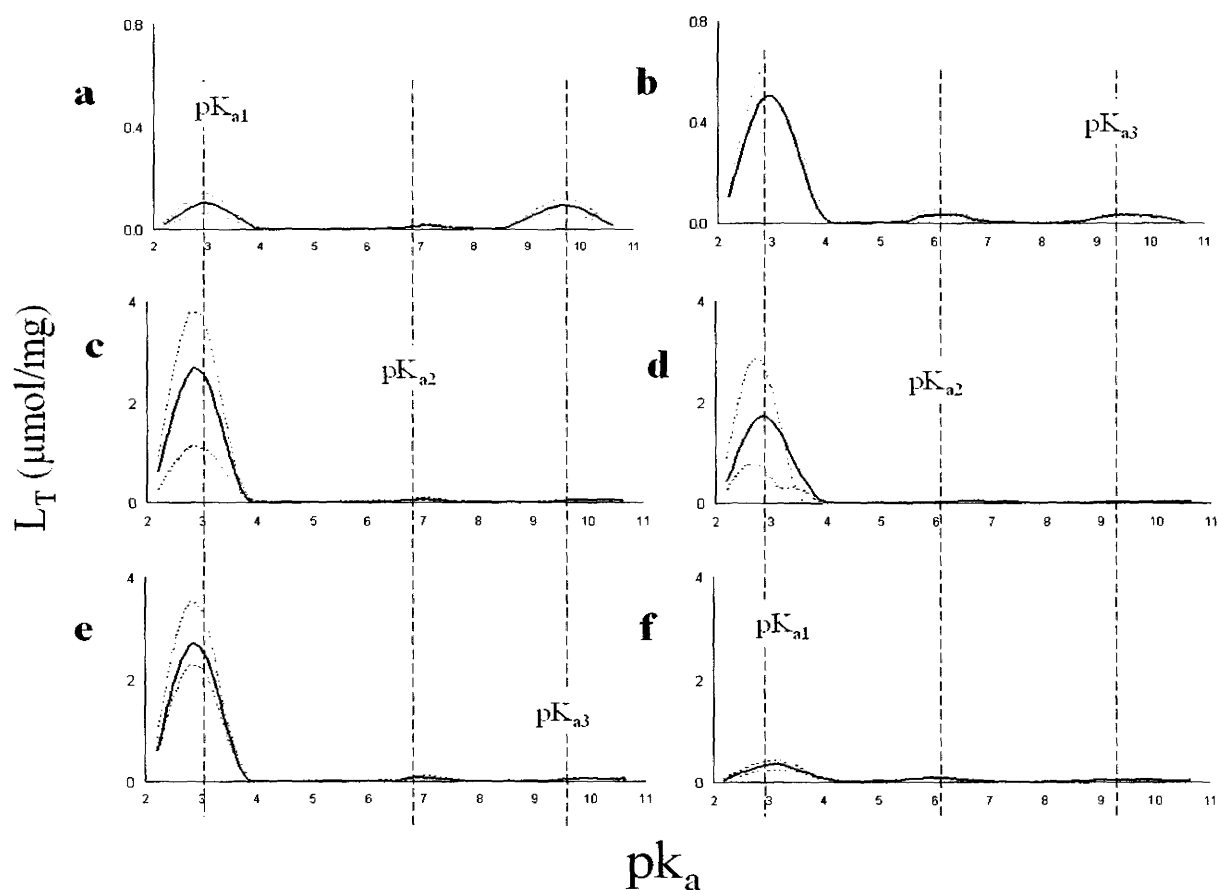


Figure 4.2. FOCUS pK_a spectra derived for $^{29}\text{SiO}_2$ (a), $^{29}\text{SiO}_2$ -cell COMP (b), naked live cells (c, LIVE_{ALL}), DEAD cells (d), $\text{LIVE}_{\text{HIGH}}$ group (e) and LIVE_{LOW} group (f); solid spectra in each sub-plot are mean $pK_a \pm 1 \sigma$ (dashed curves); vertical lines show the relative position of the pK_a for each of the 3 sites (*see text for details*).

