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Microbially assisted cementation – A biotechnological approach to improve mechanical properties of cement

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HIGHLIGHTS

- A new approach to enhance mechanical properties of cement by MICCP using soil as bacterial source.
- Use of lentil seeds as a protein source instead of peptone for cost effectiveness of the technology.
- Use of sugar as a carbon source instead of glucose to make the process cost effective.

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ABSTRACT

Microbially induced calcium carbonate precipitation (MICCP) is a promising technology used in various engineering applications. Urease produced by soil microorganisms hydrolyses urea, producing carbon dioxide which forms calcium carbonate in presence of calcium ions, which gets precipitated and enhances the mechanical properties of cementitious materials. An attempt was made to enhance the properties of ordinary Portland cement (OPC) using soil microbial solution with lentil seed (*Lens culinaris*) powder as protein source and sugar as carbon source. Bio-OPC specimens using microbial solution instead of water were prepared. Significant increase in compressive strength was observed in bio-OPC specimens as compared to the specimens prepared using water.

1. Introduction

Today, due to increase in population and urbanization, the need to provide safe structure has become a necessity. There is an increasing demand of cementitious materials due to industrial development and research. Cement is a key component in the field of construction as it is used as a binding material which maintains the stability of the structure. Nowadays many costly and innovative technologies are adopted in order to repair cracks and to increase life of the buildings. Use of commercial admixtures, water repellents, epoxy, resins, synthetic fillers and other inorganic and organic materials imparts additional cost for the construction projects. These products which are commercially available have certain drawbacks like different thermal expansion rates of the product and concrete surface, time dependent deterioration and the possibility of environmental pollution [1,2].

In nature, some bacterial species have the tendency to deposit calcium carbonate ($CaCO_3$), a phenomenon called biocementation. These depositions can prove to be a promising binder for the protection and consolidation of construction materials [3,4]. Historical structures can be preserved by using the bacterial remediation technique [5].

Application of biocementation has been investigated by various researchers in order to increase the durability of cementitious materials and restoration of buildings [6]. This microbial treatment has numerous advantages over the conventional treatments such as similar thermal expansion properties of the bacterial precipitated calcite and concrete surface; commercially viable, ecofriendly characteristics and self-healing tendency [1,2,7].

Use of microorganisms in cementitious materials leading to the process of biocementation is a new area of research in the field of concrete technology which is producing positive results [8]. In a bacterial treatment when *Sporosarcina pasteurii* at different cell concentrations in mortar was incorporated, there was 33% increase in 28 days compressive strength [7]. A study was carried out in which different types of calcium sources were used for Enterobacter sp. M2 along with various curing processes. Increase

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in compressive strength by 44% and 56% in tensile strength was noticed where for 28 days compressive strength for control and experimental specimens was observed as 37.30 N/mm^2 and 54 N/mm^2. The indirect split tensile strength for 28 days for control and experimental specimens was found as 9.40 N/mm^2 and 10.50 N/mm^2 [9]. Mukherjee et al. reported that *Bacillus megaterium* has a potential to enhance the strength of energy efficient soil cement bricks. This bacterium reduces the porosity and water absorption capacity of the bricks [10].

The effect of bacterial calcite precipitation was studied by Muynck et al. on durability of mortar and concrete. Due to the bio deposition on the specimen surface, there was a capillary water uptake reduction and permeability towards gases was also reduced [2].

Kim et al. observed that in concrete specimens, the calcium carbonate crystals precipitated were denser by *B. sphaericus* when compared with *S. pasteurii* [11]. A study by Ramachandra et al. showed an enhancement of 18% in the compressive strength of the cement mortar with *S. pasteurii and Pseudomonas aeruginosa* [5].

The bacterial strains like Bacillus *pasteurii*, *Bacillus sphaericus* and *Bacillus* sp. CT-5 can seal cracks [4,6] and also can reduce the chloride permeability and porosity of the cementitious material [4].

Muynck et al. found that *Bacillus sphaericus* have the ability to precipitate calcium carbonate and improved the durability of hardened cement paste. A comparative study between this and conventional treatments was undertaken [1,2].

Lactose mother liquor (LML), which is the dairy industry effluent was used as a growth medium for *Sporosarcina pasteurii* (NCIM 2477) in a study where increment in compressive strength of cement mortar was observed [12]. In another study, dimethyl carbonate (DMC) was used instead of bacteria and their expensive nutrients, for the production of calcium carbonate to improve the properties of concrete and porous materials [13]. Dhone et al. used an alternative cost effective soil bacterial solution with two different protein sources to study the enhancement of the compressive strength of ordinary Portland cement [14].

