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Corrosion protection of cement-based building materials by surface deposition of $CaCO_3$ by *Bacillus pasteurii*

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ABSTRACT

Bacterially induced calcium carbonate precipitation was used as a novel and environmentally friendly approach to produce a protective layer on the surface of cement-based materials in the study. The physical and chemical properties of the obtained layer were examined. X-ray diffraction analysis characterized the composition of the deposited layer and scanning electron microscopy displayed the morphology of particles. The results showed that both bacterial activity and the method of adding Ca^{2+} and urea had a profound effect on the properties of the calcium carbonate layer. A capillary water absorption test was carried out to evaluate the ability of the protective layer to improve the resistance to water penetration. Experimental results indicated that the calcium carbonate layer, obtained under the conditions of high bacterial activity, appropriate concentration of Ca^{2+}, and adding Ca^{2+} before urea to the reaction mixture, could greatly improve the water penetration resistance of the specimen surface. This type of treatment has the potential to conserve and consolidate cement-based materials.

1. Introduction

Cement-based materials, like concrete and mortar, are the most popular construction materials because of their excellent mechanical properties. Since concrete structures are exposed to corrosive substances in the environment such as Cl^-, SO_4^{2-}, CO_2 and atmospheric moisture, the maintenance and repair of such structures are important to their continuing function. Some of these corrosive substances will react with the concrete surface layer and some will penetrate inside the concrete, which will increase the material porosity and decrease its mechanical properties with a general weakening of the superficial structural strength. Consequently, surface loss and peeling happen and micro-cracks appear. In cases of more severe damages, the interior structure of the building material may disintegrate. Research has shown that concrete, which has a high permeation resistance, will not display signs of deterioration over a long time span [1]. Therefore, it is important to improve the permeation resistance of materials. The permeation properties can be used as a parameter for the comparison of the effectiveness of different surface treatments enhancing the maintenance of cement-based materials.

Nowadays, a large number of organic and inorganic products are utilized for protection and consolidation of concrete surfaces, such as water repellents, a variety of coatings and pore blockers, etc. These conventional methods of protection have, however, a number of disadvantages, such as (1) an incompatibility of the protective layer and the underlying layer due to differences in their thermal expansion coefficient; (2) disintegration of the protective layer over time; and (3) a need for constant maintenance by a treatment that is reversible and repeatable. If organic solvents are used, some may contribute to environmental pollution [2–5].

In order to avoid these disadvantages, many environmentally friendly methods have been proposed. Among these methods, bacterially induced carbonate reinforcement has been suggested as a novel and ecologically friendly strategy for the protection and consolidation of cement-based materials.

Bacterially induced calcium carbonate precipitation is related to biomineralization, which is defined as a biologically induced precipitation in local micro-environments created by organisms under conditions that allow visible extracellular chemical precipitation of mineral phases [6]. *Bacillus pasteurii* is able to precipitate calcium carbonate on its cells under appropriate microenvironmental conditions when hydrolyzing urea into ammonia and carbon dioxide [7,8]. The ammonia increases the pH and the bacterial cell surface with a variety of negative charges can induce mineral precipitation by providing a nucleation site. It is known that Ca^{2+} is used physiologically by bacteria as an essential micronutrient. But Ca^{2+} is not likely in large numbers utilized by microbial metabolic process; rather it accumulates outside the cells. Possible reactions involving the cell-surface of bacteria that lead to $CaCO_3$ precipitation can be summarized as follows [9]:

$$Ca^{2+} + \text{Cell} \rightarrow \text{Cell}-Ca^{2+} \quad (1)$$

$$\text{Cell} \xrightarrow{\text{Urea}} \text{Urease} \quad (2)$$

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$$\text{Urea} \xrightarrow{\text{Urea}} 2NH_4^+ + CO_3^{2-} \quad (3)$$

$$\text{Cell}-Ca^{2+} + CO_3^{2-} \rightarrow \text{Cell}-CaCO_3\downarrow \quad (4)$$

The aim of this study is to study the formation of a protective layer of calcium carbonate on the surface of cement-based material with the aid of bacteria, and to determine some of the factors that affect the physical and chemical properties of this layer. Capillary water absorption will be measured to determine the extent of improvement in permeation resistance of the treated cement-based material.

2. Materials and methods

2.1. Samples

The specimens used in the experiments consisted of hardened cement paste because it is easy to mould and exhibits few special properties. Ordinary Portland cement graded 42.5 was used. The specimens ($30\times30\times30$ mm) were made with a water–cement ratio (w/c) of 0.45 with no additional agents, and cured in a humid atmosphere (20 °C, 95% R.H) for 28 days prior to bacterial treatment.

2.2. Bacterial strain and culture medium

Bacillus pasteurii (DSM 33) was used in the study because of its high urease activity and its lack of pathogenicity.