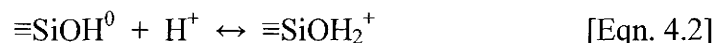
Solid	<i>n</i>	pK _{a1} Site 1	L _T 1	pK _{a2} Site 2	L _T 2	pK _{a3} Site 3	L _T 3
^c SiO ₂	4	2.99 ± (0.45)	1.16	7.00 ± (0.49)	0.20	9.67 ± (0.55)	1.26
COMP	3	2.96 ± (0.45)	5.60	6.20 ± (0.50)	0.47	9.50 ± (0.63)	0.51
LIVE _{ALL}	12	2.91 ± (0.44)	12.04	6.32 ± (0.71)	0.80	9.74 ± (0.56)	0.60
LIVE _{HIGH}	5	2.87 ± (0.41)	23.10	6.92 ± (0.42)	0.63	9.87 ± (0.46)	0.68
LIVE _{LOW}	7	3.11 ± (0.51)	4.33	6.01 ± (0.52)	0.87	9.56 ± (0.63)	0.57
DEAD	5	2.88 ± (0.43)	17.87	6.67 ± (0.42)	0.61	9.99 ± (0.49)	0.36

Table 4.1. pK_a values derived from titrated samples expressed as mean ± (1 σ) for the 3 sites identified; L_T is the total site density (μmol mg⁻¹) corresponding to each of the three pK_a sites; *n* is the number of titration replicates for each solid treatment; LIVE_{HIGH} and LIVE_{LOW} are sub-groups of LIVE_{ALL}.

4.4 Discussion

4.4.1 Colloidal SiO₂

The emergence of surface charges on amorphous silica particles is based on two chemical reactions (Sonnefeld, 1996):



from the protonation/deprotonation reactions of the surface silanol groups, $\equiv\text{SiOH}$ (where “ $\equiv$ ” symbolizes the underlying lattice) formed from the hydration of SiO₂ surfaces (Iler, 1979; Vansant, et al., 1995; Dixit and Van Cappellen, 2002). Siloxane groups ($\equiv\text{Si-O-Si}\equiv$) may also be present on amorphous SiO₂ surfaces but these sites are highly hydrophobic and unlikely to participate in surface protonation/deprotonation reactions (Nawrocki, 1997; Mendez, et al., 2003) hence they do not contribute significantly to SiO₂ surface proton charging. Positive charge accumulation via proton sorption to fully hydroxylated silanol groups ($\equiv\text{SiOH}_2^+$; Eqn. 4.2) has been shown to be negligible (Carroll, et al., 2002; Dixit and Van Cappellen, 2002) and can only occur at extreme acidities (ca. 30% at pH $\sim$ 0; Pokrovsky, et al., 2006). Therefore, at the relevant pH range investigated (i.e., pH $\sim$ 3-10) only the $\equiv\text{SiOH}^0$ and $\equiv\text{SiO}^-$ are considered relevant charge-forming species (Eqn. 4.3) on the surfaces of amorphous colloidal SiO₂. It is often difficult to ascertain the surface protonation state of SiO₂, particularly for the amorphous types. This difficulty arises largely from the random arrangement of surface

sites (e.g., silanols) on amorphous silica surfaces, resulting to a variety of charge-forming architectures (Iler, 1979; Vansant, et al., 1995; Nawrocki, 1997). Frequently, conflicting results arise from theoretical and empirical investigations of amorphous silica surfaces primarily because much of the derivations used to model amorphous silica surfaces are determined from their much known crystalline counterparts (e.g., quartz: Dove and Rimstidt, 1994; Carroll, et al., 2002), which obviously have more predictable and consistent surface architecture than the amorphous varieties (Dove and Rimstidt, 1994). Further, the silanol group itself occurs in three forms (i.e., isolated; paired or geminal: Bergna, 1994; Nawrocki, 1997) and are all typically represented on amorphous silica surfaces in varying proportions (Vansant, et al., 1995; Bergna, 1994). Therefore, the biggest challenge of interpreting the surface property of amorphous silica is that the (proton) charge-forming silanol groups can occur in different forms, and at random distribution and proportion over the entire SiO_2 interface (Vigil, et al., 1994; Vansant, et al., 1995; Nawrocki, 1997). With these caveats in mind, this work makes no attempt to distinguish the identities of the silanol types responsible for the charge formation on surface amorphous $^{\text{C}}\text{SiO}_2$. However, the following results will hopefully shed light on the general charging behavior of amorphous SiO_2 , with particular emphasis in the context of microbial silicification, and mineralizations in general.