In the study, an effort has been made to enhance the mechanical properties of cement using soil microbial solution. On the earth, the mineralization processes undertaken by bacteria are prominent everywhere [6,15]. Biocementation technique using ureolytic bacteria nowadays has become a popular method for self-healing concrete. These ureolytic bacteria can produce urease enzyme which is responsible to catalyze hydrolysis of urea thus producing carbamate and ammonia, due to which the pH of the medium increases. Various biochemical reactions occurring thereafter lead to formation of calcium carbonate as shown in Eqs. (1)–(7) [16].

Initially, in the presence of urease, urea gets hydrolyzed to form carbamate and ammonia, Eq. (1).

$$CO(NH_2)_2 + H_2O \rightarrow NH_2COOH + NH_3 \quad (1)$$

Additional one mol of carbonic acid and ammonia are formed due to hydrolyses of carbamate.

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

These products subsequently form 1 mole of bicarbonate and 2 moles of ammonium and hydroxide ions respectively.

$$H_2CO_3 \rightarrow H^+ + HCO_3^- \quad (3)$$

$$2NH_3 + 2H_2O \rightarrow 2NH_4^+ + 2OH^- \quad (4)$$

Due to the above reactions, pH is increased and because of that the bicarbonate equilibrium is shifted and carbonate ions are formed.

$$HCO_3^- + H^+ + 2NH_4^+ + 2OH^- \rightarrow CO_3^{2-} + 2NH_4^+ + 2H_2O \quad (5)$$

As the bacterial cell wall is generally negatively charged, the bacteria attract cations including Ca^{2+} from the environment which get deposited on their cell surface. Subsequently the Ca^{2+} ions react with CO_3^{2-} ions, to form $CaCO_3$ precipitation at the cell surface, which acts as a site of nucleation [16].

$$Ca^{2+} + \text{Bacterial Cell} \rightarrow \text{Bacterial Cell} - Ca^{2+} \quad (6)$$

$$\text{Bacterial Cell} - Ca^{2+} + CO_3^{2-} \rightarrow \text{Bacterial Cell} - CaCO_3 \downarrow \quad (7)$$

Significance of this research is to use ecofriendly method for enhancing the mechanical properties of cementitious materials. To improve the compressive strength of ordinary Portland cement IS 12269 (2013) [17], microbial solution has been used. The investigation has identified the effect of biomass (soil bacteria) using lentil seed (*Lens culinaris*) powder as a protein source (which contains around 25 g proteins 100 g/dry weight) [18] on the compressive strength and water absorption of ordinary Portland cement cubes. This study explores the possibility of utilizing local soil bacteria in strengthening construction materials. Also, to grow the bacteria in the laboratory, instead of the commercial peptone which is the commonly used as protein source, lentil seed powder has been used and instead of glucose as a carbon source, sugar has been used. This is done to reduce the cost of the process and make it techno-economically viable.

2. Materials and methods

2.1. Preparation of microbial solution

The microbial solution which was used in this study was prepared with the ingredients as given in the Table 1. Microbial solution was prepared in water, containing: 200 g/l Rhizospheric soil which was collected from VNIT campus (without isolating bacteria), 20 g/l lentil seed powder, 3 g/l beef extract and 0.5 g/l sugar. The mixture was incubated for 24 h at 37 °C. In order to measure the cell biomass in microbial solution, optical density (OD) was measured at wavelength 600 nm in a spectrophotometer and it was found to be 0.866. After incubation, the microbial suspension was allowed to stand. The supernatant microbial solution was separated from the soil. Urea (3 g/l) and gypsum (6 g/l) were then added to the microbial solution and this solution was then used for preparing the experimental i.e. bio-OPC specimens. As per the stoichiometry of the chemical reactions, the gypsum concentration was taken double the urea concentration.

2.2. Preparation of control I solution

Control I solution was prepared in order to study the effect of ingredients apart from soil bacteria on mechanical properties of cement. The control I solution was prepared with exactly the same composition as the microbial solution except for soil as soil was not added in this. The control I solution was used for preparing the control I specimens.

Table 1
Ingredients used in the preparation of microbial solution along with their significance.

Ingredients	Significance
Soil	Source of bacteria
Lentil seed powder	Protein source
Beef extract	Vitamin source
Sugar	Carbon source
Gypsum	Calcium source
Urea	Substrate