

Research Article

Modifying Mechanical Strength and Capillary Porosity of Portland Cement-Based Mortar Using a Biosurfactant from *Pseudomonas fluorescens*

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We characterized the effects of a biosurfactant derived from *Pseudomonas fluorescens* on slump loss, mechanical strength, capillary porosity, and bacterial colonization inside Portland cement-based mortar samples. Standard tests were used to evaluate the utility of this biosurfactant as an admixture. The addition of 1.5% biosurfactant increased the plasticity and improved the workability of fresh samples. Although compressive and flexural strengths of mortars with biosurfactant were lower than those of mortars without biosurfactant after a short curing period (28 days), the addition of biosurfactant increased the compressive strength of mortar after a long curing period (180 days), with 1% biosurfactant having the highest value. After 180 days, mortar with biosurfactant had significantly lower capillary absorption coefficient A values ($P < 0.05$) than mortar without biosurfactant. Furthermore, the addition of biosurfactant reduced the relative abundance of the mortar-deteriorating bacterial genus *Pseudomonas* (phylum Proteobacteria).

1. Introduction

Admixtures have been widely used in the cement mortar industry since ancient times because they can improve properties such as water retention, shrinkage reduction, adhesion, and plasticization [1]. The ancient Chinese used rice pasta and boiled bananas as set retarders [2]. Roman architects (84–10 B.C.) used blood and milk for air entrainment [3]. More recently, chemical or mineral admixtures like polycarboxylate [4] and fly ash [5] have gained popularity. However, chemical and mineral admixtures have harmful environmental effects and can substantially increase the cost of a construction project [6, 7].

Considering these limitations, bio-admixtures (defined as biopolymers and products derived from biotechnological processes) have attracted great attention [8, 9]. Nakamatsu et al. evaluated the use of carrageenan as a bio-admixture to considerably improve compressive strength of earth

constructions [10]. Molasses was used by Akar and Canbaz [11] as a bio-admixture to improve concrete durability. When a bio-product is evaluated for use as an admixture, researchers generally consider its impact on the mechanical strength and durability of a cementitious material. Surprisingly, effects on the porosity of the material (e.g., capillary porosity and calcium silicate hydrate (C-S-H) gel porosity) have rarely been investigated, despite the fact that the pore system is of critical importance in determining physical and mechanical properties of cement-based materials [12].

The pores of cement pastes are present at different ranges from nano- to macroscale: (1) gel C-S-H pores (below 10 nm), (2) capillary pores (10 nm–10 μ m), (3) hollow-shell pores (10 μ m–0.1 mm), and (4) air voids or bubbles (0.1–1 mm) [13]. C-S-H gel is the major product of hydration in Portland cement and can help to enhance cement-based materials' mechanical characteristics [14]. Capillary pores are primarily responsible for moisture transport processes and the

durability of mortar; thus, capillary pore characteristics have been recognized as an important index of mortar deterioration [15]. Furthermore, mortars with high porosity are more susceptible to colonization by microorganisms because the pores can absorb more water and make the mortar more suitable for microbial survival [16]. Some colonization by autotrophic microbes capable of inorganic sulfur oxidation, such as *Acidithiobacillus thiooxidans* and *Halothiobacillus neapolitanus*, has been associated with mortar biodeterioration [17]. Therefore, a better understanding of mortar pore systems could help us to better evaluate bio-products as potential admixtures in cementitious materials. We also wanted to identify the bacteria occurring inside mortar in order to confirm the presence of mortar biodeterioration promoters linked to deterioration conditions of mortar. Thus, we used high-throughput sequencing of the 16S rRNA gene to identify the bacterial species occurring in both untreated and admixture-treated mortars.

In this study, we tested the use of a lipopeptide biosurfactant derived from *Pseudomonas fluorescens* as bio-admixture. Unlike surfactants obtained by chemical synthesis, biosurfactants exhibit better biodegradability and low toxicity, making them a better environmental choice. Here, two effects of the addition of biosurfactant to mortar were tested:

- (1) The influences of the bio-admixture on different features of mortar were evaluated, including measurements of compressive and flexural strength, a slump test, and porosity characterization.
- (2) The 16S rRNA gene was sequenced for bacterial communities inside mortar in order to confirm the presence of mortar-biodeteriorating bacteria.

2. Materials and Methods

2.1. Manufacture of the Biosurfactant. The biosurfactant used in this study is produced by *Pseudomonas fluorescens* 495, a saprophyte strain extracted from the autochthonous flora of the chicory leaf *Cichorium endivia* var. *latifolium* (INRA, Jouy-en-Josas, France) [18]. It was extracted by the following process: first, the bacteria were frozen at -80°C in cryotubes. After thawing at 25°C , the bacteria were inoculated on a King's medium B agar (KBA) in Petri dishes. The Petri dishes were incubated at $20\text{--}22^{\circ}\text{C}$ under a relative humidity of 60% for 4 days. The cells were then recovered by scraping and were suspended in 150 mL of sterile deionized water. Then, the solutions were stirred vigorously for 3 minutes and the biosurfactant was recovered by centrifugation (30 minutes at $18000\times g$). It was then sterilized at 121°C for 20 minutes and stored in 5 mL aliquots, at 4°C until use.

The surface activity of the biosurfactant as well as the critical micellar concentration (CMC), *i.e.*, the surfactant concentration in a medium above which micelles form spontaneously, was determined by water surface tension measurements of the supernatant after dilutions using the Wilhelmy method and a blood pressure monitor. The CMC value is equal to $0.6\text{ g}\cdot\text{L}^{-1}$, and the surface tension value is about $27\text{ mN}\cdot\text{m}^{-1}$. The biosurfactant can therefore greatly reduce the surface tension of water [19].

The biosurfactant produced by *Pseudomonas fluorescens* 495 belongs to the class of lipopeptides or lipoproteins. It consists of a lipophilic part with a beta-carbon chain, $\beta\text{-OH-C}_{10}$, and a hydrophilic part composed of the amino acids L-Leu, D-Glu, D-AlloThr, and D-Val. The conventional dry extract, *i.e.*, the solid content of the bio-admixture, was determined according to the EN 480-8 standard. Its value, very low, is equal to 0.06%. For water/cement (W/C) ratio calculations, the biosurfactant was therefore treated as equivalent to water.

