[SI-4]

Microbiologically - Enhanced Crack Remediation (MECR)

Sookie S. Bang*and V. Ramakrishnan
South Dakota School of Mines and Technology 501 E. Saint Joseph Street, Rapid City, SD 57701

ABSTRACT

A novel approach of microbiologically-enhanced crack remediation (MECR) has been initiated and evaluated in this report. Under the laboratory conditions, *Bacillus pasteurii* was used to induce $CaCO_3$ precipitation as the microbial urease hydrolyzes urea to produce ammonia and carbon dioxide. The ammonia released in surroundings subsequently increases pH, leading to accumulation of insoluble $CaCO_3$. Scanning electron micrography (SEM) and x-ray diffraction (XRD) analyses evidenced the direct involvement of microorganisms in $CaCO_3$ precipitation. In biochemical studies, the primary roles of microorganisms and microbial urease were defined. Furthermore, the role of urease in $CaCO_3$ precipitation was characterized utilizing recombinant *Escherichia coli* that encoded *B. pasteurii* urease genes in a plasmid. Microorganisms immobilized in polyurethane (PU) polymer were applied to remediate concrete cracks. Although microbiologically- induced calcite precipitation enhanced neither the tensile strength nor the modulus of elasticity of the PU polymer, cement mortar whose crack was remediated with the cell-laden polymer showed a significant increase in compressive strength. Through detailed investigation, MECR showed an excellent potential in cementing cracks in granite, concrete, and beyond.

INTRODUCTION

In natural environments, mineral precipitation processes constantly occur at a slow rate over geologic time, plugging or selectively cementing permeable porous media (Hart *et al.*, 1960; Kantzas *et al.*, 1992). Microbial metabolic activities often contribute to selective cementation by producing relatively insoluble organic and inorganic compounds intra- and/or extracellularly, which remain in the environment for a long time even after cell death. Some microorganisms produce glycocalyx and a variety of organic polymers outside the cell wall (Lappin-Scott *et al.*, 1988; MacLeod *et al.*, 1988), while others accumulate inorganic compounds such as phosphorites, carbonates, silicates, iron, and manganese oxides in cytoplasm (Beveridge *et al.*, 1983; Ghiorse, 1984; Knoll, 1985; Ruiz *et al.*, 1988; Rivadeneya *et al.*, 1991). The importance of selective cementation has been widely recognized in Petroleum Engineering, Geological Engineering and Civil Engineering. It has been documented that cracks in rock formations surrounding oil reservoirs could be remediated by microorganisms to enhance the oil recovery (Updegraff, 1982; Finnerty and Singer, 1983).

Cracks and fissures are a common problem in building structures, pavements, and historic monuments. Methods currently used for crack remediation often use synthetic polymers that need to be applied repeatedly. We have introduced a novel technique in fixing cracks with environmentally friendly biological processes that is a continuous self-remediating process (Gollapudi et al., 1995). In the study, *Bacillus pasteurii* that is abundant in soil has been used to induce $CaCO_3$ precipitation. The long-term goal of our research is to use microbiologically-induced $CaCO_3$ in remediation of cracks and fissures in natural and man-made structures. It is therefore imperative to understand the fundamentals of microbial

*Corresponding author. tel.: 1 605 3942426; fax: 1 605 3941232; e-mail: Sookie.Bang@sdsmt.edu

participation in crack remediation and establish the methodology of remediation prior to implementing the microbiologically-enhanced crack remediation (MECR) process.

Microbiologically-induced $CaCO_3$ precipitation results from a series of complex biochemical reactions, including the concomitant participation of microorganisms, urease (urea amidohydrolase; EC 3.5.1.5), and high pH. As urease from *B. pasteurii* hydrolyzes urea to ammonia and carbon dioxide, the ammonia increases the pH of the surrounding, which in turn induces $CaCO_3$ precipitation. It is hypothesized that urease is the main component that initiates the microbial $CaCO_3$ precipitation. Urease activity is found in a wide range of microorganisms and plants, some of which produce the enzyme in large quantities (Mobley et al., 1995; Ciurli et al., 1996). In particular, *B. pasteurii* produces intracellular urease constituting close to 1% of the cell dry weight. The urease from *B. pasteurii* consists of three different subunits with two nickel atoms in individual active sites (Benini et al., 1999). Urease genes from numerous microorganisms have been sequenced and expressed in the recombinant plasmids (Maeda et al., 1993; Mordorf et al., 1994). To identify the role of urease in calcite precipitation, we have utilized *Escherichia coli* HB101 that harbors a plasmid encoding *B. pasteurii* urease genes (You et al., 1995). *B. pasteurii* and the recombinant *E. coli* were tested for the effects of acetohydroxamic acid (AHA) that functions as a competitive inhibitor of urease on microbial calcite precipitation.

Use of microbial products as a long-term remediation tool has exhibited high potential for crack cementation of various structural formations such as granite and concrete (Gollapudi et al., 1995; Stocks-Fischer et al., 1999). However, the microbiological concrete remediation requires further consideration before it can be used with confidence, mainly due to the fact that the pH of concrete remains extremely high even after it is completely cured, i.e., above 12.5. Several reinforcement materials have been considered for providing not only a protection from adverse environmental conditions in concrete but also a higher bonding strength between the crack and the concrete. An immobilization technique for remediation of cracks in concrete, where microbial cells are encapsulated in polymers, has been adopted to enclose $CaCO_3$ precipitation in the gap and to enhance the strength for selective cementation. The immobilization technique offers several advantages for concrete remediation, in which encapsulated cells retain high metabolic activities and are protected from adverse environmental conditions (O'Reilly and Crawford, 1989).