Considering that the relevant charge forming species on amorphous SiO_2 surface is the silanol group, it is most surprising that the acid-base properties of amorphous SiO_2 in this work shows two dominant surface sites: an acidic ($\text{pK}_{\text{a}1} = 2.99$; Table 4.1; Fig. 4.2) and a basic group ($\text{pK}_{\text{a}3} = 9.67$; Table 4.1; Fig. 4.2) of very similar concentrations (L_{T} ; Table 1). The basic site will be discussed first. The pK_{a} value of the most basic site

on $^{\text{C}}\text{SiO}_2$ (Site 3; Table 1) is very similar to the dissociation of silicic acid ($\text{H}_4\text{SiO}_4 \leftrightarrow \text{H}_3\text{SiO}_4^-$, $\text{pK}_a = 9.5$; Iler, 1979; Bergna, 1994; see Chap. 1, Fig. 1.2). The presence of silicic acid species would be surprising considering that the tendency for polymerized (i.e., colloidal) SiO_2 species is to lower the pK_a values on the surface $\equiv\text{SiOH}$ compared to precursor silica monomers (H_4SiO_4 ; Perry, 2003; Sahai, 2002). The presence of silica monomers (as silicic acids) in solution from possible dissolution of $^{\text{C}}\text{SiO}_2$ was ruled out because contrary to Sonnefeld et al.'s (1995) argument, dissolved silica (as molybdate-reactive) was not detected after NaOH base titration. Quite possibly, the significant proportion (~50%) of these silanol groups showing “monomer-like” pK_a is “silicic-acid chains” that are sticking out of the surface $^{\text{C}}\text{SiO}_2$. These silicic acid “hairs” or “chains”, as often encountered in the literature (Vigil, et al., 1994; Nawrocki, 1997; Laven and Stein, 2001), have been inferred or alluded to in many studies of nanometer-sized silica colloids, and have been implicated for the “anomalous” behavior of amorphous SiO_2 solids. For instance, derivation of theoretical pK_a value of bulk silanols on amorphous SiO_2 surface is in the order $\text{pK}_{a=\text{SiOH}} = 7.1 \pm 0.1$ (Iler, 1979; Nawrocki, 1997). However, literature reports of empirical silanol pK_a values range from as low as 1.5 (acidic pK_a , discussed subsequently) to as high as 10 (Nawrocki, 1994) probably due to the presence of “silicic acid-like chains” in the latter case. Interestingly, while these supposed silicic acid “hairs” are the most readily accessible for interaction to protons (or hydroxyls) in solution, their deprotonation is still “silicic acid-like” and does not occur until basic conditions ($\text{pH} \sim \text{pK}_a \sim 9.5$; Fig. 4.2; Table 4.1).

This “anomalous” behavior of silica surface silanol group is also expressed in the acidic end of the pK_a spectrum (Fig. 4.2-a). The most acidic site (pK_{a1} , Site 1; Table 1)

can be likened to surface “acidification” of silanols due to polymerization of silica monomers (Tossell and Sahai, 2000; Sahai, 2002; Perry, 2003). The mechanism for this “acidification” (i.e., lowering of pK_a value) of silanols on the formed SiO_2 solids is not exactly known, but the occurrence of this acidic site markedly contrasts amorphous silica (as $SiO_{2(s)}$) from its precursor dissolved H_4SiO_4 , and is often a source of confusion in the literature (Nawrocki, 1997; Sahai, 2002; Tossell and Sahai, 2000). Recent interpretations propose that these low pK_a silanols are of the “free” silanol forms (Vigil, 1994; Vansant, et al., 1995), while others suggest the incorporation of metals during SiO_2 synthesis lowers its surface silanol pK_a (Dove and Craven, 2005). In the same token, the slight pK_a peak at neutral pH shown by $^C SiO_2$ (Site 2; Table 4.1; Fig. 4.2-a) may likely be a variation of the surface silanol acidity mentioned previously. In other words, the possible combinations of silanol types and subsequent acidities may vary as much as the product silica itself (Nawrocki, 1991; Bergna, 1994), and any of the variations in SiO_2 synthesis histories can impact the surface characteristics of amorphous SiO_2 being investigated. While these possible interpretations are still subject to debate, results from this work are consistent with works on amorphous SiO_2 surfaces in that they acquire net negative charge at a wider range of environmental pH ($> pH\ 3$: Nawrocki, 1997; Laven and Stein, 2001; Goyne, et al., 2002). Verification of the existence of two distinct and dominant pK_a sites for silica (i.e., both acidic and basic silanols) at the pH range 3-10, confirms that amorphous silica can become even more negatively charged at higher pH conditions (pH ~ 9.5 ; Goyne et al., 2002).

