




# Linking the microstructural characteristics of mortar specimens involving non-ureolytic bacteria and their strength properties

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## ABSTRACT

Despite a growing interest in using bacteria to improve the performance of cementitious composites, the mechanisms by which bacteria favourably modify the composites remain unclear. This study investigated the microscale processes by which *Bacillus cohnii* (*B. cohnii*), a non-ureolytic bacterium, modified the properties of mortar focusing on the roles played by factors such as bacteria concentration, status (live or dead) of the cells, and water-cement ratio. Results show that the use of dead or live *B. cohnii* at  $10^5$  cells/ml of mixing water led to an increase in compressive and flexural strength at early (3 and 7) curing days, while  $10^7$  cells/ml resulted in a decrease in strength. After 28 days of curing, no significant strength improvement was observed from either dead or live cells of *B. cohnii*. Water-cement ratio did not have a significant influence on how *B. cohnii* impacted the strength of mortar. Raman spectroscopy analysis indicated the presence of calcite of higher intensity within the specimens with  $10^5$  dead cells/ml than within the control and the specimens with both dead and live cells at  $10^7$  cells/ml. F-SEM imaging confirmed the presence of calcite crystals within the specimens with  $10^5$  dead cells/ml. The dominant mechanism by which addition of *B. cohnii* cells impacted the strength of the mortar specimens was through pore-filling as evidenced by the reduction of porosity. The pore-filling benefits of *B. cohnii* addition within cementitious materials holds prospect for applications especially within aggressive environments where the need to reduce the ingress of deleterious substances can be critical.

## 1. Introduction

The use of bacteria in infrastructure materials is inspired by their ability to induce the precipitation of calcite through their metabolic activities, in a process known as Microbially Induced Calcite Precipitation (MICP). Ureolytic bacteria aid precipitation of calcite through hydrolysis of urea, alkalization, and by providing bacterial cell surfaces as nucleation sites for calcite precipitation [1]. The pathways for calcite precipitation in non-ureolytic bacteria are less clear. They include oxidation of organic salts, denitrification, photosynthesis, and by serving as nucleation sites [2–4]. Although ureolytic bacteria species may cause more rapid calcite precipitation than non-ureolytic bacteria, final yields of mineral products are comparable in both types of bacteria [5]. Moreover, urea

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hydrolysis by ureolytic bacteria releases ammonia, a pollutant, as by-product [1,2,6,7], encouraging the use of non-ureolytic bacteria for MICP.

In contrast to the vast literature on use of bacteria for self-healing concrete, few studies have considered the use of non-ureolytic bacteria species [8–16], to directly improve the strength properties of mortar/concrete. Despite the availability of several studies on the inclusion of bacteria in mortar/concrete, there are conflicting reports as to its influence on the mechanical properties of mortar/concrete. While some studies reported that the inclusion of bacteria enhanced the strength and durability of mortar/concrete [8, 11,17–23], others have reported some negative effects on setting and hardening, whilst yet other groups have shown the effect of bacteria to be insignificant [9,12,24–28]. These conflicting reports can be likely attributed to the different bacteria species [29], cement type and water-cement ratio used, the varying concentrations of bacterial cells incorporated, the inclusion of nutrients and admixtures of varying concentrations in some studies or the lack thereof in other studies, and the use of bacteria with different status-live cells, dead cells, or even cell walls and proteins secreted by bacteria. All these factors result in complex bacteria-matrix interactions which ultimately determine the performance of bacteria in mortar/concrete matrices.

A number of mechanisms have been suggested in the literature to explain why addition of bacteria modifies the properties of mortar/concrete. Some of the mechanisms put forward include: (i) calcite precipitated by bacteria (MICP) acting as binder or filler [10–15,18,22,23,25,26,28,30] (ii) bacteria behaving like organic fibre [21,30] (iii) or serving as nucleating sites for deposition of calcium silicate hydrates [8,31–33]. MICP as a mechanism for strength improvement in mortar/concrete has been dismissed by Skevi et al. [8] on the basis that the effects shown by adding bacteria can be achieved by using either dead or live cells, suggesting mechanisms not dependent on bacterial metabolism.

The aim of this study was to investigate the influence of *Bacillus cohnii*, a non-ureolytic bacterial species, on the macroscopic (i.e. compressive and flexural strength) and microscopic (porosity, element composition and morphology) properties of bacterial mortar. *B. cohnii* was used in this study because it promotes calcite precipitation through the oxidation of organic sources [34]. Also, it is known to be compatible with applications in cementitious materials as shown in earlier studies [8,35]. The objectives were to: (i) ascertain the influence of *B. cohnii* concentration on the strength and durability properties of bacterial mortar, utilising two of the most frequently used concentrations of bacterial cells in the literature,  $10^5$  and  $10^7$  cells/ml of mixing water (see Table A1 in the appendix); (ii) understand the role played by bacteria status, i.e. dead and live cells of *B. cohnii*; (iii) determine the influence of water-cement ratio on the properties of bacterial mortar; (iv) establish the link between the microscopic and the macroscopic properties of bacterial mortar; (v) identify the mechanisms by which *B. cohnii* influences the properties of mortar. The influence of other factors such as bacteria species, cement type, inclusion of nutrients and admixtures on the properties of bacterial mortar are beyond the scope of this study.

This study contributes to the small number of existing studies on the use of non-ureolytic bacteria for direct strength improvement in mortar/concrete. It identifies the pathways for strength modification by *B. cohnii*, a non-ureolytic bacterium, when incorporated in mortar specimens. Given the reduction in porosity and the strength improvement obtained from the incorporation of *B. cohnii* at early curing ages, this study encourages the adoption of *B. cohnii* as a non-polluting, low carbon additive for enhanced properties in mortar/concrete.

## 2. Materials and methods

### 2.1. Preparation of live and dead bacterial cells

The bacterial cells used in this study were the alkaliphilic and spore-forming non-ureolytic bacterium *Bacillus cohnii* DSM 6307. The bacterial cells were cultured at 30 °C, with the liquid cultures agitated at 150 rpm. For the cell culture, a combination of lysogeny broth (LB) and 100 ml/l Na-sesquicarbonate (42 g/l NaHCO<sub>3</sub> and 53 g/l Na<sub>2</sub>CO<sub>3</sub>) ensured that pH 9.5 was achieved. After the culturing phase, cell densities were determined by optical density measurements at 600 nm wavelength (OD<sub>600</sub>), which had been previously calibrated to precisely relate OD<sub>600</sub> values to viable cell counts (cells per ml). The cells then were split into live and dead sets. The dead set was subjected to autoclaving at 2 bar, 121 °C for 15 min, to kill the cells. In order to ensure the cells were indeed completely killed, a viability test was performed using the plate count method.

### 2.2. Preparation of mortar specimens

Mortar specimens were made using Portland limestone cement, CEM II/A-L 32.5R (BS EN 197-1) and standard sand (BS EN 196-1).

**Table 1**  
Mix proportions.

Bacteria concentration		Mix 1, M1 (0.50 w/c)			Mix 2, M2 (0.45 w/c)		
<i>Bacillus cohnii</i> (cells/ml)	Specimen ID	Cement (g)	Water (ml)	Sand (g)	Cement (g)	Water (ml)	Sand (g)
0	Control	450	225	1350	450	202.5	1350
$2 \times 10^5$ - live cells	$2 \times 10^5$ -L	450	225	1350	450	202.5	1350
$1 \times 10^7$ - live cells	$1 \times 10^7$ -L	450	225	1350	450	202.5	1350
$2 \times 10^5$ - dead cells	$2 \times 10^5$ -D	450	225	1350	450	202.5	1350
$1 \times 10^7$ - dead cells	$1 \times 10^7$ -D	450	225	1350	450	202.5	1350

A total of five mortar specimens each for two sets of specimens were prepared (see Table 1). These included four bacterial mortars, and one without bacterial cells that served as the control. The bacterial mortar specimens were of two different concentrations and bacterial status,  $2 \times 10^5$  dead or live cells/ml and  $1 \times 10^7$  dead or live cells/ml. Dead cells were used as they were considered in earlier studies [36,37] to possess greater binding potential in comparison to live cells. The specimens in the first set designated Mix 1 (M1) were prepared with water-cement ratio (w/c) of 0.5. Due to the suggestion in earlier studies [25,38], that the enhancement in strength gained from the inclusion of bacteria in concrete is more pronounced in higher strength concrete specimens, a second set of specimens designated Mix 2 (M2) were prepared with 0.45 w/c, to investigate the influence of bacteria inclusion in cement mortars of different compressive strength levels.

To prepare the bacterial mortar specimens, the bacterial cells were prepared to the desired concentrations by serial dilution with tap water, and mixed with cement and sand in accordance with BS EN 196-1. In this study, no nutrients or growth medium were added during casting or curing of the specimens. This was to avoid having multiple variables and interactions, and to focus on the sole influence of bacteria inclusion on the properties of the bacterial mortar specimens.

After mixing, the specimens were cast in 40 mm × 40 mm x 160 mm standard moulds and were demoulded 24 h after casting. Specimens were cured by submerging in tap water at 20 °C. The bacterial mortar specimens of different proportions were kept in different containers to avoid cross-contamination during curing. The specimens were cured for 3, 7, or 28 days before testing. A total of 90 specimens (3 replicas per specimen x 5 series x 2 w/c ratio x 3 curing days) were prepared. Figure A1 in the appendix shows samples specimens before and after flexural strength test.

### 2.3. Compressive and flexural strength tests

In accordance with EN 196-1, flexural strength tests were conducted on three replicas of each specimen. Compressive strength tests were then conducted on the split specimens from the flexural test, making a total of six replicas for each specimen. The compressive and flexural strength tests were conducted using 50 kN and 100 kN hydraulic frames, respectively.

### 2.4. Mercury intrusion porosity

A Mercury Porosimeter Pascal 440 (Thermo Scientific) was used to quantify the pore distributions and to determine the bulk

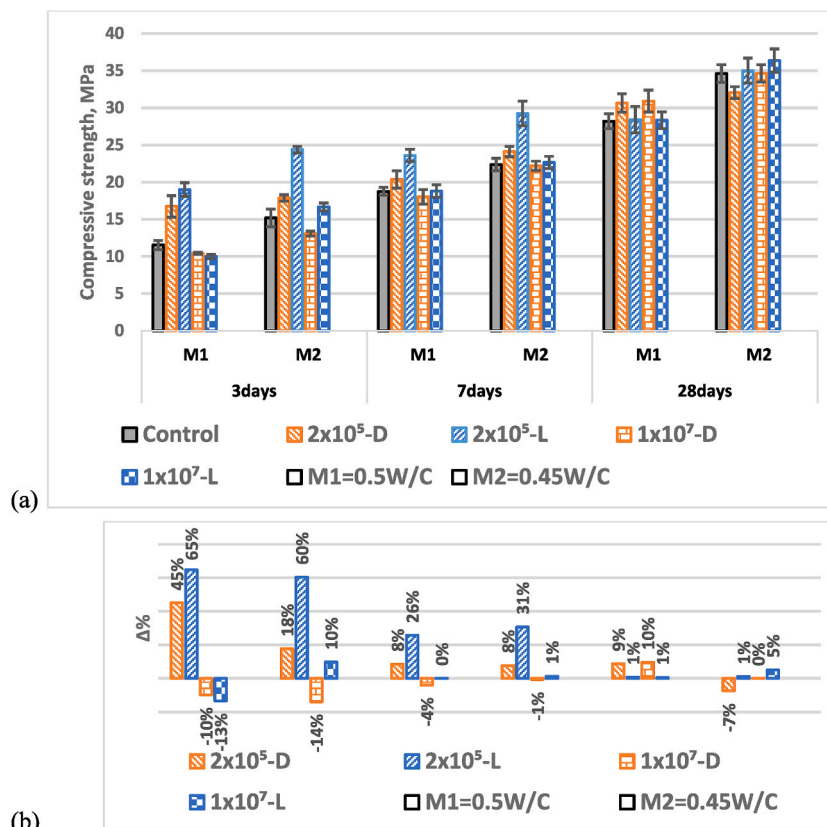


Fig. 1. (a) Compressive strength of bacterial mortars with different bacteria concentration and status (b) Change in compressive strength with respect to the control.

porosity of the specimens. The samples taken from the specimens were first degassed overnight in a vacuum and later at 50° C for 40 h before commencing the analysis. The analysis was conducted using mercury with density, surface tension and contact angle of 13.54 g/mL, 0.485 N/m and 130°, respectively, while maintaining a temperature of 19.14° C and a pressure range from 0.1 to 61 MPa.

