Refining Concrete’s micro-structure by enzymatically-induced carbonate precipitation through urease activity of bacteria

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Abstract. This paper presents the mechanism of improving the microstructure of concrete by enzymatically-induced carbonate precipitation through urease activity of bacteria Sporosarcina pasteurii. This calcium carbonate or calcite crystal precipitated during the biological activity fills the pores in the concrete imparting the concrete the dense microstructure. The presence of 10^5 per milliliter of mixing water yields high compressive strength due to precipitation of highest amount of calcium crystals in the concrete pores thereby plugging the micro and macro pores in the concrete refining the micro-structure of the concrete. SEM images, XRD plots and thermogravimetric analysis confirmed the presence of calcite mineral in the bacterial mortar specimens. The presence of spores of Sporosarcina pasteurii in the bacterial mortar specimens of 365 days old are found through phase contrast microscopic images. This confirms the sporulation ability of bacteria Sporosarcina pasteurii. Concrete specimens induced with bacteria showed considerably less water absorption capacity compared to normal specimens. This decrease in water absorption capacity of bacteria induced concretes is credited to the reduction of pores in the concrete due to calcite mineral precipitation filling the pores reducing the typical pore radius of concrete. Average pore diameter in bacteria incorporated concrete decreases drastically due to calcite mineral precipitation in the pores because of microbial metabolic activity. The total pore volume also decreased in case of bacteria incorporated concrete samples.

Keywords: Bacterial concrete, MICP, durability, Sporosarcina pasteurii, bio-mineralization

1. Introduction

Bio-mineralization is a chemical process during bacterial metabolic activity yielding minerals in the crystalline form. Microbial induced calcite precipitation (MICP) deposits calcium carbonate in the concrete pores and cavities by urease enzyme in bacteria by hydrolyzing the urea which is a calcium based system. Different species of bacteria producing urease enzyme are used for calcite precipitation such as B. subtilis, Sporosarcina pasteurii (formerly Bacillus pasteurii), Bacillus sphaericus, Bacillus cophnni, Aerobacter aerogenes, Shewanella, B. megaterium, B. thuringiensis, Bacillus sp. CR2, Deleya halophila, Kocuria flava CR1, Methylocystis parvum, Myxococcus xanthus, Proteus mirabilis, Halmonas
2. Objectives

The objective of the study is to refine the concrete’s micro-structure by enzymatically-induced carbonate precipitation through urease activity of bacteria. To demonstrate the efficacy of the enhanced micro-structure various experiments are conducted such as determination of optimum cell count to be used for effective production of calcium carbonate crystals, water absorption capacity and porosity studies. High quantities of calcite mineral presence is observed using SEM, XRD and thermo-gravimetric images.

3. Methodology

3.1 Cell Concentration

This section of study identifies the most optimum cell count required to produce maximum amount of calcium carbonate crystals. So various cell counts of Sporosarcina pasteurii solutions are prepared and used as mixing water to prepare concrete mortar cubes and assessed for their compressive strength to determine the cell concentration of bacteria Sporosarcina pasteurii. The presence of $10^5$ per milliliter of mixing water yields high compressive strength due to precipitation of highest amount of calcium crystals in the concrete pores thereby plugging the micro and macro pores in the concrete refining the micro-structure of the concrete.

![Figure 1. Optimum cell count for maximum calcite crystals precipitation](image)

3.2 Factors affecting the properties of bacterial concrete

The investigations are carried out to understand the role of each constituent of bacterial concrete and their synergic effect.
Table 1. Factors affecting the properties of bacterial concrete

| Cement mortar cube sample made with | Cement Mortar Cube Compressive strength (MPa) @ 28 days | % increase |
|-------------------------------------|--------------------------------------------------------|------------|
| 1. Distilled water                  | 54.3                                                   | -          |
| 2. *Sporosarcina pasteurii* + Nutrient broth solution | 56.2                                                   | 3.5        |
| 3. Nutrient broth solution only     | 55.1                                                   | 1.5        |
| 4. *Sporosarcina pasteurii* + Nutrient broth + CaCO₃ supplement solution | 65.3                                                   | 20.3       |
| 5. *Sporosarcina pasteurii* + Nutrient broth + 5% bwc Calcium lactate additive | 68.3                                                   | 25.8       |
| 6. *Sporosarcina pasteurii* + Nutrient broth + CaCO₃ supplement solution + 5% bwc Calcium lactate additive | 68.4                                                   | 25.9       |
| 7. Distilled water and 5% bwc Calcium lactate additive | 60.3                                                   | 11.1       |

