Microbial calcite, a bio-based smart nanomaterial in concrete remediation

S.S. Banga*, J.J. Lipperta, U. Yerrab, S. Mulukutla and V. Ramakrishnanb

aDepartment of Chemical and Biological Engineering; bDepartment of Civil and Environmental Engineering, South Dakota School of Mines and Technology, Rapid City, SD 57701, USA

(Received 29 December 2009; final version received 6 January 2010)

The concept of developing a biosealant in concrete remediation is based on unique microbial metabolic processes. A common soil microorganism, Sporosarcina pasteurii, can induce CaCO3 precipitation in the surroundings in response to environmental cues such as high pH and available nutrients and minerals. A new biomolecule, microbial calcite is introduced as a smart nanomaterial for self-healing concrete – its effects on concrete performance were evaluated with regard to surface crack remediation and durability enhancement. For crack remediation, S. pasteurii cells immobilized on porous glass beads, SiranTM, were applied to cracks and tested for stiffness and compressive strengths. For durability tests, cement mortar beams prepared with bacteria were subjected to freeze–thaw cycles and examined for mean expansions and weight changes. Overall performance of the concrete was significantly enhanced by treatment with microbial calcite in simulated concrete cracks and cement mortar beams.

Keywords: biosealant; microbial calcite; smart nanomaterial; Sporosarcina pasteurii; immobilization; concrete remediation

1. Introduction

Use of a microbial-based product as biosealant for enhancement of concrete performance has introduced a revolutionary concept to the concrete industry [1,2]. This microbial carbonate is a metabolic byproduct of Sporosarcina pasteurii (formerly known as Bacillus pasteurii), an endospore-forming soil microorganism. Microbiologically induced calcium carbonate (CaCO3) precipitation (MICCP) results from cascade reactions triggered by S. pasteurii urease (urea amidohydrolase; EC 3.5.1.5) that hydrolyzes urea to produce ammonia and carbon dioxide. The ammonia increases the pH in the surroundings to induce CaCO3 precipitation [3]. Such ureolytic microorganisms (including S. pasteurii) not only initiate calcite precipitation, but also serve as nucleation sites for calcite crystals in association with other factors such as Ca2+ ions, dissolved inorganic carbon, pH, and temperature in the medium [2,4–6]. Although microbial carbonate deposition, a type of biomineralization, occurs constantly in diverse ecosystems as part of biogeochemical cycling, use of such environmentally friendly biomaterials in structural remediation and rehabilitation is undoubtedly innovative.

Application of biomolecules such as inorganic as well as organic polymers in conservation of natural and man-made structures has drawn worldwide attention [7–10]. When
compared to organic materials that are susceptible to microbial degradation, inorganic polymers are more resistant and persist in the environment for a prolonged period. In particular, microbial calcite has gained its credence as a smart biomolecule that could protect structural integrity, remediate cracks, and enhance the overall performance of concrete in response to environmental changes [11–13].

In our earlier studies [11], sand-mixed microorganisms were applied in concrete cracks where alkaline pH (>12.5) was a potential threat to bacterial growth. To overcome such adverse conditions associated with a high pH environment, we employed an immobilization technique using a prepolymer of hydrophilic polyurethane (PU), where \textit{S. pasteurii} cells were mixed for matrix encapsulation [14]. We observed that the compressive strength of the concrete remediated with PU-immobilized cells increased approximately 12% in seven days, but only 3% in 28 days. A relatively low increase of the compressive strength from the longer treatment (28 days) might have been caused by diffusional limitations of substrates as well as a significant reduction in the number of viable cells in PU matrices. Nevertheless, our investigation on the PU-immobilized urease enzyme demonstrated that the PU matrix not only provided an additional nucleation site for CaCO$_3$ precipitation but also protected urease activity from temperature changes and proteolytic enzyme hydrolysis often associated with such environments [3].

