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Effect of calcifying bacteria on permeation properties of concrete structures

V. Achal · A. Mukherjee · M. S. Reddy

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Abstract Microbially enhanced calcite precipitation on concrete or mortar has become an important area of research regarding construction materials. This study examined the effect of calcite precipitation induced by *Sporosarcina pasteurii* (Bp M-3) on parameters affecting the durability of concrete or mortar. An inexpensive industrial waste, corn steep liquor (CSL), from starch industry was used as nutrient source for the growth of bacteria and calcite production, and the results obtained with CSL were compared with those of the standard commercial medium. Bacterial deposition of a layer of calcite on the surface of the specimens resulted in substantial decrease of water uptake, permeability, and chloride penetration compared with control specimens without bacteria. The results obtained with CSL medium were comparable to those obtained with standard medium, indicating the economization of the biocalcification process. The results suggest that calcifying bacteria play an important role in enhancing the durability of concrete structures.

Keywords Calcite precipitation · *Sporosarcina pasteurii* · Industrial waste · Corn steep liquor · Permeability · Chloride penetration

Introduction

The long-term durability of concrete is affected to a large extent by its permeability. Concrete with high permeability provides ready access for both water and harmful substances, resulting in deterioration of either concrete or steel reinforcement embedded in the concrete or a combination of both [13]. Chloride-induced corrosion of reinforcing steel is one of the most pressing problems worldwide that the construction industry is facing today.

The most common cause of deterioration in reinforced concrete is the transport of aggressive gases and/or liquids into concrete from the surrounding environment followed by physical and/or chemical reactions within its internal structure, possibly leading to irreversible change [7, 15]. Therefore, the permeation properties, rather than mechanical properties, are the important factors to study in relation to concrete durability. A layer of calcium carbonate deposition on the concrete surface might be helpful in reducing the concrete's permeability.

Considerable research on calcium carbonate precipitation by bacteria has been performed by using ureolytic bacteria such as *Sporosarcina pasteurii* (formerly known as *Bacillus pasteurii*) [1, 11]. These bacteria are able to influence the precipitation of calcium carbonate by the production of urease enzyme. This enzyme catalyzes the hydrolysis of urea to CO_2 and ammonia, resulting in an increase of the pH and carbonate concentration in the bacterial environment. On the basis of this property of urease, microbially induced calcium carbonate precipitation on concrete has been demonstrated [20]. A large number of *Bacillus* species participate in calcite precipitation in the environment by producing the urease enzyme. In addition, bacterial net cell surface charge is negative and draws cations from the environment, including Ca^{2+}, to deposit on the cell surface.

For the remediation of damaged structural formations, a method has been developed by employing selective microbial plugging in which their metabolic activities

V. Achal · A. Mukherjee · M. S. Reddy (✉)
Department of Biotechnology, Thapar University, Patiala 147 004, India
e-mail: msreddy@thapar.edu; vasu70@yahoo.com

promote precipitation of calcium carbonate in the form of calcite [14]. The effectiveness of calcite precipitation as a protective film in reducing the corrosion rate of the underlying mild steel was determined [12]. Microbial mineral precipitation (biodeposition) technologies have already been used for consolidation of sand columns [1, 2, 20], for repair of limestone monuments [11, 21, 23], and to a lesser extent for remediation of cracks in concrete [5, 6, 20].

However, for successful commercialization of the technique, economical alternatives for the medium ingredients that cost as high as 60% of the total costs must be developed [16]. Researchers have hitherto used standard nutrient media like nutrient broth or yeast extract medium to grow the bacteria. The reported nutritional profile for the calcite-forming bacterium *S. pasteurii* indicated a high preference for protein-based media [17]. Process economics reveal that the use of inexpensive, high-protein-containing industrial wastes such as corn steep liquor (CSL) or lactose mother liquor (LML) not only reduce the overall production cost but also eliminate an environmental pollutant [1].

The present study aimed to reduce the water absorption and permeability of building materials to enhance the durability of mortar or concrete cubes by using microbially induced calcite precipitation. These studies were performed by using CSL as nutrient source and compared with commercially available standard media for the bacterial growth.

