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Investigation into the optimal bacterial concentration for compressive strength enhancement of microbial concrete

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HIGHLIGHTS

- Significant calcite precipitation obtained without external calcium source.
- Optimum compressive strength enhancement at intermediate cell concentration.
- Crack healing and water absorption most efficient at highest cell concentration.
- Reasons for the existence of the optimum concentrations provided.

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ABSTRACT

The efficient crack healing capability of microbial concrete leads to an improvement in its various mechanical properties such as compressive strength, water absorption and water permeability. Studies on microbial concrete have reported that the enhancement of the compressive strength is maximum at a particular bacterial concentration, which is not necessarily the highest amongst the considered levels of bacterial concentrations. So far, the reason for the existence of such an optimal bacterial concentration for the increase in the compressive strength of concrete remains unexplored. In this paper, an attempt has been made to establish the cause of the presence of this optimal bacterial concentration. Three different bacterial concentrations of Bacillus *subtilis* have been used in this study, namely 10^3 cells/ml, 10^5 cells/ml and 10^7 cells/ml of water. Results indicate that though the higher bacterial concentration of 10^7 cells/ml is more efficient for crack healing, the best performance in compressive strength enhancement is achieved with the bacterial concentration of 10^5 cells/ml. It is seen that for a given bacterial type and mortar mix, the different calcite precipitation patterns inside the mortar matrix at varying levels of bacterial concentrations constitute the reason for the existence of the optimal bacterial concentration for compressive strength enhancement.

1. Introduction

The prime strategy of microbial concrete relies on microbial induced calcite precipitation at the cracks, thereby precluding the deterioration of concrete, as well as of reinforcement, due to ingression of harmful substances like chloride, sulphate, moisture, etc. Amongst the various aspects of microbial concrete, the thrust of research so far has chiefly been on the types of bacteria used for crack healing in concrete [1–7], on the survivability of bacteria inside the concrete matrix [8–10] and on the compatibility of bacteria with different types of cement replacing material in concrete, namely flyash, cement kiln dust etc. [11,12].

Since healing of micro-cracks enhances the compressive strength and other mechanical properties of concrete, some researchers have also been investigating the effect of the addition of bacteria on the compressive strength of concrete. Generally, strength enhancement has been reported [13–16], though reduction in strength has also been obtained [17]. A review of these studies indicates that researchers who have dealt with strength enhancement have considered bacterial concentrations in the range of 10^3–10^7 cells/ml, whereas researchers who have focused more on crack healing usually have used higher bacterial cell concentrations (10^7–10^9 cells/ml). Various studies have also reported the existence of an optimum bacterial concentration for maximizing the compressive strength of microbial concrete. Though it is evident that higher concentration of bacteria leads to higher calcite precipitation [18], it is seen that the optimum concentration for compressive strength enhancement is not necessarily the highest

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considered cell concentration. Ghosh et al. [13] investigated seven different bacterial concentrations of the *Shewanella* species (10–10^7 cells/ml) and obtained the optimal bacterial concentration to be 10^5 cells/ml, with a corresponding 25% increase in compressive strength. Chahal et al. [16] used three bacterial concentrations (10^3, 10^5 and 10^7 cells/ml) and found that the maximum increment of 22% in compressive strength of flyash concrete was also attained at the cell concentration of 10^5 cells/ml of Sporoscarcina *pasteurii*. Sarkar et al. [19] showed that even for the genetically modified Bacillus *subtilis*, the maximum compressive strength was achieved at the cell concentration of 10^5 cells/ml. Further, Andalib et al. [14] used five cell concentrations (viz. 10×10^5–50×10^5 cfu/ml) of Bacillus *magaterium* and reported that the optimum concentration for strength enhancement was at 30×10^5 cfu/ml. Thus in all these works, the maximum compressive strength enhancement was attained at an intermediate value of the range of considered bacterial concentrations. Apart from the above, in a single instance as reported in literature, the optimum concentration for compressive strength enhancement was obtained at the highest considered level of bacterial cell concentration. Kumari et al. [20] used three different cell concentrations (10^5, 10^5 and 10^7 cfu/ml) of Bacillus *conhii* and found that the maximum strength enhancement of 49.18% occurred at the bacterial cell concentration of 10^7 cells/ml.

Several researchers who have focused on crack healing with a single high bacterial concentration have also presented results on the compressive strength of the microbial concrete samples. Khaliq et al. [5] reported a 12% increase in the compressive strength by using lightweight aggregate with Bacillus *subtilis* at bacterial cell concentration of 3×10^8 cells/cm^3, while Jonkers et al. [17] stated a 10% decrease in the compressive strength at 6×10^8 cells/cm^3 of bacterial spores. These results indicate that high cell concentrations have a negative impact on the enhancement of compressive strength in microbial concrete.