*bwc – by weight of cement

It can be observed that the concrete cube strength made with normal distilled water (sample 1) is 54.3 MPa at 28 days of curing. When bacteria and nutrients suspension is used as mixing water instead of normal distilled water there is no significant improvement in compressive strength but when calcium nutrient supplement is added to bacteria and nutrients broth, the improvement of strength is very substantial due to abundant availability of calcium source for calcite mineral precipitation which will eventually fills the pores in the cementitious materials leading to dense microstructure. Since calcium supplement in the form of nutrient is expensive so an inexpensive calcium source such as calcium lactate is added to mortar dry mixture (5% bwc) to facilitate the mechanism of bio-mineralization in bacterial concrete. This study also proves that the expensive component calcium nutrient supplement can be replaced with calcium lactate to obtain similar strength development. Table 1 shows the effect of each constituent of bacterial concrete on the strength development and it proves that addition of calcium complement will facilitate favorable environment for the bacterial activity.

3.3 Scanning Electron Microscopic studies

Scanning electron microscopic studies on various samples made with various cell counts of *Sporosarcina pasteurii* showed that 10⁵ cell count of *Sporosarcina pasteurii* addition to concrete precipitates maximum amount of calcite crystals giving the concrete optimal dense micro-structure as shown in figure 2.
Figure 2. SEM images of cement mortar specimens made with various counts of *Sporosarcina pasteurii* cells.
3.4 X-ray diffraction studies

X-ray diffraction studies shows that *Sporosarcina pasteurii* incorporated concrete has high amount of calcite mineral in its microstructure which can be observed by the spikes of a high peaks indicating presence of calcium mineral as shown in figure 3b.

![Diffractogram of normal cement mortar specimens](image)

**Figure 3a.** Diffractogram of normal cement mortar specimens
Figure 3b. Diffractogram of bacteria incorporated mortar specimen’s shows the abundant presence of Ca\(^{2+}\) and precipitation was inferred to as calcite (CaCO\(_3\)) crystals

3.5 Thermo-gravimetric Studies

Thermo-gravimetric analysis shows that Sporosarcina pasteurii incorporated concrete has high amount of calcite mineral so on heating beyond 591°C makes calcite mineral to decompose into lime and carbon dioxide so there is sudden weight loss in the concrete as shown in figure 4.
Figure 4. TGA Results for cement mortar specimens with and without bacteria showing weight loss and change in weight loss per °C

3.6 Sporulation Test

The presence of spores of *Sporosarcina pasteurii* in the samples of bacteria of 365 days old are found through phase contrast microscopic images. This confirms the sporulation ability of bacteria *Sporosarcina pasteurii*. 
**Figure 5.** Phase contrast microscopic images shows white calcium carbonate crystals formation

### 3.7 Permeation properties

This section investigates the water absorption capacity and porosity of bacteria induced concrete samples conforming to standard ASTM C 642-13.