Polyurethanes (PU) have been widely used as a vehicle for immobilization of enzymes and whole cells because of its mechanically strong and biochemically inert characteristics (Fukushima et al., 1978; Wang and Ruchenstein, 1993). PU makes open cell foam as a result of condensation of polycyanates (R-CNO) and polyols (R-OH). Typically, porous matrices of PU not only increase the surface areas but also minimize the diffusion limitation for substrates and products, which is a common disadvantage of polymers currently used for encapsulation such as acrylamide, alginate, and carrageenan (Klein and Kluge, 1981; Bang and Pazirandeh, 1999). Although metabolic activities of cells remain high in PU matrices, it is however uncertain whether the rate of cell growth remains the same (O'Reilly and Crawford 1989; Sumino et al, 1992).

This paper summarizes our findings on the calcite precipitation induced by microorganisms and the feasibility of using microbiologically-induced $CaCO_3$ in concrete crack remediation. Data on the mineral cementation by scanning electron microscopy (SEM) and x-ray diffraction (XRD) quantitative analysis present physical evidence on microbial participation, which are detailed by the biochemical studies of microbiological calcite precipitation. This report also includes the results of the molecular investigation of the urease function in $CaCO_3$ precipitation utilizing recombinant microorganisms and the application of immobilization technology in concrete crack remediation.

MATERIALS AND METHODS

Microorganisms and Experimental Conditions

Microorganisms used for the study are *Bacillus pasteurii* ATCC 11859 (Bethesda, MD) and two recombinant *Escherichia coli* HB101 (*supE44 recA ara14 proA2 lacY1 galK2*) strains containing plasmids pBU11 and pBR322, respectively (You et al., 1995). The recombinant *E. coli* strains were provided by S.D. Kim (Yeungnam University, Korea). Details of the growth conditions of microorganisms and the experimental procedures for urease assay, $CaCO_3$ precipitation, ammonia production, XRD, and SEM are described elsewhere (Stocks-Fischer et al., 1999; Bang et al., 2001; Ramachandran et al., 2001; Bachmeier et al., 2001). Water-based prepolymer of polyurethane (HYPOL® 2000, 3000) was provided by the Hampshire Chemical Corp. (Boston, MA). All other essential chemicals used were purchased from Aldrich Chemicals (St. Louis, MO).

RESULTS

Evidence of microbiologically-induced $CaCO_3$ precipitation

Previously, we reported that the granite cracks remediated with a mixture of sand and *B. pasteurii* cells showed a significant increase in compressive strength (Gollapudi, 1995; Zhong and Islam, 1995). Table 1 summarizes the results of XRD quantitative analyses of sand samples that were tested under different experimental conditions. The most abundant compound was clearly quartz, the main component of sand. Microbiologically-induced $CaCO_3$ crystals were identified as calcite, not aragonite that is more common in seawater or magnesium-rich aqueous solutions (Berner, 1975; Rivadeneyra *et al.*, 1991). Calcite constituted 30.2% of the total weight of the sand samples plugged by bacteria, but none was detected in the samples without live cells. In the sand samples treated with medium, urea was consumed by growing bacteria (sample 4), while unmetabolized urea was detected in the samples with killed or no bacteria. It is noteworthy that a small amount of Ca^{2+} (8.3%) was crystallized as a form of gehlenite in the presence of killed bacteria. Details of the consolidated sand samples examined under SEM are depicted in Fig. 1, where distinct calcite crystals embedded with microorganisms are found between and on the surface of sand grains. Rod-shaped bacteria were prominent in all sediment samples and appeared fossilized as intact bacilli within the calcite crystals. The presence of crystalline calcite associated with bacteria suggests that bacteria served as nucleation sites during the mineralization process.

Table 1. XRD quantitative analysis of the final weight fractions[a] of sand samples[b].

[a]Numbers represent an average of weight fraction values obtained from energy-dispersive XRD quantitative analysis; ( ), variance errors; Trace, <0.001; ND, not detected.

[b]Sample 1, untreated sand; Sample 2, sand treated with medium; Sample 3, sand treated with killed *B. pasteurii* and medium; Sample 4, sand treated with *B. pasteurii* and medium.

Sample	Quartz SiO_2	Calcite $CaCO_3$	Gehlenite $Ca_2Al(Al,Si)_2O_7$	Mullite $Al_6Si_2O_{13}$	Hematite Fe_2O_3	Goethite α-FeO(OH)	Urea CH_4N_2O
1	0.960 (0.010)	ND	ND	0.039 (0.010)	Trace	ND	ND
2	0.954 (0.009)	ND	ND	0.032 (0.008)	0.003 (0.001)	ND	0.011 (0.003)
3	0.892 (0.015)	ND	0.083 (0.011)	0.012 (0.003)	Trace	0.003 (0.001)	0.030 (0.005)
4	0.683 (0.065)	0.302 (0.066)	ND	0.015 (0.004)	Trace	ND	ND