2.2. Sample Preparation. We prepared the mortars following NF EN 196-1 standard. A constant sand content of 1350 g per 450 g cement was used. Water content (including biosurfactant) was set at W/C ratio of 0.5. Based on NF EN 934-2 standard, the biosurfactant solution should be classified as an admixture. The content of biosurfactant in samples ranged from 0.5–2.5% of cement mass. Mortar compositions are given in Table 1.

Mortar samples were prepared using common Portland cement, CEM I 52.5 R CE CP2 NF (containing 95% clinker and 5% other constituents), in the form of $4\times 4\times 16\text{ cm}^3$ blocks, removed from molds after 24 hours and stored in a curing chamber at $23\pm 2^{\circ}\text{C}$ and relative humidity of above 90% for periods of varying length (28, 120, and 180 days). The relative amounts of each of the Portland cement minerals, including the amount of calcium sulfate, and the total amount of alkali metals (Na and K) are given in Table 2.

The mortar tests included four parts: (1) a slump test of mortar samples was performed before curing; (2) flexural and compressive strength mechanical tests were conducted 28, 120, and 180 days after curing; (3) after mechanical tests, some parts of samples (around $4\times 4\times 4\text{ cm}^3$) were selected for open porosity and capillary absorption tests; and (4) after curing 180 days, mortar samples were selected for bacterial community analyses.

2.3. Mechanical Evaluation. We wanted to test the effects of the biosurfactant on the rheological and mechanical properties of mortar in order to evaluate its utility as an admixture. Slump tests were performed to estimate the workability of mortars at different biosurfactant concentrations. Mechanical strength properties (compressive and flexural strength) were also compared across different biosurfactant concentrations and curing times.

To obtain the slump values of the freshly prepared mortar samples, we used a mini-cone with height of 150 mm and top and bottom diameters of 50 and 100 mm, respectively. Slump measurements were conducted at room temperature ($21\pm 2^{\circ}\text{C}$). The difference between final and initial mortar heights after removing mini-cone was measured as slump. After measuring initial slump, slump heights were measured at 15, 30, 60, 90, 120, and 150 minutes after the cone was removed. The whole test took 150 min. Each biosurfactant-treated sample had three replicates.

The Instron 3384 system (Instron, Norwood, MA, USA) was used to conduct both flexural and compressive strength tests. Load cell capacity and load measurement accuracy

TABLE 1: Compositions of mortar samples.

Cement (g) normal Portland cement	Sand (g) CEN standard sand	Water (g)	Biosurfactant (g)	Water/cement ratio	Biosurfactant concentration (%)
		225	0	0.5	0
		222.75	2.25	0.5	0.5
450 ± 1	1350 ± 5	220.5	4.5	0.5	1
		218.25	6.75	0.5	1.5
		216	9	0.5	2
		213.75	11.25	0.5	2.5

TABLE 2: Chemical composition of normal Portland cement: CEM I 52.5 N CE CP2 NF.

Composition	95% clinker 5% secondary constituent ^a
	SiO ₂ 20.2
	Al ₂ O ₃ 5.0
	Fe ₂ O ₃ 3.6
	CaO 63.2
	MgO 1.5
	SO ₃ 3.5
	K ₂ O 0.9
	Na ₂ O 0.1
	C ₃ S 63.5
	C ₃ A 7.6
	C ₄ AF 11.0
Oxide composition (wt.% ± 0.1%)	
Cement minerals in the clinker (wt.% ± 0.1%)	

^aAccording to EN 197-1, the secondary constituent in cement is a mixture of calcium sulfate anhydrite and semihydrate.

were 150 kN and ±0.5% of reading values, respectively. Two test speeds of 1 and 2 mm/min with the accuracy of ±0.2% were applied for flexural and compressive strengths, respectively. Three mortar samples from each mixture group were prepared following NF EN 196-1 standard for flexural strength tests. Then, the broken sample pieces from flexural strength test were used for compressive strength tests. Finally, average compressive and flexural strength values were calculated from the test results.

2.4. Open Porosity. Water accessible porosity was measured following NF P 18-459 standard. Using this technique, open porosity of materials can be estimated. The weights of mortar samples were measured under saturated surface-dry (SSD) and under water conditions. All biosurfactant treatments were performed in three replications. Test samples were dried at 50°C in an oven until their weights were fixed. Porosity was measured as the weight difference between water-saturated and oven-dry conditions:

$$p = \frac{W_{\text{ssd}} - W_{\text{d}}}{W_{\text{ssd}} - W_{\text{w}}} \times 100, \quad (1)$$

where p (%) is porosity, W_{ssd} (g) is the weight of the fully saturated sample in air, W_{w} (g) is the weight of the fully saturated sample in water, and W_{d} (g) is the oven-dry weight. This method has been previously used to measure water accessible porosity of cement-based materials [20–22].

2.5. Capillary Water Absorption. Capillary porosity of mortar samples was estimated using the capillary water

absorption coefficient as described in standard procedures (EN ISO 15148, EN 105-18). A porous material allows for the movement of water through its surface by diffusion, which is caused by capillary force balanced by the forces of friction, inertia, and gravity [23]. A porous material changes in weight over time until it reaches the water saturation condition [24]. During hydration, a linear relationship exists between the surface area of the porous material multiplied by the square root of time (\sqrt{t}) and the mass of absorbed water [25]. The slope of this relationship is defined as water absorption coefficient A , with the formula:

$$A = \frac{\Delta M}{S\sqrt{t}} = \frac{M_t - M_i}{S\sqrt{t}}, \quad (2)$$

where ΔM is absorbed water mass, M_t is mortar sample weight after time t , M_i is the initial weight of the mortar samples, and S is the surface area of mortar in contact with water.

Mortar samples were dried in an oven at 65°C until they reached a constant weight and then were placed in a dry container for cooling down to laboratory temperature. The test of capillary porosity was performed by placing mortar samples on geotextile fabric over a plastic support, shallowly immersing the bottom 3 mm of the samples in distilled water, and taking weight measurements at regular intervals. Each biosurfactant treatment had three replicates.