As an alternative to the PU polymer, this investigation has examined the immobilization of \textit{S. pasteurii} on the surface of Siran$^{\text{TM}}$, sintered porous glass beads. Because of its chemically inert and physically durable nature, Siran$^{\text{TM}}$ has been used for surface immobilization of diverse biomolecules including cells and tissues [15,16]. Prior to cell immobilization, the Siran$^{\text{TM}}$ beads were activated with 3–aminopropyltrimethoxysilane (APTMS) and glutaraldehyde in sequence, where the aminosilane served as a coupler and the glutaraldehyde as a cross-linker. Surface immobilization was achieved through the covalent bonding between the cell and glutaraldehyde [17]. The main advantage of surface immobilization is to minimize diffusional limitations by allowing a large number of cells to interact with their environment with little restriction. However, there is a potential danger that the cells immobilized on Siran$^{\text{TM}}$ beads might be vulnerable to physical, chemical, and biological components present in surrounding environments [18].

Cracks and fissures are common in concrete structures. Due to its low tensile strength, cracking in concrete is inevitable and constantly occurs as part of the aging process. Freezing and thawing of concrete can be especially detrimental to structures if water freezes while trapped in the pore system of aggregates. Pressure exerted by the frozen water on the internal structure of the concrete results in cracking if it exceeds the tensile strength of the concrete. Although a variety of chemical products are currently available for enhancement of concrete performance, most of these chemicals require special attention before or after use because of their toxicity upon exposure to the air [19–21]. Earlier, we observed promising results from cement mortar mixture containing \textit{S. pasteurii} [11]. The mortar showed a significant increase in compressive strength compared to controls lacking bacteria. This observation indicates that microbial calcite precipitation continues to proceed as long as nutrients for microbial growth and ingredients for calcite precipitation are available, supporting the notion that concrete reinforced with \textit{S. pasteurii} is thriving and self-healing.

In this study, the performance of the bio-based sealant, calcite, in concrete remediation was investigated in two aspects: (1) crack remediation using \textit{S. pasteurii} immobilized on Siran$^{\text{TM}}$ and (2) durability of cement mortar beams cast with \textit{S. pasteurii} in response to environmental temperature changes.
2. Materials and methods

2.1. Microorganisms and growth conditions

*Sporosarcina pasteurii* ATCC 11859 was used for this study. *S. pasteurii* was cultured in ATCC 1832 (BPU) medium containing 10.0 g tryptone, 5.0 g yeast extract, 4.5 g tricine, 5.0 g ammonium sulfate, 2.0 g glutamic acid, and 10.0 g urea per liter of distilled water (pH 8.6), which was filter-sterilized. Experiments for the CaCO3 precipitation were carried out in liquid medium (urea–CaCl2) containing 3 g of nutrient broth (Bacto), 20 g of urea, 10 g NH4Cl, and 2.12 g of NaHCO3 (equivalent to 25.2 mM) per liter. The pH of the medium was adjusted to 6.0 prior to autoclaving. After autoclaving, filter-sterilized CaCl2 was added to yield a final concentration of 25.2 mM. Details of experimental conditions for the growth and maintenance of *S. pasteurii* are described elsewhere [2,3].

2.2. Immobilization of *S. pasteurii* on Siran™ beads

Immobilization of *S. pasteurii* on Siran™ beads included two steps: (1) preparation of the beads by acid washing and linking with ligands, APTMS and glutaraldehyde, and (2) cell immobilization on Siran™ beads.

2.2.1. Preparation of Siran™ beads

The Siran™ beads (average diameter 1–2 mm) were purchased from Jaeger Biotech Engineering (Costa Mesa, CA). Prior to the cell immobilization, the glass beads were prepared following the modified method developed by Shriver-Lake et al. [18]. Beads were washed with acid solutions using 1:1 (v/v) concentrated HCl/absolute methanol and concentrated H2SO4 for 30 min each in sequence. Each acid wash was followed by rinsing with distilled water six times during rotary shaking at 30 rpm at room temperature. The beads were then boiled in distilled water for 30 min. The acid-washed beads were dried at 80°C overnight in a single layer and kept in the oven up to seven days prior to further treatment. Then, beads were coupled with silane in a solution of 4% APTMS and then cross-linked with glutaraldehyde (2% in distilled water). The final rinse was done with sterile phosphate buffer (100 mM Na2H/NaH2PO4, 1 mM EDTA, pH 7.7), in which beads were stored up to 24 h at 4°C before immobilization.