4.4.2 Cyanobacterial cell treatments

Both the unmineralized live and dead cyanobacterial cells' acid-base proton-binding properties consistently showed three pK_a peaks (Fig. 4.2; Table 2.1). The most acidic sites are interpreted as carboxylic sites ($pK_a = 2.87\text{-}3.11$). The intermediate sites likely correspond to phosphoryl ($pK_a = 6.01\text{-}6.92$), and the most basic sites are possibly amine or hydroxyl groups ($pK_a = 9.56\text{-}9.99$). Similar surface sites were inferred from other cyanobacterial strains (e.g., *Calothrix*: Phoenix, et al., 2002; Yee, et al., 2004; Benning, et al., 2004), and are consistent with the known values for molecular analogs of functional groups known to occur on microbial cells (Van der Wal, et al., 1997; Cox, et al., 1999; Smith and Ferris, 2003; Hong and Brown, 2006). As mentioned earlier, although the abundances of these sites on microbial surfaces may vary across all strains, the possible suites of chemical identities of these functional sites are usually consistent. The summation of total binding sites (ΣL_T ; Table 4.2) for the unmineralized cells (both live and dead) in this work is about an order of magnitude higher than those determined from *Calothrix sp.* cyanobacterium (Phoenix, et al., 2002) and have far higher relative proportion of carboxylic sites (Table 4.2; compared to ca. 60% in *Calothrix sp.*; Phoenix, et al., 2002). This result show that the surfaces of the *Synechococcus*-type cyanobacteria in this work are more acidic, and will tend to deprotonate (i.e., acquire negative surface charges) at a wider range of pH values ($> \text{pH } 4$).

Comparison of the total site densities of cyanobacteria and $^c\text{SiO}_2$ (L_T ; Fig. 4.2; Table 4.1) clearly shows that microbial surfaces have higher binding sites per mg of solid (up to a factor of ~ 10) than do SiO_2 . From a surface sorbent perspective, this result is significant for dissolved metal pollutant mitigation. Higher functional site density on

microbial (cyanobacteria) versus mineral (SiO_2) surfaces is the fundamental reason why cells are more effective scavengers for counter-charged ions such as dissolved metals compared to SiO_2 minerals (Fortin, et al., 1997; Cox, et al., 1999; Ledin, 2000; Warren and Haack, 2001). Interestingly, the dominant, most acidic functional site between the unmineralized cyanobacteria (both live and dead) and amorphous SiO_2 surfaces has very similar pK_a values (Fig. 4.2; Table 4.1). This result means that both surfaces will acquire net negative charges at circumneutral pH waters; therefore, electrostatic repulsion between these two like-charged surfaces is apparent. In the context of cell silicification by colloidal SiO_2 , the acquisition of negative charges on both interfaces will hinder their effective coordination due to charge repulsion, and is consistent with what has been shown in this work that cell- $^{\text{C}}\text{SiO}_2$ biomineralization is not promoted at pH ~ 7 due to these overriding repulsive forces (see Chap. 3).

