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Biocementation of Concrete Pavements Using Microbially Induced Calcite Precipitation

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In this study, the feasibility of introducing calcite-forming bacteria into concrete pavements to improve their mechanical performance was investigated. *Lysinibacillus sphaericus* WJ-8, which was isolated in a previous study and is capable of exhibiting high urease activity and calcite production, was used. When analyzed via scanning electron microscopy (SEM) and X-ray diffraction, WJ-8 showed a significant amount of calcite precipitation. The compressive strength of cement mortar mixed with WJ-8 cells and nutrient medium (urea with calcium lactate) increased by 10% compared with that of the controls. Energy dispersive x-ray spectroscopy analyses confirmed that the increase in strength was due to the calcite formed by the WJ-8 cells.

Keywords: Microbially induced calcite precipitation, concrete pavement, calcite-forming bacteria, cement mortar

Introduction

The performance of concrete pavements is affected by crack formation induced by heat of hydration and long-term drying shrinkage in the concrete slabs [1]. Once open to traffic, the concrete pavement becomes more susceptible to cracking owing to the vehicle load. A study based on this problem was conducted to assess the improvement in the mechanical property of concrete by filling its cracks using the calcite precipitation effect of ureolytic bacteria [2]. It was reported that the introduction of calcite-forming bacteria increased the self-healing ability and strength of construction materials in the architectural field, in which the effect of climatic conditions was relatively minimal. In China, the calcite precipitation effect of bacterial vegetative cells was used to repair stone buildings [3]. Santhosh *et al.* [4] showed that ureolytic calcium carbonate precipitation by *Sporosarcina pasteurii* was effective in increasing the compressive strength of concrete and repairing concrete cracks. Additionally, it had the ability to reduce the permeability and chemical intrusion of concrete. Ghosh *et al.* [5] mixed the cement mortar with calcite-forming bacteria and performed microstructural analysis using techniques such as environmental scanning electron microscopy (SEM), image analysis, electron probe microanalysis, and X-ray diffraction (XRD).

The calcite is precipitated because of the following factors: (i) calcium ion (Ca^{2+}) concentration, (ii) dissolved inorganic carbon, and (iii) pH favorable to bacterial metabolism [9]. The hydrolysis of urea by bacterial vegetative cells provides a favorable chemical environment for calcite formation [6]; calcite crystals are precipitated on the surface of vegetative cells through a chemical reaction. Stocks-Fischer *et al.* [7] described that urea ($CO(NH_2)_2$) is decomposed into ammonia (NH_3) and carbonic acid (H_2CO_3) by urea-decomposing bacteria, as shown in Eqs. (1) and (2). Then, NH_3 and H_2CO_3 are diffused into the solution surrounding the vegetative cells. These products are then converted into ammonium ion (NH_4^+), bicarbonate (HCO_3^-), and hydroxide by reaction with water (Eqs. (3) and (4)). As pH increases, the bicarbonate is converted into carbonate ion (CO_3^{2-}), as shown in Eq. (5). Because the bacterial cell wall is negatively charged, positively charged calcium ions are attracted toward it (Eq. (6)). Then, calcium ions react with the carbonate ion, leading to calcite precipitation onto the cell surface (Eq. (7)).

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

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

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

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

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

$$Ca^{2+} + Cell \leftrightarrow Cell - Ca^{2+} \quad (6)$$

$$CO_3^{2-} + Ca^{2+} \leftrightarrow CaCO_3 \quad (7)$$

In the present study, we investigated the calcite-forming ability of bacteria, which were mixed with cement mortar. Through the compressive strength test and energy dispersive x-ray spectroscopy (SEM-EDX) analyses, the increase in cement-mortar durability due to calcite-forming bacteria was confirmed.

Materials and Methods

Microorganism and Growth Medium

The bacterial strain *Lysinibacillus sphaericus* WJ-8, capable of exhibiting high urease activity and calcite production, had been isolated in a previous study [8, 9] and was cultivated under aerobic conditions in YA broth (20 g/l yeast extract, 10 g/l ammonium sulfate; pH 7). *L. sphaericus* WJ-8 was selected on the basis of its high tolerance to environmental stresses (60°C, 3 M NaCl, and pH 12).

Calcite Precipitation

Bacterial culture was grown overnight in YAU broth (20 g/l yeast extract, 10 g/l ammonium sulfate, 20 g/l urea; pH 7) at 30°C and 200 rpm. Samples of the culture broth were collected at different time intervals, and the bacterial cells were separated by filtration through a 0.22-µm syringe filter. The filtrate (500 µl) was added to 500 µl of calcium lactate dihydrate solution (100 mM). The mixture was centrifuged at 16,179 ×*g* for 5 min at 30°C to collect the precipitate. The precipitate was dried for 24 h at 50°C and weighed.