2.2.2. Preparation of cells and immobilization

Pilot cultures of *S. pasteurii* were grown overnight in 10 ml of BPU medium at 30°C, then transferred to 100 ml fresh BPU medium and grown until the late exponential stage. Cells were harvested by centrifugation (Sorvall RC26 Plus) at 10,000 × g for 10 min. The cell pellet was washed three times with saline and the cell concentration was determined by reading the optical density (OD) at 600 nm using a spectrophotometer (Beckman Coulter DU600). For whole cell immobilization, 400 treated beads were added in a flask containing 20 ml of cell suspension (8.0 × 10⁸ cells/ml) and incubated for one hour at 4°C on a rotary shaker at 30 rpm. OD readings of the cell suspension were taken before and after immobilization, from which the difference in cell numbers was calibrated into the cell concentration immobilized on Siran™ beads (cells/bead).

2.3. Microbial CaCO3 precipitation

Free and immobilized *S. pasteurii* cells were inoculated to a final concentration of 1 × 10⁶ cells/ml in urea–CaCl2 medium for CaCO3 precipitation experiments. Culture samples were taken out at intervals to measure pH changes and determine CaCO3 precipitation using the EDTA titration method [22]. Details of the EDTA titration procedure for *S. pasteurii*–induced insoluble CaCO3 were described previously [2].

S.S. Bang et al.
2.4. Concrete remediation

2.4.1. Crack remediation using Siran™-immobilized S. pasteurii

Stiffness and compressive strength tests were carried out to evaluate the performance of MECR in concrete. Cement mortar cubes and beams were cast according to ASTM C 305 specifications. Cement mortar beams of 25.4 × 25.4 × 152 mm were used for the stiffness tests, where the slope of the load–deflection curve from a flexure test (ASTM C 348) was taken as the stiffness value (kN/m). Cement mortar cubes of 50.8 × 50.8 × 50.8 mm were used for the compressive strength tests (ASTM C 109). The width and depth of the artificial cracks were 3.175 mm and 12.7 mm in beams, respectively, and 3.175 mm and 25.4 mm in cubes, respectively. Nylon mesh was drawn around the concrete crack to secure the beads during the treatment. Each crack was filled with varying concentrations of Siran™-immobilized cells, 0, 6.1 × 10⁷, 6.1 × 10⁸, 1.2 × 10⁹, and 3.1 × 10⁹ cells/cm³, respectively. Blank beads without cells were mixed with cell-laden beads to attain the desired cell concentrations. Specimens loaded with different concentrations of immobilized cells were incubated separately in urea–CaCl₂ medium at room temperature, with fresh medium being replaced every seven days. Stiffness and compressive strength tests were independently performed after seven and 28 days of the treatment. All specimens including control sets were prepared in triplicate.

2.4.2. Performance of cement mortar mixed with S. pasteurii toward freeze-thaw cycles

Durability of concrete prepared with S. pasteurii was evaluated following ASTM C 666, a standard protocol for testing the resistance of concrete to rapid freezing and thawing. A total of four cement mortar beams (dimensions of 76.2 × 76.2 × 285.8 mm) were cast according to ASTM C 305, out of which two were mixed with bacteria (8.6 × 10⁸ cells/cm³) and two without bacteria (control). After the specimens were demolded, they were cured in urea–CaCl₂ medium for seven days, then air-cured for 14 days. Prior to freeze–thaw testing experiments, specimens were immersed in saturated limewater at 23 ± 1.7°C for 48 h. The freezing and thawing test was started by placing the specimens in thawing water. Target temperatures were 4.4°C for thawing and −17.8°C for freezing with the temperature range not fluctuating more than 1.1°C below and 2.2°C above the target temperatures. Specimens were removed at intervals of 30 cycles during the thawing phase and measured for length and weight changes for a total of 300 cycles. Mean expansions and weight changes were calculated based on the measurements taken prior to freeze–thaw cycles.