Materials and methods

Microorganism and media

The bacterial strain *Sporosarcina pasteurii* (Bp M-3) capable of producing high urease activity was used in this study. This strain was developed after exposure to UV radiation and regenerated on high-urea-containing medium to adapt high urease activity. This strain was deposited at the Institute of Microbial Technology, Chandigarh, India (accession number MTCC5428). The procedure for the improvement of strain is discussed in Achal et al. [2]. Microbiological calcite precipitation and permeability tests were carried out in the CSL medium (1.5% v/v) and standard nutrient broth (NB). The chemical composition of CSL consists of 45–50% solids, 5.8% carbohydrates, 24% proteins, 1.0% fats, 8.8% minerals, and trace amounts of vitamins [3], and commercially available standard nutrient broth (NB) consisted of (g/l) casein hydrosylates 15.0, peptone 5.0, and NaCl 5.0 g/l (HiMedia, Mumbai, India). Corn steep liquor (CSL) was collected from the corn wet milling industry (Bharat Starch Industries Ltd, Yamunanagar, Haryana, India). Both media were supplemented with 2% urea and 25 mM $CaCl_2$. The pH was adjusted to 6.5 with 1 N HCl prior to autoclaving without urea and $CaCl_2$. Filter-sterilized urea and $CaCl_2$ were added later.

Microbial cementation

Calcite production ability of *S. pasteurii* (Bp M-3) was studied in NB and CSL media. One milliliter of overnight grown Bp M-3 culture was inoculated to 100 ml of NB and CSL media supplemented with 2% urea and 25 mM $CaCl_2$. The bacteria were grown at 37°C with continuous aeration at 120 rpm till its OD_{600} reached 1.0. The calcite precipitated in the medium was filtered through 0.45-μm membrane filter which also filters the bacterial cells. The calcite retained on the membrane was dried at 50°C overnight and expressed as dry weight (mg)/cell dry mass (mg).

The microbial sand plugging was performed as described in Achal et al. [1]. Briefly, 50 ml of *S. pasterurii* (Bp M-3) culture (10^7 cells/ml) was mixed with 100 g sterilized river sand. The sand was sterilized prior to use to eliminate the indigenous microflora by autoclaving at 121°C for 1 h, and this process was repeated thrice at an interval of 24 h. Sand slurry containing bacterial culture was packed into a plastic column (height 15 in.; diameter 3 in.), and the bottom side of column was blocked by using Whatman filter paper. A control reaction was packed in a column in which sterile sand was mixed with media alone (without cells). All columns were fed continuously with the corresponding media separately at room temperature to mimic the natural environmental conditions. Flow rate was measured by measuring the volume of media that came out of the columns per minute. The experiment was terminated after 10 days and allowed to dry at room temperature. The sand columns were divided into three layers (upper, middle, and lower), and each layer was individually ground and sieved through a 45-μm-diameter mesh prior to calcite estimation. Precipitated calcite from each layer was measured by EDTA titration method [4].

Water absorption test

To determine the increase in resistance towards water penetration, a sorptivity test, based on the RILEM 25 PEM (II-6), was carried out. A cement mortar cube mold of 70.6 mm was used, as per IS 4031-1988. Ordinary Portland cement, fine aggregate (medium-sized natural/river sand), and crushed stone coarse aggregate with maximum size of 20 mm were used in concrete. The ratio of cement/sand/coarse aggregate was 1:1.54:2.86. Water/cement ratio as well as bacterial culture (10^7 cells/ml) to cement ratio was maintained at 0.47. Cement mortar cubes without the

addition of bacteria served as control. After being demolded, all cubes were cured in corresponding medium, i.e., CSL and NB media for 28 days for surface treatment. The cement mortar specimens were dipped in water of height 10 ± 1 mm. At regular time intervals (0.25, 0.5, 1, 1.5, 3, 5, 8, 24, 72, 96, 120, 144, and 168 h), the specimens were removed from the water and weighed, after drying the surface with a dry towel. Immediately after the measurement, the test specimens were submerged again. The sorptivity coefficient, k (cm $s^{-1/2}$), was obtained by using the following expression:

$$Q/A = k\sqrt{t}$$

where Q is the amount of water absorbed (cm^3), A is the cross section of the specimen that was in contact with water (cm^2), and t is the time (s); Q/A was plotted against the square root of time to calculate k from the slope of the curve.

