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Significant indicators for biomineralisation in sand of varying grain sizes

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HIGHLIGHTS

- Rate of MICP in sand of varying grain sizes is reported.
- Rate of deposition varies greatly with sizes of sand particles.
- pH and electrical conductivity can serve as indicators for onset of MICP.
- MICP inside the grains can be calibrated to unutilized urea and calcium ions.
- Approximate expressions for carbonate deposition have been reported.

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ABSTRACT

Microbially induced calcium carbonate precipitation (MICP) is emerging as a sustainable technology for improved construction materials. In this technique, calcium carbonate is deposited in the pores of substrates such as sand and concrete. As the technology moves from laboratory to field new metrics and field measurement techniques are required to ensure that the results achieved at the laboratory are replicated. In the scaled up technique it is not possible to collect samples from deep inside the substrate for direct measurement. This paper explores some indirect indicators of MICP that can be easily monitored in large scale applications. Bacterial fluid has been passed through sand columns of varying grain sizes. The rate of flow is monitored for ten days. The effluent has been tested for pH and electrical conductivity to evaluate their potential for ensuring MICP. The residual urea and calcium in the effluent has been monitored to have a quantitative estimate of MICP. At the end of the tests the quantity of carbonate deposited in the sand columns is measured. A correlation between the residual reactants and the carbonate deposition is developed. An approximate expression for different grain sizes has been developed. This study demonstrates the utility of indirect measurement techniques as flow rate, pH, conductivity, urea and calcium consumption as potential indicators of efficient MICP in sand media.

1. Introduction

Research on microbially induced calcium carbonate precipitation (MICP) is gaining momentum in civil engineering. Taking inspiration from examples in related areas such as geological formations [1] and repair of limestone monuments [2] civil engineers have explored MICP as a means of improving materials of construction that are either granular [3] or porous [4]. There can be mainly two types of benefits of MICP – (1) stabilisation of granular materials by binding the grains together through the deposition of mainly calcium carbonate (with some variations of calcium compounds) in the inter-granular spaces [5–11]; and (2) MICP in a porous material such as concrete to alter its pore structure and impede diffusion of deleterious substances into it [12–16]. Understandably, there is considerable overlap in the two lines of research. Some applications in granular materials also attempt to alter diffusion paths [17] and some investigations on porous media have reported gain in strength [5,7,18]. The main advantage of the technique over established methods is that unlike other binders (such as cement), MICP takes place in ambient conditions with little consumption of fuel or emission of greenhouse gases. Moreover, some of the chemical agents (synthetic polymers, for example) can be toxic. MICP, on the other hand, uses benign soil bacteria with no risk of toxicity.

In case of porous monolithic materials such as limestone, mortar and concrete, MICP is performed either immersing the substrate in the bacterial fluid or the fluid is sprayed on the surface. Understandably, MICP remains restricted within a few millimetres

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from the surface. Several experiments have reported that MICP has reduced water absorption [14], moisture permeability [3], chloride ion permeability [16], drying shrinkage [4], reinforcement corrosion [19–21], and freeze thaw cracking [11,13]. Ramakrishnan et al. [4] found increase in resistance of concrete to alkali, freeze thaw attack, drying shrinkage and reduction in permeability upon application of bacterial cells. De Muynck et al. [12] enhanced the permeability characteristics of mortar by *B. sphaericus* cells. Achal et al. [16] treated mortar cubes with *Bacillus* sp. CT-5 and reported nearly six times less absorption of water as compared to untreated specimens. They also studied the effect of *Bacillus pasteurii* on water permeability in concrete cubes and found the reduction in penetration of water was more significant on the top as compared to sides. Ramchandran et al. [22] observed the increase in compressive strength of cement mortar cubes at 7 and 28 days by using various concentrations of *B. pasteurii*. They found that increase in strength resulted from the presence of adequate amount of organic substances in the matrix due to microbial biomass. Ghosh et al. [23] studied the positive potential of *Shewanella* on compressive strength of mortar specimens and found that the greatest improvement was at cell concentration of 10^5 cells/ml for 3, 7, 14 and 28 days interval. They reported an increase of 17% and 25% after 7 and 28 days. In case of granular material the bacterial fluid can flow through the inter-granular spaces. Thus, MICP can reach deep inside the material. Laboratory experiments have demonstrated the potential of MICP in improvement of soil strength [3,7,24], reduction of permeability [25], stabilization of slopes [6,26], improved resistance to liquefaction [27] and also suppression of dust [28]. MICP forms "cohesive bridges" between grains of sand through the deposition of calcium carbonate crystals increasing the stiffness of the material and it also reduces permeability [10].