2.5. Scanning electron microscopy (SEM)

Following a modified procedure of Bang and Pazirandeh [23], S. pasteurii-laden bead samples were prepared for SEM analysis before and after CaCO₃ precipitation. The beads were placed in 1–2 ml of fixing solution (nine parts 100 mM cacodylate buffer, pH 7.2, one part glutaraldehyde) for 45–60 min on ice. Beads were washed with 1–2 ml of 100 mM cacodylate buffer (pH 7.2) for 2–3 min followed by washing with distilled water for 1 min. Beads were then dehydrated using four successive concentrations of ethanol (50%, 70%, 80%, and 100%) for 15 min each. Free cells were prepared in the same manner with the addition of centrifugation at 8000 rpm for 2 min for each step of dehydration. After complete dehydration with 100% ethanol, both samples were mounted on glass slides and stored separately in a desiccator until SEM analysis. Free cells and treated beads were gold-coated and examined with a Zeiss Supra 40 VP field emission scanning electron microscope. However, the samples of concrete and untreated beads were not fixed or gold-coated prior to SEM analysis. Elemental compositions of the remediated concrete surface were analyzed with an energy dispersive X-ray spectroscopy (EDS) system attached to the SEM.
3. Results

3.1. Concrete crack remediation

3.1.1. Cell immobilization on Siran™ beads

SEM images of *S. pasteurii* and Siran™ beads in relation to MICCP are presented in Figure 1. The image in Figure 1a includes *S. pasteurii* grown overnight in BPU medium, showing bacilli with an average 0.58 μm diameter and 2.90 μm length. In Figure 1b, a sintered glass bead (Siran™) with approximately 2 mm in diameter shows the distinct porous surface. Detailed SEM images of the surface of a Siran™ bead after cross-linker treatment and *S. pasteurii* immobilization are included in Figures 1c and 1d, respectively. Siran™ beads without the initial acid wash showed rougher surfaces than those with the pretreatment (data not shown). The total surface area of a bead is estimated to be $1.1 \times 10^8 \ \mu m^2$ (Jaeger Biotech Eng.). Based on the number of cells shown in a given area of the SEM picture (Figure 1d), the total cell count on a treated bead was estimated to range between $6.1 \times 10^5$ and $7.7 \times 10^6$ cells per bead.

Figure 1. Scanning electron micrographs of: (a) *S. pasteurii*, bar, 1 μm; (b) a Siran™ bead, bar, 1 mm; (c) the surface of a Siran™ glass bead after treatments with coupler and cross-linker, bar, 10 μm; (d) Siran™ bead immobilized with *S. pasteurii*, bar, 10 μm; (e) *S. pasteurii*-induced CaCO₃ crystals grown on the bead, bar, 20 μm; (f) an enlarged section of CaCO₃ crystals embedded with *S. pasteurii*, bar, 5 μm.
However, a relatively consistent average of $2 \times 10^6$ cells per bead was obtained through the OD readings before and after immobilization. Throughout this study, cell concentration on the bead was determined using OD values. To obtain a final cell concentration of $1 \times 10^6$ cells/ml used for the CaCO$_3$ precipitation reaction, an average of five beads were placed in 10 ml of urea–CaCl$_2$ medium. As evidenced in Figures 1e and 1f, calcite precipitation induced by *S. pasteurii* occurs on the surface of the beads and within the pores. Calcite crystals were mostly in rhombohedral structures with an overall spherical shape (Figure 1e), detailing dendritic filaments about 0.2 μm in diameter within calcitic matrix (Figure 1f) embedded with rod-shaped cells.