Water impermeability test

For use in the water impermeability test, concrete cubes of dimension 150 mm (M20 grade) were prepared with grown culture of Bp M-3 in both media separately, and all specimens were cured in the corresponding media at room temperature as mentioned above. After being cured, all the specimens were air-fried for 12–16 h and firmly secured in position in the impermeability test apparatus (AIMIL India Ltd, Delhi, India) and analyzed as per DIN 1048. Atmospheric pressures of 1, 3, and 7 bar were applied sequentially for 24 h each. At the end of the test, each specimen was removed and split into two halves for determination of the water penetration depth. Resistance to penetration is a measure of impermeability of concrete.

Rapid chloride permeability test

This test was conducted in accordance with ASTM C1202 by using the PROOVE'it instrument (Denmark). It measures the ease with which concrete allows electric charge to pass through and gives an indication of its resistance to chloride ion penetration. Cylindrical concrete samples of diameter 100 mm and thickness 50 mm were prepared with and without *S. paterurii* (Bp M-3). The ratio of cement/sand/coarse aggregate was 1:1.32:3.29. The water to cement ratio as well as bacterial culture (10^7 cells/ml) to cement ratio was maintained at 0.47. After being demolded, all samples were cured in corresponding medium, i.e., CSL and NB media for 28 days followed by chloride permeability test. After being cured, all the samples were air-fried for 12–16 h and subjected to a potential difference of 60 V. The total charge that passed through the samples was determined (expressed in terms of coulombs) at the end of 6 h. Chloride penetrability is directly proportional to the charge passed. A reduction in this total charge value indicates better resistance to chloride ion penetration and lower permeability.

Statistical analysis

The data were analyzed by analysis of variance (ANOVA), and the means were compared by Tukey's test. All the analyses were performed by using GraphPad Prism (4.1) software.

Results and discussion

Microbial cementation

Sporosarcina pasteurii (Bp M-3) precipitated a significant level of calcite in both NB and CSL media. It produced 1.55 mg calcite/cell dry mass (mg) in CSL medium, while calcite production was 1.3 mg in NB medium. All sand columns prepared with *S. pasteurii* (Bp M-3), using CSL and NB media were found to be tightly packed except the control sand column (after removing the plastic, it lost its form and collapsed). The flow rate of media was measured in the sand columns for 10 days. It was clearly observed that flow rate changed over time as cementation and pore plugging progressed in the case of columns containing bacterial cells. The initial flow rate was recorded as 15.6 ml/min, while at the end of 10 days it was 13 ml/min in the case of control column. The flow was completely clogged at day 8 in the case of the column fed with CSL medium, while for NB medium complete clogging occurred at day 10 in bacteria-treated columns (Fig. 1). All sand columns with bacterial cells were found to be tightly packed regardless of the media used (Fig. 2). The influence

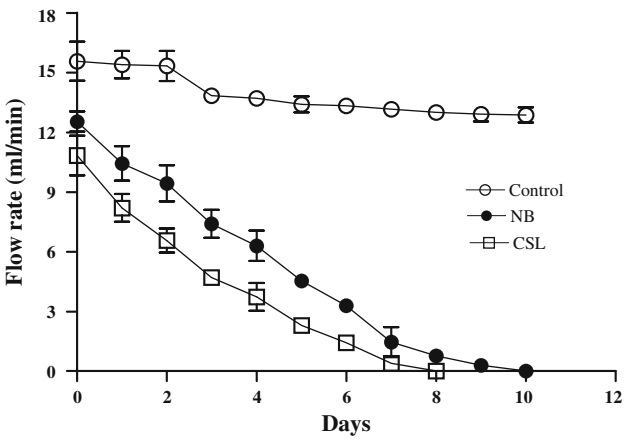

Fig. 1 Flow rate of media from sand columns with respect to time plugged by *Sporosarcina pasteurii* (Bp M-3)