2.5. Field emission scanning electron microscopy (FE-SEM) and electron diffractive X-ray (EDX)

The influence of bacterial cells on the morphology and elemental composition of bacterial mortar specimens were analysed using a scanning electron microscope (SEM, JOEL JSM-6480LV) with a magnification capacity of up to 30,000×. An EDX detector (Oxford Instruments) attached to the SEM was used to analyse the elemental composition of the specimens.

2.6. Raman spectroscopy

The molecular structure of the phases in the bacterial mortar specimens in this study were determined using Raman spectroscopy. Raman spectra for the specimens were obtained over a range of expected vibration frequencies, from 200 to 1500 cm<sup>-1</sup>, using an inVia confocal Raman microscope with a full spectrum of laser sources.

3. Results

3.1. Compressive strength

3.1.1. Effect of bacteria concentration, status and water-cement ratio on compressive strength

Fig. 1 shows the compressive strength of all bacterial mortar at the different bacteria concentrations and status – dead or live, water-cement ratios and at all three ages. The effects of bacteria concentration and status on compressive strength are presented in Figs. 2 and 3, respectively. At the early stage of curing (3 and 7 days), bacterial mortar specimens with 2 × 10<sup>5</sup> cells/ml of mixing water exhibited higher compressive strength values in comparison to both the control specimens and the specimens with 1 × 10<sup>7</sup> cells/ml, regardless of cell status (Fig. 2a and b). The compressive strength values for the specimens with 1 × 10<sup>7</sup> cells/ml were in general lower than the control after 3 days of curing, and not significantly different from the control at 7 days of curing (p > 0.05). At 28 days of curing, the inclusion of bacteria in the mortar specimens at both concentrations yielded the least modification in compressive strength in comparison to the 3 and 7 curing days (Fig. 2a–c). The difference in the compressive strength values of the bacterial mortar specimens from the control was within 10 % (Fig. 1b).

In contrast to observations made by Andalib et al. [25,38], the influence of bacteria on strength was similar for both water cement ratios considered. It was observed that the bacterial mortar specimens with 2 × 10<sup>5</sup> live cells/ml specimens yielded slightly higher compressive strength in comparison with those with dead cells, after 3 and 7 days of curing (p < 0.05) (Fig. 3a and b). This trend was not found for the specimens with 1 × 10<sup>7</sup> cells/ml where in general (except for M2 specimens at 3 days), no significant difference was obtained from the inclusion of dead or live cells.

3.1.2. Effect of bacteria concentration on compressive strength development of bacterial mortar

To determine the influence of bacteria on the strength development of the bacterial mortar specimens, Table 2 shows the strength

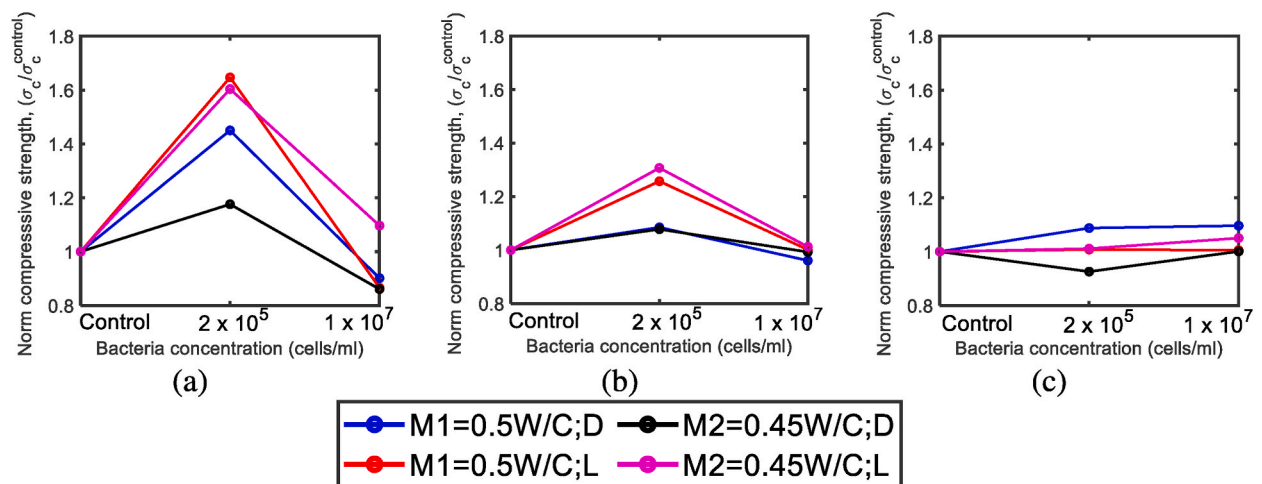


Fig. 2. Influence of bacteria concentration on normalised compressive strength,  $\sigma_c/\sigma_c^{control}$ : (a) 3 days of curing (b) 7 days of curing (c) 28 days of curing. The influence of bacteria concentration on compressive strength is presented using the normalised compressive strength to facilitate comparison with the control specimen.

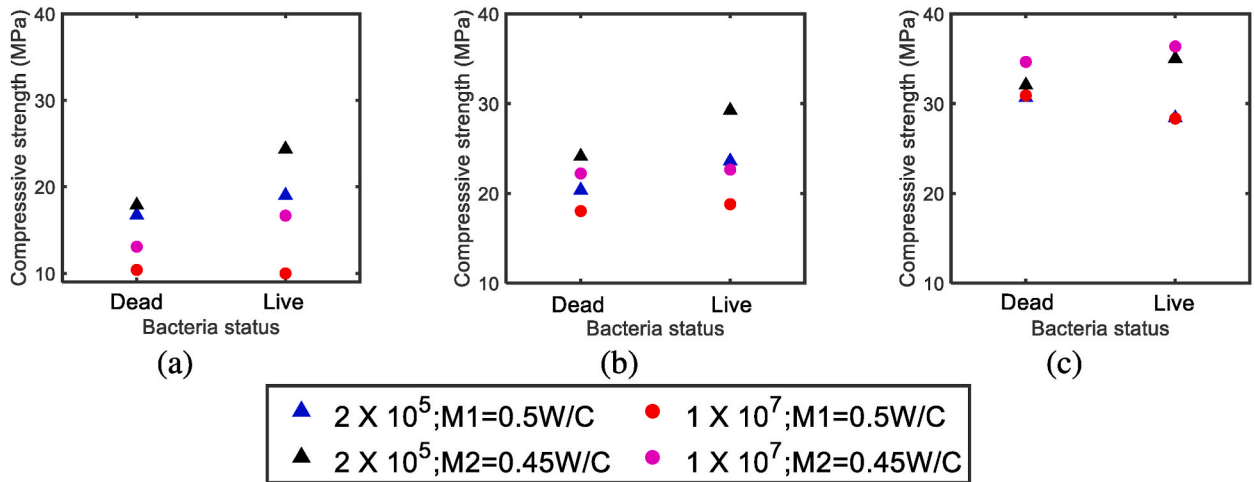


Fig. 3. Influence of bacteria status on compressive strength: (a) 3 days of curing (b) 7 days of curing (c) 28 days of curing.

development, i.e. the ratio of the compressive strength for each curing day to the 28th-day value. At 3 days of curing,  $2 \times 10^5$  cells/ml specimens had an accelerating effect on compressive strength development while strength development progressed at a slower rate in the  $1 \times 10^7$  cells/ml specimens. However, at 7 and 28 days of curing, compressive strength gain was higher in the  $1 \times 10^7$  cells/ml specimens and lower in the  $2 \times 10^5$  cells/ml specimens. These trends may suggest that, with  $2 \times 10^5$  cells/ml, bacteria act as a nucleating site where more strength-enhancing minerals and crystals are precipitated, hence, the higher early strength observed in comparison to the control. However, at  $1 \times 10^7$  cells/ml, the bacterial cells might have overwhelmed the mortar matrices thereby having a detrimental effect on strength development, especially at the early stages of curing, but more beneficial at later stages, presumably due to the morphological transformation of the mineral phases as the specimens age. Further discussions on the factors responsible for the trends obtained are presented in Section 4.

Our experimental data agree with earlier reports in the literature. Ramachandran et al., [30] reported a significant increase in the compressive strength of Portland cement mortar containing lower concentration of live cells, especially at early days of curing. The authors also observed a decrease in compressive strength for specimens containing both live and dead cells when the concentration and curing time increased, and attributed the trend to a possible interference of the bacterial biomass with strength development within the mortar. Bundur et al. [26] also reported an increase in the compressive strength of bacterial mortar specimens in comparison to the control at 7 days, and similar strength values by day 56. From the data presented in the current study, when bacteria concentration of  $1 \times 10^7$  cells/ml was used, strength gain was initially slower than the control after 3 days of curing, but became accelerated as curing progressed. The reverse was the case for the specimens with  $2 \times 10^5$  cells/ml of bacteria, indicating that strength development in bacteria mortar specimens may vary with bacteria concentration.

### 3.2. Flexural strength

#### 3.2.1. Effect of bacteria concentration and water-cement ratio on flexural strength

Fig. 4 shows the flexural strength of the bacterial mortar specimens plotted against their compressive strength. Fig. 5 shows the effect of bacteria concentration on the flexural strength. A linear root-based relationship is obtained between flexural and compressive strength for the control and bacterial mortar specimens alike, following established relationship in the literature [39]. Our data show that addition of bacteria modifies the flexural strength of mortar in a similar manner as the compressive strength, with the  $2 \times 10^5$  cells/ml specimens exhibiting higher flexural strength than the  $1 \times 10^7$  cells/ml specimens, especially at early curing ages (Fig. 5a and b). It was observed that the  $1 \times 10^7$  live cells/ml M2-specimens exhibited a significantly higher flexural strength than the control at day 28 ( $p = 0.028$ ) (Fig. 5c). This observation aligns with a suggestion that a higher concentration of bacterial cells may result in the bacteria acting as fibres whose impact may be detrimental to strength development at early stages but more beneficial at later stages

Table 2  
Compressive Strength development.