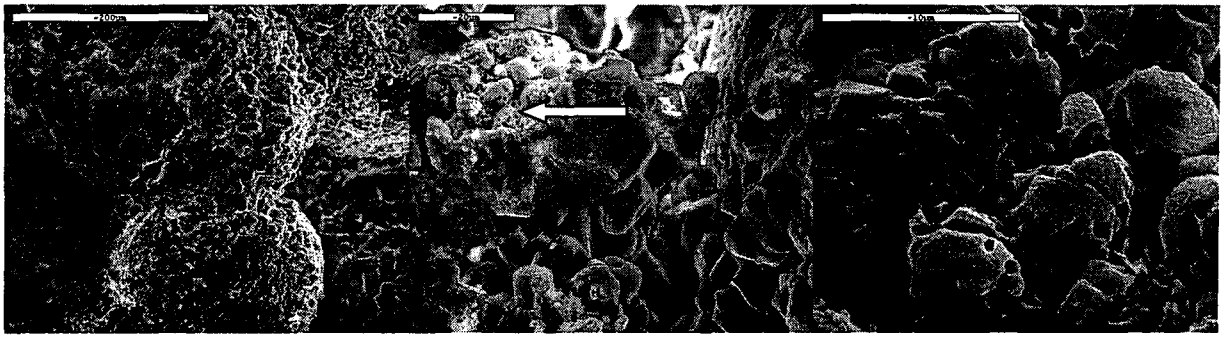

Figure 1. Scanning electron micrographs of microbiologically-induced calcite precipitation. ***a.*** Calcite crystals have formed over the sand particles. Bar, 200 μm. ***b.*** Details of crystal formations shown in ***a.*** At this magnification, the fine structure of the crystallites is easily discerned and evident. Bar, 20 μm. ***c.*** Magnification of the area marked with an arrow in ***b*** shows *B. pasteurii* embedded in the crystals. Bar, 10 μm.

Biochemistry of microbiologically-induced $CaCO_3$ precipitation

Fig. 2 presents the patterns of calcite precipitation, cell growth, ammonia production, and pH at an initial concentration of 25.2 mM $CaCl_2$ in the presence of live and killed cells (1×10^6 cells ml^{-1}). The microbiological $CaCO_3$ precipitation began approximately at pH 8.3 and was completed at 9.2, consolidating 98% of the initial concentration of Ca^{2+}. Calcium carbonate precipitation appeared to be correlated with the growth of *B. pasteurii* and was completed within 16 hours following inoculation. However, ammonia was released continuously even during the stationary phase of cell growth. The pH of the medium also increased slowly as the ammonia production increased. In the presence of killed cells, there were no detectable changes in calcite precipitation, ammonia production, and pH.

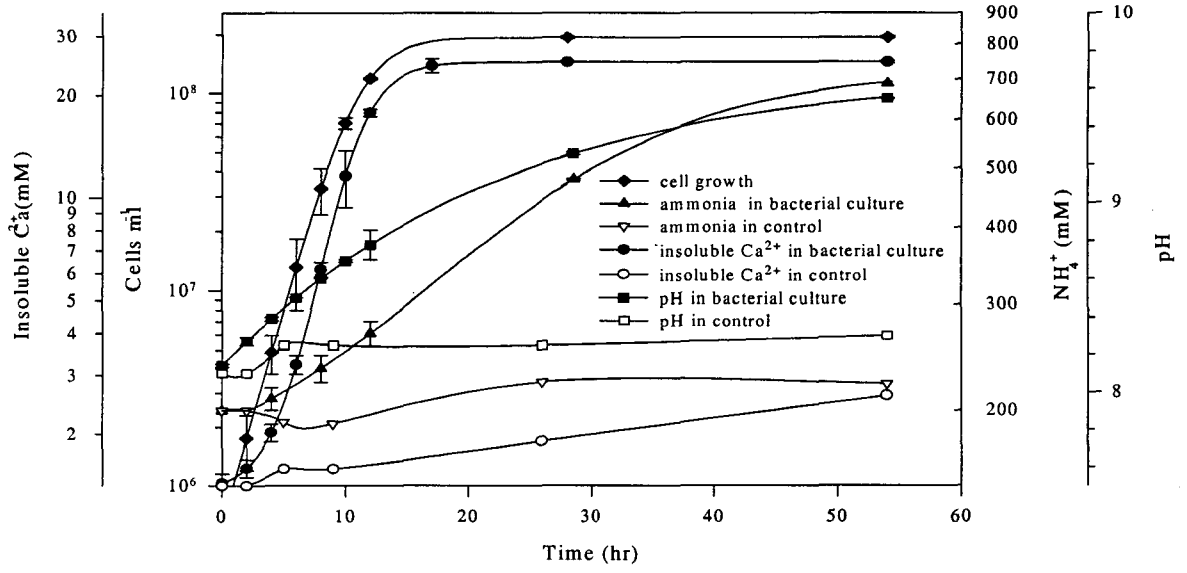

Figure 2. Profiles of $CaCO_3$ precipitation, NH_4^+ production, and pH changes in the presence of live and killed *B. pasteurii* (inoculum size, 1.0 x 10^6 cells ml^{-1}) in urea-$CaCl_2$ medium. Each point represents the average of duplicate assays.

Participation of urease in calcite precipitation

A urease inhibitor, acetohydroxamic acid (AHA), was used to identify the role of urease in calcite precipitation. Fig. 3 shows the effects of AHA on the calcite precipitation induced by *B. pasteurii* and a recombinant *E. coli* (pBU11) with the urease genes. *E. coli* (pBR322) encoding no urease genes served as the negative control. In the absence of AHA, the recombinant *E. coli* induced calcite precipitation even though its precipitation level was not as high as that by *B. pasteurii*, whereas there was no detectable calcite precipitation by *E. coli* (pBR322). Similar trends were also noted for the ammonia production by the same cells (data not included), corresponding to the patterns observed in calcite precipitation. In the presence of AHA, *B. pasteurii* or *E. coli* (pBU11) induced little calcite precipitation. Subsequently, there was neither pH increase nor ammonia release under the condition where AHA inhibited the urease activity in the cell. However, the turbidity of both cultures containing AHA still increased, indicating that the cell growth was not affected.