Fig. 2 Microbial sand plugging formed by *Sporosarcina pasteurii* (Bp M-3)

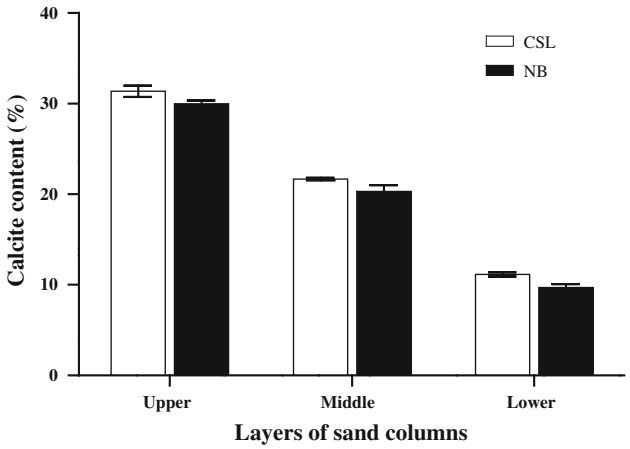

Fig. 3 Calcite content in different layers of sand columns consolidated with *Sporosarcina pasteurii* (Bp M-3) in CSL and NB media

of microbial cementation on granular behavior is dependent on the ability of microbes to freely move throughout the pore space and on sufficient particle to particle contact [8]. The calcite content was measured at three levels of the column. It ranged between 33% at top level and 7% at the bottom (Fig. 3). Calcite precipitation occurs predominantly in the areas close to the surface of the sand column. This is due to the fact that the facultative anaerobic bacterial cells like Bp M-3 grow at a higher rate in the presence of oxygen and consequently induce active precipitation of $CaCO_3$ around the surface area [3, 22]. While comparing the

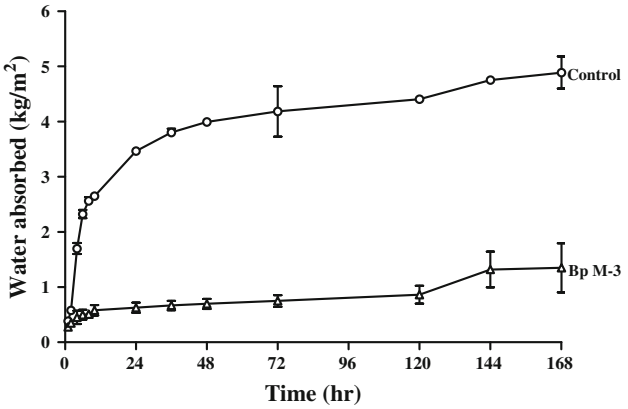

Fig. 4 Influence of microbially induced calcite precipitation on the rate of water absorption versus time for mortar cubes

media, on calcite precipitation in sand, we found that the samples with CSL as nutrient source had marginally higher content of calcite compared with the standard nutrient broth (Fig. 3). These results suggest that CSL can be used as an alternative nutrient source for biocalcification; such a replacement can dramatically reduce the process cost. Moreover, a potential environmental hazard is recycled beneficially.

Water absorption test

Figure 4 shows the influence of the microbially induced calcite precipitation on the water absorption rate in mortar cubes. Over a period of 168 h, the cubes with bacterial cells (Bp M-3) absorbed nearly five times less water than the control cubes. Presence of bacteria resulted in a significant decrease of the water uptake compared with control specimens. Similar observations were also made by De Muynck et al. [10].

The deposition of a layer of calcium carbonate crystals on the surface resulted in a decrease of the permeation properties. As a consequence, the ingress of harmful substances may be limited. Nemati and Voordouw [18] noticed a decrease in the permeability of sandstone cores after injecting $CaCO_3$-forming reactants. From these experiments, it is clear that the deposition of a layer of carbonate crystals on the surface by bacterial isolate has the potential to improve the resistance of cementitious materials towards degradation processes.