Specimen ID	Mix 1, M1 (0.50 w/c)			Mix 2, M2 (0.45 w/c)		
	3 days (%)	7 days (%)	28 days (%)	3 days (%)	7 days (%)	28 days (%)
Control	40.9	66.6	100	43.9	64.6	100
$2 \times 10^5$ -L	66.9	83.1	100	69.6	83.5	100
$1 \times 10^7$ -L	35.3	66.3	100	45.8	62.3	100
$2 \times 10^5$ -D	54.6	66.4	100	55.8	75.2	100
$1 \times 10^7$ -D	33.7	58.3	100	37.7	64.1	100

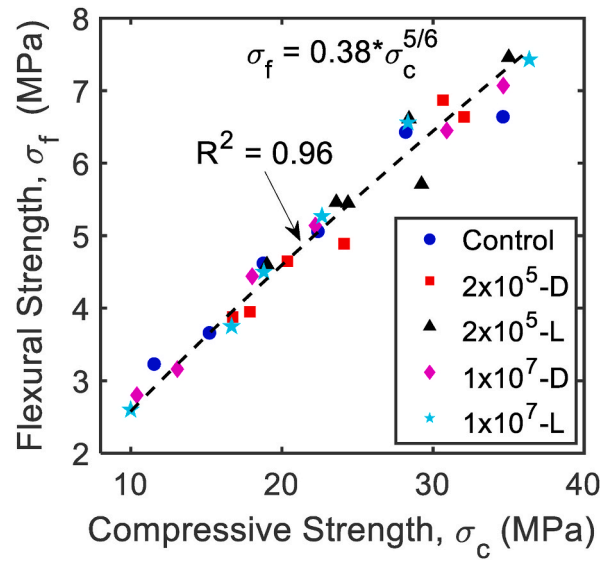


Fig. 4. Relationship between flexural and compressive strength for bacterial mortar.

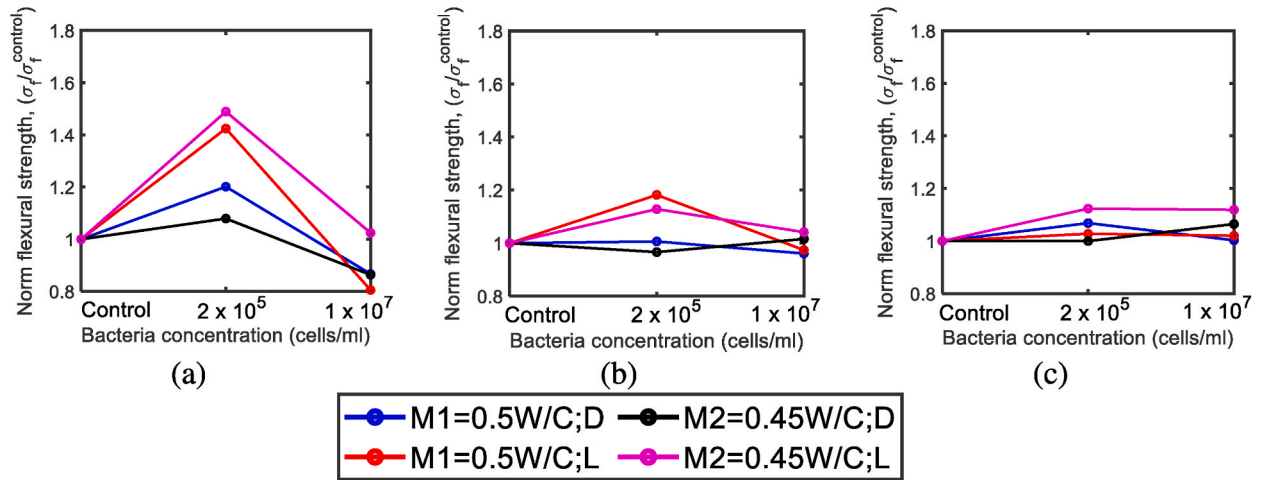


Fig. 5. Influence of bacteria concentration on normalised flexural strength,  $\sigma_f/\sigma_f^{control}$ : (a) 3 days of curing (b) 7 days of curing (c) 28 days of curing. The influence of bacteria concentration on flexural strength is presented using the normalised compressive strength to facilitate comparison with the control specimen.

[30].

### 3.2.2. Effect of bacteria concentration on flexural strength development of bacterial mortar

Similar to the observation made for the compressive strength, flexural strength development (Table 3) was in general slowest in the

Table 3  
Flexural Strength development.

Specimen ID	Mix 1, M1 (0.50 w/c)			Mix 2, M2 (0.45 w/c)		
	3 days (%)	7 days (%)	28 days (%)	3 days (%)	7 days (%)	28 days (%)
Control	50.2	71.9	100	55.1	76.2	100
2 x 10 <sup>5</sup> -L	69.6	82.6	100	73.1	76.6	100
1 x 10 <sup>7</sup> -L	39.6	68.6	100	50.5	70.9	100
2 x 10 <sup>5</sup> -D	56.4	67.7	100	59.5	73.7	100
1 x 10 <sup>7</sup> -D	43.3	68.9	100	44.8	72.8	100

$1 \times 10^7$  cells/ml specimens at 3 days of curing; higher strength increment was attained in these specimens at 7 and 28 days of curing. We observed that the specimens attained a higher percentage of their 28-day flexural strength at 3 and 7 days of curing, than their 28-day compressive strength, again supporting the suggestion that the bacterial cells may have acted as flexural strength-enhancing fibres as the specimens age.

### 3.3. Porosity

Fig. 6 shows the bulk porosity data from the MIP analysis conducted for the bacterial mortar specimens at 7 days of curing. The specimens prepared after 7 days of curing were preferred for the MIP analysis since the least difference was observed in the 28-day strength of the bacterial mortar in comparison to the control (see Fig. 2c). As expected, the porosity values for the M1 (0.50 w/c) specimens are higher than the values for the M2 (0.45 w/c) specimens, since porosity increases with an increase in water-cement ratio. The bacterial mortar specimens were in general less porous than the control specimens, except for the  $2 \times 10^5$  dead-cells/ml specimen with 0.5 w/c which had a higher porosity value than the control. This specimen however had a lower porosity value than the control at 28 days of curing (see Figure B1 in Appendix B), suggesting that its 7-day porosity value may be an outlier.

The influence of bacteria inclusion on porosity was less pronounced within the M2 specimens in comparison to the M1 specimens. It is possible that increased porosity within specimens with higher water-cement ratio may provide more spaces for bacteria and precipitated calcite to occupy [40], thereby modifying the porosity of the specimens. These data agree with earlier studies where bacterial mortar specimens had lower porosity values than the control [9,13,18,20]. The bacterial cells may have acted as micro-particles/microfibres which filled the void spaces within the mortar matrices. They might also have served as nucleating sites for calcite precipitation thereby occupying void spaces within matrices [11,13,17,28].

It was observed that the  $2 \times 10^5$  live-cells/ml specimens, which had the lowest porosity values, also had the highest compressive and flexural strength values. The highest porosity value corresponds with the lowest compressive strength among the M2 specimens but, curiously, not among the M1 specimens, which further supports the case that the 19.2% porosity value obtained for the  $2 \times 10^5$  dead-cells/ml specimen may be an outlier. The insignificant difference in the 7-day strength properties of the specimens with  $1 \times 10^7$  cells/ml and the control ( $p > 0.05$ ) (Fig. 2b), despite their lower porosity, indicate that the higher bacteria concentration might have acted as microparticles which filled pore spaces but were not potent enough to cause a significant increase in strength.

In order to establish the relationship between strength and porosity for the studied specimens, the compressive strength and the flexural strength, were plotted against porosity in Figs. 7–8. Following earlier studies in which empirical relationships were established between porosity and strength for concrete/mortar [41], an exponential fit for all data points in Figs. 7–8 is included, to quantify the degree to which strength relates with porosity for all specimens considered. The data points for M1 (0.50 w/c) and M2 (0.45 w/c) specimens are in blue and red colours, respectively, to identify the distinct trends between the two sets of specimens. For the M2 specimens (red data points), an increase in porosity is accompanied by a decrease in strength, in agreement with Chen et al. [41]. The reduction in porosity and increase in strength observed specifically for the M2 specimens with  $2 \times 10^5$  cells/ml may be attributed to the filling of pores within the mortar matrices as the bacterial cells acted as microparticles or as nucleating sites for calcite precipitation.

Porosity correlates poorly with the strength parameters for the M1 specimens (blue data points). The higher water-cement ratio for the M1 specimens resulted in higher porosity (see Fig. 6), possibly based upon a non-uniform pore size distribution, which may have caused a haphazard precipitation of minerals particularly within the pores of the specimens with  $1 \times 10^7$  cells/ml. Despite yielding a reduction in porosity, higher concentrations of bacteria may result in non-uniform distribution of bacteria microparticles and calcite crystals within pores which is detrimental to strength development [42]. A number of earlier studies showed that although  $10^5$  cells/ml yielded optimum strength, the optimum surface-level pore filling and reduction in water absorption was derived from  $10^7$  cells/ml bacteria inclusion [13,43]. It is also possible that the filling of surface-level pores in the M1 specimens with  $1 \times 10^7$  cells/ml may have reduced the amount of water absorbed by the specimens during curing, such that the cement hydration process that will produce strength-enhancing minerals was hampered.

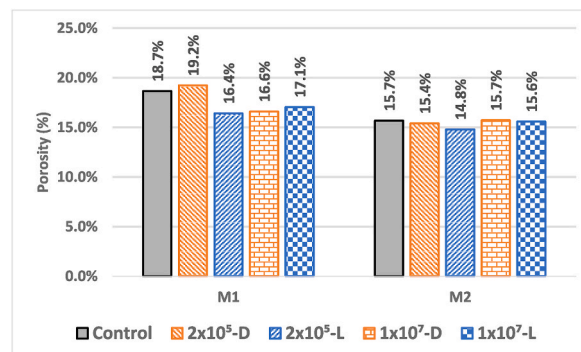


Fig. 6. Porosity of bacterial mortar specimens after 7 days of curing.

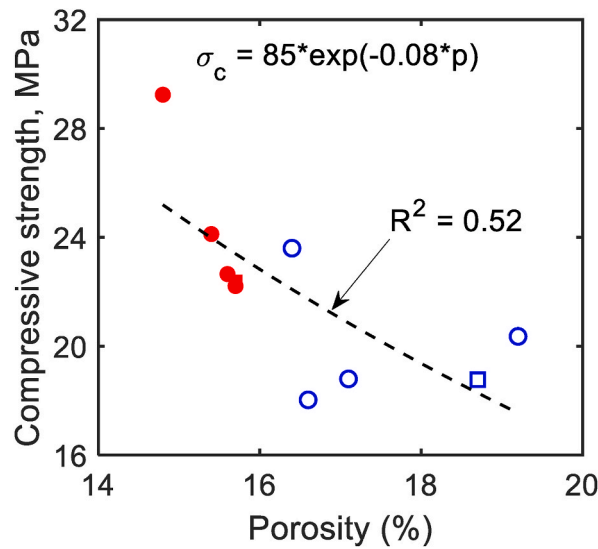


Fig. 7. Relationship between compressive strength and porosity for control (square data points) and bacterial mortar (circle data points) specimens. M1 and M2 specimens are represented in hollow-blue and filled-red data points, respectively.

