MODIFYING CONCRETE PROPERTIES THROUGH BACTERIOGENIC MINERAL PLUGGING

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ABSTRACT

Microorganisms are incredibly diverse and include bacteria, fungi, archaea and some microscopic plants and animals such as plankton. Numerous bacterial species participate in the precipitation of mineral carbonates in various natural environments, including soils, geological formations, freshwater, oceans and saline lakes. Bacteria are believed to affect carbonate precipitation both through affecting local geochemical conditions and by serving as potential, nucleation sites for mineral formation. A novel technique for the remediation of damaged structural formations has been developed by employing a selective microbial plugging process, in which metabolic activities promote precipitation of calcium carbonate in the form of calcite. Recently, microbial mineral precipitation resulting from metabolic activities of some specific microorganisms in concrete to improve the overall behavior of concrete has become an important area of research. It has been hypothesized that almost all bacteria are capable of $CaCO_3$ production because precipitation occurs as a byproduct of common metabolic processes such as photosynthesis, ammonification, de-nitrification, sulfate reduction and anaerobic sulphide oxidation. In this paper, an overview of bacteria, their types based on the classification has been presented along with the development of concrete incorporated with microorganism *Bacillus subtilis JC3* (developed at JNTU Centre for Environment Lab) and reports the research findings about modified properties of microbial concrete.

Keywords: *Bacterial concrete, Bioremediation, Bacillus subtilis, self-healing, biomineralization*

INTRODUCTION

Microbial mineral precipitation resulting from metabolic activities of some specific microorganisms in concrete to improve the overall behavior of concrete has become a considerable area of research. These bacteria are able to influence the precipitation of calcium carbonate by the process of Ammonification (Ammo acid degradation). Precipitation of calcium carbonate crystals occurs by heterogeneous nucleation on bacterial cell walls once super-saturation is achieved [1]. The application of concrete is rapidly increasing worldwide and therefore the development of bacterial mediated concrete is urgently needed for environmental reasons. As presently, about 8% of atmospheric carbon dioxide emission is due to cement production, mechanisms that would contribute to longer service life of concrete structures would make the material not only more durable but also self repair, i.e., the autonomous healing of cracks in concrete. The potential of bacteria to act as self healing agent in concrete has proven to be a promising future. This field appears to be more beneficial as bacterial concrete appears to produce more substantially more crack plugging minerals than control specimens (without bacteria). A promising sustainable repair methodology is currently being investigated and developed in several laboratories, i.e., a technique based on the application of mineral producing bacteria. The application for ecological engineering purposes is becoming increasingly popular as is reflected by recent studies where bacteria were applied for removal of chemicals from waste water streams , for bioremediation of contaminated soils and removal of green house gases from landfills . The applicability of specifically mineral producing bacteria for sand consolidation and limestone monument repair and filling of pores and cracks in concrete have been recently investigated[2]. In all these studies so far, bacteria were externally applied on cracked concrete structures or test specimens, i.e., as surface treatment or repair system. An integrated healing agent would save manual inspection and repair and moreover increase structure durability. Addition of such an agent to the concrete mixture would thus save both money and the environment as less maintenance and use of environmental friendly repair material is needed. Microbial carbonate precipitation (bio-deposition) decreases the permeation properties of concrete [3]. Hence, a deposition of a layer of calcium carbonate on the surface of concrete resulted in a decrease of water absorption and porosity. Bacteria-based self-healing concrete is produced by incorporating spores of bacteria of a special kind (*Bacillus subtilis* JC3), in the concrete matrix at the stage of preparation of the concrete by mixing the spore suspension in concrete mixing water. When crack is formed water enters the crack subsequently the homogeneously distributed bacterial spores in hardened concrete matrix gets activated and germinate to become metabolically active vegetative cells that are able to convert the organic nutrient compounds into insoluble inorganic calcium carbonate based minerals[4].

BIOMINERALIZATION

Bio-mineralization is defined as a biologically induced precipitation in which an organism creates a local micro-environment with conditions that allow optimal extracellular chemical precipitation of mineral phases [4]. Numerous diverse microbial species participate in the precipitation of mineral carbonates in various natural environments including soils, geological formations, fresh water bio films, oceans and saline lakes. The precise role of the microbes in the carbonate precipitation process is still not clear. Almost all bacteria are capable of calcium carbonate precipitation [5]. A novel technique for the remediation of damaged structural formations has been developed by employing a selective microbial plugging process, in which metabolic activities promote precipitation of calcium carbonate in the form of calcite [6]. Biomineralization of calcium carbonate is one of the strategies to remediate cracks in building materials. In nature, microorganisms can induce calcite mineral precipitation through nitrogen cycle either by ammonification of amino acids/ nitrate reduction/ hydrolysis of urea [7]. The binding strength of the precipitated crystals is highly dependent on the rate of carbonate formation and under suitable conditions it is possible to control the reaction to generate hard binding calcite cement (or bio-cement) [8].