Biocement Mortar

Calcium lactate and urea were used as components of the culture medium because nutrient components are necessary for bacterial growth and calcite formation [10]. The powdery medium was completely dissolved in water at 80°C, and the medium (15 g/l calcium lactate and 20 g/l urea) was cooled down to ambient temperature before mixing. Hydraulic cement (KSL 5201) and standard sand (KSL ISO 679) were mixed with water to form the mortar. Table 1 shows the mixing proportion of the cement mortar. Six types of test specimens were fabricated, including normal mortar samples (control), mortar test samples mixed with calcium lactate and urea medium, and mortar test samples mixed with WJ-8 cells. *Escherichia coli* KCCM41300, which has no ureolytic activity, was used as a negative control. The ambient temperature and moisture of the laboratory at the mixing time point ranged between 13°C and 18°C and between 33% and 42%, respectively. The flow of the mixed mortar measured according to KSL 5111 ranged between 110 and 140 mm.

Compressive Strength of Biocement Mortar

The cement mortar mixed with WJ-8 cells was subjected to compressive strength tests to confirm the influence of calcite precipitation on cement mortar strength. The biocement mortar sample was cured for 7 and 28 days in environmental chamber (20°C and 90% RH) and the compressive strengths were measured by the KSL 5105 testing method.

SEM-EDX and XRD

Modified SEM and XRD methods were employed to observe calcite production. Control and biocement mortar samples were coated with platinum. Field emission SEM (S-4300; Hitachi, Japan) with an EDX detector (EDAX, The Netherlands) was also used to observe calcite in the biocement mortar samples. Control and biocement mortar samples were crushed and calcite formation was confirmed by XRD analysis. Each sample was analyzed in an

Table 1. Mixing proportions of cement mortars.

No.	Treated	Flow (mm)	Temp. (°C)	Humidity (%)	Water/cement ratio	Cement/aggregate ratio
1	Normal cement mortar	118	17.7	41.6	1:3	1:2
2	Calcium-lactate (15 g/l) + Urea (20 g/l)	133	15.0	41.6		
3	WJ-8 (3.1×10^8 CFU/ml)	118	16.0	38.0		
4	WJ-8 (3.1×10^8 CFU/ml) + Calcium-lactate (15 g/l) + Urea (20 g/l)	136	16.0	38.0		
5	*E. coli* (3.1×10^8 CFU/ml)	117	13.4	33.0		
6	*E. coli* (3.1×10^8 CFU/ml) + Calcium-lactate (15 g/l) + Urea (20 g/l)	140	13.4	33.0		

XRD (DMAX-2500; Rigaku, Japan); each spectrum was scanned from 2θ = 10° to 2θ = 90°.

Results and Discussion

Calcite Crystal Morphology and Structure

Mineralization, which refers to producing minerals, is often used in civil engineering. In biomineralization, living organisms participate in the process. They produce minerals more specifically, an inorganic mineral phase in the presence of a biopolymer [11]. Fig. 1 shows the morphology of WJ-8 cells and calcite. The colonies of WJ-8 cells, which had grown excessively, were observed by SEM at 9,000× magnification (Fig. 1A). At 20,000×, the WJ-8 cells were observed to be encircling mineral precipitates (Fig. 1B). Finally, it could be confirmed that the white precipitate produced by the six test specimens contained minerals. The activity of microbial cementation on granular behavior is dependent on the ability of microbes to freely move either by injection throughout the pore space or by sufficient particle-particle contacts so that cementation will occur. These conditions require a balanced relationship between the microbe size and the pore between the sand particles.

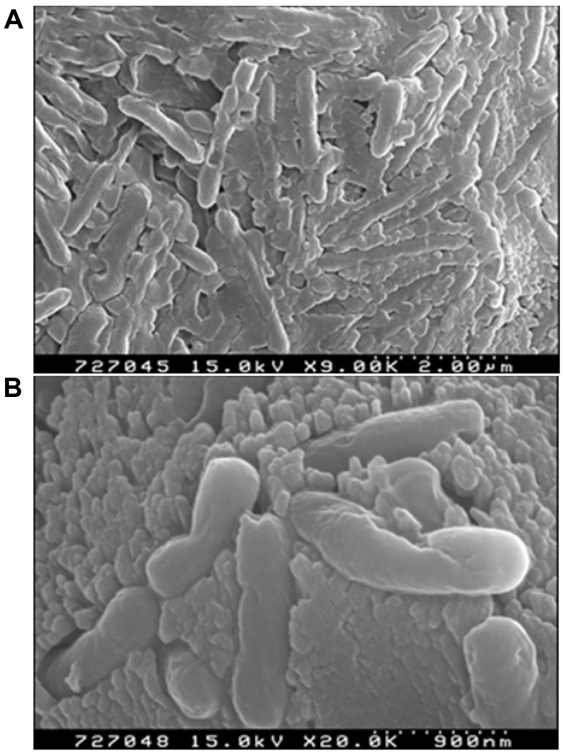

Fig. 1. Microscopic observation of *L. sphaericus* WJ-8 by SEM. (**A**) Bacterial cell and (**B**) calcite.