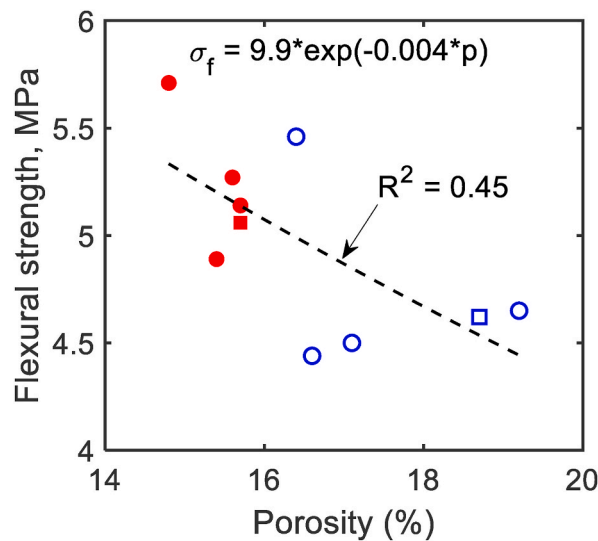


Fig. 8. Relationship between flexural strength and porosity for control (square data points) and bacterial mortar (circle data points) specimens. M1 and M2 specimens are represented in hollow-blue and filled-red data points, respectively.

### 3.4. Elemental composition of mortars

Table 4 shows the elemental composition as determined by the EDX microanalysis for the M1 specimens at 7 days of curing. EDX analysis was only conducted for the M1 specimens since the porosity data (see Fig. 6) suggests bacterial inclusion had more influence

**Table 4**  
Elemental composition for bacterial mortar specimens (Atomic %).

	C	O	Na	Mg	Al	Si	S	K	Ca	Fe	Ca/Al	Ca/Si
Control	13.84	57.01	0.04	0.41	1.17	7.84	0.94	0.14	18.17	0.46	15.52	2.32
2 x 10 <sup>5</sup> -D	16.41	56.97	0.03	0.45	1.60	7.78	1.01	0.00	15.45	0.32	9.74	1.99
2 x 10 <sup>5</sup> -L	16.14	58.15	0.00	0.40	1.28	7.37	1.04	0.20	15.01	0.38	11.79	2.05
1 x 10 <sup>7</sup> -D	13.28	59.23	0.07	0.45	1.29	7.39	1.01	0.30	16.64	0.38	13.16	2.26
1 x 10 <sup>7</sup> -L	11.72	57.58	0.00	0.28	1.51	9.10	1.31	0.21	17.97	0.34	11.87	2.02

on the microstructure of the M1 specimens than the M2 specimens. The data in Table 4 represent the mean of the elemental compositions from two different sites for each specimen. For cementitious materials, the calcium/aluminium ratio (Ca/Al) is correlated with the presence of calcium aluminosilicate hydrate, C-(A-)S-H phases [44], which is responsible for the durability of cementitious matrices. A low Ca/Al ratio indicates the presence of higher amounts of C-(A-)S-H phases. Ca/Al ratio is highest for the control specimen and lowest for the bacterial mortar specimens with  $2 \times 10^5$  cells/ml, indicating the potential for enhanced durability performance for the  $2 \times 10^5$  cells/ml specimens. Also, the presence of Al significantly accelerates hydration and promotes the formation of highly polymerised and cross-linked C-(A-)S-H products; higher Al/Si ratio indicates more stable C-(A-)S-H products [45,46]. Higher Al and Al/Si values were obtained for the bacterial mortar specimens in comparison to the control, indicating the formation of more stable C-(A-)S-H products in the specimens. These observations are in agreement with the porosity of the specimens (see Fig. 6).

Earlier studies on cement hydration have indicated a correlation between calcium/silicon ratio (Ca/Si) and the degree of silica polymerisation during the formation of a calcium silicate hydrate network [47,48]. Ca/Si ratio determines the precipitation of calcium silicate hydrate (C-S-H) phases; the presence of C-S-H phases with low Ca/Si ratio has been correlated with an increase in compressive strength due to the formation of denser C-S-H morphology [49,50]. The control specimen had higher Ca/Si ratio than the bacterial mortar specimens. Within the bacterial mortar specimens, the highest Ca/Si ratio was found for the specimen with  $1 \times 10^7$  dead cells/ml while the lowest was found for the specimen with  $2 \times 10^5$  dead cells/ml. The lower bacteria concentration in the specimens with  $2 \times 10^5$  cells/ml may have provided nucleating sites for the formation of C-S-H phases with lower Ca/Si ratio necessary for early-age strength development (Figs. 2 and 5). In contrast, higher concentrations of bacterial cells especially in the specimen with  $1 \times 10^7$  dead cells/ml may have yielded the formation of C-S-H phases with higher Ca/Si ratio, thereby resulting in retarded early strength development.

The calcium content in the specimens with  $2 \times 10^5$  cells/ml is the lowest followed by the specimens with  $1 \times 10^7$  cells/ml and then the control. Decrease in calcium content has been associated with the precipitation of calcite crystals [33]. The presence of calcite within the bacterial mortar specimens is investigated in Sections 3.5 and 3.6.

### 3.5. Calcium carbonate formation

Raman spectroscopy was used to determine and distinguish between the three crystalline polymorphs of calcium carbonate ( $\text{CaCO}_3$ ), i.e. calcite, vaterite and aragonite within the mortars. Calcite is the primary and most thermodynamically stable of the three polymorphs while aragonite and vaterite occur as secondary products [51,52]. There are three further metastable phases of  $\text{CaCO}_3$ , i.e. amorphous calcium carbonate, calcium carbonate hexahydrate and calcium carbonate monohydrate, which are precursors to the crystalline polymorphs [53]. Raman spectroscopy yields the atomic (phonon) vibrational frequencies in the crystals of the polymorphs, thereby making it possible to distinguish between them [54].  $\text{CO}_3^{2-}$  molecules in the polymorphs are classified into four fundamental vibrations,  $v_1 - v_4$ , used to distinguish between the polymorphs [51,54]. The Raman active vibration modes are  $v_1$  ( $1085 \text{ cm}^{-1}$ ),  $v_3$  ( $1450 \text{ cm}^{-1}$ ) and  $v_4$  ( $1085 \text{ cm}^{-1}$ ) for calcite;  $v_1$  ( $1085 \text{ cm}^{-1}$ ),  $v_2$  ( $852\text{-}854 \text{ cm}^{-1}$ ),  $v_3$  (very weak) and  $v_4$  (double-degenerate  $700$  and  $704 \text{ cm}^{-1}$ ) for aragonite;  $v_1$  (doublet at  $1074$  and  $1090 \text{ cm}^{-1}$ ),  $v_2$  and  $v_3$  (very weak), and  $v_4$  (doublet  $740$  and  $750 \text{ cm}^{-1}$ ) for vaterite

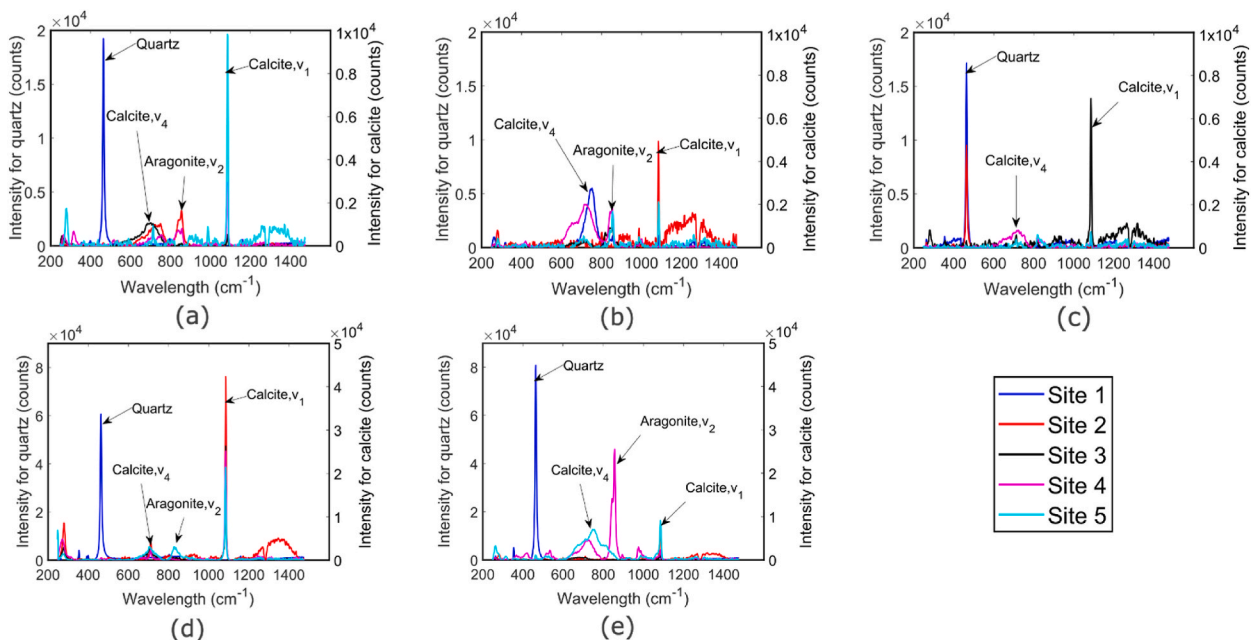


Fig. 9. Raman spectrum after 7 days of curing for (a) control (b)  $2 \times 10^5$  live cells/ml (c)  $1 \times 10^7$  live cells/ml (d)  $2 \times 10^5$  dead cells/ml (e)  $1 \times 10^7$  dead cells/ml.

[51–53]. In the lattice region, Raman bands occur at 280, 205 and 300  $\text{cm}^{-1}$  for calcite, aragonite and vaterite, respectively [51–53].

Fig. 9 shows the Raman spectra for the M1 specimens after 7 days of curing. Given that the crystals within the microstructure of the specimens may be non-uniformly distributed, the analysis was conducted on five different randomly selected sites for each specimen, to ensure the observations made are representative. Raman band 462  $\text{cm}^{-1}$ , characteristic of quartz [51], was found in both the control and bacterial mortar specimens (except in  $2 \times 10^5$  L), with higher intensity than calcite, and plotted to the left of Fig. 9. Table 5 shows the Raman vibration modes for the carbonate phases determined in the specimens. While vibration mode  $\nu_1$  (1085  $\text{cm}^{-1}$ ) was found in all specimens, the intensity was highest within the specimen with  $2 \times 10^5$  dead cells/ml (Fig. 9d), in comparison to the specimens having  $1 \times 10^7$  cells/ml and the control. The presence of calcite was also indicated in the vibration mode  $\nu_4$  (711  $\text{cm}^{-1}$ ) which was obtained for all specimens, although with lower intensities in the control and the specimen with  $1 \times 10^7$  live cells/ml. All specimens (except in  $1 \times 10^7$  D) had the lattice vibration mode (280  $\text{cm}^{-1}$ ) which is characteristic of calcite. Other phases of  $\text{CaCO}_3$  such as aragonite, vaterite and amorphous  $\text{CaCO}_3$  appeared to be present in the specimen with  $2 \times 10^5$  live cells/ml. The dominant calcite vibration mode ( $\nu_1$ ) generally occurred with similar frequency and intensities in the control (Fig. 9a) in comparison with the specimens having  $1 \times 10^7$  cells/ml (Fig. 9c and e). This may explain why they exhibited similar compressive strength values after 7 days of curing (see Fig. 2c).