One of the most significant results of this work that needs to be highlighted is the apparent variability induced by titrating living microbial cells compared to their dead counterparts (Fig. 4.2; Table 4.1). In most acid-base potentiometry of live microbial systems, cells are almost always assumed to be viable but non-functioning due to the absence of nutrients during titration runs (Claessens, et al., 2004; Guine, et al., 2007). However, it is not uncommon for investigations of surface acid-base properties of living cells that only the most “ideal” (i.e., reproducible replicates) results are presented (Dr. Elizabeth Haack, pers. comm.) while the proton-binding variabilities encountered from titrating live cells are typically faulted to instrumentation failure. In this work, the most apparent difference between the live and dead titration result is the existence of two groups of carboxylic sites ($\text{LIVE}_{\text{HIGH}}$ and LIVE_{LOW} ; Table 4.1; Fig. 4.2). The $\text{LIVE}_{\text{HIGH}}$

group has similar percentage distribution of the three sites identified (Table 4.2) compared to the dead systems; however, relative to the dead cyanobacterial systems, live cells in the LIVE_{LOW} group show far less acidic site concentration (i.e., carboxylic; Table 4.2). There is reason to believe that the proton binding variability shown by living cells, expressed as variable total surface site densities (L_T ; Table 4.1) are real, and induced by the activities of living cells. If the dead cells were considered as inactivated surfaces, it appears that the LIVE_{HIGH} group are not doing any sort of counter-metabolic response because of their similarities both in absolute (Fig. 4.2; Table 4.1) and relative (Table 4.2) surface site concentrations with the DEAD systems. For low L_T values such as the low carboxylic site density shown by the LIVE_{LOW} group (Fig. 4.2; Table 4.1; Table 4.2), the charge excess value $b = C_b - C_a + H - OH$ (Eqn. 4.1; left hand side) should be close to zero. Because $(C_b - C_a)$ expression is negative (i.e., upward titration), the term $(H - OH)$ should be positive for b to be close to zero (Dr. Scott Smith, pers. comm.). Therefore, to account for the “missed” carboxylic sites in the LIVE_{LOW} group, extraneous H^+ must be going out into the bulk solution to neutralize the OH^- titrant added. If proton pumping is invoked as the likely cyanobacterial (counter) metabolic response similar to those shown for other live cell investigations (Urrutia et al., 1992), then the live cyanobacteria is working against a proton concentration gradient. Although the cyanobacteria used in this work are not acidophilic, a similar proton-pumping mechanism has been shown for an acidophilic algae, leading to further acidification of the cell interfacial region via H^+ efflux (Gross, 2000).

Naked Cell	ΣL_T	% Carboxylic	% Phosphoryl	% Amine/Hydroxyl
LIVE _{ALL}	13.45	89.56	5.94	4.50
LIVE _{HIGH}	24.41	94.63	2.57	2.80
LIVE _{LOW}	5.77	75.08	15.07	9.85
DEAD	18.85	94.82	3.26	1.92

Table 4.2. Recalculated percentage distribution of inferred organic functional sites on naked cyanobacteria for live (both LIVE_{ALL} and LIVE_{LOW} groups) and dead cell suspensions; ΣL_T ($\mu\text{mol mg}^{-1}$) is the sum of L_T values for all three monoprotic sites (*see Table 4.1*).

Qualitative comparison of the pK_a peaks of the $^{29}\text{SiO}_2$, live cyanobacteria and the SiO_2 -cyanobacteria composite shows relative changes in the pK_a peak evolution from the fully exposed live cyanobacterial surface (Fig. 4.3, dashed line) to a purely inorganic SiO_2 surface (Fig. 4.3, solid line), and the intermediate mineral-organic composite peak (Fig. 4.3, bold line). There was little shift in the position of the pK_{a1} peaks of the most acidic sites (i.e., $pK_{a1\text{LIVE}} \sim pK_{a1\text{SiO}_2} \sim pK_{a1\text{COMP}}$; Table 4.1) from either naked live cells (carboxylic) or SiO_2 (“acidic” silanol) end members, to the $pK_{a1\text{COMP}}$; however, there was a considerable reduction in the naked live cell carboxylic $pK_{a1\text{LIVE}}$ upon evolution to $pK_{a1\text{COMP}}$ after being encrusted with silica (Fig. 4.3-a). The reduction of the acidic pK_{a1} peak in the live naked cell spectra after being encrusted with SiO_2 to form the composite solids indicates that SiO_2 interacted with the cyanobacterial surfaces directly via these acidic carboxylic sites (Fig. 4.3-a). On the basic end of the pK_a spectrum ($pK_{a3} \sim 9.5$; Fig. 4.3-b, boxed area in Fig. 4.3-a), the cell- SiO_2 composite surface showed roughly the same pK_{a3} position as the starting inorganic (SiO_2) mineral and organic surfaces of living cyanobacteria. However, as with the carboxylic site scenario for the live cyanobacteria (Fig. 4.3-a), there was also a reduction in peak intensity of the SiO_2 most basic site (Fig. 4.3-b; solid line; $pK_{a3\text{SiO}_2}$) upon formation of the cell- SiO_2 composite (Fig. 4.3-b; bold line; $pK_{a3\text{COMP}}$). The relative peak reduction on surface SiO_2 upon evolution to SiO_2 -cell composite suggests that the “basic” silanol groups coordinated directly with the carboxylic sites of the naked, live cyanobacterial surfaces. This direct cell-mineral coordination inferred from the reduction of the acidic and basic functional groups of the naked live cyanobacterial and SiO_2 surfaces, respectively is consistent with what has been shown from similar acid-base surface characterization of other bacterial and mineral

