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Use of carbonate precipitating bacteria to reduce water absorption of aggregates

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HIGHLIGHTS

- Bacterial treatment reduced water absorption of both lightweight and normal-weight aggregates.
- Bacteria *S. pasteurii* resulted in a more decrease in the uptake of water in LWA compared to *Bacillus subtilis*.
- Bacterial activity on the aggregates was proven through SEM and XRD analysis.

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ABSTRACT

This paper presents the results of an experimental investigation carried out to evaluate the influence of two types of bacteria, namely *Sporosarcina pasteurii* and *Bacillus subtilis*, with different cell concentrations (10^6, 10^7, 10^8 cells.ml^{-1}) on the water absorption of four types of concrete aggregates. Surface deposition of calcium carbonate crystals was found to decrease water absorption by 20–30%, depending on the type of bacteria and aggregate porosity. The use of ureolytic gram-positive bacteria *S. pasteurii* resulted in a more pronounced decrease in the uptake of water by the aggregates. The reduced water absorption observed was possibly due to deposition on the bacteria cell walls in the pores. The results show that the aggregates retained their properties and yielded the same results after about 20 days, indicating that the deposits remained over this time period. The XRD and SEM analysis indicated the formation of calcite in bacterial aggregates.

1. Introduction

Concrete is made up of three basic materials (cement, water, and aggregate). The low cost and availability of these materials in addition to the simple construction of concrete have made concrete one of the most widely used building materials in the world [1]. A special subtype of structural concrete is the lightweight aggregate concrete. A major problem of lightweight aggregate concrete is the water absorption of the aggregate in the cement paste. As the aggregate content accounts for 70–75% of the concrete, any improvement in aggregate properties is expected to have an improve effect on concrete properties. Carbonate precipitate induced by bacteria has been regarded as an environment-friendly and inexpensive material with a promising potential for a wide range of engineering applications. The application of bacteria in concrete gives rise to the deposition of carbonates on their surfaces, which nowadays forms a potential field of research in concrete technology. Bacteria are very small in size, their relative surfaces are very large compared to any other form of life, and they induce carbonates to deposit on their surfaces [2,3]. They, therefore, provide a large contact surface area that can interact with the surrounding environment [4]. *Sporosarcina pasteurii* and *Bacillus subtilis* are common soil bacteria and have also been found to be able to precipitate calcium carbonate given a calcium source and urea through the process of biological cementation; much researches has been carried out using these gram positive bacteria in concrete [5–8]. All these studies have been conducted on concrete properties like improvement of compressive strength, decrease in water absorption and improving the durability of concrete; however, it seems their ability of deposition can also be exploited to reduce aggregate water absorption as well. Increasing aggregate density has a direct impact on increasing concrete density which is an important concrete property in certain concretes such as lightweight aggregate concrete. A method is, therefore, sought that can fill the pores among the aggregate grains while the change in pallid density is not serious. Moreover, the method should be capable of investigating the effect of carbonate

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precipitate on the aggregate induced by the bacteria in an environment-friendly manner.

The first studies on the properties of calcite deposits were conducted in 1995. Gollapudi et al. used this technique to reduce the porosity of soil with a high permeability by injecting bacteria into soil. For this purpose, they mixed a calcium chloride solution containing urea and carbonic acid directly with the soil and compressed the mixture in a sand column. They found that the pores and cracks in the column were closed [9].

Remarkable studies have also been carried out to develop methods that exploit the potential of mineral-producing bacteria for improving concrete properties. Earlier promising results obtained from using different bacteria to repair the concrete cracks [10–11], improve compressive strength of concrete [11–12], and improve the durability of concrete [13–15] by using bacteria in the concrete mix or on the concrete surface.

Muynck et al. indicated that the durability of mortar specimens with different porosity levels was affected by bacterial carbonate precipitation. They utilized different types of bacteria to find that surface deposition of calcium carbonate crystals decreased water absorption by 70–85% depending on the specimen porosity and the type of bacteria used [13]. Some recent studies on bacteria have directed their efforts at extending the life of the organism [14,15]. It has been found that extended bacterial life obviously leads to enhanced mineral deposition. Moreover, aggregate properties improved by bacterial deposition before it is used in the concrete mix will expectedly yield better results.

Based on the results reported in the above-mentioned studies, and due to the reduced permeability of biologically treated concrete, the current study was designed to utilize microbial carbonate precipitation in order to reduce aggregate water absorption. The study was conducted in two stages. The first stage involved the investigation of the impact of carbonate depositions on the water absorption of four types of aggregate of different sizes and two different bacteria used at three different cell concentrations. Of special interest in this stage was the duration of the expected impact. In the second stage of the study, efforts were made to determine whether the deposits have a long lasting effect on the water absorption of the aggregates. To the best of our knowledge, this is the first study specifically dedicated to achieving improved aggregate quality by adopting a biological strategy.

