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SHORT COMMUNICATION

Biocalcification by *Bacillus pasteurii* urease: a novel application

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Abstract Biocalcification, also known as microbiologically induced calcite precipitation (MICP), is a phenomenon involving the activity of the enzyme urease. A large number of soil microorganisms exhibit urease-producing ability. A novel application of MICP to improve properties of bricks by a soil bacteria *Bacillus pasteurii* NCIM 2477 was studied. Most of the deterioration of brick structures takes place because of the presence of moisture. Deposition of calcite on the surface and in voids of bricks reduces the water absorption substantially. A favorable effect of microbes to improve the durability of bricks by reducing water absorption was demonstrated as a novel concept in this paper.

Keywords *Bacillus pasteurii* · Microbiologically induced calcite precipitation (MICP) · Urease · X-ray diffraction (XRD)

Introduction

Biocalcification is a process in which calcite is formed in the soils or civil structures due to action of microbes, especially urease-producing organisms [12]. This phenomenon, known as microbiologically induced calcite precipitation (MICP), is dependent on the urease enzyme activity, and a large number of soil microorganisms are found to contribute to the process. Undesirable effects of biofilm formation resulting in biodeterioration in civil works have gained attention and have been extensively studied [2, 13, 14, 18], whereas this paper reports the useful role of a urease-producing microorganism to induce biocalcification in bricks. An endospore-forming soil microorganism, *Bacillus pasteurii*, has been used as the urease producer. A few studies on calcite precipitation for strengthening cement concrete [1], plugging of sand [16], remediation of cracks in granite [7] and ornamental stone [4] have been reported. Bricks are the basic building blocks for many civil structures; however, to the best of our knowledge, there is no work reporting on improvement in properties of bricks by MICP. This paper reports this novel application. The concept of microbial calcite precipitation was investigated in bricks. The high porosity of bricks allows the penetration of water along with ions, such as chlorides, having a detrimental corrosive effect. A significant reduction of the water absorption capacity of bricks because of microbial calcite precipitation showed encouraging results.

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Methods

Screening of the microbial cultures for urease production

Bacillus pasteurii NCIM 2477, *Brevibacterium ammoniagenes* ATCC 6871 and *Bacillus lentus* 2466-NCIB 8773, procured from the National Collection of Industrial Microorganisms (NCIM) Pune, were used in screening studies to select the maximum urease producer. The experiment was carried out by inoculating 50 ml of nutrient broth (NB) in a 250-ml Erlenmeyer flask with a 2% cell suspension of the respective culture, having an optical density (OD) of 1.5 at 660 nm. This OD was maintained for all further experiments unless otherwise mentioned. These cultures were maintained on nutrient agar slants, stored at 4°C in a refrigerator and subcultured every month.

Microbial calcite deposition in bricks

Bricks were procured from a construction site (Parel, Mumbai), and plastic containers were purchased from a local shop. Bricks were oven dried at 100°C for 6 h, allowed to cool at room temperature and weighed.

Nutrient broth and brain heart infusion (BHI) media were used for this study. A seed culture was prepared having an OD of 1.1 at 660 nm using the respective media. The media were inoculated, and the dried bricks were immersed in the inoculated medium. After 24-h incubation, membrane sterilized urea–$CaCl_2$ (2% urea, 0.3% $CaCl_2$) solution was added aseptically to the medium. After 4 weeks, bricks were removed, dried at room temperature, weighed and tested for water absorption capacity. For control, bricks were cured in water for 4 weeks. Calcium carbonate precipitation was confirmed by XRD analysis of the precipitate collected from the tubs with NB and BHI.

Water absorption test

The bricks were cured for 4 weeks, saturated overnight in water and weighed. The bricks were then dried in an oven at 100°C for 24 h, cooled and weighed again. Water absorption was calculated by using following formula:

$$\%\ \text{Water absorption} = \frac{(W_{\text{saturation}} - W_{\text{Oven dried}})}{W_{\text{Oven dried}}} \times 100 \qquad (1)$$

where $W_{\text{saturation}}$ is the weight of bricks after saturation in water for 24 h, and $W_{\text{Oven dried}}$ is the weight of bricks after oven drying for 24 h.

Results and discussion

Biocalcification or MICP has been proposed as a novel strategy for strengthening and remediation of several civil structures. It involves the breakdown of urea into ammonia and carbon dioxide by the enzyme urease, with the resultant precipitation of carbonate ions as calcium carbonate. Such a novel application illustrates the need to enhance urease production by various methods using these microorganisms.

Urea is widely distributed in nature [3, 17]. Urease (urea amidohydrolase—EC 3.5.1.5) catalyzes the hydrolysis of urea to ammonia and carbon dioxide; it is synthesized by plants, algae, fungi and bacteria [6, 9, 15]. It is a nickel-based metalloenzyme first isolated from seeds of the jack bean plant in 1926 [5]. Most of the studies have utilized urease obtained from the jack bean, which is expensive, emphasizing the need to obtain urease from a non-conventional, unutilized and cheaper source for versatile applications [10]. Hydrolysis of one molecule of urea results in the release of two molecules of ammonia and one molecule of carbon dioxide [11].