2.6. Identification of Bacteria inside Mortar Samples

2.6.1. DNA Extraction and DNA Sequence Analysis. After 180 days in the curing chamber, mortar samples were broken

in a ventilated cabinet, and then mortar pieces (3 g) from the surface 1 cm were selected and kept in sterile iceboxes for subsequent analyses. To extract the total genomic DNA of mortar samples, the UltraClean Soil DNA Isolation Kit (MO BIO, Carlsbad, CA, USA) was employed according to the instructions of the manufacturer. Extracts were checked by 1.5% agarose gel electrophoresis before PCR amplification.

The primers 806R (5'-GGACTACNNGGGTATC-TAAT-3') and 341F (5'-CCTAYGGGRBGCASCAG-3') were used to amplify the V3 and V4 regions of bacterial 16S rRNA [26]. PCR amplifications were conducted in triplicate 20 μ L reactions containing 0.25 mM of each dNTP, 0.2 μ M of each primer, 10 ng DNA, 0.4 μ L of Pfu polymerase, and 1 \times Fast Pfu Buffer (TransGen Biotech, Beijing, China). The reaction conditions were as follows: an initial melting step at 95°C for 2 min, 25 cycles of 95°C for 30 s, 55°C for 30 s, and 72°C for 30 s, followed by a final extension step of 72°C for 5 min. Amplicons were visualized on 2% agarose gels in order to assess the quality and check the size of PCR products. When successful, the right bands were removed and purified using the Axygen DNA Gel Extraction Kit (Corning Life Sciences, Corning, NY, USA). 16S rRNA gene libraries were made using the TruSeq DNA PCR-Free Sample Preparation Kit (Illumina, San Diego, CA, USA) and sequenced with the Illumina HiSeq2500 (PE250) platform at Novogene Co., Beijing, China.

2.6.2. Bioinformatic Analysis. The Quantitative Insights Into Microbial Ecology (QIIME) pipeline ver.1.9.0 was used to process the obtained raw sequence data. Only raw sequences that perfectly matched Illumina barcodes were included [27]. The sequences were trimmed of primers and barcodes, PANDAseq was used to merge paired-end reads [28], USEARCH ver. 8.0.1623 [29] was used to denoise sequences, and UCHIME was applied to check for chimeras [30]. Operational taxonomic units (OTUs) were defined by the MOTHUR program at cutoff levels of 3%. High quality sequences were aligned with publically available sequences using Muscle software [31], in order to identify the phylogenetic position of the bacterial communities. A circular genus-level phylogenetic tree based on a distance matrix was visualized using the Interactive Tree of Life (iTOL) online tool (<https://itol.embl.de/>). Vegan package ver.2.3-4 was used to calculate the richness (number of OTUs, ACE, and Chao1) and evenness (Shannon diversity index H) of bacterial communities.

2.7. Statistical Analysis. Two-way ANOVA was performed using SPSS Statistic ver. 22 (IBM, Armonk, NY, USA) to identify statistically significant treatment effects of biosurfactant concentration and curing period on mechanical strength, capillary water absorption coefficient A , and open porosity of the mortar samples. In order to test the significance of biosurfactant concentration on slump loss, one-way ANOVA was conducted. Significance was defined as $P < 0.05$.

Correlation between capillary absorption coefficient A and the relative abundance of bacterial phyla was calculated using Pearson's correlation in SPSS Statistics. A correlation

network of Pearson's values was assembled using Cytoscape ver. 3.6.0, and a heatmap of the correlation network was plotted using the "pheatmap" R-package (r-project.org).

3. Results

3.1. Slump Tests. Slump tests were carried out to evaluate the effect of biosurfactant concentration on the fresh state applicability of mortar. All slump values of mortar samples decreased over time, with or without biosurfactant. All slump values decreased rapidly in the early 30 min, and then slump rates slowed down. This phenomenon can be explained by the hydration process of Portland cement. Immediately upon adding water, calcium silicates (C_3S) and tricalcium aluminate (C_3A) are hydrated. In the first minutes, Ca^{2+} is absorbed on the silicon-rich surface of C_3S particles [32]. This gives the C_3S particles a positive charge, which increases the repulsive force between particles. The hydration of C_3A particles is also strongly exothermic and accelerates the movement of particles [33]. Therefore, mortar samples have high workability and high slump values during early hydration. Over time, more C_3S particles react with water to form $Ca(OH)_2$ and C-S-H, and C_3A can also act with $Ca(OH)_2$ to form C_4AH_n , which can inhibit the workability and flowability of mortar samples [34].

Mortar samples containing 1.5% and 2.5% biosurfactant had significantly higher slump values in the first 15 min, compared with the untreated mortar samples (Table 3). Mortar samples with 1.5% biosurfactant had an initial slump larger than 43 mm. However, slump values of mortar with 1.5% biosurfactant did not differ significantly from those of the controls after 30 min. Unexpectedly, the slump value of mortar with 2.5% biosurfactant was significantly lower than those of controls after 120 min. This suggested that 2.5% biosurfactant accelerated stiffening of the mortar with the progress of hydration after 120 min. Therefore, an admixture of biosurfactant at the proper dosage (1.5%) improved workability, but overdosage of the biosurfactant resulted in lower workability.

3.2. Compressive and Flexural Strengths. The compressive strengths of mortars that were cured in the curing chamber for 28, 120, and 180 days are shown in Figure 1. Two-way ANOVA showed that compressive strength was greatly influenced by biosurfactant dose ($P < 0.05$) and by curing time ($P < 0.01$). After 120 days, mortars with 2% and 2.5% biosurfactant had significantly higher compressive strengths (68.3 and 68.1 MPa, respectively) than samples without biosurfactant ($P < 0.05$). After 180 days, the compressive strength of mortar with biosurfactant was higher than that of the control group, and mortar with 1% biosurfactant had the highest value (67.51 MPa).

Interestingly, the compressive strength of mortar samples without biosurfactant significantly decreased with curing time, from 64.63 MPa at 28 days to 61.37 MPa at 180 days ($P < 0.05$).

This can be explained by the fact that length of C-S-H gel decreases with extended curing time [35]. Additionally,

TABLE 3: Slump values of CEM I based mortars with biosurfactant (BS) concentrations of 0%, 0.5%, 1.5%, and 2.5%.