CHEMISTRY OF BIO-CALCIFICATION BY *BACILLUS SUBTILIS* JC3

Bacterial calcium carbonate precipitation results from both passive and active nucleation. Passive carbonate nucleation occurs from metabolically driven changes in the bulk fluid environment surrounding the bacterial cells. This increases the mineral saturation and induces nucleation. In the Ammo acid degradation driven system, this occurs from an increase in pH due to ammonification. Active carbonate nucleation occurs when the bacterial cell surface is utilized as the nucleation site. The cell clusters exhibit a net electronegative charge which favors the adsorption of Ca^{2+} ions. The Ca^{2+} ions attract CO_3^{2-} and HCO_3^- ions, which will eventually form calcium carbonate precipitates [9]. Although it is known that there are many different types of bacteria capable of calcium carbonate precipitation, it has been hypothesized that there are specific attributes of certain bacteria that promote and affect CaCO3 precipitation more than others [10]. It has already been noted that cell walls have an inherent electronegative charge that affect the binding of certain ions, but the extracellular polymeric substance associated with biofilms may also be involved. Biofilm cells are contained in the extracellular polymeric substance matrix and may exhibit an overall negative charge. This negative charge is important in trapping metal ions. Strain *Bacillus subtilis* JC3, selected for the present study, was distinguished as aerobic alkaliphilic spore-forming soil bacteria. The medium used to grow *Bacillus subtilis* JC3 was based on peptone, NaCl , yeast extract. The pure culture was isolated from the soil sample of JNTU.

Microbiologically induced calcium carbonate precipitation occurs via more complicated processes than chemically induced precipitation. In nutrients medium, it is possible that individual microorganisms produce ammonia as a result of amino acids degradation to create an alkaline micro-environment around the cell. The high pH of these localized areas, without an initial increase in pH in the entire medium, commences the growth of $CaCO_3$ crystals around the cell. Specific proteins present in biological extracellular polymeric substances cause the formation of different calcium carbonate polymorphs. Some bacteria and fungi can induce precipitation of calcium carbonate extracellularly through a number of processes that include photosynthesis, ammonification, denitrification, sulfate reduction and anaerobic sulphide oxidation. Although all the Bacillus strains were capable of depositing calcium carbonate, differences occurred in the amount of precipitated calcium carbonate on agar plate colonies.

Oxidative deamination of amino acids by *Bacillus subtilis* JC3 is temperature dependent and that the highest calcite precipitation rates occurred near the point of critical saturation. *B. Subtilis* JC3 member of the genus Bacillus is Gram-positive, rod-shaped, endospore forming bacteria commonly found in soil; precipitate calcium carbonate ($CaCO_3$) in its micro-environment by the ammonification of amino acids into ammonium (NH_4^+) and carbonate (CO_3^{2-}) ions. Microbiologically induced (also called ‘‘bacteriogenic’’) calcite carbonate precipitation by *Ammonification* (Ammo acid degradation**)** comprises of series of complex biochemical reactions. Amino acids released during proteolysis (the process of enzymatic breakdown of proteins by the microorganisms with the help of proteolysis enzymes) undergo deamination in which nitrogen containing amino (-NH_2) group is removed. Thus, process of deamination which leads to the production of ammonia is termed as "ammonification". The process of ammonification is mediated by *Bacillus subtilis* JC3. Ammonification usually occurs under aerobic conditions (known as oxidative deamination) with the liberation of ammonia (NH_3) or ammonium ions (NH_4) when dissolved in water .The biochemical reactions of ammonification in peptone based medium is represented as follows

$$Ca^{2+} + \textit{B.subtilis}\text{ Cell} \rightarrow \textit{B.subtilis}\text{ Cell- }Ca^{2+}$$

$$CH_3CH(NH_2)COOH\text{ (Peptone)} + \tfrac{1}{2}O_2 \text{ --------->} C_2H_2 + H_2CO_3 + NH_3$$

$$H_2CO_3 \text{ ---------->} H^+ + HCO_3^-$$

$$NH_3 + H_2O \text{ --------> } NH_4^+ + OH^- \text{ (pH increase)}$$
$$HCO_3^- + H^+ + OH^- \text{ ----------> } CO_3^{2-} + H_2O$$
$$\textit{B.subtilis} \text{ Cell- } Ca^{2+} + CO_3^{2-} \rightarrow \textit{B.subtilis} \text{ Cell- } CaCO_3$$

Upon examination, bacterial cells were shown encased in calcite crystals, which indicated that the bacteria acted as a nucleation site for the mineralization process, an example of active nucleation.