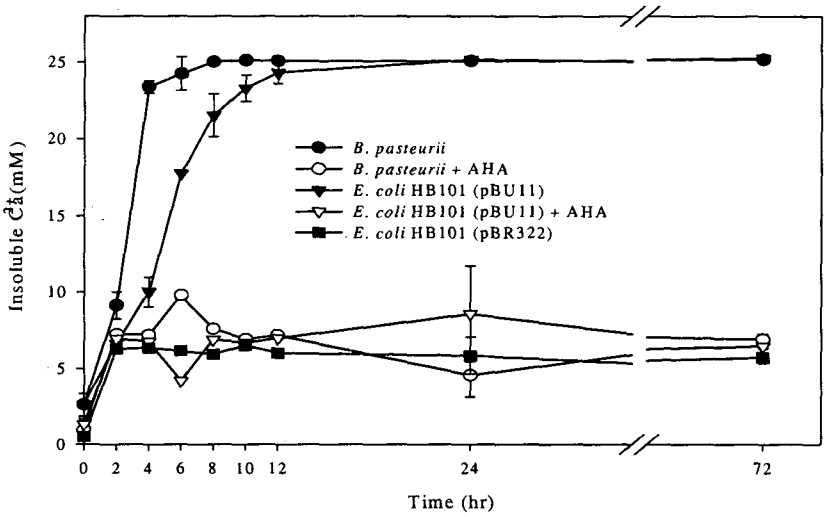

Figure 3. Effects of acetohydroxamic acid (AHA) on urease-induced calcite precipitation by *B. pasteurii* and the recombinant *E. coli* HB101 strains.

Calcite precipitation induced by immobilized cells

Upon polymerization, PU foam was pliable and elastic with open-cell structure of matrices (Fig. 4a). Micrographs (Figs. 4b-4c) showing cell-laden PU matrices indicate that immobilization caused no apparent morphological damage to the cells and microorganisms were entrapped throughout the polymer matrices where cells were adhered or embedded with some clumping. As shown in Figs. 4d and 4e, calcite precipitation occurred throughout the entire matrices, including the inside of pores as well as the surface areas. It was also apparent that calcite crystals grew around the microorganisms and PU matrices (Fig. 4f).

Fig. 5 depicts the patterns of calcite precipitation and ammonia production by free and immobilized cells. At 5×10^7 cells ml^{-1}, calcite precipitation by both free and immobilized cells was completed within 4 hours in medium, where 98% of the soluble Ca^{2+} became insoluble. There appear no significant differences in the rate of calcite precipitation by free and immobilized microorganisms when they are in the same concentration. As shown in the figure, controls including PU with no cells and killed cells show neither calcite precipitation nor ammonium production. Viable cell counts in plates with aliquots of the urea-$CaCl_2$ medium containing PU-immobilized cells indicated that there was no apparent cell leakage throughout the incubation.

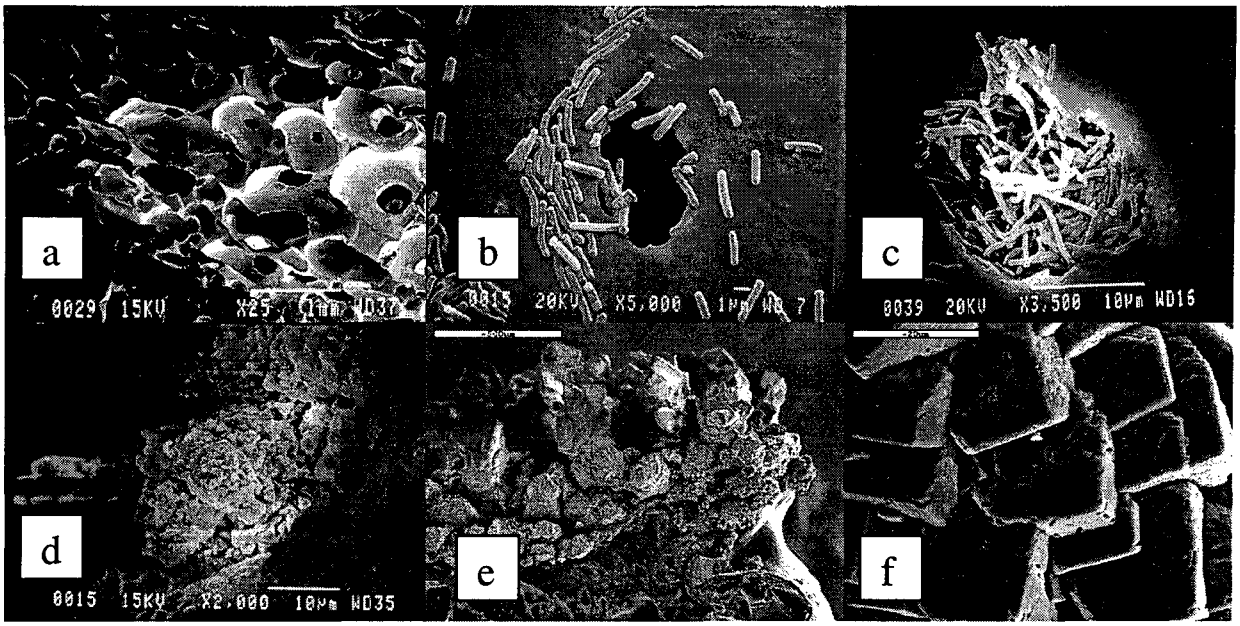

Figure 4. Scanning electron micrographs of calcite precipitation induced by *B. pasteurii* immobilized in PU. ***a.*** Porous PU matrix without microbial cells showing open-cell structures. Bar, 1 mm. ***b.*** Distribution of microorganisms on the PU surface. Bar, 1 µm. ***c.*** Microorganisms densely packed in a pore of the PU matrix. Bar, 10 µm. ***d.*** Calcite crystals grown in the pore (shown in ***c***) of the PU matrix. Bar, 10 µm. ***e.*** Calcite crystals grown extensively over the PU polymer. Bar, 500 µm. ***f.*** Magnified section pointed with an arrow in ***e*** shows crystals embedded with microorganisms. Bar, 20 µm.