It is thus observed that the optimum bacterial concentration for the increase in compressive strength lies between 10^5–10^7 cells/ml for all considered bacteria, whereas enhanced crack healing occurs at higher bacterial cell concentrations of 10^8–10^9 cells/ml. Further, the rate of calcite precipitation is dependent upon the type of bacteria and the concentration of the bacteria. However, the reason for the reduction of compressive strength at higher bacterial concentrations is not currently understood. It is true that the main purpose of microbial concrete is crack healing, but it should be without adversely affecting the compressive strength. Therefore, it is necessary to identify the reason for the existence of the optimal concentration for compressive strength enhancement of microbial concrete, which can lead to an appropriate selection of bacterial concentration as per the requirement. An external calcium source like calcium lactate, calcium oxide, calcium glutamate etc. is often used to enhance the self-healing efficiency of microbial concrete [17,21,22]. However, various researchers have also obtained improvement in the mechanical properties of concrete by the addition of bacteria alone [13,23].

The aim of the current study is to explore the reasons behind the existence of an optimum bacterial concentration for the enhancement of compressive strength of microbial concrete. To avoid the influence of any external effects on the precipitation of bacteria as well as on the mechanical properties of concrete, no external calcium source has been used here. In this study, firstly, the effect of three different bacterial concentrations on the compressive strength and water absorption of mortar samples is examined. This is followed by an investigation of the calcite precipitation on the mortar cube surface, at different bacterial cell concentrations, through crack healing analysis and surface pore-healing analysis. Next, a water penetration test is performed to comprehend the effect of bacterial concentration on moisture transport through the mortar cubes. Thereafter, Scanning Electron Microscopy (SEM) is employed to examine the calcite precipitation at the inner concrete matrix. Finally, based on the results, a schematic diagram of calcite precipitation patterns at different bacterial concentrations inside the mortar matrix is provided to explain the existence of the optimal bacterial concentration for compressive strength enhancement.

2. Materials and test methods

2.1. Materials

Bacillus *Subtilis* (MTCC 441) obtained from the Microbial Type Culture Collection and Gene Bank, India, is used in this study. The culture was grown in a nutrient broth made with Beef Extract 1.0 gm/l, Yeast Extract 2.0 gm/l, Peptone 5.0 gm/l, NaCl 5.0 gm/l and distilled water (pH = 7.0). After 5–6 days of inoculation, about 10 μl from the culture medium was taken on a Haemocytometer and counted under the microscope. This was followed by serial dilution to obtain the required bacterial concentrations. The live bacterial cells obtained from the pre-culture were added to water at different cell concentrations, namely 10^3 cells/ml, 10^5 cells/ml and 10^7 cells/ml.

A total of 123 mortar samples were prepared by using Ordinary Portland cement (OPC) of grade 43 and locally available river sand. Mortar cubes of dimension 70.6 mm × 70.6 mm × 70.6 mm were prepared for both control and bacterial mortar samples. The cement to sand ratio was taken as 1:3 (by weight) and water to cement ratio was fixed at 0.4 (by weight). The samples were removed from the moulds after 24 h and cured at room temperature (27 °C) in fresh water.

2.2. Compressive strength test

The compressive strength tests of the control and the bacterial mortar cubes were performed at the age of 3, 7 and 28 days of curing, in a 2000 kN capacity compression testing machine.

2.3. Water absorption test

For water absorption test, initially the mortar cubes were oven dried at 105 °C for 24 h and the dry weight measured ($= W_{\text{oven dried}}$). The samples were then kept in a saturated condition in water at room temperature for 24 h and weighed again ($= W_{\text{saturated}}$). The water absorption was then calculated by using the following formula.

$$\text{water absorption}(\%) = \frac{W_{\text{saturated}} - W_{\text{oven dried}}}{W_{\text{oven dried}}} \times 100 \quad (1)$$

The water absorption tests of the control and the bacterial mortar samples were performed after 3, 7 and 28 days of curing.

2.4. Self-healing study at mortar surfaces

2.4.1. Crack healing

To evaluate the self-healing efficiency at different bacterial concentrations, crack healing in microbial concrete was analysed. At 28 days of curing, the mortar samples were loaded on the compression testing machine. When visible cracks appeared on the surface, the loading was stopped. Thereafter, the widths of the cracks were measured by the crack-measuring instrument. The widths of the cracks varied from 0.1 mm to 1.2 mm. Next, the cracked samples were submerged in water. For crack healing quantification, the crack widths were measured after 3, 7 and 28 days of curing. Further, Scanning Electron Microscopy (SEM) and Energy Dispersive Spectroscopy (EDS) were carried out to examine the shape and morphology of the healing product. Additionally, X-ray Diffraction (XRD), with a Cu anode (40 kV and 40 mA) and scanning from 10° to 80° (2θ) at the rate of 0.02° s^{-1} at room temperature, was employed to analyse the chemical composition of the healing product.

2.4.2. Surface pore-healing

The effect of different bacterial concentrations on the calcite precipitation rate on the concrete surface was evaluated by analysing the healing of the surface pores of the mortar samples. This was carried out by capturing the digital surface images of the samples immediately after demoulding and again after submerging the samples in water for 7 days and 28 days. The images were analysed by pixel analysis in Matlab version R2015b. All pixels were represented by Red, Green and Blue (RGB) components where the values of the intensities range from 0 to 255. Thus, point (0, 0, 0) stands for black and (255, 255, 255) stands for white. By analysing the pixels in and around a pore as shown in the Fig. 1, it was observed that the values of the RGB components in the pore area ranges from (0, 0, 0) to (120, 120, 120). The latter set of values was fixed after carrying out pixel analysis of a large number of surface