### 3.6. Microstructure

Fig. 10 show FE-SEM images of the microstructure of the bacterial mortar specimens at low, medium and high magnifications. The low magnification ( $\times 2000$ ) was intended to show the surface-level landscape of the imaged samples; the medium magnification ( $\times 5000$ ) was intended to identify the presence of calcium silicate hydrate minerals at a deeper level than the low magnification. The high magnification ( $\times 10000$ ) was intended to explore the potential presence of imprints from rod-shaped bacterial cells within the specimen matrices as found by Refs. [40,55,56], and to find crystals, particularly rhombohedral crystals of calcite [54,57,58] that might have formed within pores. Needle-like ettringite structures and foil-shaped calcium silicate hydrate minerals can be observed in all specimens at medium and high magnifications (see Fig. 10e). However, in general, the morphology for the specimens with  $2 \times 10^5$  cells/ml (Fig. 10d–i) appear denser than the control (Fig. 10a–c) and the specimens with  $1 \times 10^7$  cells/ml (Fig. 10j–o). This is in agreement with SEM images presented by Ramin et al. [25] and Mondal and Ghosh [13] which showed that the morphology of deeper sections of specimens with  $10^7$  bacterial cells/ml appeared more porous than the specimens with  $10^5$  bacterial cells/ml and the control. This may be due to the higher bacteria concentration acting as microparticles and as nucleating sites for calcite precipitation which filled pores at the surface level possibly limiting the amount of water available for cement hydration within the inner sections. A lack of adequate water for cement hydration may hamper the formation of dense calcium silicate hydrate (C-S-H) gels. The specimens with  $2 \times 10^5$  cells/ml which had low calcium/silicon ratio (see Table 4) may have promoted the formation of denser C-S-H gels.

Fig. 11 shows FE-SEM images (at 10000 and 20000 magnifications) for the specimen with  $2 \times 10^5$  dead cells/ml which exhibited the highest Raman band intensity for calcite (see Fig. 9). The images show the presence of rhombohedral calcite crystals also found in earlier studies involving ureolytic bacterial cells [1,12,33,40,56,59]. No visible imprints of rod-shaped bacterial cells were found in the specimens, as expected, given the bacteria are dead and cannot proliferate. However, the presence of the rhombohedral calcite crystals suggests that the precipitation of calcite was induced by the presence of bacterial cells, in agreement with the findings from the Raman spectroscopy analysis (see Section 3.5). Also, the bacterial cells may have been embedded within the crystals as the crystals grow larger than the bacteria [33], or hidden behind the visible structures within the morphologies such that they are not visible even at high magnification. While calcite may have been present in the presence and absence of bacteria as suggested by the Raman spectroscopy analysis (see Fig. 9), Mitchell and Ferris [33] suggested that the morphology of the calcite differs in the absence of bacteria, and that larger calcite crystals were found in the presence of bacteria. The morphology of calcite precipitated was shown to depend on bacterial activity, the availability of oxygen, the pH and the concentration of  $\text{Ca}^{2+}$  present in the environment [60–62].

## 4. Discussion

The objective of this study was to ascertain the influence of *B. cohnii* inclusion on the strength and microstructural properties of mortar, considering factors such as bacteria concentration, bacteria status and water-cement ratio, while establishing the mechanisms by which the presence of bacteria modifies the properties of cement mortar. By showing that the inclusion of *B. cohnii* yielded a reduction in the porosity and an increase in early age strength of mortar, this study encourages the adoption of *B. cohnii* as a non-polluting, low carbon additive for enhanced properties in mortar/concrete.

**Table 5**  
Observed Raman frequencies ( $\text{cm}^{-1}$ ) and assignment of carbonate phases identified in studied specimens.

Vibration mode	Phase	Present in (specimens)
1085 ( $\nu_1$ )	Calcite or Aragonite	All
855 ( $\nu_2$ )	Aragonite	All except $1 \times 10^7$ L
750 ( $\nu_4$ )	Vaterite	$2 \times 10^5$ L
723 ( $\nu_4$ )	Amorphous calcium carbonate	$2 \times 10^5$ L
711 ( $\nu_4$ )	Calcite	All
280 (lattice)	Calcite	All except $1 \times 10^7$ D

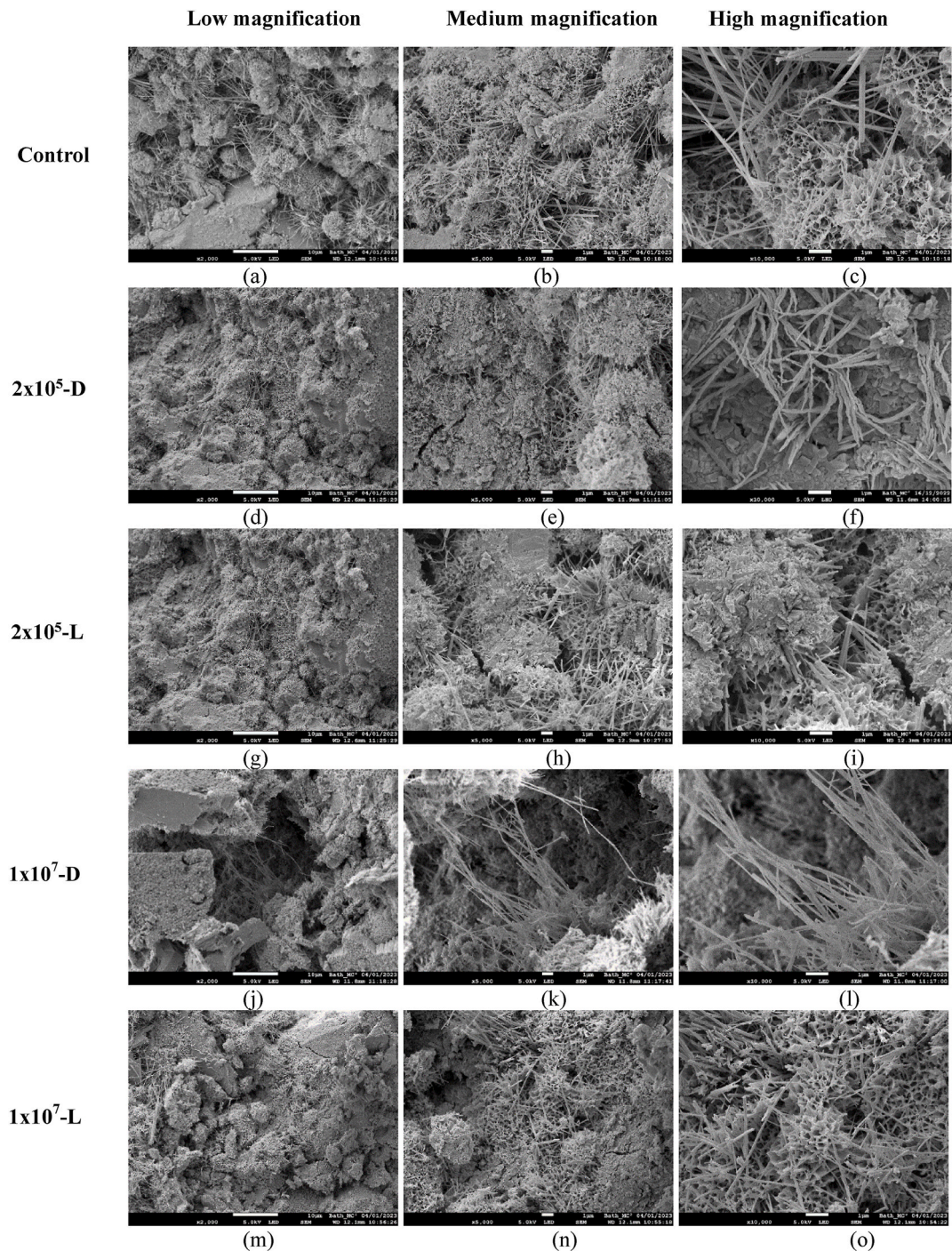


Fig. 10. FE-SEM images after 7 days of curing (a-c) Control (d-f)  $2 \times 10^5$ -D (g-i)  $2 \times 10^5$ -L (j-l)  $1 \times 10^7$ -D (m-o)  $1 \times 10^7$ -L at low magnification at low, medium and high magnifications.

#### 4.1. Influence of bacteria concentration on the modification of mortar properties

In earlier studies involving ureolytic bacteria, cell concentrations varying from  $10^1$ - $10^{14}$  (see Table 1) have been investigated to determine the optimum concentration for mortar/concrete. Most of these studies reported  $10^5$  cells/ml as the optimum concentration for various bacteria types, both in the absence of nutrients [15,16,18,24,43,63,64], and when nutrients were included as part of mixing water [25,28,65]. Yet, a few studies suggested  $10^7$  cells/ml as the optimum cell concentration for strength [59,66], and for water

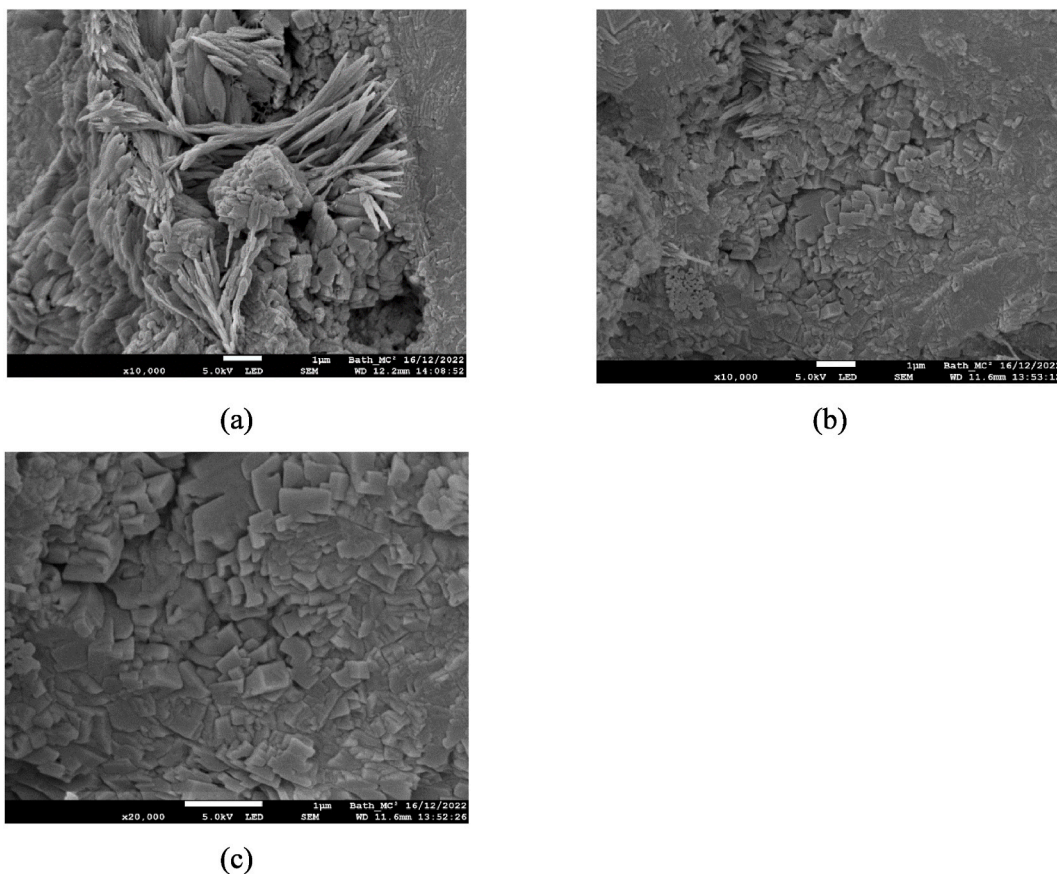


Fig. 11. FE-SEM images for  $2 \times 10^5$ -D at 7-days of curing: (a) Site 1 on sample (b) Site 2 on sample (c) Site 3 on sample.