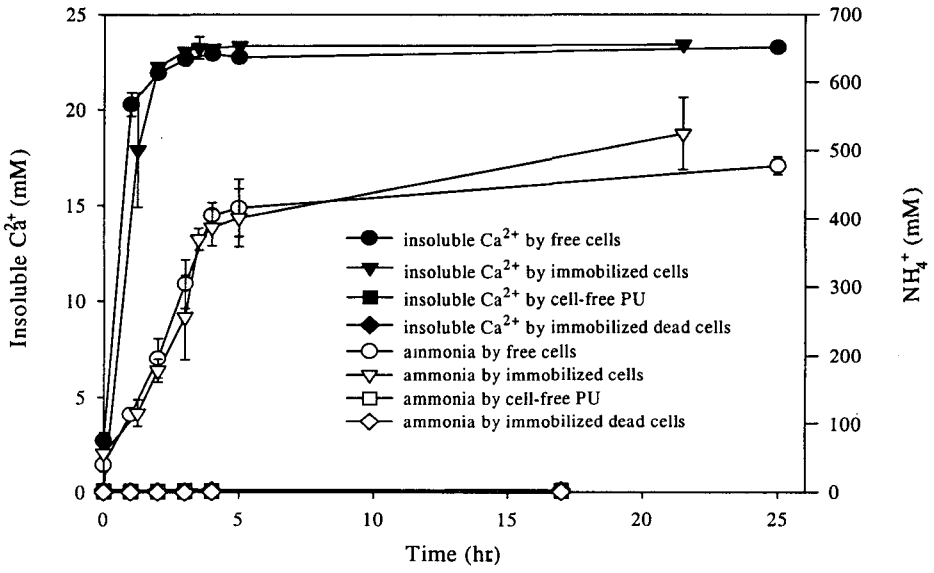

Figure 5. Patterns of calcite precipitation and ammonia production by free and immobilized *B. pasteurii* (inoculum size, 5 x 10^7 cells ml^{-1}).

Variations of the tensile strength and the stiffness of the PU foam with respect to the duration of the incubation in urea-$CaCl_2$ medium are shown in Figure 6. Immediately after immersion in medium, there was a 42% increase in tensile strength of the foam with cells (21.18 psi) compared to that of the foam without (14.94 psi). However, the tensile strength decreased with extended incubation period in medium. Most of the decrease took place within the first ½ day, eventually approaching gradually to the final decrease of 21% at the end of the 7-day incubation. On the contrary, there was no significant change in the tensile strength of the foam without cells. At the end of the 7-day incubation, the overall tensile strength of PU with cells was slightly higher than that of PU without cells. Results from the stiffness (modulus of elasticity) tests of the foams are similar to those of tensile strength tests. Same trends were also found in the modulus of elasticity of the foam except that the modulus of the PU with cells was slightly less than that of PU without cells at the end of the 7-day incubation.

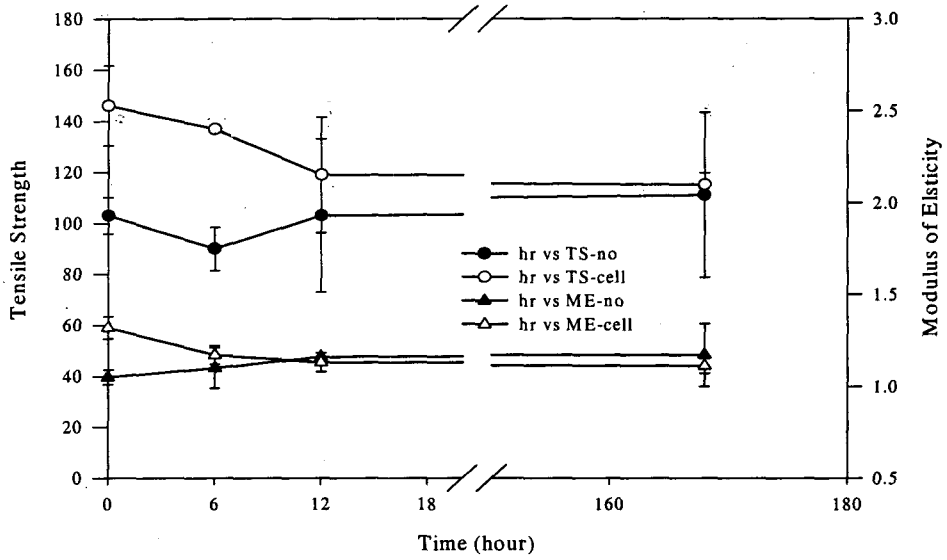

Figure 6. Effects of microbiologically-induced calcite precipitation on tensile strength and modulus of elasticity of polyurethane

Application Immobilized Cells in Concrete Crack Remediation

In order to examine the effects of MECR, simulated cracks (d = 2.54 cm, w = 0.312 cm) in cement mortar cubes (5.08 × 5.08 × 5.08 cm) were loaded with PU-encapsulated *B. pasteurii* and treated with urea-$CaCl_2$ medium for 7 and 28 days, respectively. Fig. 7 depicts the changes in compressive strength of the concrete cubes treated with different concentrations of immobilized microorganisms when compared to the control cubes treated with cell-free PU polymer. The highest compressive strength was obtained with the cubes that were remediated with a concentration of 5×10^9 immobilized cells $crack^{-1}$ for 7 days. With a longer incubation period (28 days), the increase in strength of all microbiologically-remediated cubes was found to be marginal when compared to that with 7-day incubation. It was also observed that the PU polymer with or without cells became less rigid when incubated longer than 7 days in urea-$CaCl_2$ medium. Nonetheless, there was no apparent change in compressive strength of the control cubes even for a prolonged incubation period in medium.