Biocement Mortar

Biocement is produced by microbially induced calcite precipitation in the extent between the particles of a granular material by draining a liquid solution containing alkaliphilic bacteria, urea as the substrate, and calcium as the ion [12]. The flow of normal cement mortar (118 mm) was lower than that of the sample mixed with calcium lactate- and urea-containing medium (136 mm).

The compressive strengths of the mortar mixed with calcium lactate- and urea-containing medium were 7% (7 days cure) and 5% (28 days cure) higher than those of normal mortar, as shown in Fig. 2. However, the compressive strengths of the mortar samples mixed with WJ-8 or *E. coli* KCCM41300 without medium were similar to those of the control samples. The compressive strengths of mortar mixed with WJ-8 and medium were 10% (7 days cure) and 11% (28 days cure) higher than those of the control samples. The mortar mixed with *E. coli* KCCM41300 and medium showed a slight increase in compressive strengths by 3% (both 7 and 28 days cure) as compared with that of the control samples. Biocementation was shown to be effective in binding sand matrix to form biocement of adequate compressive strength [13, 14]. It was also used for the improvement of the compressive strength of other cementitious materials. Compressive strength improvement of up to 25% was noticed when biocementation was achieved by mixing *Shewanella* sp., isolated from a hot spring, in mortar [15]. Achal *et al*. [16] reported that biocementation was used in conjunction with conventional cement for making mortars. The bacterial cells grown in broth were added to the mixture of sand and cement, where the bacterial culture-to-cement ratio was 0.47 for 70.6-mm cubes, and a similar formulation was adopted to make

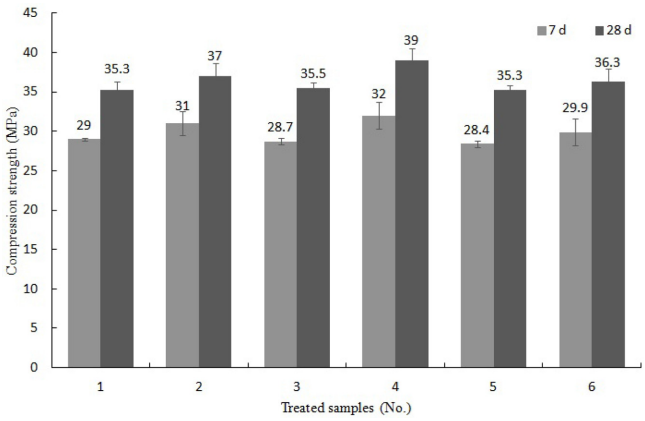

Fig. 2. Compressive strength of biocement mortar samples. The sample numbers match with the numbers in Table 1.

concrete samples with the addition of coarse aggregates [16]. Such cementitious specimens showed 17–36% higher compressive strength [13, 16], and resistance to water permeability was improved four times [16].

The cement mortar specimen was crushed to a size smaller than 5 mm to conduct SEM-EDX analyses. Normal mortar showed a smooth surface on which particles could hardly be observed (Fig. 3A), whereas the mortar mixed with WJ-8 and medium showed particles, which were expected to be calcite, forming an irregularly rough surface (Fig. 3B). EDX analysis was performed on the crushed cement mortar that had been analyzed by SEM. Calcite formation in the mortar was investigated by measuring the mass and atomic weight of carbon (C). The carbon component consisting of calcite was not found in the normal mortar samples (Fig. 4A). On the contrary, the carbon component was detected in mortar mixed with WJ-8 and medium (Fig. 4B). Table 2 shows EDX analysis results of control normal mortar, in which the percentages of both the mass and atomic weight of carbon were 0. For the mortar mixed with WJ-8 and medium, the percentages of both the mass and atomic weight of carbon were 16.7% and 25.1%, respectively. Thus, based on the results of SEM-EDX analyses, the major component of the particles observed on the surface of mortar mixed with WJ-8 and medium was confirmed to be calcite. The primary role of microorganisms in carbonate precipitation is mainly owing to their ability to create an alkaline environment (high pH and increased dissolved inorganic carbon) through their various physiological activities [17]. When biocementation is attempted in conjunction with other binders, such as cement, the high alkalinity of these materials poses an additional challenge. In such applications, microbes with a high alkaline pH tolerance must be used.