3.1.2. Calcite precipitation induced by Siran™-immobilized *S. pasteurii*

Patterns of CaCO$_3$ precipitation by free and Siran™-immobilized *S. pasteurii* in relation to pH changes in urea–CaCl$_2$ medium are illustrated in Figure 2. The symbols represent mean values of calcite precipitation measurements (triplicate samples) of free and immobilized cells at a given pH. It is interesting to note that free cells initiated CaCO$_3$ precipitation at lower pH (approximately 7.7), whereas immobilized cells did not obtain equivalent precipitation until pH of approximately 8.2. It appears that the free cells nearly completed CaCO$_3$ precipitation at pH 8.15 where immobilized cells had no precipitation yet. In practice, the Siran™-immobilized system will provide a protection from the drastic pH changes of the medium in the pores of the bead. It is possible that in Siran™-immobilized systems, glutaraldehyde molecules unattached to the cells might remain on the bead and react with positively charged ions in the medium to produce electrostatic interactions. Subsequently, the microenvironment surrounding the immobilized cells becomes less charged. This could be the

![Figure 2](image-url)
reason why the pH changes in medium significantly affect the free cell system, and not the Siran™-immobilized system. This also suggests that the pH of the bulk medium does not necessarily correspond to the pH of the medium in the pores of the beads.

3.1.3. Concrete crack remediation using Siran™-immobilized S. pasteurii

Figure 3 shows a comparison of stiffness and compressive strength of the concrete specimen cracks, which were remediated with Siran™-immobilized S. pasteurii for seven and 28 days in urea–CaCl₂ medium. Error bars represent standard deviations for triplicate values in the data. The stiffness was found to improve in correlation with cell concentration, where the maximum increase in stiffness was nearly 12% at a cell concentration of 6.1 × 10⁸ cells/cm³. However, there were little changes in the stiffness value of the specimens that were treated for a longer period and/or with cell concentrations of higher than 6.1 × 10⁸ cells/cm³. For the compressive strength, the highest increase in strength (up to 24%) was measured after 28 days in the cement mortar cubes remediated with the highest concentration of S. pasteurii (3.1 × 10⁹ cells/cm³). However, taking into account the standard deviation values, the overall compressive strength values did not improve significantly with cell concentrations above 6.1 × 10⁸ cells/cm³. It appears that in compressive strength test data, specimens treated for a longer period (28 days) performed better than those treated for a short period, although the concentration of 6.1 × 10⁸ cells/cm³ would serve as the optimum concentration for the best performance in stiffness and compressive strength of the immobilized S. pasteurii-remediated concrete.

3.2. Performance enhancement of cement mortar beams prepared with S. pasteurii

Effects of temperature changes, freezing and thawing, on the concrete structure were examined in relation to changes in the percent mean expansion (Figure 4a) and the percent weight change (Figure 4b). Symbols in Figure 4a represent average data values of two beams made with and without bacteria at every 30 cycles of freezing and thawing, whereas the bars in Figure 4b represent average data values of percent weight changes of two beams with and without bacteria at 0 and 300 cycles of freezing and thawing. Significant reductions (44–73%) in the mean expansion of bacteria-laden mortar beams compared to control specimens were observed throughout the test cycles (Figure 4a). The mean expansion of control specimens was 0.054% when exposed to 300 cycles of freeze–thaw, whereas that of
bacteria-laden beams was only 0.030%. As shown in Figure 4b, specimens made with bacteria retained 98% of the original weight after 300 cycles of freezing and thawing, whereas those without bacteria retained only 69% of the original weight.

SEM investigation of *S. pasteurii*-laden cement mortar beams subjected to the repeated freeze–thaw cycles revealed an interesting phenomenon (Figure 5a). A new layer (surface II) formed over the surface of the cement mortar beam (surface I). Elemental compositions of surface I strongly resemble those of the original cement material (Figure 5b), whereas elemental compositions of surface II are predominantly calcite components (Figure 5c). It appears that this second coating on the surface of cement beams formed an impermeable

Figure 4. Durability tests of cement mortar beams cast with \((8.6 \times 10^8 \text{ cells/cm}^3)\) and without bacteria and subjected to 300 freeze–thaw cycles: (a) percent mean expansion; (b) percent weight change.