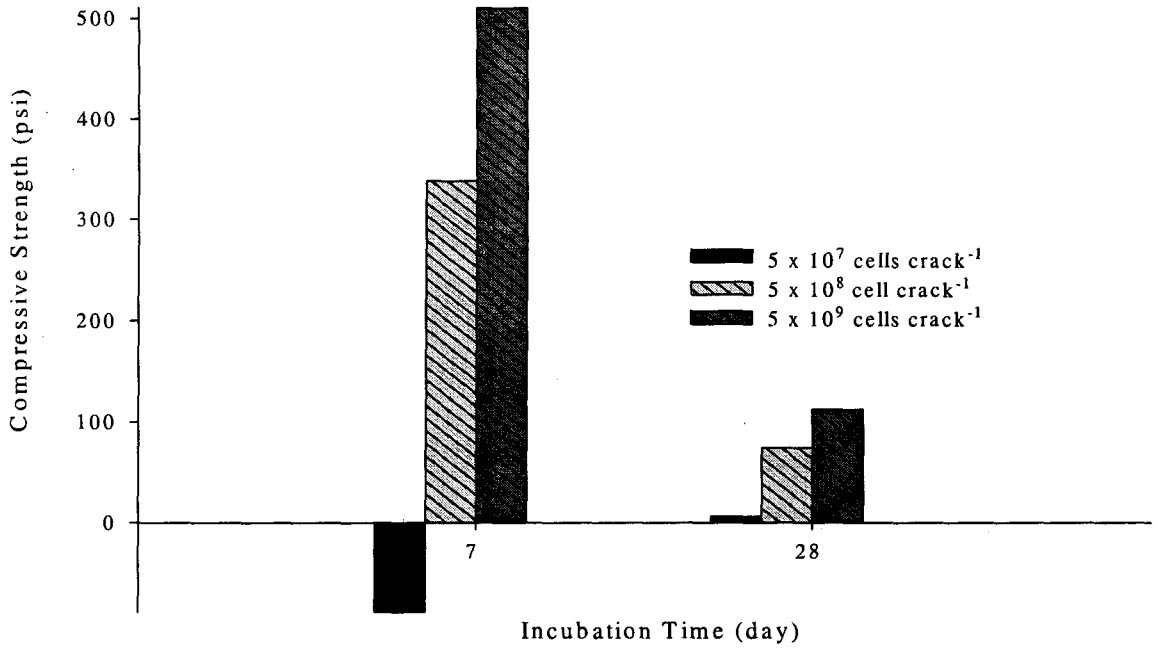

Figure 7. Increase of compressive strengths of concrete with cracks remediated with different concentrations of *B. pasteurii* encapsulated in PU.

DISCUSSION

Role of microorganisms in $CaCO_3$ precipitation

The overall equilibrium reaction of calcite precipitation can be described below.

$$Ca^{2+} + CO_3^{2-} \rightleftharpoons CaCO_3\downarrow \quad (1)$$

The solubility of $CaCO_3$ is a function of pH and affected by ionic strength in the aqueous medium (Stumm and Morgan, 1981). In urea-$CaCl_2$ medium that supports microbial growth, NH_4^+ and Cl^- also react with OH^- and H^+, respectively, at different pH, further interfering with chemically-induced $CaCO_3$ precipitation. Microbiologically-induced $CaCO_3$ precipitation occurs via far more complicated processes than chemically-induced precipitation. The bacterial cell surface with a variety of ions could nonspecifically induce mineral deposition by providing a nucleation site (Ferris *et al.*, 1986, 1987). Especially, Ca^{2+} is not likely utilized by microbial metabolic processes, rather accumulates outside the cell (Silver *et al.*, 1975). In medium, it is possible that individual microorganisms produce ammonia as a result of enzymatic urea hydrolysis to create an alkaline micro-environment around the cell. The high pH of these localized areas, without a significant increase in pH in the entire medium at the beginning, apparently commences the growth of $CaCO_3$ crystals around the cell. Possible biochemical reactions in urea-$CaCl_2$ medium to precipitate $CaCO_3$ at the cell surface can be summarized as follows.

$$Ca^{2+} + Cell \longrightarrow Cell\text{-}Ca^{2+} \quad (2)$$

$$Cl^- + HCO_3^- + NH_3 \rightleftharpoons NH_4Cl + CO_3^{2-} \quad (3)$$

$$Cell\text{-}Ca^{2+} + CO_3^{2-} \longrightarrow Cell\text{-}CaCO_3\downarrow \quad (4)$$

In addition, the results of kinetic studies render an explanation that the rate of $CaCO_3$ precipitation correlates with the cell growth and urease exhibits higher enzymatic activities and stronger affinity to urea at higher pH levels (pH 8-9) where calcite precipitation is favorable. Observations from the XRD analysis and electron micrography have also added credence to our previous study results (Gollapudi *et al.*, 1995; Zhong and Islam, 1995), providing conclusive evidence of direct participation of *B. pasteurii* in calcite formation. Undoubtedly, *B. pasteurii* not only provides a nucleation site for calcite precipitation but also creates an alkaline environment inducing further precipitation of calcite. Findings from this study provide additional supports to the notion that *in-situ* implementation of microbial mineral plugging might provide a practical means for bioremediation of porous media.