BS concentration (% by weight of cement)	Slump value (mm)						
	0 min	15 min	30 min	60 min	90 min	120 min	150 min
0	32.33 ± 2.52a	12.20 ± 0.20a	9.23 ± 0.85a	7.13 ± 0.45a	5.37 ± 0.95a	4.83 ± 0.40ab	1.12 ± 0.37a
0.5	34.73 ± 1.84ab	13.47 ± 2.67a	10.13 ± 3.10a	7.43 ± 1.14a	7.13 ± 0.98a	5.30 ± 0.16b	0.87 ± 0.12a
1.5	43.63 ± 3.35bc	26.27 ± 1.56b	10.83 ± 1.95a	8.77 ± 1.15a	6.27 ± 1.86a	3.5 ± 0.90ac	0.67 ± 0.25a
2.5	39.47 ± 3.00c	25.87 ± 2.00b	15.67 ± 3.16a	9.63 ± 3.21a	5.77 ± 1.11a	2.80 ± 0.1c	0.80 ± 0.10a

One-way ANOVA (Tukey’s HSD test, $P < 0.05$) was used to determine the effect of BS on slump of mortar samples at a given time. Different lowercase letters indicate significant differences among BS concentrations at same time.

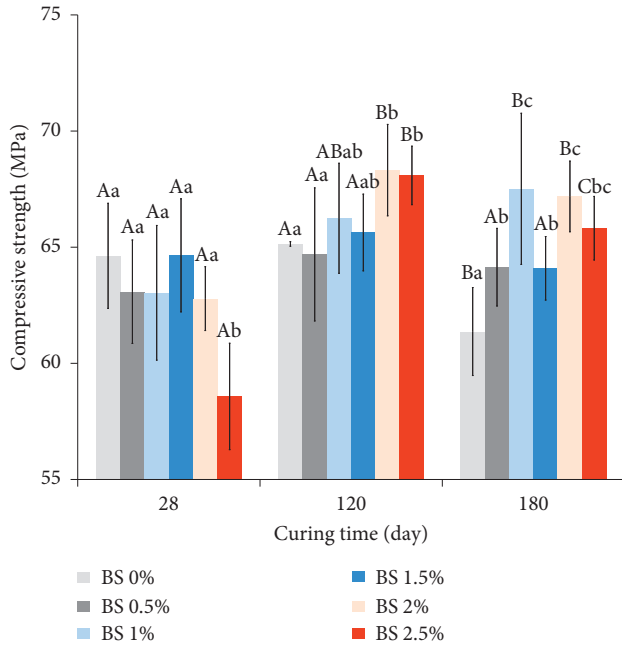


FIGURE 1: Effect of biosurfactant (BS) concentration on the compressive strength of mortars at curing periods of 28, 120, and 180 days. Tukey’s HSD test was performed to differentiate the significance of treatment. Statistically significant differences are noted by different letters. Capital letters indicate significant difference among curing times at the same biosurfactant concentration, and lowercase letters indicate significant difference among concentrations at the same curing time.

under high humidity (90%), the internal pore volume of cement-based materials expands as curing time increases [36]. The shrinkage of C-S-H gel and an increase in internal pore volume will lead to a reduction in the compressive strength of cement-based materials [35, 36]. Thus, a long curing time under high humidity can result in a slight decrease in compressive strength. All of the tested mortar samples exceeded the standard compressive strength (52.5 MPa) of CEM I 52.5 R CE CP2 NF. However, the addition of 1%, 2%, and 2.5% biosurfactant significantly increased the compressive strength of mortar samples with curing times of 28 to 180 days ($P < 0.05$, Figure 1). This suggests that the addition of biosurfactant could reduce damage to mortar that is cured for a long time under high humidity conditions.

Figure 2 shows the flexural strengths of mortars that were cured in water for 28, 120, and 180 days. Two-way

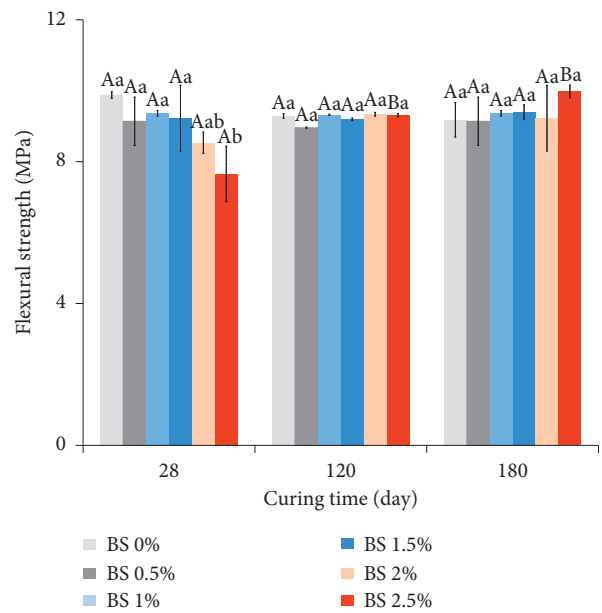


FIGURE 2: Effect of biosurfactant (BS) concentration on the flexural strength of mortars at curing periods of 28, 120, and 180 days. Tukey’s HSD test was performed to differentiate the significance of treatment. Statistically significant differences are noted by different letters. Capital letters indicate significant difference among curing times at the same biosurfactant concentration, and lowercase letters indicate significant differences among biosurfactant concentrations at the same curing time.

ANOVA showed that biosurfactant dose and curing time had no significant effects on flexural strength ($P > 0.05$). However, the interaction of curing period and biosurfactant dose was significant ($P < 0.05$). The flexural strength of cement-based materials depends on the water/cement ratio, curing conditions, and the admixture because these factors can affect the hydration process [37, 38]. After curing for 28 days, the flexural strength of mortar with 2.5% biosurfactant was lower than in untreated mortar samples, possibly because biosurfactant had slowed the hydration process of the mortar [38]. The flexural strengths stayed between 8.9 and 9.3 MPa after 120 days and between 9.0 and 9.9 MPa after 180 days at all biosurfactant concentrations (Figure 2). Interestingly, with 2.5% biosurfactant, curing for 120 and 180 days had a positive effect on flexural strength, compared with 28 days ($P < 0.05$). These results were consistent with the compressive strength tests. The addition of biosurfactant may alter the hydration process of cement particles and

strengthen mortars cured for a long time under high humidity condition.