Figure 5. (a) Scanning electron micrograph of a layer (surface II) newly formed over the cement mortar beam (surface I) cast with *S. pasteurii* and treated with urea–CaCl\(_2\) medium, bar, 100 μm; EDS analyses of (b) surface I and (c) surface II.
layer, which contributed to resistance of the cement structure to adverse environmental conditions such as freezing and thawing and subsequently increased structural durability with reduced mean expansions and weight changes.

4. Discussion

As evidenced in SEM images (Figure 1), *S. pasteurii* cells immobilized on Siran™ glass beads serve as nucleation sites for the growth of CaCO₃ crystals, a typical rhombohedral structure. In our earlier studies, cuboidal crystals of CaCO₃ were more frequently found in calcite precipitation induced by *S. pasteurii* cells and *S. pasteurii* urease [2,3], compared to the spherical amorphous crystal shapes induced by ureolytic subsurface bacteria [24]. It was also reported that these morphological variations in CaCO₃ crystals were influenced by the ionic strength, saturation level, temperature, and nucleation site availability in the solution [25].

In the Siran™-immobilized system, it is hypothesized that glutaraldehyde molecules remaining on the beads without being attached to the cells react as positively charged ions in urea–CaCl₂ medium to cause electrostatic interactions between the substrate and microbial urease [26]. As a result, the microenvironment around the immobilized cells is less affected by NH₄⁺ ions than that around the free cells. A shift of pH to a higher level due to the localized influx of microbiologically produced NH₄⁺ ions apparently initiates the growth of CaCO₃ crystals around the free-cell surface without a significant increase in the pH in the bulk medium. Therefore, *S. pasteurii*-induced CaCO₃ precipitation can commence at lower pH in the free cell system than in the immobilized system, where the electrostatic interactions on the Siran™ bead surface coated with cross linkers may delay the calcite precipitation.

As noted earlier [14], the compressive strength of concrete remediated by PU-immobilized *S. pasteurii* decreases as the length of treatment increases, possibly due to the diminished cell viability during the prolonged incubation period. Although there are some disadvantages associated with surface immobilization due to the susceptibility of immobilized cells to environmental changes compared to matrix immobilization, the Siran™ immobilization system with the porous surface might have protected the cells from high pH in concrete. Based on the results of this study, Siran™ surface immobilization may be considered to be more effective than the PU matrix immobilization in microbial crack remediation.

In our laboratories, microbial plugging of concrete cracks was examined with *S. pasteurii* cells applied with sand [11], PU [14], and Siran™ beads (this study). Figure 6 summarizes the specific compressive strengths exerted by individual cells in relation to cell concentrations loaded to cracks by these three applications. It is noteworthy that the trends were similar regardless of the system used for concrete crack remediation. The specific compressive strength (Pa/cell) decreased as the cell concentration and overall compressive strength increased. In particular, the largest amount of cells was immobilized on Siran™ beads mainly because the porosity of the beads increased the total surface area allowed for cell adherence. However, metabolic activities of the individual cells may be negatively affected as the microbial population increases within the given space on Siran™ beads. Furthermore, it becomes inevitable that as MICCP continues, the extracellular accumulation of Ca²⁺ and alkaline pH in surroundings hinders passage of substrates and metabolites to lower the overall metabolic performance of the individual *S. pasteurii* cells.

One of the most detrimental effects on concrete in environments is temperature change, especially undulating temperature cycling in which the water freezes and thaws. Concrete subjected to these repeated cycles may deteriorate rapidly due to the hydraulic pressure from water molecules trapped in pores of the concrete matrix. Mulukutla [27] examined the effects of the repeated freeze–thaw cycles on cement mortar beams prepared with *S. pasteurii* cells.
by comparing the changes in pulse velocity, mean expansion, weight, and durability and further identified that S. pasteurii-induced calcite effectively protected the integrity of concrete from adverse environmental conditions such as the alkali aggregate and sulfate attack. Our current study clearly demonstrates that a new layer of calcite has coated the cement surface, which might have contributed to durability enhancement by reducing the mean expansion and weight changes due to the temperature fluctuations in the surroundings.