surface end-members, and their evolved mineralized composites (e.g., *S. putrefaciens* – Fe-oxyhydroxide: Smith and Ferris, 2003).

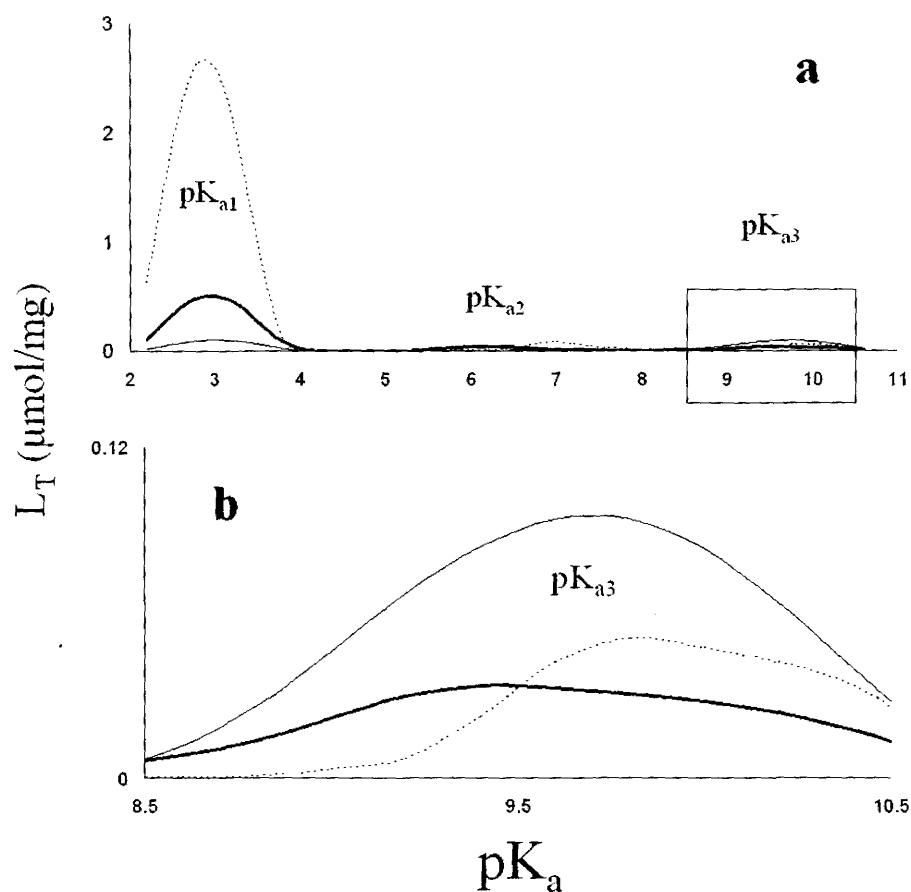


Figure 4.3. Mean pK_a spectra (a) for living cells (LIVE_{ALL}, dashed curve), $^c\text{SiO}_2$ (solid curve) and SiO₂-cell composite (bold curve); (b) zoomed pK_a spectra for the same set of curves from boxed area in (a).