3.3. Porosity Measurements

3.3.1. Open Porosity. The open porosity accessible for mortar water content was in the range of 13.9–14.5% (Table 4). One-way ANOVA revealed that biosurfactant dose had no significant effect on open porosity of mortar samples ($P > 0.05$).

3.3.2. Capillary Absorption Coefficient A. The rate of capillary absorption in mortar samples varied over time (Figure 3). Early in the capillary absorption experiment, the value of $\Delta M/S$ exhibited a linear increase as \sqrt{t} increased. Later in the experiment, the rate of capillary absorption slowed down. Finally, the mass of all mortars increased to a constant value over time. Capillary water absorption coefficient A values were calculated using equation (2) and are shown in Table 5. After 28 days, lower concentrations of biosurfactant (0.5%, 1%, 1.5%, and 2%) did not produce significant changes in the coefficient A value ($P < 0.05$), but 2.5% biosurfactant significantly increased the value of coefficient A ($P < 0.05$) compared to mortar without biosurfactant. After 180 days, all mortars with biosurfactant showed a significant decrease in coefficient A value ($P < 0.05$) compared to untreated mortar samples. It is interesting to note that the mortar samples with 1% biosurfactant had the lowest value ($121 \text{ kg}/(\text{m}^2 \cdot \text{min}^{1/2})$) after 180 days curing. A low coefficient A value indicates that the mortar has a low water absorption ability and a dense cement matrix [39]. Densification of a cement matrix inhibits water immobilization inside bulk and consequently decreases water absorption [40]. Additionally, the durability of cement-based materials is linked to water absorption, so a decrease of water absorption will extend service life and durability [39]. Since the addition of biosurfactant in our study significantly reduced water absorption in mortar cured for 180 days, it appears that addition of biosurfactant can increase the durability of mortar.

3.4. Characterization of Bacterial Diversity inside Mortars. Some bacteria species, such as sulfur-oxidizing bacteria, have been found to be important promoters of concrete biodegradation. Thus, identification of the bacteria inside mortar can be used to confirm the presence of mortar biodeterioration promoters and to indicate deterioration conditions. Given that bacterial colonization inside mortar takes time, mortar samples that were cured for 180 days were chosen for bacterial community analysis.

A total of 60,000–80,000 validated sequences were obtained from bacteria inside mortar samples (Table 6). Each community included 1100–1700 operational taxonomic units (OTUs). The coverage estimator showed that the sequences covered 82–88% of the bacterial communities. The ACE and Chao1 estimators calculated the richness of the bacterial communities, while the Shannon diversity index

TABLE 4: Open porosity of mortars with different concentrations of biosurfactant (BS) at curing times of 28, 120, and 180 days.

BS concentration (%)	Curing time (days)		
	28	120	180
0	14.2 ± 0.1a	14.1 ± 0.1a	14.0 ± 0.3a
0.5	14.2 ± 0.3a	14.2 ± 0.2a	13.9 ± 0.1a
1	14.3 ± 0.2a	14.1 ± 0.1a	13.9 ± 0.2a
1.5	14.5 ± 0.1a	14.1 ± 0.2a	14.1 ± 0.3a
2	14.4 ± 0.2a	13.9 ± 0.1a	13.9 ± 0.3a
2.5	14.3 ± 0.1a	14.1 ± 0.2a	13.9 ± 0.1a

Note. One-way ANOVA (Tukey's HSD test, $P < 0.05$) was used to test the effect of BS on open porosity of mortars. Different lowercase letters indicate significant differences among BS concentrations with same time.

(H) gave information about the richness and evenness of the communities. Mortars with 1% biosurfactant had a lowest richness and diversity of bacterial communities among all mortars.

A total of 42 phyla and 100 classes of bacteria were identified. The relative abundances of the ten commonest phyla are shown in Figure 4. Phylum Proteobacteria represented the most abundant group in mortars with or without biosurfactant, followed by Firmicutes, Actinobacteria, Bacteroidetes, and Gemmatimonadetes. These results agree with those of a previous study using DNA sequencing and classical cultivation methods to characterize the microbial communities in and on concrete, in which Actinobacteria and Proteobacteria were the most abundant groups [41]. Biosurfactant application noticeably reduced the relative abundance of Proteobacteria from 74.77% in the control group to around 56.14% in samples with 0.5% added biosurfactant, whereas the relative abundance of Actinobacteria increased from 15.76% in the control group to 28.76% in samples with 0.5% added biosurfactant.

A circular genus-level phylogenetic tree based on a distance matrix is shown in Figure 5. The outer ring shows the relative abundance of each genus in mortars with different concentrations of biosurfactant (0%, 0.5%, 1%, and 2.5%). The genus *Pseudomonas* (Gammaproteobacteria) dominated the bacterial communities, followed by *Sphingobium*, *Lactobacillus*, *Rhodococcus*, and *Acinetobacter*. Some *Pseudomonas* species grow autotrophically by reducing sulfur compounds with acid production, which can lead to deterioration of cement-based materials [42]. Some species of *Lactobacillus* have the ability to secrete biogenic organic acids that dissolve the cement paste matrix [43]. *Acinetobacter*, a genus of nitrifying bacteria, includes several species that can severely deteriorate cement-based materials by production of nitric acid [44]. The relative abundance of *Pseudomonas*, the dominant genus in these samples, was higher in mortars without biosurfactant than in those with biosurfactant, showing that the addition of biosurfactant reduced the amounts of certain biodegrading bacteria, which might decrease the rate of biodeterioration over time. However, biodeterioration of cement-based materials caused by microorganisms is a complex process which is still poorly understood. Future community studies of mortar