Role of the microbial urease in calcite precipitation

The role of the microbial urease was defined from the data (Fig. 3) that the calcite precipitation by *B. pasteurii* and *E. coli* (pBU11) expressing *B. pasteurii* urease was inhibited in the presence of a urease inhibitor and a significant amount of calcite precipitation was induced by *E. coli* (pBU11), whereas little was induced by *E. coli* (pBR322) lacking urease genes. These observations support our assumption that the urease enzyme is a primary factor that initiates microbiologically-induced calcite precipitation. In detail, reaction 1 produces calcium carbonate and protons in aqueous medium where CO_3^{2-} primarily stays as HCO_3^-.

$$Ca^{2+} + HCO_3^- \longrightarrow CaCO_3 + H^+ \quad (5)$$

In biological systems, many calcareous organisms couple calcification to their metabolic assimilation processes to scavenge protons (McConnaughey and Whelan, 1996). In urease-based reactions, NH_3 released by the enzymatic hydrolysis of urea uses the protons generated from the calcite precipitation (reaction 5) to produce NH_4^+.

$$NH_3 + H^+ \longrightarrow NH_4^+ \quad (6)$$

The subsequent increase of pH in surrounding medium due to the presence of ammonia ions and the additional release of CO_2 from the enzymatic urea hydrolysis further accelerate the rate of the urease-induced calcite precipitation. Thus, an active participation of urease is of essence in biochemical calcite precipitation.

Concrete crack remediation

It was hypothesized at the beginning of the study that calcite precipitation in the polymer matrices might influence the physical characteristics of PU, particularly enhancing the tensile strength and the stiffness. However, microbiologically-induced calcite precipitation in PU matrices did not yield any increase in tensile strength of the foam. Although the reason for this phenomenon is not yet clearly identified, it appears that the precipitation by calcite results in no specific chemical bonding between $CaCO_3$ and the foam, rather in simple accumulation of $CaCO_3$ in the matrices. In general, the increases in tensile strength and stiffness depend on the bond between the precipitated calcium carbonate and the PU foam. The initial increases in the strength and elastic modulus, however, indicate that there is a possible nonspecific interaction between the precipitated calcite and the foam.

We observed previously that the use of a mixture of sand and microorganisms in concrete remediation increases the compressive strength (Ramachandran et al., 2001). However, the microbiological calcite precipitation occurred mainly close to the surface area of the crack where dense growth of calcite crystals embedded with cells was observed. In this study, it is observed that PU-immobilized *B. pasteurii* used for the remediation of concrete cracks induces calcite precipitation

throughout the matrices. The major advantage of using PU in *B. pasteurii* encapsulation is that its matrix can provide the microorganisms with a means of protection from the extremely alkaline environment of concrete, while serving as additional nucleation sites for calcite crystals. Microbiologically-induced calcite remains intact in PU matrix mainly because of the high pH of concrete, where the solubility of $CaCO_3$ is extremely low.

In summary, the positive potential of MECR that has been demonstrated in our study offers an interesting concept of the crack remediation technique in various applications. For instance, biological metabolic products can be utilized not only in crack remediation but also in sealing the gaps in various structures. The latter application of the technique can utilize the immobilized urease instead of the whole cell. In practice, the urease-laden PU polymer is placed in cracks to which a subsequent application of the substrate induces calcite precipitation. To explore possibilities of using the immobilized urease in crack remediation, detailed biochemical studies are currently under investigation.

ACKNOWLEDGEMENTS

This research was funded by grants from the National Science Foundation (CMS-9412942; -9802127; INT-0002608). Authors express sincere appreciation Dr. S. D. Kim for providing the recombinant urease. Authors acknowledge the vital contribution of Dr. E. F. Duke of the Engineering and Mining Experiment Station at the South Dakota School of Mines and Technology for his technical assistance in SEM and XRD.

REFERENCES

Bachmeier, K., Williams, A.E., Warmington, J., and Bang, S.S. 2001. Urease activity in microbiologically-induced calcite precipitation. J. Biotechnol. in press

Bang, S.S., Galinat, J.K., and Ramakrishnan, V. 2001. Calcite precipitation induced by polyurethane immobilized *Bacillus pasteurii*. Enz. Microbial Tech. 28, 404-409

Bang, S.S. and Pazirandeh, M. 1999. Physical properties and heavy metal uptake of encapsulated *Eshcerichia coli* expressing a metal binding gene (NCP). J. Microencapsul. 16, 489-499

Benini, S., Rypniewski, W., Wilson, K.S., Miletti, S., Ciurli, S., and Mangani, S. 1999. A new proposal for urease mechanism based on the crystal structures of the native and inhibited enzyme from *Bacillus pasteurii:* why urea hydrolysis costs two nickels. Structure, 7, 205-216

Berner, R.A. 1975. The role of magnesium in the crystal growth of calcite and aragonite from sea water. Geochim. Cosmochim. Acta. 39, 489-504

Beveridge, T.J., Meloche, J.D., Fyfe, W.S., and Murray, R.G.E. 1983. Diagenesis of metals chemically complexed to bacteria: laboratory formation of metal phosphates, sulfides, and organic condensates in artificial sediments. Appl. Environ. Microbiol. 45, 1094-1108

Ciurli, S., Marzadori, C., Benini, S., Deiana, S., and Gessa, C. 1996. Urease from the soil bacterium *Bacillus pasteurii:* immobilization on Ca-polygalacturonate. Soil Biol. Biochem. 28, 811-817

Ferris, F. G., Beveridge, T. J. and Fyfe, W. S. 1986. Iron-silica crystallite nucleation by bacteria in a geothermal sediment. Nature, 320, 609-611