In summary, the current study has demonstrated that concrete treated with microbial calcite performs better than untreated concrete, exhibiting increased strength and stiffness through crack remediation and reduced mean expansion and weight changes after repeated freeze–thaw cycles. The major role of microbial calcite in remediation of concrete cracks and internal matrices is to function as a biosealant comprised of inorganic calcite in protection of the concrete from adverse environmental conditions by reducing permeability of porous matrices. Especially, this type of inorganic surface protection can resist additional biological degradation that occurs frequently in environments and persists for a prolonged period. In recent years, in addition to concrete remediation, microbial calcite has been employed in a wide array of applications for soil improvement by reducing liquefaction potential [28], strengthening mechanical properties [29], and controlling fugitive dusts [30]. However, there are many challenges associated with the application of such biologically-induced molecules, and further studies are absolutely necessary to identify variable environmental parameters that might affect the integrity of bio-based sealant.

Acknowledgments
The authors thank Dr. Edward F. Duke from the Engineering and Mining Experiment Station at the South Dakota School of Mines and Technology for his technical assistance with SEM and EDS analyses. This research was funded by the National Science Foundation (Grant numbers: CMS-9802125, INT-0002608, and CMS-0301312).
References

[1] U.K. Gollapudi, C.L. Knutson, S.S. Bang, and M.R. Islam, *A new method for controlling leaching through permeable channels*, Chemosphere 30 (1995), pp. 695–705.

[2] S. Stocks-Fischer, J.K. Galinat, and S.S. Bang, *Microbiological precipitation of CaCO₃*, Soil Biol. Biochem. 31 (1999), pp. 1563–1571.

[3] K.L. Bachmeier, A.E. Williams, J.R. Warminston, and S.S. Bang, *Urease activity in microbiologically-induced calcite precipitation*, J. Biotech. 93 (2002), pp. 171–181.

[4] F. Hammes and W. Verstraete, *Key roles of pH and calcium metabolism in microbial carbonate precipitation*, Rev. Environ. Sci. Biotech. 1 (2002), pp. 3–7.

[5] F. Hammes, N. Boon, J. de Villiers, W. Verstraete, and S.D. Siciliano, *Strain-specific ureolytic microbial calcium carbonate precipitation*, Appl. Environ. Microbiol. 69 (2003), pp. 4901–4909.

[6] S. Baskar, R. Baskar, L. Mauclaire, and J.A. McKenzie, *Microbially induced calcite precipitation in culture experiments: Possible origin for stalactites in Sahastradhara caves, Dehradun, India*, Curr. Sci. India 90 (2006), pp. 58–64.

[7] F.A. McLeod, H.M. Lappin-Sott, and J.W. Costerton, *Plugging of a model rock system by using starved bacteria*, Appl. Environ. Microbiol. 54 (1988), pp. 1365–1372.

[8] P. Tiano, L. Biagiotti, and G. Mastromei, *Bacterial biomediated calcite precipitation for monumental stones conservation*, J. Microbiol. Meth. 36 (1999), pp. 139–145.

[9] C. Rodriguez-Navarro, M. Rodriguez-Gallego, K. Ben Chekroun, and M.T. Gonzalez-Munoz, *Conservation of ornamental stone by Myxococcus xanthus induced carbonate biomineralization*, Appl. Environ. Microbiol. 69 (2003), pp. 2182–2193.

[10] C. Rodriguez-Navarro, C. Jimenez-Lopez, A. Rodriguez-Navarro, M.T. Gonzalez-Muñoz, and M. Rodriguez-Gallego, *Bacterially mediated mineralization of vaterite*, Geochim. Cosmochim. Acta 71 (2007), pp. 1197–1213.

[11] S.K. Ramachandran, V. Ramakrishnan, and S.S. Bang, *Remediation of concrete using microorganisms*, ACI Mater. J. 98 (2001), pp. 3–9.

[12] V. Ramakrishnan, R.K. Panchalan, and S.S. Bang, *Bacterial concrete – a concrete for the future*, in *Serviceability of Concrete*, F. Barth, ed., American Concrete Institute, 2005, pp. 37–54.