After the success of laboratory scale experiments in-situ soil strengthening at the field (bio-grouting) has been attempted where the biological fluid is injected in the soil. It has been observed that although the technique works well for surface treatments, coarse grained materials and mixed-in-place applications, but in case of fine grained materials the injection well clogs rapidly [3]. The optimum pressure to avoid clogging has been investigated and it was observed that a flow rate of 350 ml/h could avoid clogging of a 5 m long tube [7]. Harkes et al. [25] reported that injection of bacterial fluid followed by cementation fluid gave a more homogeneous distribution of bacterial activity and calcite precipitation. Van Paassen et al. [10] observed that in large scale experiments (treatment volume of up to 100 m^3), use of bio-grouting is technically feasible under conditions common to what is found in practice [26]. Large scale laboratory tests on dike reinforcement using biocementation have already been carried out. Burbank et al. [27] reported formation of around 1% $CaCO_3$ in near surface and 1.8–2.4% calcite below 90 cm upon subjecting the soils on the shore Snake river, USA with ureolytic microbes. Chu et al. [28] and Stabnikov et al. [29] reported considerable reduction in permeability, improved shear strength of soil and reduction of seepage rate due to formation of impermeable microbial carbonate crust. Bang et al. [30] recently showed the potential of MICP by ureolytic bacteria to suppress dust. In case of sand plugs, Kantzas et al. [31] reported that sand consolidation by *B. pasteurii* reduced porosity by up to 50% and permeability by up to 90% in the areas where cementation took place.

In field applications it is imperative to ensure that MICP happens in the desired depths of the granular material. However, it is not feasible to collect samples from deeper areas and test the amount of deposition. Thus, laboratory experiments must identify reliable indirect measurement techniques. This paper explores a few indicators of MICP in a granular substrate. It is already mentioned that the bacterial fluid is passed through the granular material for MICP. When the fluid has passed through the grains and leaves the media it can be collected easily. The effluent can be analysed for estimating MICP inside the media. This paper attempts to arrive at two indicators from the analysis, (a) confirmation of MICP happening and (b) quantity of deposition. For both goals simple chemical analyses that can be easily performed at site have been adopted. To understand the principle a brief description of the process of MICP is included.

1.1. MICP

Although there are a few variations, the popular process in MICP needs an ureolytic bacterium, urea, calcium chloride and nutrients. In the presence of ureolytic bacteria, 1 mol of urea is hydrolysed intra-cellularly to 1 mol of ammonia and 1 mol of carbonate (Eq. (1)), which spontaneously hydrolyses to form additional 1 mol of ammonia and carbonic acid (Eq. (2)) [32].

$$CO(NH_2)_2 + H_2O \xrightarrow{\text{bacteria}} NH_2COOH + NH_3 \quad (1)$$

$$NH_2COOH + H_2O \rightarrow NH_3 + H_2CO_3 \quad (2)$$

These products equilibrate in water to form bicarbonate, 1 mol of ammonium and hydroxide ions, which raise the pH.

$$H_2CO_3 \rightarrow 2H^+ + 2CO_3^{2-} \quad (3)$$

$$NH_3 + H_2O \rightarrow NH^{4-} + OH^- \quad (4)$$

In the presence of calcium ions this results in calcium carbonate precipitation (Eq. (5)), once a certain level of super-saturation is reached

$$Ca^{2+} + CO_3^{2-} \rightarrow CaCO_3 \ (K_{SP} = 3.8 \times 10^{-9}) \quad (5)$$

K_{SP} is the solubility product in Eq. (5).

Deposition of calcium carbonate can happen in the inter-granular spaces or in the pores. Justifiably, the bacterial media must have access to the site for a successful deposition at the desired location. The dichotomy is that the solution that is supposed to block the gap must use the same gap to reach the site of deposition. Due to deposition, the gaps are likely to get progressively narrower. As a result, flow through them will reduce. Clearly, the topological distribution of grains plays a very important role. This paper investigates the rate of deposition in a sand media of varying grain sizes. It also demonstrates the efficacy of indirect indicators such as consumption of urea (Eq. (1)) and that of calcium (Eq. (5)) for estimating MICP inside the grains.

2. Experimental and methods

2.1. Microbial growth conditions

Alkaliphilic strain of urease producing bacteria *Bacillus megaterium* (SS3) isolated from calcareous soils (pH 10.5) collected from Anantapur district of Andhra Pradesh, India was used in this study. This strain was selected because of its efficacy to produce high amounts of urease (UA) and carbonic anhydrase (CA) in the experimental system [33,34]. Following [8], the strain was maintained in the nutrient broth (Peptone 10 g/L, Beef extract 10 g/L, Sodium chloride 5 g/L) prior to use. To perform calcium carbonate precipitation, the culture was grown in nutrient broth (Himedia, India) supplemented with filter sterilized 2% urea (w/v) and 25 mM $CaCl_2$ (Calcification media). The pH of the media was adjusted to 6.5 with 1N HCl prior to autoclaving without urea and $CaCl_2$. Filter-sterilized urea and $CaCl_2$ was added later in the experiment with a final pH of 8.

2.2. Preparation of sand columns

Locally available clean, dry, well graded, natural river sand was used in the present study (Table 1). The sand was sieved through varying sized meshes (0.1 mm, 0.2 mm, 0.5 mm, 0.75 mm, 1 mm, 1.5 mm, 2 mm) and each fraction was collected separately. 0.2, 0.5, 1 and 1.5 mm fractions were autoclaved in order to remove the indigenous bacterial flora and this process was repeated thrice at an interval of 24 h [35]. *B. megaterium* SS3 was inoculated into 500 ml conical flasks containing 100 ml Nutrient broth media supplemented with 2% urea (pH 8·0) and incubated