Water impermeability test

The water impermeability test results for concrete are presented in Table 1. These results indicated that the permeability of the concrete cubes prepared with bacterial cells was lower than that of the control irrespective of media used, with CSL being more effective than standard

Table 1 Water penetration in concrete prepared with bacterial cells in different media

Sample	Top penetration (mm)	Side penetration (mm)
Control	32.67 ± 2.52a	40.00 ± 3.00a
Bp M-3 (in CSL)	8.22 ± 1.79c	17.47 ± 2.33c
Bp M-3 (in NB)	10.83 ± 2.64b	24.74 ± 3.25b

Values bearing a different letter in the same column are significant at $P < 0.05$. All values are mean ± SD ($n = 3$)

NB media in the case of the bioremediated cubes. The lower permeability of the bioremediated cubes was probably due to a denser interfacial zone formed because of calcite precipitation between the aggregate and the concrete matrix compared with that of the control. The resistance to water is measured as penetration depth at the top and the sides of the concrete cubes. The penetration at the sides is higher than that at the top. This is due to better compaction and closing of pores at the top. Bacterial intervention dramatically reduced the depth of penetration regardless of the media. This demonstrates the profound effect of the proposed process on the permeability of concrete. This can be extremely beneficial in remediating porous or deteriorated concrete.

Research has indicated that a concrete which is low in permeation properties lasts longer without exhibiting signs of distress and deterioration [19]. Table 1 shows that the bacterial intervention was far more effective on the top than on the sides. Understandably, calcite precipitates better on the top surface because of gravity. Consistent with previous tests, biocalcification using CSL showed better performance than the standard media.

Rapid chloride permeability test

ASTM C1202-05 specifies the rating of chloride permeability of concrete based on the charge passed through the specimen during a 6-h testing period. The resistance of concrete to chloride penetration increased with microbially induced calcite precipitation. The permeability class type was "moderate" for control concrete specimens, whereas when specimens were treated with bacterial cells, the class changed to "low" irrespective of media used. For control samples, the average charge passed was 3,177 C, whereas for samples prepared with bacterial cells in NB and CSL media it was 1,019 and 1,185 C, respectively. Thus, bacterial intervention dramatically reduces the chloride permeability of concrete. Permeability of concrete depends on the pore structure of concrete, while electrical conductivity or resistivity of concrete is determined by both pore structure and the chemistry of the pore solution. The results appear to be consistent with water impermeability tests. Thus, bacterial deposits were beneficial in reducing both permeability and porosity.

De Muynck et al. [9] analyzed the cost of biodeposition treatment which mainly depends on the price of the microorganisms and the price of the nutrients. The calculated price of 1 kg lyophilized bacteria was about US $1,500 (1,100 €) and 2–3 g per m^2 is applied which costs about US $4 (3 €) per m^2. The cost of nutrients is estimated to be about US $250 (180 €) per kg. The dosage for biodeposition on concrete surface generally ranges between 0.04 and 0.08 kg per m^2, bringing the cost of nutrients to US $7–15 (5–10 €) per m^2. For a successful commercial process the cost of nutrients is very high. However, in the present study we performed the biocalcification using an industrial effluent which is otherwise a potential environmental hazard. Corn steep liquor can typically be available locally with a price of nearly US $2 (1.5 €) per liter, which is very economic compared with standard nutrient medium and this brings the biodeposition cost to US $0.5–1.0 (0.3–0.7 €) per m^2. The performance of CSL was significantly better than standard laboratory nutrients in terms of microbial concrete production. Hence, CSL offers an economic advantage over the standard nutrient medium and the overall process cost reduces dramatically.

Conclusion

This identified the positive effects of *S. pasteurii* (Bp M-3), calcifying bacteria, on the permeability of concrete. Bacterial deposits increased the impermeability to both water and other aggressive substances. This paper also demonstrates the use of industrial wastes such as corn steep liquor as the nutrient source for the bacteria. The development of the microbial addition will provide the basis for an alternative and high-quality concrete sealant that is cost effective and environmentally safe, and ultimately lead to enhancement in the durability of building materials.

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