The culture medium for preparing bacterial stock cultures and for bacterial growth contained the following ingredients: beef extract (3 g l^{-1}), peptone (5 g l^{-1}), and urea(20 g l^{-1}); to which 15 g l^{-1} agar was added to obtain a solid medium for the stock culture.

De-ionized water was used throughout the experiments to avoid the influence of contaminating ions.

2.3. Bio-deposition experiments

The culture medium (pH = 7.0) lacking urea, in a Erlenmeyer flask, was sterilized by autoclaving for 25 min at 121 °C and then was inoculated with *B. pasteurii* from the stock culture. Then the Erlenmeyer flask was put on the rotary shaker (30 °C, 170 rpm). 24 h later, the bacteria medium was poured out from the Erlenmeyer flask and was poured into a 250 mL beaker, followed by adding a cement specimen, urea solution and calcium nitrate solution into the beaker. Finally, the beaker was sealed with gauze and was kept under the stationary condition. In the experiments, the initial concentration of urea and Ca^{2+} was kept identical.

In the bio-reinforced process, the influence of three factors, including the addition sequence of Ca^{2+} and urea, bacterial activity and concentration of Ca^{2+}, on physical and chemical properties of the precipitated layer was studied. The experiments were designed as shown in Table 1.

Bacteria in three different growth periods show different bacterial activity. In the experiments, bacteria in the log phase, the stationary phase and the decline phase, were used. The initial concentrations of Ca^{2+} in each subgroup were 0.3 mol/L, 0.2 mol/L and 0.1 mol/L respectively. Two different addition methods of Ca^{2+} and urea were taken. One was adding $Ca(NO_3)_2$ and urea together, the other was adding $Ca(NO_3)_2$ an hour before urea.

After the additions of the cement specimens and the urea and calcium nitrate, all nine subgroups of cultures were then incubated in a stationary condition under room temperature for 7 days. At the end of that incubation, the depositions on the surfaces of the cement specimens in the beakers were analyzed for their thickness, composition, morphology, and water penetration resistance.

2.4. X-ray diffraction (XRD) analysis

X-ray diffraction was used to determine the mineralogical composition of newly formed $CaCO_3$ crystals. The diffractometer used was a Thermo model ARLX'TRA with a Cu anode (45 kV and 35 mA), scanning from 5 to 80°2θ. Precipitation obtained from the treated surfaces of the specimens was added to the sample holder.

2.5. Thickness analysis (metalloscope)

The thickness of the $CaCO_3$ layer formed on the cement specimens was obtained by using an OLYMPUS BHM metalloscope. Prior to observation, four surfaces adjacent to the treated surface were ground to be even. Then each of the adjacent surface was put under the lens and photos were taken and scaled. By means of Photoshop and Image Proplus softwares, the average thickness of the layer could be obtained.

2.6. Scanning electron microscope (SEM) analysis

The morphology of the bacterially induced calcium carbonate formed on the specimen surface was observed using a JEOL JSM-5610LV scanning electron microscope at accelerating voltage 15 kV. The technique of secondary electron imaging (SEI) was employed for electron micrography. The treated surface was gold coated before examination.

2.7. Capillary water absorption of treated surface

A capillary water absorption test was carried out, to compare the water penetration resistance between treated and untreated specimens. The specimens were dried at 45 °C in an oven until mass changes came to less than 0.1% at 24 h intervals. Prior to the test, all specimens were coated with wax at four sides adjacent to the treated surface to ensure unidirectional absorption through the treated surface. The specimens were weighed as initial weights before their exposure to water and then were immersed in the depth of 10 ± 1 mm of water, with the treated, unwaxed surface facing downwards. At regular time intervals (5 min, 10 min, 20 min, 30 min, 60 min, 90 min, 120 min, 180 min, and 240 min), the specimens were taken out of the water and weighed after drying the surfaces with a wet towel. After the weighing, the specimens were immediately returned to the water.

Table 1
Details of experiments of groups A, B and C.

		Bacterial activity (bacteria in growth period)	Addition sequence of $Ca(NO_3)_2$ and urea	Initial concentration of Ca^{2+} in each beaker
Group A	Subgroup 1	In log phase	Adding $Ca(NO_3)_2$ and urea together	0.3 mol/L
	Subgroup 2			0.2 mol/L
	Subgroup 3			0.1 mol/L
Group B	Subgroup 1	In stationary phase	Adding $Ca(NO_3)_2$ first, 1 h later, adding urea	0.3 mol/L
	Subgroup 2			0.2 mol/L
	Subgroup 3			0.1 mol/L
Group C	Subgroup 1	In decline phase	Adding $Ca(NO_3)_2$ first, 1 h later, adding urea	0.3 mol/L
	Subgroup 2			0.2 mol/L
	Subgroup 3			0.1 mol/L