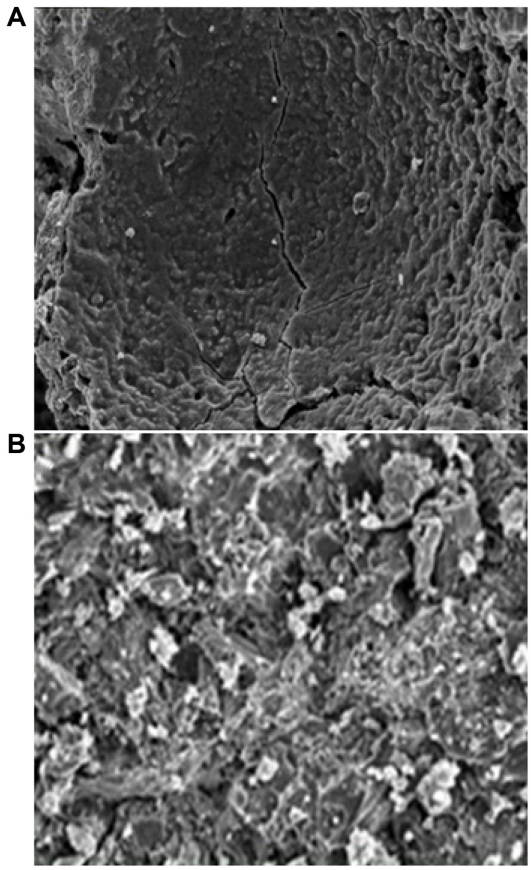

Fig. 3. Microscopic observation of mortar by SEM.
(**A**) Cement mortar (without *L. sphaericus* WJ-8), and (**B**) biocement mortar (with *L. sphaericus* WJ-8).

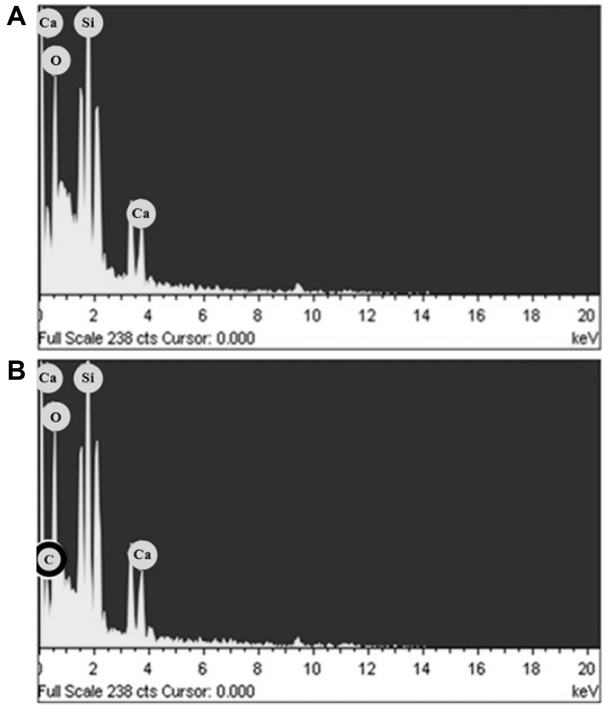

Fig. 4. EDX analysis of cement mortar (**A**) and biocement mortar (**B**).

Table 2. Results of EDX analysis of cement mortar.

	Control (cement mortar)		Biocement mortar	
Element	Mass (%)	Atomic weight (%)	Mass (%)	Atomic weight (%)
C	0.00	0.00	16.66	25.14
O	49.83	65.55	46.08	52.27
Si	35.60	26.50	29.59	19.12
Ca	14.57	7.95	7.67	3.47
Total	100.00	100.00	100.00	100.00

In this study, the change in mechanical property of cement mortar mixed with calcite-forming bacteria, which were isolated from a section of concrete pavement, was studied. In a previous study [8], the survivability of the bacteria in the concrete pavement environment was investigated to understand the feasibility of its application in road construction. In the laboratory, the mineral precipitation reaction by the vegetative cells of calcite-forming bacteria was observed by SEM analysis. The precipitate was confirmed to be calcite from the result of XRD analysis. The compressive strength of cement mortar mixed with *L. sphaericus* WJ-8 vegetative cells and medium increased by around 10%. The SEM-EDX results confirmed that the increase in strength was because of the effect of calcite formation. To improve the long-term mechanical performance of concrete pavement, a study on the self-survival of bacteria in a cement concrete environment is necessary. Subsequent studies on self-healing and crack filling by injection into or spraying on the concrete pavement slabs will be performed based on the results of this study.

Acknowledgments

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