[13] W. De Muynck, D. Debrouwer, N. De Belie, and W. Verstraete, *Bacterial carbonate precipitation improves the durability of cementitious materials*, Cement Concr. Res. 38 (2008), pp. 1005–1014.

[14] S.S. Bang, J.K. Galinat, and V. Ramakrishnan, *Calcite precipitation by polyurethane-immobilized Bacillus pasteurii*, Enzym. Microb. Tech. 28 (2001), pp. 404–409.

[15] A.K. Sun, J. Hong, and T.K. Wood, *Modeling trichloroethylene degradation by a recombinant pseudomonad expressing toluene ortho-monoxygenase in a fixed-bed bioreactor*, Biotech. Bioeng. 59 (1998), pp. 40–51.

[16] P. Srivastava and S. Kundu, *A comparative evaluation of cephalosporin C production using various immobilization modes*, J. Gen. Appl. Microbiol. 44 (1998), 113–117.

[17] M. Pazirandeh, E. Goldman, L.C. Shriver-Lake, S.S. Bang, and A. Singh, *Bacterial and enzyme systems for the bioremediation of heavy metals and other pollutants*, Recent Res. Dev. Microbiol. 4 (2000), pp. 683–700.

[18] L.C. Shriver-Lake, W.B. Gammeter, S.S. Bang, and M. Pazirandeh, *Covalent binding of genetically engineered microorganisms to porous glass beads*, Anal. Chim. Acta 470 (2002), pp. 71–78.

[19] M. Ibrahim, A.S. Al-Gahtani, M. Maslehuddin, and A.A. Almusallam, *Effectiveness of concrete surface treatment materials in reducing chloride-induced reinforcement corrosion*, Constr. Build. Mater. 11 (1997), pp. 443–451.

[20] K.G. Babu and D.S. Babu, *Behaviour of lightweight expanded polystyrene concrete containing silica fume*, Cement Concr. Res. 33 (2003), pp. 755–762.

[21] B. Kumar, G.K. Tike, and P.K. Nanda, *Evaluation of properties of high-volume fly-ash concrete for pavements*, J. Mater. Civ. Eng. 19 (2007), pp. 906–911.

[22] American Water Works Association, *EDTA titrimetric method*, in *Standard Methods for the Examination of Water and Wastewater*, 20th ed., 1998, 2340B.

[23] S.S. Bang and M. Pazirandeh, *Physical properties and heavy metal uptake of encapsulated Escherichia coli expressing a metal binding gene (NCP)*, J. Microencapsulation 16 (1999), pp. 489–499.

[24] Y. Fujita, F.G. Ferris, R.D. Lawson, F.S. Colwell, and R.W. Smith, *Calcium carbonate precipitation by ureolytic subsurface bacteria*, Geomicrobiol. J. 17 (2000), pp. 305–318.

[25] D.C. Harris, *Quantitative Chemical Analysis*, 4th ed., W.H. Freeman and Company, New York, 1995.
[26] F. Shiraishi, Diffusional and electrostatic effects on apparent kinetic parameters of reactions catalyzed by enzyme immobilized on the external surface of transport, J. Ferment. Bioeng. 79 (1995), pp. 373–377.

[27] S. Mulukutla, Properties and performance of bacterial cement mortar, M.S. thesis, South Dakota School of Mines and Technology, 2002.

[28] J.T. DeJong, M.B. Friyges, and K. Nusslein. Microbially induced cementation to control sand response to undrained shear, J. Geotech. Geoenviron. Eng. 11 (2006), pp. 1381–1392.

[29] V.S. Whiffin, L.A. van Paassen, and M.P. Harkes, Microbial carbonate precipitation as a soil improvement technique, Geomicrobiol. J. 24 (2007), pp. 417–423.

[30] S.S. Bang, S. Bang, S. Frutiger, L.M. Nehl, and B.L. Comes, Application of novel biological technique in dust suppression, TRB 88th Annual Meeting, Washington, DC, 2009.