2. Experimental program

2.1. Bacteria and their growth conditions

Two different bacterial strains, *Bacillus Subtilis* (*Bacillus. Sb*) (PTCC 1715; BGSC 1A747) and *Sporosarcina pasteurii* (S. *pasteurii*) (PTCC 1645; DSM 33, ATCC 11859, CCM 2056, NCIB 8841, NCTC 4822) were primarily applied to selected aggregates. Earlier studies have reported these bacteria to possess the ability to precipitate calcium carbonate given a calcium source and urea through the process of biological cementation [16–21].

For the purposes of this study, the bacteria were cultured in a liquid medium nutrient (the culture was taken from Merck Company and consisted of 5.0 g peptone of Quelab Company and 3.0 g meat extract per liter of distilled water). Furthermore, 1.5% agar was added into another liquid medium with the same ingredients to produce a solid medium. The media thus produced were supplemented with 1 N HCl and pH was adjusted to 7.0. The mixture was initially sterilized in an autoclave for 30 min at 121 °C before it was allowed to cool to room temperature (25 °C).

According to supplier recommendations for culturing of *S. pasteurii* strain, 10 ml of filter-sterilized 20% urea solution passed through a sterile 0.22 mm filter (Jet Biofil) was added aseptically post-autoclaving to 100 ml of the cooled molten peptone/meat extract medium. It should be noted that the whole culturing process was performed under sterile conditions. The cultures were then incubated at 37 °C on a shaker incubator operating at 150 rpm for 48 h. The bacterial cells were subsequently harvested by centrifuging (at 6000 rpm for 10 min). The 48-h old cells were washed twice in the saline solution.

2.2. Gram staining

Gram staining is the method of differentiating bacterial species into one of two large groups (Gram-positive and Gram-negative) based on their stain ability as well as the chemical and physical properties of their cell walls. It is one of the basic foundations on which bacterial identification is built. Earlier studies have identified *Bacillus subtilis* and *Sporosarcina pasteurii* used in the current study as gram-positive bacteria which allow carbonate calcite to precipitate because of their cell wall properties [22–27]. In the current study, the authors performed the Gram staining test to ensure that no gram-negative or other microorganisms were present in the bacterial cultures. The bacteria were picked up from the solid culture made before and placed on a clean glass slide using a sterile loop. The slide's temperature was fixed by passing it several times over a flame; care was taken so that the slide would not get too hot to avoid staining artifacts or damages to the normal morphology of the bacteria. Subsequently, the staining procedure was carried out in four steps. The slide was first flooded with crystal violet ($C_{25}N_3$-$H_{30}Cl$) (2 mol of crystal violet to be sure that the whole part of slide was impregnated with crystal violet) for 60 s followed by washing with tap water. Then, the slide was flooded this time with Gram's iodine for 90 s, which caused the iodine to bind to crystal violet and to traps it in the cell. This was followed by a second round of washing with tape water. The sample was then carefully decolorize using 95% ethanol until the thinnest parts of the smear became colorless; the sample was washed with water again. Finally, the sample was flooded with safranin, pink color (10% Fuchsine), for 60 s before it was washed with water. The slide glass was then air dry. The micrographs in Fig. 1 were obtained using an Olympus BH2 microscope.

2.3. Sample preparation

To ensure that the samples truly represented the right choice of total aggregates in terms of size and granulation, proper samples were taken from 10 random parts of each aggregate stockpile as per ASTM D75 [28]. The standard method for sample preparation was used to mix aggregates with the bacterial solution on a flat surface for three times before they were placed in a cone shape container and finally pressed into a flattened cylinder. The aggregate specimens were then divided into four parts and the two on opposite sides of the cylinder were discarded. The procedure was repeated until specimens of the aggregate were obtained each 500 g in weight. Table 1 presents the details of the aggregate specimens.

2.4. Classification of specimens

In order to investigate the performance of the aggregates cured with the bacteria solution, the specimens were classified into five groups, each group comprising aggregates submerged in solutions of different bacterial types and with different exposure times to bacteria (aging). The main classification details of the specimens in each group are provided in Table 1. A description of each also follows. It should be noted that aggregates of similar type and size with similar type of bacteria were labeled with similar codes followed by different numbers in parenthesis indicating their expo-