Ferris, F.G., Fyfe, W.S. and Beveridge, T. J. 1987. Bacteria as nucleation sites for authigenic minerals in a metal-contaminated lake sediment. Chem. Geol. 63, 225-232

Finnerty, W.R. and Singer, M.E. 1983. Microbial enhancement of oil recovery. Biotechnol. 1, 47-54

Fukushima, S., Nagai, T., Fujita, S., Tanaka, A., and Fukui, S. 1978. Hydrophilic urethane prepolymers: convenient materials for enzyme entrapment. Biotechnol. Bioeng. 20, 1465-1469

Ghiorse, W.C. 1984. Biology of iron- and manganese-depositing bacteria. Ann. Rev. Microbiol. 38, 515-550

Gollapudi, U.K., Knutson, C.L., Bang, S.S., and Islam, M.R. 1995. A new method for controlling leaching through permeable channels. Chemosphere, 30, 695-705

Hart, R.T., Fekete, T., and Flock, D.L. 1960. The plugging effect of bacteria in sandstone systems. Can. Mining Metallug. Bull. 53, 495-501

Kantzas, A., Ferris, F.G., Stehmeier, L., Marentette, D.F., Jha, K.N., and Mourits, F.M. 1992. A novel method of sand consolidation through bacteriogenic mineral plugging (CIM 92-46). *In* Proceedings of the CIM 1992 Annual Technical Conference, Vol. 2, pp. 1-15, Petroleum Society of CIM, Calgary Canada

Klein, J. and Kluge, M. 1981. Immobilization of microbial cells in polyurethane matrices. Biotechnol. Lett. 3, 65-70

Knoll, A. H. 1985. The distribution and evolution of microbial life in the late proterozoic era. Ann. Rev. Microbiol. 39, 391-417

Lappin-Scott, H.M., Cusack, F., and Costerton, J.W. 1988. Nutrient resuscitation and growth of starved cells in sandstone cores: a novel approach to enhanced oil recovery. Applied Environ. Microbiol. 54, 1373-1382

MacLeod, F.A., Lappin-Scott, H.M., and Costerton, J.W. 1988. Plugging of a model rock system by using starved bacteria. Appl. Environ. Microbiol. 54, 1365-1372

Maeda, M., Hidaka, M., Nakamura, A., Masaki, H., and Uozumi, T. 1993. Cloning, sequencing, and expression of thermophilic *Bacillus* sp. Strain TB-90 urease gene complex in *Escherichia coli*. J. Bact 176, 432-442

McConnaughey, T.A. and Whelan, J.F. 1997. Calcification generates protons for nutrient and bicarbonate uptake. Earth-Sci. Rev. 42, 95-117

Mobley, H.L.T., Island, M.D., and Hausinger, R.P. 1995. Molecular biology of microbial ureases. Microbiol. Rev. 59, 451-480

Morsdorf, G., Weinmann, P., and Kaltwasser, H. 1994. Nucleotide sequence of the three genes on a urease encoding DNA-fragment from *Bacillus pasteurii*. Genbank accession number X78411

O'Reilly, K.T. and Crawford, R.L. 1989. Degradation of pentachlorophenol by polyurethane-immobilized *Flavobacterium* cells. Appl. Environ. Microbiol., 55, 2113-2118

Ramachandran, S.K., Ramakrishnan, V., and Bang, S.S., 2001. Remediation of concrete using microorganisms. ACI Materials J. 98, 3-9

Rivadeneyra, M.A., Delgado, R., Quesada, E., and Ramos-Cormenzana, A. 1991. Precipitation of calcium carbonate by *Deleya halophila* in media containing NaCl as sole salt. Cur. Microbiol. 22, 185-190

Ruiz, C., Monteoliva-Sanches, M., Huertas, F., and Ramos-Cormenzana, A. 1988. Calcium carbonate precipitation by several species of *Myxococcus*. Chemosphere, 17, 835-838

Silver, S., Toth, K., and Scribner, H. 1975. Facilitated transport of calcium by cells and subcellular membranes of *Bacillus subtilis* and *Escherichia coli*. J. Bact. 12, 880-885

Stocks-Fischer, S., Galinat, J.K., and Bang, S.S. 1999. Microbiological precipitation of $CaCO_3$. Soil Biol. Biochem. 31, 1563-1571

Stumm, W. and Morgan, J. J. 1981. Aquatic Chemistry, John Wiley & Sons, NY

Sumino, T., Nakamura, H., Mori, N., Kawaguchi, Y., and Tada, M. 1992. Immobilization of nitrifying bacteria in porous pellets of urethane gel for removal of ammonium nitrogen from waste-water. Appl. Microbiol. Biotechnol. 36, 556-560

Updegraff, D.M. 1982. Plugging and penetration of petroleum reservoir rock by microorganisms. *In* Proceedings of 1982 International Conference on Microbial Enhancement of Oil Recovery, pp. 80-85, U.S. Dept. of Energy, Oklahoma

Wang, X. and Ruchenstein, E. 1993. Preparation of porous polyurethane particles and their use of enzyme immobilization. Biotechnol. Prog. 9, 661-665

You, J.H., Song, B,H., Kim, J.H., Lee, M.H., and Kim, S.D. 1995. Genetic organization and nucleotide sequencing of the *ure* gene cluster in *Bacillus pasteurii*. Mol. Cell. 5, 359-369

Zhong, L. and Islam, M.R. 1995. A new microbial plugging process and its impact on fracture remediation (SPE 30519). *In* Proceedings of the 70th Annual Technical Conference and Exhibition of the Society of Petroleum Engineers, pp. 703-715, Dallas, Texas