APPLICATIONS OF BACTERIA IN CONCRETE

Microbial concrete as an alternative surface treatment for concrete

An important measure to protect concrete against damage is diminishing the uptake of water [10]. Many of the physical and chemical deterioration mechanisms of concrete are related to m aggressive substances present in aqueous solution. Surface treatments play an important role in limiting the infiltration of water. Broad arrays of organic and inorganic products are available in the market for the protection of concrete surfaces, such as a variety of coatings, water repellents and pore blockers. But these means of protection beside their favorable influences even show disadvantageous aspects such as: Degradation over time, Need for constant maintenance, Different thermal expansion coefficient of the treated layers, Use of certain solvents contributes to environmental pollution as well.

Microbial concrete as concrete crack remediation/healing

When cracks appear in the concrete, the possibility for corrosion of the embedded steel arises which could eventually ruin the integrity of the structure. Without immediate attention, the cracks can expand and cause extensive damage. Current forms of concrete crack remediation are structural epoxy, resins, epoxy mortar, and other synthetic filler agents. These synthetic solutions often need to be applied more than once as the cracks expand. Clearly there is a need for an effective, long-term, environmentally safe method to repair cracks in concrete structures. Several research groups have investigated the possibility of bio-mineralization as an effective method to remediate cracks and fissures in concrete structures. Cracks filled with a mixture of *B. subtilis* and sand showed a significant increase in compressive strength and stiffness, compared to cracks without cells. Microscopy confirmed the presence of calcite crystals and cells near the surface of the cracks.

Table 1: Microbial Concrete applications

Applications	Types of bacteria
Microbial concrete as crack healer	*Bacillus pasteurii, Bacillus subtilis , Deleya halophila, Halomonas eurihalina, Myxococcus Xanthus, Bacillus megaterium*
Microbial concrete as surface treatment	*Bacillus subtilis , Bacillus sphaericus*
Microbial concrete as water purifier	*Bacillus subtilis, Bacillus sphaericus,Thiobacillus*

Table 2: Overview of various Construction Materials made using MICP

Application	Microorganism	Metabolism	Nutrients	***Reference***
Biological mortar	*Bacillus cereus*	oxidative deamination of amino acids	Growth media (peptone, extract yeast, KNO3, NaCl) + CaCl2.2H2O, Actical, Natamycine	*(Muynck et al., 2010)*
Crack in concrete remediation	*Bacillus subtilis*	Hydrolysis of urea	Nutrient broth, urea, CaCl2.2H2O, NH4Cl, NaHCO3	*(Santhosh et al., 2001)*
Crack in concrete remediation	*Bacillus sphaericus*	Hydrolysis of urea	Extract yeast, urea, CaCl2.2H2O	*(Belie, 2010)*
Bacterial concrete	*Bacillus subtilis*	Hydrolysis of urea	Nutrient broth, urea, CaCl2.2H2O, NH4Cl, NaHCO3	*(Santhosh et al., 2001)*
Bacterial concrete	*Bacillus subtilis*	oxidative deamination of amino acids	*Peptone: 5 g/lit., NaCl: 5 g/lit., Yeast extract: 3 g/lit.*	***(M V Seshgiri Rao et al., 2010) JNTU Hyderabad***

Table 3: Microorganism used for Calcium Carbonate Precipitation in Concrete

Type of microorganism	System	Crystal type	*Reference*
Photosynthetic organism : *Synechococcus GL24*	Meromictic lake	Calcite ($CaCO_3$)	*(Douglas and Beveridge, 1998)*
Photosynthetic organism : *Chlorella*	Lurcene Lake	Calcite ($CaCO_3$)	*(Dittrich, 2004)*
Sulfate reducing bacteria: *Isolate SRB LVform6*	Anoxic hypersaline lagoon	Dolomite (Ca(Mg) CO_3)	-
Nitrogen cycle *Bacillus subtilis*	Urea degradation in synthetic medium	Calcite ($CaCO_3$)	*(Mc.Connaughey, 2000)*
Nitrogen cycle *Bacillus cereus*	Ammonification and nitrate reduction	Calcite ($CaCO_3$)	*(Castanier et al., 1999)*
Nitrogen cycle Bacillus subtilis JC3	Ammonification (Ammo acid degradation)	*Calcite ($CaCO_3$)*	***(M V Seshagiri Rao and Ch Sasikala, 2010) JNTU Hyderabad***