**Table 2.** Water Absorption at regular time intervals of normal and bacteria induced concrete

| Measurement Intervals ti (min) | Normal Concrete | Bacteria induced concrete |
|-------------------------------|-----------------|---------------------------|
|                               | M20             | M40                       | M20             | M40             |
|                               | mo= 2.49 kg     | mo= 2.51 kg               | mo= 2.51 kg     | mo= 2.53 kg     |
|                               | mi(kg)           | Mi(%)                     | mi(kg)           | Mi(%)                     |
| 0                             | 2.49             | 0.00                      | 2.51             | 0.00                      |
| 15                            | 2.51             | 0.80                      | 2.55             | 1.59                      |
| 30                            | 2.59             | 4.02                      | 2.56             | 1.99                      |
| 60                            | 2.60             | 4.42                      | 2.58             | 2.79                      |
| 90                            | 2.61             | 4.82                      | 2.58             | 2.79                      |
| 180                           | 2.62             | 5.22                      | 2.58             | 2.79                      |
| 480                           | 2.62             | 5.22                      | 2.58             | 2.79                      |
| 1440                          | 2.63             | 5.62                      | 2.58             | 2.79                      |
| 2880                          | 2.63             | 5.62                      | 2.58             | 2.79                      |

**Table 3.** Permeation properties of controlled and bacteria incorporated concrete specimens for different grades

|                                | Controlled Concrete | Bacteria incorporated concrete |
|--------------------------------|---------------------|--------------------------------|
| Water Absorption Capacity (WAC) (%) | M20 | M40 | M20 | M40 |
|                                | 5.62 | 2.79 | 2.79 | 1.19 |
| Porosity, P                    | 0.14 | 0.07 | 0.04 | 0.03 |
| Decrease in Porosity           | -    | -    | 72%  | 57%  |
Concrete specimens induced with bacteria showed considerably less water absorption capacity compared to normal specimens. This decrease in water absorption capacity of bacteria induced concretes is credited to the reduction of pores in the concrete due to calcite mineral precipitation filling the pores reducing the typical pore radius of concrete.

3.8 Pore Structure Analysis

Brenauer- Emmett-Teller method (BET) Nitrogen (N2) nitrogen adsorption method is adopted to analyze the pore structure of the bacteria induced concrete in terms of specific surface area, pore size distribution, and pore volume conforming to DIN 66131.

Table 4. Pore diameter, Total pore surface area, Total pore volume and porosity of bacteria incorporated concretes

|                        | M20 Controlled | M20 Bacterial | M40 Controlled | M40 Bacterial |
|------------------------|----------------|---------------|----------------|---------------|
| Average Pore Diameter (nm) | 19.121 | 3.386 | 5.084 | 3.097 |
| Total Pore Surface Area (m²/g) | 1.747 | 5.047 | 3.346 | 5.088 |
| Total pore volume (cc/g) | 0.0161 | 0.0071 | 0.0137 | 0.0057 |
| Porosity (%) | 1.6 | 0.7 | 1.37 | 0.57 |

Average pore diameter in bacteria incorporated concrete decreases drastically due to calcite mineral precipitation in the pores because of microbial metabolic activity. The total pore volume also decreased in case of bacteria incorporated concrete samples. Porosity in bacteria incorporated concretes is reduced by 20 to 60%. Total Pore volume in bacteria incorporated concretes is reduced by 20 to 60%.

4.0 Conclusions

The following conclusions are derived from the above experimental investigations-

1. The presence of 10⁵ per milliliter of mixing water yields high compressive strength due to precipitation of highest amount of calcium crystals in the concrete pores thereby plugging the micro and macro pores in the concrete refining the micro-structure of the concrete.

2. SEM images, XRD plots and thermo-gravimetric analysis confirmed the presence of calcite mineral in the bacterial mortar specimens

3. The presence of spores of *Sporosarcina pasteurii* in the bacterial mortar specimens of 365 days old are found through phase contrast microscopic images. This confirms the sporulation ability of bacteria *Sporosarcina pasteurii*.

4. Concrete specimens induced with bacteria showed considerably less water absorption capacity compared to normal specimens. This decrease in water absorption capacity of bacteria induced concretes is credited to the reduction of pores in the concrete due to calcite mineral precipitation filling the pores reducing the typical pore radius of concrete.

5. Average pore diameter in bacteria incorporated concrete decreases drastically due to calcite mineral precipitation in the pores because of microbial metabolic activity. The total pore volume also decreased in case of bacteria incorporated concrete samples. Porosity in bacteria incorporated concretes is reduced by 20 to 60%. Total Pore volume in bacteria incorporated concretes is reduced by 20 to 60%.
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