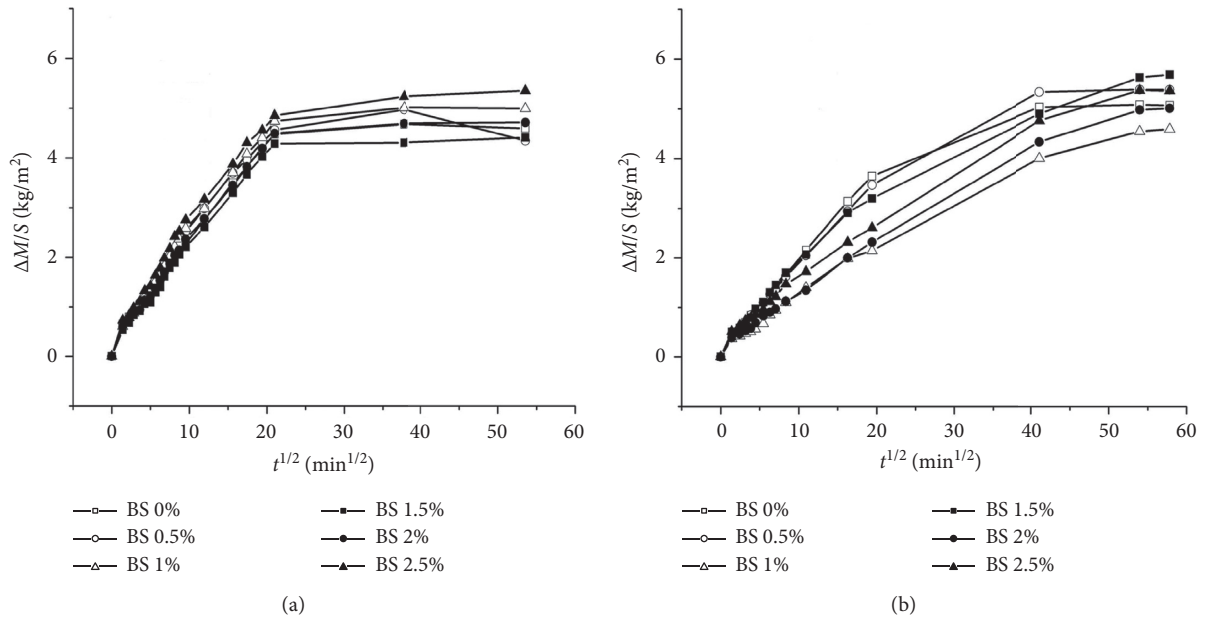


FIGURE 3: Cumulative mass of capillary water uptake versus time for mortars with different concentrations of biosurfactant (BS) at curing periods of 28 days (a) and 180 days (b).

TABLE 5: Capillary water absorption coefficient A of tested mortars.

	BS concentration (%)	Equation $M_s = A\sqrt{t}$	Capillary water absorption coefficient A ($\text{kg}/(\text{m}^2 \cdot \text{min}^{1/2})$)	R^2
28 days	0	$y = 0.226x$	$0.226 \pm 0.058\text{Aa}$	0.98
	0.5	$y = 0.238x$	$0.238 \pm 0.067\text{Aab}$	0.96
	1	$y = 0.242x$	$0.242 \pm 0.170\text{Aab}$	0.97
	1.5	$y = 0.215x$	$0.215 \pm 0.033\text{Aa}$	0.98
	2	$y = 0.226x$	$0.226 \pm 0.020\text{Aa}$	0.98
	2.5	$y = 0.254x$	$0.254 \pm 0.045\text{Ab}$	0.96
180 days	0	$y = 0.194x$	$0.194 \pm 0.015\text{Ba}$	0.99
	0.5	$y = 0.186x$	$0.186 \pm 0.005\text{Bb}$	0.97
	1	$y = 0.121x$	$0.121 \pm 0.026\text{Bc}$	0.97
	1.5	$y = 0.182x$	$0.182 \pm 0.067\text{Bb}$	0.97
	2	$y = 0.127x$	$0.127 \pm 0.058\text{Bc}$	0.97
	2.5	$y = 0.151x$	$0.151 \pm 0.007\text{Bd}$	0.91

Two-way ANOVA (Tukey’s HSD test, $P < 0.05$) was used to test the effect of biosurfactant (BS) concentration and curing time on capillary water absorption coefficient A . Statistically significant differences are noted by different letters. Different capital letters indicate significant differences among curing times at the same BS concentration; different lowercase letters indicate significant differences among BS concentrations at the same curing time.

TABLE 6: Number of OTUs, richness estimators ACE and Chao1, and Shannon diversity index H for bacterial communities inside mortars with different biosurfactant (BS) concentrations.

Samples with different BS concentration (%)		Number of valid sequences	Number of OTUs	ACE	Chao1	Shannon H	Coverage (%)
0%	1	80,684	1693	3509	3497	1.87	83.98
	2	82,486	1537	3745	3643	1.91	86.48
0.5%	1	79,000	1450	3086	3009	1.71	87.95
	2	80,273	1520	3126	3130	1.82	87.48
1%	1	64,178	1198	2821	2833	1.40	84.26
	2	68,726	1264	3073	2754	1.52	85.91
2.5%	1	80,071	1409	2831	2735	1.81	82.29
	2	82,652	1652	3065	3083	1.71	84.52

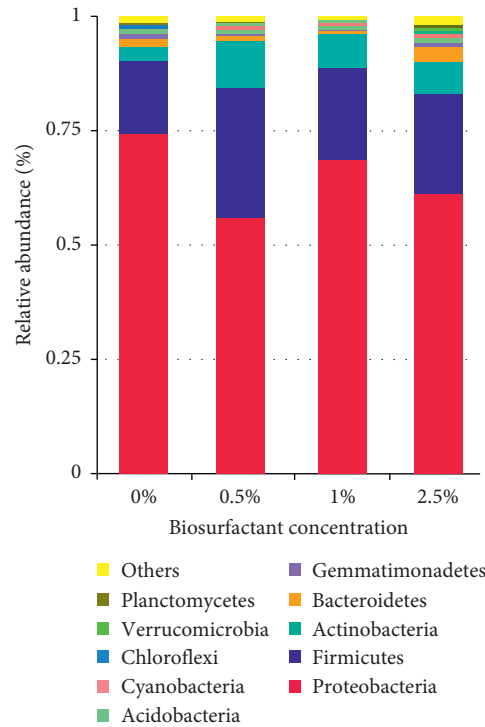


FIGURE 4: Relative abundances of the top ten bacterial phyla found in mortar samples with different biosurfactant concentrations.

bacteria based on high-throughput sequencing data should improve our understanding of biodegradation in mortar.