The surface-dependent chemical identities and concentrations of these functional sites on both organic and mineral surfaces, and their composite mixture indicate that the potential reactivity of these various solids is dictated largely by their interfacial characteristics. Organic (i.e., cell) interfaces have much higher density of functional sites compared to SiO₂ mineral surfaces (L_T , Table 4.1; Table 4.2), while the composite cell - SiO₂ mineral matrix shows L_T values intermediate between the two end-members. Interestingly, because the cell-silica composite surfaces contain remnant organic and mineral site functionalities, they behave as transitional (mineral-organic) surfaces akin to diatoms (Gélabert, et al., 2004). Diatom biomineral frustules were shown to possess higher surface coordination sites (being a composite of organic macromolecules and precipitated SiO₂: Perry, 2003) compared to abiotic SiO₂ surfaces (Dixit and Van Cappellen, 2002). Not surprisingly, functionalizing mineral surfaces with organic layers (akin to a composite) has been the main thrust of materials science research which seeks novel synthetic, organo-mineral “hybrids” for a variety of applications (Mann, 1995; Saoudi, et al., 1997; Mann, 2001).

Fully-exposed cells contain more surface site functionalities available for chemical interaction with ions in solution; and are therefore better templates for ion sequestration than do composite matrices or abiotic mineral surfaces. From a pollution mitigation perspective, mineralized/encrusted cells therefore are less effective sorbents compared to unmineralized cells, and show reactivities intermediate between a mineral and microbial surface due to reduction of these organic functional sites. In the context of biomineralization (i.e., silicification), results from this work are consistent with what was shown in Chapter 2 that the propensity for microbes to biosilicify is governed by the

amount of exposed organic (i.e., cell) surfaces. The degree of prior silica coverage of microbial mats reduces their biomineralization propensity, consistent with what was shown from the reduced site densities of cell-mineral composite analogs compared to fully exposed cell surfaces shown in this work (Fig. 4.3).

Lastly, the inferred chemical coordination of SiO_2 and cyanobacterial surfaces resulting from the acid-base characterization of SiO_2 -cell composite and its end-members (fully exposed cell and abiotic SiO_2) shows that the most basic silanol site of SiO_2 ($\text{pK}_{\text{a3SiO}_2}$; Fig. 4.3-b) coordinated directly with the most acidic cell surface site (carboxylic; pK_{aLIVE} ; Fig. 4.3-a). At the onset, this result appears contradictory to what was proposed for perceptible cell biosilicification mechanism proposed in Chapter 3, i.e., that the colloidal SiO_2 acidic (silanol) groups coordinated directly with the acidic (carboxylic) sites on cyanobacterial surfaces (via H-bonding; see Chap. 3, Eqn. 3.1). It should be noted that the concentration of starting colloidal silica solution used to prepare the composite solids for acid-base characterization in this chapter is highly supersaturated ($^{\text{C}}\text{SiO}_2 = 1 \text{ M}$), and would precipitate abiotically at $\text{pH} \sim 7$ with or without the presence of templating cyanobacterial surfaces. However, it is argued in Chapter 2 that to qualify for a perceptible microbially-mediated biosilicification, conditions must be that the rate of colloidal SiO_2 precipitation is sluggish enough for microbial participation. Further, a distinct overlay of microbially induced biosilicification occurs by direct chemical coordination of the acidic silanol groups with the corresponding acidic sites on cell surfaces (see Chap. 3, Fig. 3.10). It is proposed that the pK_{a} - derived mechanism for silica coordination to cell surfaces via the basic and acidic functionalities, respectively

shown in this chapter (Fig. 4.3) best describes the silicification pathway observed in silica supersaturated waters such as hydrothermal systems.