absorption [13,43]. In this study where *B. cohnii* was used, the optimum concentration within the two concentrations considered was  $10^5$  cells/ml for both strength and porosity, in agreement with earlier studies involving ureolytic bacteria. The use of  $10^7$  cells/ml of *B. cohnii* often yielded a decrease or an insignificant influence on strength, in the present study and in the study by Hui et al. [9]. This is in contrast to two other earlier studies involving *B. cohnii* where  $10^7$  cells/ml is regarded as the optimum concentration [8,10]. It is unclear whether batch-to-batch variations in *B. cohnii* cells could impact the performance of bacterial mortar/concrete. While the inclusion of bacterial cells in mortar/concrete can be beneficial, the use of bacteria concentrations that are too high could overwhelm cementitious matrices with strength-inhibiting by-products of cement hydration, thereby resulting in unintended negative outcomes. Overwhelming amount of bacterial cells incorporated into mortar mixes by Su et al. [17] and Bundur et al. [26] increased setting time and retarded the early hydration process of the mortar.

#### 4.2. Influence of bacteria status – live or dead – on the modification of mortar properties

A number of studies involving ureolytic and non-ureolytic bacterial cells in mortar/concrete have considered the use of dead cells alongside live/vegetative cells [8,9,30]. Dead cells are prepared by killing live cells with autoclaving or UV-light [9]. The activity of live bacterial cells might determine the secretion of an organic matrix which regulates the precipitation of calcite and vaterite [60]. Like live cells, dead cells provide nucleating sites for the precipitation of calcite and other beneficial minerals [8]. In fact, dead cells may sometimes exhibit greater binding capabilities in comparison to live cells [36,37].

In this study, it was shown that both dead and live cells promoted the precipitation of calcite (see Sections 3.5 and 3.6), and yielded higher strength in agreement with [8,30], and lower porosity values, in comparison with the control, especially at early ages. It was observed that the specimens with  $2 \times 10^5$  live cells/ml specimens yielded slightly higher compressive strength in comparison with those with dead cells, after 3 and 7 days of curing ( $p < 0.05$ ). This may be explained by the capacity of live cells to provide a more potent nucleating site, owing to the more negative surface charge in the live cells of *B. cohnii* than dead cells, as shown in the zeta potential analysis conducted by Skevi et al. [8]. Further studies are required to fully unravel the link between viability status, cell surface physico-chemical properties and crystal nucleation.

### 4.3. Influence of curing age on the modification of mortar properties

While some studies show that the inclusion of bacteria in mortar/concrete resulted in an increase in strength which continued to increase with the curing age of the specimens [17,18,24,25,64], others showed a diminishing influence of bacteria inclusion with time [9,26,30,67,68]. The decrease in the potency of bacteria to cause an increase in strength was attributed to a decomposition of bacterial cells over time, thereby releasing cellular contents such as proteins and peptidoglycan which might weaken mortar/concrete matrices by interfering with the cement hydration process [9,12,30].

In this study, higher early strength was obtained for the specimens with  $2 \times 10^5$  cells/ml at 3 and 7 days of curing, while the least difference was obtained between the specimens and the control at 28 days of curing. In the study conducted by Bang et al. [68], the insignificant difference obtained between the strength of bacterial specimens in comparison to the control was attributed to lower amounts of viable cells. Although calcite precipitation occurred as early as within 4–16 h with or without a Urea-CaCl<sub>2</sub> medium [67, 68], the amount of viable cells left in the matrices of bacterial mortar specimens decreases over time as found in earlier studies [19,31]. Mitchell and Ferris [33] also showed that the rate of calcite precipitation increased until day 2 and then decreased thereafter. The decline in strength enhancement observed in the specimens with  $2 \times 10^5$  live cells/ml after 3 days of curing may be due to the decrease in the amount of viable cells with time.

Mortar matrices containing autoclaved (killed) cells can yield better strength properties than the control, as the cells act as nucleating sites for the precipitation of strength-enhancing crystals. However, the decomposition of both dead and live cells over time can release substances deleterious to continuous strength development. Bundur et al. [26] showed that while live cells helped to maximise calcite precipitation at early ages (up to day 7), specimens with dead cells had similar calcite content as with live cells and the control, by the 28th day of curing.

The specimens with  $1 \times 10^7$  cells/ml exhibited retarded strength development at early curing ages, and yielded similar or slightly higher strength than the control at 28 days of curing (see Tables 2 and 3). It is possible that decomposed matter from the high concentration of bacterial cells was in sufficiently large quantity to act as fibre which slightly enhanced flexural strength at later stages. It has been suggested that dead cells can serve as organic fibres yielding better strength performance [30]. In particular, the 28-day flexural strength for the M2 specimens with  $1 \times 10^7$  cells/ml were slightly higher in comparison with the control. The presence of fibre in cementitious matrices limits crack width and provides structural rigidity under flexural loading, and may be responsible for observed increase in flexural strength.

### 4.4. Influence of water-cement ratio on the modification of mortar properties

Water-cement ratio is one of the factors that have been identified to influence the performance of bacterial cells in mortar/concrete [8,13,25,38]. The increased porosity derived from a higher water-cement ratio could provide more room for the calcite precipitated by bacteria as they act as nucleating sites. Therefore, it is expected that the enhancement in strength derived from bacterial cells should be more pronounced as water-cement ratio increases, although the overall strength for cementitious matrices decreases with an increase in water-cement ratio. On the other hand, the higher porosity in specimens with higher water-cement ratio may increase the optimum number of bacterial cells required to precipitate crystals in sufficient quantity to fill pores [13].

In this study, the influence of water-cement ratio on the properties of bacterial mortar was more pronounced for porosity than for strength. The porosity of M1 (0.50 w/c) bacterial mortar specimens were lower than the control (see Fig. 6), while smaller difference was observed for the M2 specimens (0.45 w/c). The higher water-cement ratio and pore spaces in the M1 specimens may have provided more room for precipitated calcite to fill, hence the more pronounced porosity modification observed. In terms of strength modification by bacteria inclusion (see Fig. 1), the M2 specimens (0.45 w/c) did not significantly outperform the M1 specimens (0.50 w/c), similar percentage differences were observed from both water to cement ratios. This is contrary to the findings from Andalib et al. [25, 38] who showed that the increment in compressive strength derived from the use of bacteria in concrete continued to rise as the water-cement ratio decreased, due to more calcite produced within the tiny concrete pores and cavities. Instead, it is more plausible that a reduction in water-cement ratio will provide minimal pore space for calcite precipitation.

### 4.5. Mechanism by which addition of bacteria modifies mortar properties

The modification of mortar/concrete matrices by the presence of ureolytic bacteria has been attributed to the precipitation of calcite (MICP) or as a result of bacterial cells providing nucleating sites for the precipitation of minerals including C-S-H and C-A-S-H [12–15,31–33]. However, from the few studies involving non-ureolytic *B. cohnii* in mortar/concrete, Kumari et al. [10] suggested MICP as the mechanism by which the bacteria improve the strength of mortar, while Skevi et al. [8] and Chaurasia et al. [11] argued in favour of other minerals such as C-S-H and C-H nucleated by the presence of bacterial cells.

Although the formation of calcite is indicated in bacterial mortar and control specimens considered in this study (see Figs. 9 and 11), the higher calcite intensity obtained specifically for the bacterial mortar specimens containing  $2 \times 10^5$  cells/ml, and the lower

porosity derived from bacterial mortar specimens in comparison to the control, indicate calcite formation as a possible mechanism by which presence of *B. cohnii* cells modifies the properties of mortar. Given that addition of both live or dead cells gave similar results, this calcite formation likely occurred as a result of the cells acting passively as nucleating sites for calcite precipitation. Pore-filling effects of calcite crystals and bacterial cells acting as microparticles have the capacity to reduce porosity and increase the compressive strength of cement mortar as observed in this study. Furthermore, the formation of C-S-H phases with lower Ca/Si ratio as indicated in the element composition analysis (see Table 4) may also be responsible for the higher early strength obtained for the specimens with  $2 \times 10^5$  cells/ml.

## 5. Conclusion

This study contributes to the small number of existing studies on the use of non-ureolytic bacteria for direct strength improvement in mortar/concrete by investigating the influence of *B. cohnii*, a non-ureolytic bacterium, on the strength and microstructural properties of mortar. The mechanisms by which presence of *B. cohnii* modifies the properties of mortar and the roles played by factors such as bacteria concentration, bacteria status, water-cement ratio on the modification were investigated. The factors considered have not been combined as done in the present study, in the few studies in the literature where *B. cohnii* has been incorporated in mortar/concrete. The following are the conclusions drawn from the study:

Both dead (autoclaved) and live *B. cohnii* cells are capable of enhancing the early strength of mortar, likely by serving as nucleating sites for mineral precipitation. Given the short life span of live cells within the aggressive alkaline environment of cement, live and dead cells may impact the properties of mortar/concrete in similar manner in the long term as both live and dead cells decompose over time.

The identified mechanisms by which presence of *B. cohnii* cells modifies the properties of cement mortar include the formation of calcite crystals and the ability of bacterial cells to act as microparticles which fill pores and reduce porosity, and the precipitation of dense C-S-H minerals with low Ca/Si ratio. Other possible mechanisms which could not be verified in this study include decomposed bacteria matter acting as fibres especially when they are in large quantity, and the formation of strength-enhancing minerals other than C-S-H through the interactions of the bacterial cells with ions in their environment.

The high early strength derived from the use of  $10^5$  cells/ml holds prospects for the use of *B. cohnii* for early strength development in pozzolana mortar/concrete materials where early strength development is slow. Superior durability properties can be benefited from the use of *B. cohnii* in lightweight cementitious materials where the need to reduce porosity can be critical. Ultimately, the findings of this study encourage the adoption of *B. cohnii* as a non-polluting, low carbon additive for enhanced properties in mortar/concrete.