4. Discussion

4.1. Optimal Concentration of the Biosurfactant as Bio-Admixture. The results of the slump loss tests showed that an optimal concentration of biosurfactant (around 1.5%) increased mortar workability and indicated that the biosurfactant could act as a plasticizer in mortar. The working mechanism of an anionic surfactant as a plasticizer to improve slump loss of mortar has been widely investigated [45, 46]. The most commonly accepted explanation is that anionic surfactant molecules adhere to the surface of cement grains and the electrostatic repulsion among the cement grains disperses the flocculated cement [47]. The addition of biosurfactant may change the physical interactions among cement grains during early hydration, altering the workability of mortar.

It should be noted that after a short curing period (28 days), the flexural and compressive strengths of mortar samples with 2.5% biosurfactant were lower than those of mortars without biosurfactant ($P < 0.05$). Thus, while the addition of biosurfactant does increase mortar strength, there is an optimum limit beyond which an increase in dosage does not increase the strength [48].

4.2. Correlation between Mechanical Strength, Capillary Absorption, and Bacterial Abundance. Capillary pores (0.003–10 μm) play the decisive role in durability of cement-based materials [49]. The amount and distribution of

capillary pores are affected by curing time, curing conditions, addition of admixture, etc. [50]. In this study, longer curing time decreased the water absorption coefficient A of mortars, whether biosurfactant was used or not. The lower absorption value of mortar samples (180 days curing) indicates a lower capillary porosity of the samples, attributable to the longer curing time. Curing for 180 days made the mortar samples much denser than those with only 28 days of curing [49].

After curing for 28 days, mortar with 2.5% biosurfactant had a considerably greater A value compared to mortar without biosurfactant ($P < 0.05$). Similarly, the mechanical strength results showed that after a short curing period (28 days), both compressive and flexural strengths of mortar with 2.5% biosurfactant were lower than those of mortars without biosurfactant ($P < 0.05$). However, after a long curing time (180 days), the addition of biosurfactant resulted in a decrease in the value of coefficient A and a corresponding increase in compressive strength. Similar results about the relationship between porosity and mechanical strength of construction materials have been obtained by other researchers [49]. For example, Yao et al. [49] reported that the colonization of *Limnoperna fortunei* on concrete resulted in an increase in capillary pores of different sizes, an increase in water absorption, and a decrease in compressive strength. In the cement hydration process, tricalcium silicate can react with water and give calcium silicate hydrates (C-S-H gel), an important component of cement hydrate that contributes the most to the mechanical properties of mortar prepared from cement [51]. Here, the application of biosurfactant significantly improved the compressive strength of mortar compared to

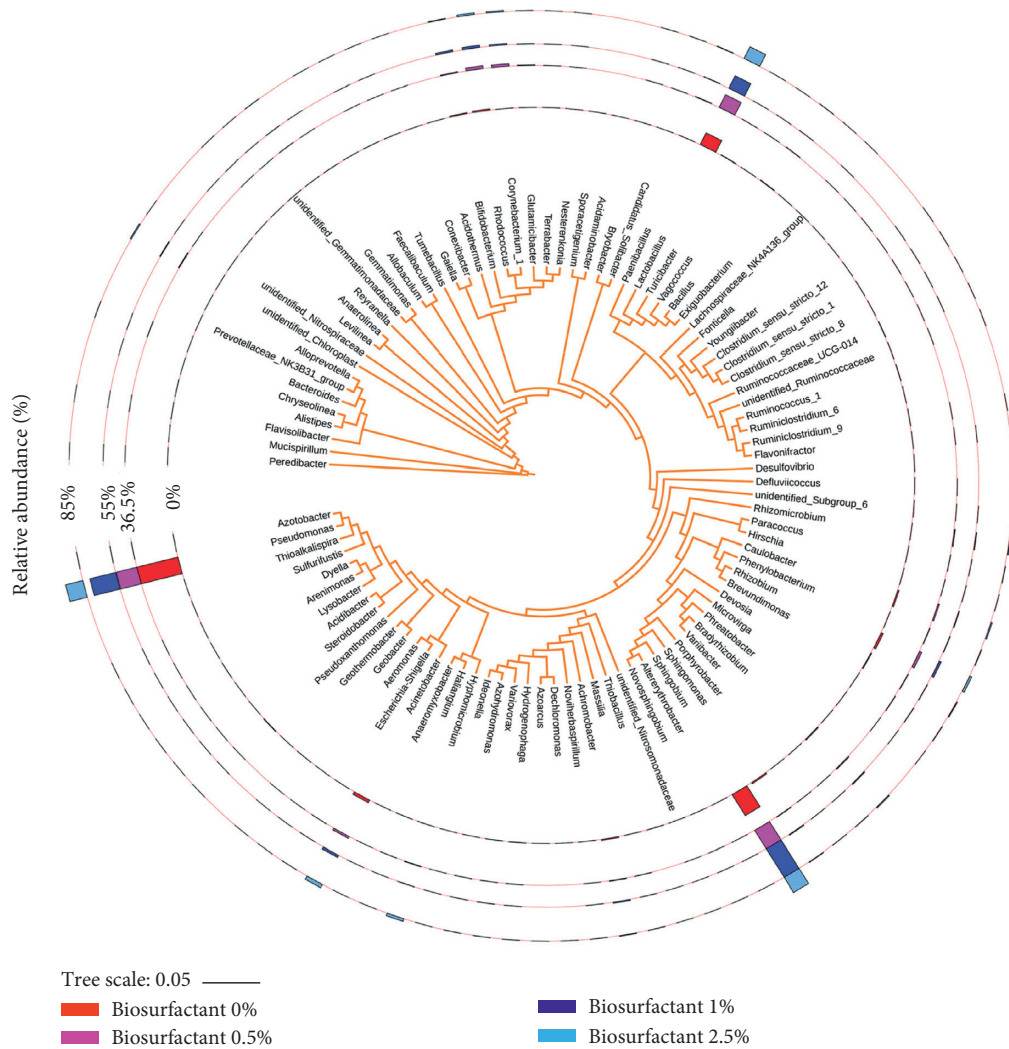


FIGURE 5: Circular maximum likelihood phylogenetic tree at the level of genus. The consecutive circles from the center to the periphery represent mortars with biosurfactant concentrations of 0%, 0.5%, 1%, and 2.5%, respectively. The outer bands show the relative abundance of the top 100 bacterial genera found in the mortar samples.

the control group after a long curing time (180 days). It is possible that the use of biosurfactant alters cement hydration rate, changes C-S-H gel density, and thus modifies the mechanical properties of mortar samples [52]. Thus, to study C-S-H structure and morphology by microscopic observation is our future research direction.