EFFECT OF BIOGENIC TREATMENT ON CONCRETE PROPERTIES

Experimental investigation reports confirm that incorporation of bacteria into concrete modifies the mechanical and durability properties of concrete as presented below:

Optimum Cell Concentration for Promotion of Maximum Mineral Precipitation

For cell concentration of 10^5 cells per ml of mixing water the compressive strength of bacteria incorporated cement mortar specimens is maximum (increased by about 17%) at age 28 days as shown in Table 4. Above 1x 10^5 cells per ml of mixing water reduces the strength due to presence of organic matter above the limits permitted as per IS 456.

Table 4: Effect of *Bacillus subtilis* JC3 cell concentration on Compressive Strength (MPa)

Cell concentration/ml of mixing water	Average Compressive Strength (Mpa) ± S.D					
	7 days	% Increase relative to control	14 days	% Increase relative to control	28 days	% Increase relative to control
Nil (control)	37.32 ± 0.42	-	44.1±0.66		51.81±0.10	-
10^4	41.68±0.52	11.68	45.23±0.85	2.56	58.02±0.72	11.99
10^5	45.02±0.22	20.63	49.21±0.91	11.59	61.79±0.68	16.15
10^6	43.09±0.36	15.46	47.69±0.32	8.14	57.21±0.49	10.42
10^7	40.11±0.58	7.48	45.97±0.44	4.24	54.66±0.89	5.51

Compressive Strength

The Compressive Strength of bacteria incorporated concrete showed significant increase by nearly 25% in all grades of concrete proposed for all ages considered as shown in Table 5 because the voids and pores are sealed up by mineral precipitation due to bacteria.

Table 5: Strength Characteristics of Controlled and Bacterial Concrete mixes at various ages

STRENGTH STUDIES								
	Controlled Concrete				Bacterial Concrete			
Grade →	M20	M40	M60	M80	M20	M40	M60	M80
Age	Average Compressive Strength (MPa) ± S.D							
28 days	28.18±21	52.01±96	72.61±71	93.8±88	32.74±50	61.06±87	94.21±96	119.2±46
60 days	32.44±56	56.47±56	79.26±58	98.35±95	37.97±09	64.92±66	102.9±80	125.6±27
90 days	33.27±42	57.96±55	83.59±32	107.57±49	40.4±42	66.83±29	108.6±38	138.14±61

Ultrasonic pulse velocity

Ultrasonic pulse velocity values as listed in Table 6 increased with bacteria induction into concrete due to enhanced pore structure and microstructure of hardened bacterial concrete of all grades. From the results it is observed that the reduction in pores in bacterial concrete improves the surface integrity of concrete, improves its homogeneity good bonding and reduces the probability of cracks.

Table 6: Ultrasonic pulse velocity measurements

	Controlled Concrete				**Bacterial Concrete**			
Grade ⟶	**M20**	**M40**	**M60**	**M80**	**M20**	**M40**	**M60**	**M80**
Age	**Ultrasonic Pulse Velocity** (km/sec)							
28 days	4.26	4.49	4.89	5.13	4.77	4.93	5.22	5.94
60 days	4.36	4.53	4.92	5.19	4.83	4.99	5.36	6.02
90 days	4.41	4.61	4.99	5.33	4.91	5.02	5.41	6.05

CONCLUSIONS

Microbial mineral precipitation appears to be a promising technique. The type of bacterial culture and medium composition had a profound impact on calcium carbonate crystal morphology. The use of pure culture resulted in a more pronounced effects. Metabolic activities of some specific microorganisms in concrete are responsible to improve the performance of concrete. It has been hypothesized that almost all bacteria are capable of $CaCO_3$ production because precipitation occurs as a byproduct of common metabolic processes such as photosynthesis, ammonification, denitrification, sulfate reduction and anaerobic sulphide oxidation. Even the effect of bacteria on various parameters in concrete proves to be beneficial development. Based on the studied properties like compressive strength, permeability, water absorption, chloride ingression, the microbial mineral precipitation appears to be a promising technique at this state of development.

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