## CRedit authorship contribution statement

**Peter Adesina:** Writing – original draft, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Bianca J. Reeksting:** Writing – review & editing, Methodology, Conceptualization. **Susanne Gebhard:** Writing – review & editing, Validation, Methodology, Funding acquisition, Conceptualization. **Kevin Paine:** Writing – review & editing, Validation, Supervision, Project administration, Methodology, Funding acquisition, Conceptualization.

## Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Professor Kevin Paine reports financial support was provided by Engineering and Physical Sciences Research Council. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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## Appendix A. Sample specimens

Figure A1 shows sample specimens in this study before and after flexural strength test.

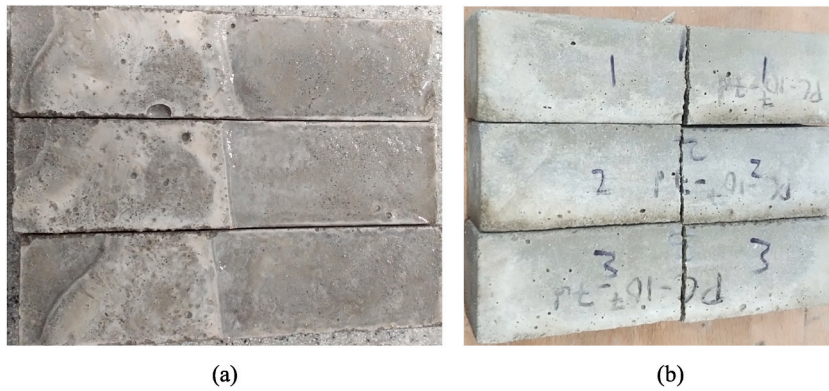


Fig. A1. (a) Sample bacterial mortar specimen before flexural strength test (b) Sample bacterial mortar specimen after flexural strength test.

**Table A1**  
Concentration of bacterial cells used in mortar/concrete matrices in the literature

Bacteria concentration (cells/ml)	Bacteria type and references	Result
$10^1 - 10^2$	<i>B. subtilis</i> [15,16]; <i>E. coli</i> [63]; <i>Shewanella specie</i> [18,64]	Negligible/Marginal positive effect
$10^3 - 10^4$	<i>B. megaterium</i> [25]; <i>Shewanella specie</i> [18,64]; <i>B. pasteurii</i> also known as <i>S. pasteurii</i> [28,30,43]; <i>B. subtilis</i> [13–16]; <i>phylum Firmicutes</i> [65]; <i>E. coli</i> [63]	Moderate positive effect
$10^5 - 10^6$	<i>B. megaterium</i> [22,25]; <i>ACRN3</i> and <i>ACRN5</i> [24]; <i>Shewanella specie</i> [18,64]; <i>B. pasteurii</i> also known as <i>S. pasteurii</i> [26,28,31,43,58,68,69]; <i>B. cohnii</i> [8,10]; <i>Bacillus specie</i> [38,55]; <i>B. sphaericus</i> [59]; <i>B. subtilis</i> [13–16]; <i>phylum Firmicutes</i> [65]; <i>B. Acetophenoni</i> & <i>B. Odysseyi</i> [66]; <i>B. aerius</i> [70]	Optimal effect
$10^7 - 10^8$	<i>B. megaterium</i> [11,19,25]; <i>B. cohnii</i> [8–11]; <i>Shewanella species</i> [18,64]; <i>ACRN3</i> and <i>ACRN5</i> [24]; <i>B. subtilis</i> [12,13]; <i>B. pasteurii</i> also known as <i>S. pasteurii</i> [11,28,31,58,68]; <i>B. sphaericus</i> [40]; <i>Spore-forming bacteria</i> [27]; <i>Bacillus specie</i> [55]; <i>B. sphaericus</i> [59]; <i>B. Acetophenoni</i> & <i>B. Odysseyi</i> [66]; <i>Neisseria perflava</i> [23]	Optimal/Strong positive effect
$10^9 - 10^{10}$	<i>B. cohnii</i> [8]; <i>B. pasteurii</i> also known as <i>S. pasteurii</i> [58,68]; <i>B. sphaericus</i> [59]	Sub-optimal positive effect
$10^{13} - 10^{14}$	<i>B. mucilaginosus</i> [17]	Marginal/Negative effect

**Appendix B. Mercury Intrusion Porosity**

Figure A1 shows the MIP data for the control M1 specimen and the bacterial mortar with  $2 \times 10^5$  dead cells/ml at 7 and 28 days of curing. As expected, porosity decreased with the age for both the control and the bacterial mortar. The bacterial mortar specimen had higher porosity than the control at 7 days of curing. This was unexpected, and considered an outlier, given that other bacterial mortar specimens had lower porosity values than the control at 7 days (see Fig. 6). The reduction in porosity is attributed to the formation of crystals and minerals from the bacterial cells acting as nucleating sites for calcite precipitation. The lower porosity obtained from the bacterial mortar specimen in comparison to the control, at 28 days, supports the consideration of the porosity value at 7 days as an outlier.

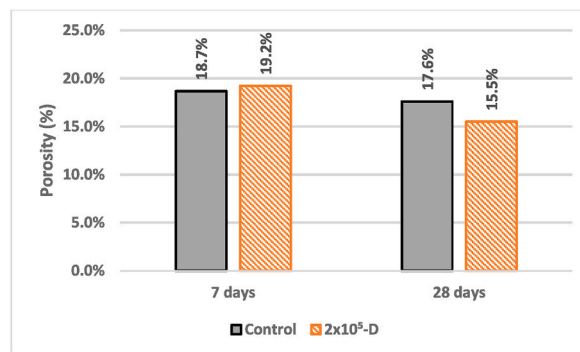


Fig. B1. Porosity of specimens at 7 and 28 days of curing

## Data availability

Data will be made available on request.

## References

- [1] N. De Belie, J. Wang, Bacteria-based repair and self-healing of concrete, *J Sustain Cem Mater* 5 (1) (2015) 35–56.
- [2] M. Fahimzadeh, P. Pasbakhsh, L.S. Mae, J.B.L. Tan, R.K.S. Raman, Multifunctional, sustainable, and biological non-ureolytic self-healing systems for cement-based materials, *Engineering* 13 (2022) 217–237, <https://doi.org/10.1016/j.eng.2021.11.016> [Internet].
- [3] T.D. Hoffmann, B.J. Reeksting, S. Gebhard, Bacteria-induced mineral precipitation: a mechanistic review, *Microbiol.* 167 (4) (2021).
- [4] M. Seidel, C. Hamley-Bennett, B.J. Reeksting, M. Bagga, L. Hellmann, T.D. Hoffmann, et al., Metabolic insights into microbially induced calcite formation by bacillaceae for application in bio-based construction materials, *Environ. Microbiol.* 27 (4) (2025).
- [5] B.J. Reeksting, T.D. Hoffmann, L. Tan, K. Paine, S. Gebhard, In-depth profiling of calcite precipitation by environmental bacteria reveals fundamental mechanistic differences with relevance to application, *Appl. Environ. Microbiol.* 86 (7) (2020).
- [6] W. De Muynck, N. De Belie, W. Verstraete, Microbial carbonate precipitation in construction materials: a review, *Ecol. Eng.* 36 (2) (2010) 118–136.
- [7] H. Jonkers, T. Arjan, M. Gerard, C. Oguzhan, S. Erik, Application of bacteria as self-healing agent for the development of sustainable concrete, *Ecol. Eng.* 36 (2010) 230–235.
- [8] L. Skevi, B.J. Reeksting, T.D. Hoffmann, S. Gebhard, K. Paine, Incorporation of bacteria in concrete: the case against MICP as a means for strength improvement, *Cem. Concr. Compos.* 120 (April) (2021) 104056, <https://doi.org/10.1016/j.cemconcomp.2021.104056> [Internet].
- [9] T.Y. Hui, L. Skevi, B. Reeksting, S. Gebhard, K. Paine, The effect of bacteria on early age strength of CEM I and CEM II cementitious composites, *Sustain. Times* 14 (2) (2022).
- [10] C. Kumari, B. Das, R. Jayabalan, R. Davis, P. Sarkar, Effect of nonureolytic bacteria on engineering properties of cement mortar, *J. Mater. Civ. Eng.* 29 (6) (2017) 1–9.
- [11] L. Chaurasia, V. Bisht, L.P. Singh, S. Gupta, A Novel Approach of Biomineralization for Improving Micro and macro-properties of Concrete, 195, *Constr Build Mater*, 2019, pp. 340–351, <https://doi.org/10.1016/j.conbuildmat.2018.11.031> [Internet].
- [12] R. Pei, J. Liu, S. Wang, M. Yang, Use of bacterial cell walls to improve the mechanical performance of concrete, *Cem. Concr. Compos.* 39 (2013) 122–130, <https://doi.org/10.1016/j.cemconcomp.2013.03.024> [Internet].
- [13] S. Mondal, A. (Dey) Ghosh, Investigation into the optimal bacterial concentration for compressive strength enhancement of microbial concrete, *Constr. Build. Mater.* 183 (2018) 202–214, <https://doi.org/10.1016/j.conbuildmat.2018.06.176> [Internet].
- [14] S. Basha, L.K. Lingamgunta, J. Kannali, S.K. Gajula, R. Bandikari, S. Dasari, et al., Subsurface endospore-forming bacteria possess bio-sealant properties, *Sci. Rep.* 8 (1) (2018) 1–13, <https://doi.org/10.1038/s41598-018-24730-3> [Internet].
- [15] S. Jena, B. Basa, K.C. Panda, N.K. Sahoo, Impact of *Bacillus subtilis* bacterium on the properties of concrete, *Mater. Today Proc.* 32 (xxxx) (2020) 651–656, <https://doi.org/10.1016/j.matpr.2020.03.129> [Internet].
- [16] M. Sarkar, D. Adak, A. Tamang, B. Chattopadhyay, S. Mandal, Genetically-enriched microbe-facilitated self-healing concrete-a sustainable material for a new generation of construction technology, *RSC Adv.* 5 (127) (2015) 105363–105371.
- [17] Y. Su, J. Feng, P. Jin, C. Qian, Influence of bacterial self-healing agent on early age performance of cement-based materials, *Constr. Build. Mater.* 218 (2019) 224–234.
- [18] P. Ghosh, S. Mandal, B.D. Chattopadhyay, S. Pal, Use of microorganism to improve the strength of cement mortar, *Cement Concr. Res.* 35 (10) (2005) 1980–1983.
- [19] V. Achal, X. Pan, N. Özyurt, Improved strength and durability of fly ash-amended concrete by microbial calcite precipitation, *Ecol. Eng.* 37 (4) (2011) 554–559.
- [20] S.A. Abo-El-Enin, A.H. Ali, F.N. Talkhan, H.A. Abdel-Gawwad, Application of microbial biocementation to improve the physico-mechanical properties of cement mortar, *Hous Building Natl Res Cent* 9 (1) (2013) 36–40.
- [21] V. Achal, A. Mukherjee, P.C. Basu, M.S. Reddy, Lactose mother liquor as an alternative nutrient source for microbial concrete production by *Sporosarcina pasteurii*, *J. Ind. Microbiol. Biotechnol.* 36 (3) (2009) 433–438.
- [22] S. Krishnapriya, G.P.A. Venkatesh Babu D, Isolation and identification of bacteria to improve the strength of concrete, *Microbiol. Res.* 174 (2015) 48–55.
- [23] R. Syarif, R.I. Chaerun, S.K. Chaerun, S.H. Prasetyo, R.K. Wattimena, Construction biotechnology: improving mortar properties through calcium carbonate precipitation using a novel strain of the bacterium *Neisseria perflava*, *Discov Civ Eng* 1 (1) (2024), <https://doi.org/10.1007/s44290-024-00047-1> [Internet].
- [24] M.J.C. Alonso, C.E.L. Ortiz, S.O.G. Perez, R. Narayanasamy, San Miguel G. Fajardo, J. del, H.H. Hernández, et al., Improved strength and durability of concrete through metabolic activity of ureolytic bacteria, *Environ. Sci. Pollut. Res.* 25 (22) (2018) 21451–21458.
- [25] R. Andalib, M.Z. Abd Majid, M.W. Hussin, M. Ponraj, A. Keyvanfar, J. Mirza, et al., Optimum concentration of *Bacillus megaterium* for strengthening structural concrete, *Constr. Build. Mater.* 118 (2016) 180–193, <https://doi.org/10.1016/j.conbuildmat.2016.04.142> [Internet].
- [26] Bundur Z. Basaran, M.J. Kirisits, R.D. Ferron, Biomineralized cement-based materials: impact of inoculating vegetative bacterial cells on hydration and strength, *Cement Concr. Res.* 67 (2015) 237–245, <https://doi.org/10.1016/j.cemconres.2014.10.002> [Internet].
- [27] M. Luo, C. Qian, Influences of bacteria-based self-healing agents on cementitious materials hydration kinetics and compressive strength, *Constr. Build. Mater.* 121 (2016) 659–663, <https://doi.org/10.1016/j.conbuildmat.2016.06.075> [Internet].
- [28] N. Chahal, R. Siddique, A. Rajor, Influence of bacteria on the compressive strength, water absorption and rapid chloride permeability of concrete incorporating silica fume, *Constr. Build. Mater.* 37 (2012) 645–651, <https://doi.org/10.1016/j.conbuildmat.2012.07.029> [Internet].
- [29] A.M. Ahmad, J. Zhang, B. Liu, X. Guohao, T. Xiaoyi, G. Haoying, et al., Synergistic effect of composite bacteria on self-healing process of concrete crack, *Case Stud. Constr. Mater.* 20 (November 2023) (2024) 1–14, <https://doi.org/10.1016/j.cscm.2024.e03028> [Internet].
- [30] S.K. Ramachandran, V. Ramakrishnan, S.S. Bang, Remediation of concrete using micro-organisms, *ACI Mater. J.* 98 (2001) 3–9.
- [31] Z.B. Bundur, A. Amiri, Y.C. Ersan, N. Boon, N. De Belie, Impact of air entraining admixtures on biogenic calcium carbonate precipitation and bacterial viability, *Cement Concr. Res.* 98 (April) (2017) 44–49.
- [32] S. Douglas, T.J. Beveridge, Mineral formation by bacteria in natural microbial communities, *FEMS Microbiol. Ecol.* 26 (2) (1998) 79–88.
- [33] A.C. Mitchell, F. Grant Ferris, The influence of *Bacillus pasteurii* on the nucleation and growth of calcium carbonate, *Geomicrobiol. J.* 23 (3–4) (2006) 213–226.
- [34] J. Xu, W. Yao, Z. Jiang, Non-ureolytic bacterial carbonate precipitation as a surface treatment strategy on cementitious materials, *J. Mater. Civ. Eng.* 26 (2014) 983–991.
- [35] L. Tan, B. Reeksting, I. Justo-Reinoso, V. Ferrandiz-Mas, A. Heath, S. Gebhard, et al., The effect of oxygen and water on the provision of crack closure in bacteria-based self-healing cementitious composites, *Cem. Concr. Compos.* 142 (February) (2023) 105201, <https://doi.org/10.1016/j.cemconcomp.2023.105201> [Internet].
- [36] F.G. Ferris, W.S. Fyfe, T.J. Beveridge, Metallic ion binding by *Bacillus subtilis*: implications for the fossilization of microorganisms, *Geology* 16 (1988) 149–152.
- [37] K.O. Konhauser, Diversity of iron mineralization, *Earth-Science Rev.* 43 (1998) 91–121.
- [38] R. Andalib, M. Abd Majid, A. Keyvanfar, A. Talaiekhoozan, M.W. Hussin, A. Shafaghhat, et al., Durability improvement assessment in different high strength bacterial structural concrete grades against different types of acids, *Sadhana Acad. Proc. Eng. Sci.* 39 (6) (2014) 1509–1522.
- [39] American Concrete Institute, Building Code Requirements for Structural Concrete (ACI 318-19) and Commentary. Farmington Hills (MI), 2019.
- [40] W. De Muynck, D. Debrouwer, N. De Belie, W. Verstraete, Bacterial carbonate precipitation improves the durability of cementitious materials, *Cement Concr. Res.* 38 (7) (2008) 1005–1014.
- [41] X. Chen, S. Wu, J. Zhou, Influence of porosity on compressive and tensile strength of cement mortar, *Constr. Build. Mater.* 40 (2013) 869–874, <https://doi.org/10.1016/j.conbuildmat.2012.11.072> [Internet].