Urease-producing soil bacteria catalyze hydrolysis of urea to produce ammonia and CO_2. The enzymatic hydrolysis of urea is generally described as [9]:

$$H_2N\text{–}CO\text{–}NH_2 + H_2O \xrightarrow{\text{Urease}} 2NH_3 + CO_2 \qquad (2)$$

The ammonia that is released by urea hydrolysis results in an increase of pH in the surrounding medium wherein supplemented mineral ions (Ca^{2+} and ${CO_3}^{2-}$) may precipitate out as $CaCO_3$. This process of precipitation is a complex mechanism and is a function of the cell concentration, ionic strength and pH of medium. Thus, the media for the growth of the microorganism are supplemented with a calcium source, such as calcium chloride, which is precipitated as calcium carbonate by the following complex set of reactions:

$$Ca^{2+} + \text{Cell} \rightarrow \text{Cell–}Ca^{2+} \qquad (3)$$

$$Cl^- + HCO_3^- + NH_3 \rightarrow NH_4Cl + CO_3^{2-} \qquad (4)$$

$$\text{Cell–}Ca^{2+} + CO_3^{2-} \rightarrow \text{Cell–}CaCO_3 \downarrow \qquad (5)$$

Screening of the urease producer

From the three standard microbial cultures used for the screening, it was found that *B. pasteurii* showed the maximum urease production (Fig. 1) and was hence used for further studies.

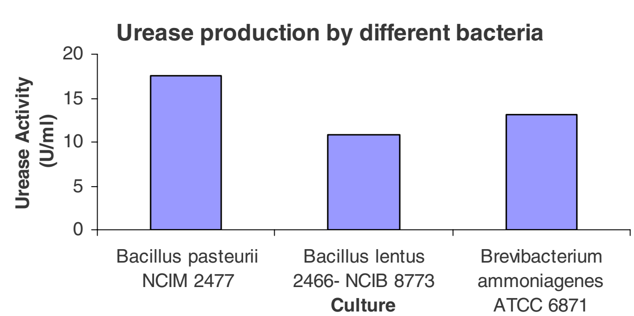

Fig. 1 Urease production by different bacterial cultures

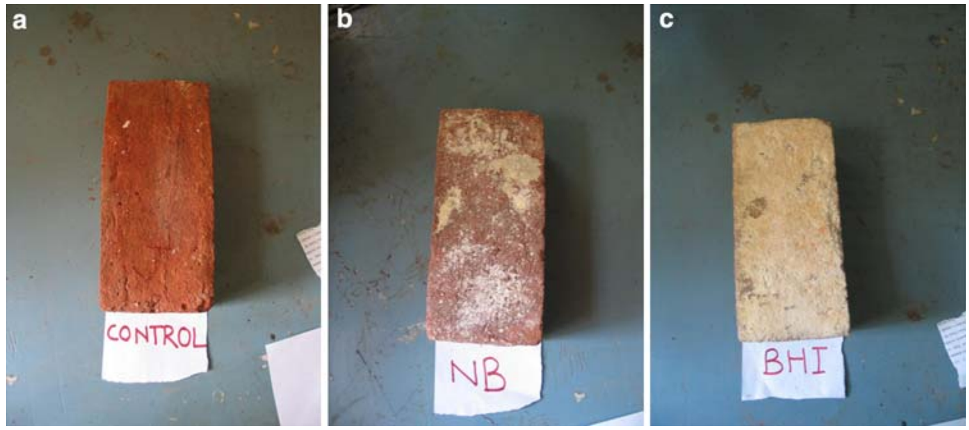

Fig. 2 Microbially induced calcite deposition in bricks. **a** Control, **b** NB and **c** BHI