4.5 Conclusion

Fitting experimental acid-base titration data using continuous binding site model to derive the pK_a spectra for chemically heterogeneous solids consisting of unmineralized live and dead cyanobacteria, amorphous colloidal silica and cell SiO_2 -mineralized composite showed a surface-dependent proton binding properties. The chemical identities inferred from the proton-binding constants of the organic surfaces are consistent with other suites of experimentally-derived molecular analogs for organic functional groups known to occur on microbial surfaces (carboxylic, phosphoryl and amine/hydroxyl groups). Acid-base modeling of colloidal silica particles also verified the existence of two dominant possible variants of silanol groups occurring simultaneously on silica surfaces within the titratable pH range (3-10). Quantification of the surface functional site densities dependent on the type (i.e., organic or mineral) or extent of organic surface exposure showed decreasing total site concentration in the order: unmineralized cells > cell- SiO_2 mineralized composite > inorganic SiO_2 . Intuitively, this same trend indicating decreasing surface functional site density dependent on the nature of the surface (organic vs. mineral), also follows their relative reactivities.

Because microbial cells are living organisms, they are capable of inducing cell-related changes surrounding the interfacial region due to perturbations such as changes in pH during acid-base titrations. As a consequence, it may not be straightforward to interpret the proton-binding characteristics of living cell suspensions, particularly with

regard to the relative concentrations of the inferred organic functional groups. The information presented in this work cannot address definitively the nature of these cellular mechanisms resulting to varied surface site densities among live cells; however, results highlight the need to better elucidate the mechanisms and implications of investigating the acid-base surface chemistry of living cells.

CHAPTER 5: CONCLUSIONS

The research carried out and presented in this thesis broadened our understanding on biomineralization, particularly the microbial surface- and metabolic- induced silica mineralization. The combined results from laboratory experimentations of natural mat consortia and pure isolate of cyanobacterial analogs showed that microbial silicification, qualified as a distinct biomineralization process, can be promoted under a specific set of geochemical conditions.

Microbial mediation in SiO_2 mineralization reflects a number of interconnected geochemical factors including acid pH and silica saturation conditions that provide thermodynamically favorable but sluggish kinetics with respect to abiogenic silica polymerization, as well as the requirement for colloidal SiO_2 , rather than dissolved silicic acid species as was previously thought from current literature information. Perceptible microbially induced biosilicification via colloidal silica pathway is primarily regulated by surface charge effects, and is dependent on the speciation of charge-forming moieties on both microbial surfaces and mineralizing silica nano-phases. As such, system pH naturally exerts a control on the protonation state of both the mineralizing cell sorbent and the aqueous SiO_2 sorbate. This work has shown definitively that microbial biosilification is possible at acidic conditions, when the interfacial repulsive charge from both cell and SiO_2 colloids is reduced. The proposed mechanistic direct chemical bonding of silica on cell surfaces to form stable inner-sphere complexes occurs under conditions where charge repulsion arising from both surfaces is overcome. The broader implication of this result is that the silica immobilization and subsequent biomineralization of microbes follows the same surface-charge dependence on both cell

and silica surfaces, much like any other sorbent-sorbate phases regardless if these mineralizing interfaces are organic (i.e., cell) or inorganic (i.e., mineral) in nature. Intuitively, if specific chemical coordination between silica sorbate and cell sorbent surface functional groups is the mechanism invoked for biosilicification, then the reactivity or propensity of microbial surfaces to biomineralize is dependent on the amount of exposed functional sites available for solute coordination. This surface functional-site-dependent reactivity for biomineralizing microbes is clearly expressed by the intermediate silicification propensities of silica-coated microbial composites relative to fully exposed, unmineralized cells and the purely abiotic SiO_2 mineral template.

While microbial biosilicification can be promoted solely as a surface induced mineralization phenomenon, putative evidence from live, metabolizing cells showed intensified biosilicification propensities than do inactivated dead cells. Unlike their pure mineral sorbent counterparts, microbial cells are living entities capable of inducing metabolic-related modifications either on the cell surface architectural array (e.g., growth of ultrastructural projections) or the surrounding interfacial region (pH regulation) to counter perturbations such as changes in solution chemistries. The impact of this cell/interfacial modification arising from cell-related activities is that interpretations from conventional modeling (e.g., acid-base titrations) of cell surface reactivities may not be straightforward. Clearly, more work needs to be done to better elucidate the mechanisms and the consequences of actively metabolizing cells leading to varied microbial reactivities and propensities to biomineralize. However, this work opens the possibilities for a more sensitive approach to potentially distinguish a biological signature in

microbially-induced silicification process, and extend our ability to interpret microbial-mineral interactions in general.

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