The absorption coefficient was found to be positively correlated with the phyla Proteobacteria and Firmicutes (Figure 6), which accounted for 85%–92% of the total bacteria found in our mortar samples. *Pseudomonas* (Proteobacteria) was the most abundant genus of mortar-associated bacteria identified in this study. High capillary pores can provide more space for colonization by these bacteria [53]. Furthermore, acid compounds produced by these bacteria deteriorate cement-based materials and increase porosity [43, 44]. Previous studies have reported that some species of *Pseudomonas* reduce sulfur compounds by acid production, which can lead to deterioration of construction materials [42]. And some nitrifying *Pseudomonas* and *Acinetobacter* species, which survive on the substrate

ammonium sulfate, colonize concrete surfaces via floating dust and rain [44, 54]. These bacteria can severely deteriorate building materials by the production of nitric acid [44]. *Lactobacillus* (Firmicutes) was another abundant genus in the studied mortar samples, and some species of *Lactobacillus* can secrete biogenic organic acids to dissolve the cement paste matrix [43]. As acid products are formed, the hydration compounds (e.g., C-S-H, CaCO₃, and Ca(OH)₂) of cement-based materials are eroded, and consequently, the materials gradually lose structure and decrease in compressive strength [49, 55].

It is worth to note that under high humidity, biodeterioration of mortar mainly originates from biogenic organic acids produced by the commonest bacterial phyla. Because the addition of 1% biosurfactant appears to decrease bacteria richness and diversity, this concentration of biosurfactant may reduce mortar biodeterioration caused by biogenic inorganic and organic acids. The prophylactic effect of the biosurfactant could be utilized in the marine environment to increase the durability of concrete.

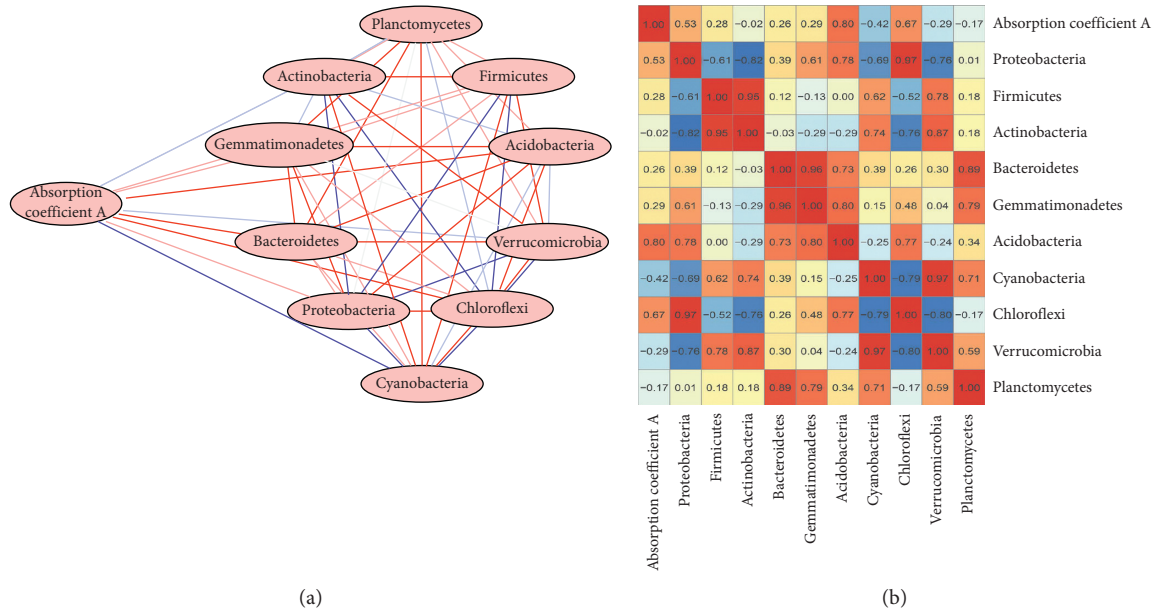


FIGURE 6: The correlation between capillary absorption coefficient A and abundance of bacterial phyla is shown in a network (a). Positive correlations are displayed with red lines and negative correlations with blue lines. The darker the color, the higher the correlation. The heatmap (b) shows the correlation values.

5. Conclusions

This work aimed to characterize the influence of a biosurfactant on the mechanical strength, capillary porosity, and bacterial colonization inside of Portland cement-based mortar samples. The following conclusions may be drawn:

- (1) The addition of biosurfactant at an optimal concentration (around 1.5%) improves the applicability of freshly prepared samples through a plasticizing action.
- (2) After a short curing period (28 days), the compressive and flexural strengths of mortar with 2.5% biosurfactant were lower than those of mortars without biosurfactant ($P < 0.05$). However, after 120 days, treatments with 2% and 2.5% biosurfactant had significantly higher compressive strength ($P < 0.05$). After 180 days, the compressive strengths of all mortars with biosurfactant were higher than those of the control group, and mortar with 1% biosurfactant had the highest value. Therefore, it seems that the addition of biosurfactant may enhance the compressive strength of mortar samples after long curing periods.
- (3) At any dosage level, the addition of biosurfactant did not influence the open porosity. However, after the longest curing period (180 days), the use of biosurfactant significantly decreased the coefficient A value ($P < 0.05$), compared to mortar samples without biosurfactant. Therefore, the addition of biosurfactant decreases capillary porosity after a long curing period.
- (4) The use of 1% biosurfactant resulted in a lower richness and diversity of bacterial communities that can lead to mortar deterioration. The addition of 1% biosurfactant to mortar may therefore decrease biodegradation of mortar.

Data Availability

The data used to support the finding of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that there are no conflicts of interest.

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Supplementary Materials

Supplementary Figure: the experimental procedures with two purposes—(1) investigating the influence of the bio-admixture on different properties of mortar samples including slump test, flexural and compressive strength measurements, and porosity determination, with the aim to confirm the ability of the biosurfactant to be used as an admixture in cementitious materials; (2) investigating the abundance and the diverse bacterial communities inside mortars in order to elucidate the relationship between the mortar porosity and bacteria community colonization. (*Supplementary Materials*)

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