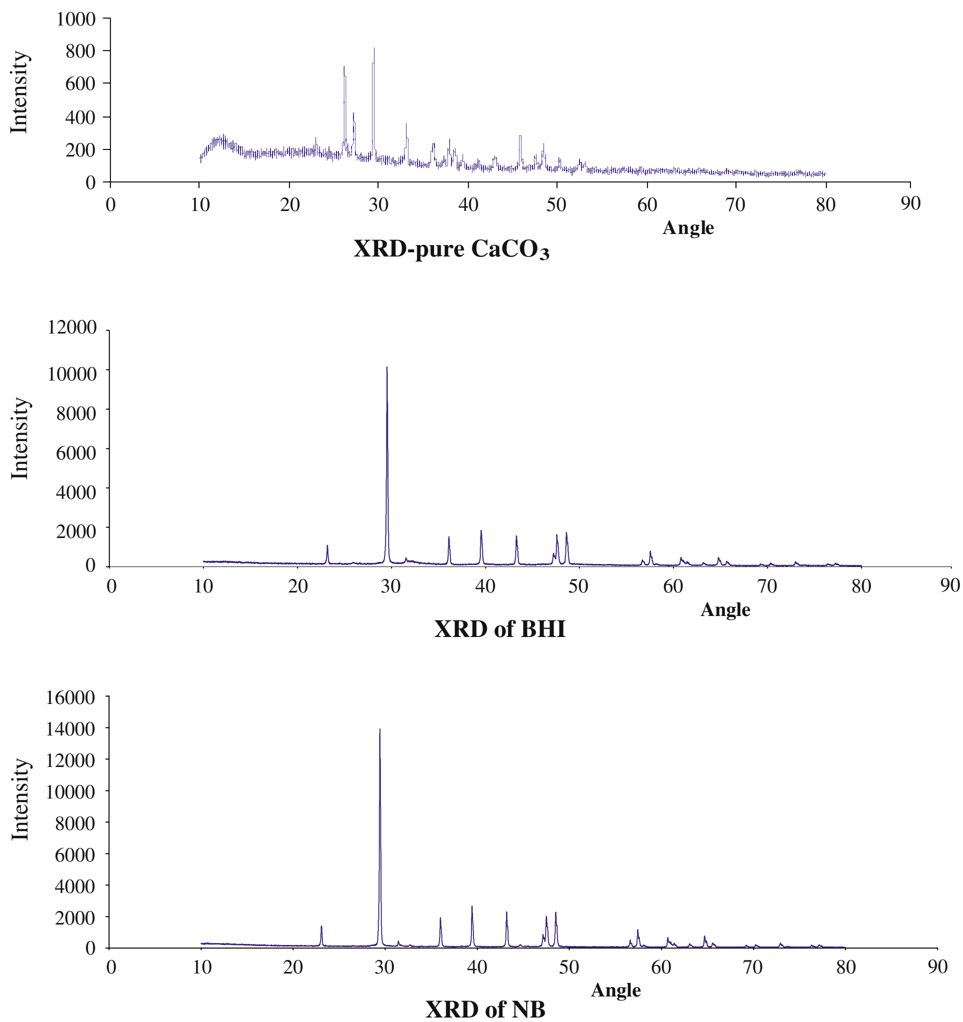

Fig. 3 XRD analysis of laboratory grade $CaCO_3$ and precipitate from a tub containing BHI and NB

Microbial calcite deposition in bricks

Microbial metabolic activities often contribute to selective cementation by producing relatively insoluble organic and inorganic compounds. Calcite precipitation is one such process that is induced due to microbial metabolic activities. Urease plays a key role in the deposition of the calcite in the form of precipitate. A layer of white deposit was clearly observed on the surface of the bricks (Fig. 2) as well as on the inner surface of the curing tub. The precipitates were characterized by XRD (Fig. 3; Table 1) by using calcium carbonate as standard. It can be clearly

Table 1 Comparative study of '*d*' spacing and its relative intensities obtained from X-ray diffractogram of laboratory-grade $CaCO_3$, calcite and precipitate from BHI and NB media

Lab $CaCO_3$		Calcite		BHI		NB	
d Spacing	I/I_0	*d* Spacing	I/I_0	*d* Spacing	I/I_0	*d* Spacing	I/I_0
3.03541	100	3.04	100	3.02595	100	3.02411	100
1.87735	15.5	1.88	19	1.87.197	15.62	1.87064	18.65
2.10370	7.06	2.10	21	2.08844	14.9	2.08975	13.86
2.28742	6.25	2.29	24	2.27778	17.72	2.27829	16.69
1.97826	20.3	1.92	16	–	–	1.90864	22.61

Table 2 Water absorption test for bricks

Brick samples	% Water absorption	% Reduction in water absorption
Control	24.97	–
NB	21.47	14.1
BHI	13.84	44.5

seen from the XRD graphs that all the major peaks were at the same 2θ. The spectra when compared with the standard JCPD files showed that microbial calcium carbonate precipitation in both the media was in the form of calcite. Detailed study of carbonate bio-mineralization by XRD and SEM also indicated that calcite is the dominant mineral phase form when the bacteria are present [8]. Brick samples were analyzed for their water absorption capacity, and the results are depicted in Table 2. It can be seen that the absorption of water by bricks cured in media was lower than that by a control sample, indicating that pores on the surface are blocked because of calcite deposition, thus preventing water and other pollutants from penetrating into the body of bricks. Thus, this process of bio-mineralization retarding the water absorption in bricks may be effective in enhancing the durability and longevity of civil structures, and it may be of immense help in the preservation of monumental structures constructed with bricks.

Conclusions

Microbiologically induced calcite precipitation was found effective in increasing brick strength by reducing water absorption up to 45%. Among different microbial cultures, *B. pasteurii* NCIM 2477 was found to be the highest producer of urease and was an effective inducer of calcite deposition on the surface of the bricks. The reduction of water absorption was attributed to biocalcification on the surface, leading to a reduction in permeability and a subsequent decrease in the diffusion of water and other corrosive ions. The water absorption capacity of bricks was lowest for BHI-cured bricks (~14%) compared to the control samples (25%). However, use of BHI on a large scale would make the process very costly, showing the need to develop cheaper media that also enhance the production of urease. In conclusion, biocalcification by *B. pasteurii* urease would be an effective method for increasing the durability of bricks. However, more work is needed for developing a cost-effective process.

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