- [42] A. Al Qabany, K. Soga, C. Santamarina, Factors affecting efficiency of microbially induced calcite precipitation, *J. Geotech. Geoenviron. Eng.* 138 (8) (2012) 992–1001.
- [43] F. Ameri, P. Shoaie, N. Bahrami, M. Vaezi, T. Ozbakkaloglu, Optimum rice husk ash content and bacterial concentration in self-compacting concrete, *Constr. Build. Mater.* 222 (2019) 796–813, <https://doi.org/10.1016/j.conbuildmat.2019.06.190> [Internet].
- [44] E.K. Ezugwu, J. Calabria-Holley, K. Paine, Physico-mechanical and morphological behavior of hydrothermally treated plant fibers in cementitious composites, *Ind. Crops Prod.* 200 (2023), <https://doi.org/10.1016/j.indcrop.2023.116832> [Internet].
- [45] R.J. Myers, E. L'Hôpital, J.L. Provis, B. Lothenbach, Effect of temperature and aluminium on calcium (alumino)silicate hydrate chemistry under equilibrium conditions, *Cement Concr. Res.* 68 (2015) 83–93.
- [46] H. Zheng, Y. Duan, M. Li, D. Hou, P. Wang, J. Chen, et al., Reaction molecular dynamics study of calcium aluminosilicate hydrate gel in the hydration deposition process at the calcium silicate hydrate interface : the influence of Al/Si, *J. Build. Eng.* 86 (February) (2024) 1–15, <https://doi.org/10.1016/j.jobe.2024.108823> [Internet].
- [47] J.J. Beaudoin, L. Raki, R. Alizadeh, A 29Si MAS NMR study of modified C-S-H nanostructures, *Cem. Concr. Compos.* 31 (8) (2009) 585–590, <https://doi.org/10.1016/j.cemconcomp.2008.11.004> [Internet].
- [48] J. Calabria-Holley, K. Paine, S. Papatzani, Effects of nanosilica on the calcium silicate hydrates in Portland cement-fly ash systems, *Adv. Cement Res.* 27 (4) (2015) 187–200.
- [49] W. Kunther, S. Ferreiro, J. Skibsted, Influence of the Ca/Si ratio on the compressive strength of cementitious calcium-silicate-hydrate binders, *J. Mater. Chem. A* 5 (33) (2017) 17401–17412.
- [50] B. Lothenbach, K. Scrivener, R.D. Hooton, Supplementary cementitious materials, *Cement Concr. Res.* 41 (12) (2011) 1244–1256, <https://doi.org/10.1016/j.cemconres.2010.12.001> [Internet].
- [51] S. Martínez-Ramírez, S. Sánchez-Cortés, J.V. García-Ramos, C. Domingo, C. Fortes, M.T. Blanco-Varela, Micro-Raman spectroscopy applied to depth profiles of carbonates formed in lime mortar, *Cement Concr. Res.* 33 (12) (2003) 2063–2068.
- [52] C. Tang, T.C. Ling, K.H. Mo, Raman spectroscopy as a tool to understand the mechanism of concrete durability—A review, *Constr. Build. Mater.* 268 (xxxx) (2021) 121079, <https://doi.org/10.1016/j.conbuildmat.2020.121079> [Internet].
- [53] L. Black, Raman spectroscopy of cementitious materials, *Spectrosc. Prop. Inorg. Organomet. C* 40 (2009) 72–127.
- [54] G. Behrens, L. Kuhn, R. Ubig, A. Heuer, Raman spectra of vateritic calcium carbonate, *Spectrosc. Lett.* 28 (6) (1995) 983–995.
- [55] V. Achal, A. Mukerjee, M. Sudhakara Reddy, Biogenic treatment improves the durability and remediates the cracks of concrete structures, *Constr. Build. Mater.* 48 (2013) 1–5, <https://doi.org/10.1016/j.conbuildmat.2013.06.061> [Internet].
- [56] W. De Muynck, K. Cox, N. De Belie, W. Verstraete, Bacterial carbonate precipitation as an alternative surface treatment for concrete, *Constr. Build. Mater.* 22 (5) (2008) 875–885.
- [57] I. Justo-Reinoso, A. Heath, S. Gebhard, K. Paine, Aerobic non-ureolytic bacteria-based self-healing cementitious composites: a comprehensive review, *J. Build. Eng.* 42 (May) (2021) 102834, <https://doi.org/10.1016/j.jobe.2021.102834> [Internet].
- [58] S.S. Bang, J.J. Lippert, U. Yerra, S. Mulukutla, V. Ramakrishnan, Microbial calcite, a bio-based smart nanomaterial in concrete remediation, *Int J Smart Nano Mater* 1 (1) (2010) 28–39.
- [59] K.K. Sahoo, A.K. Sathyan, C. Kumari, P. Sarkar, R. Davis, Investigation of cement mortar incorporating *Bacillus sphaericus*, *Int J Smart Nano Mater* 7 (2) (2016) 91–105, <https://doi.org/10.1080/19475411.2016.1205157> [Internet].
- [60] Q. Chunxiang, W. Jianyun, W. Ruixing, C. Liang, Corrosion protection of cement-based building materials by surface deposition of CaCO<sub>3</sub> by *Bacillus pasteurii*, *Mater Sci Eng C* 29 (4) (2009) 1273–1280.
- [61] A.M. Ahmad, J. Zhang, L. Bing, P. Tan, X. Deng, Unlocking the efficacy of cement-shell encapsulation for microbial self-healing process of concrete cracks, *J. Mater. Res. Technol.* 32 (June) (2024) 2733–2742, <https://doi.org/10.1016/j.jmrt.2024.08.133> [Internet].
- [62] F. Hou, D. Shen, Y. Diao, H. Guo, J. Wang, Preliminary study on in-situ spore germination at different crack depths in bacteria-based self-healing concrete, *Cem. Concr. Compos.* 162 (April) (2025) 1–17, <https://doi.org/10.1016/j.cemconcomp.2025.106140> [Internet].
- [63] M. Sarkar, N. Alam, B. Chaudhuri, B. Chattopadhyay, S. Mandal, Development of an improved *E. coli* bacterial strain for green and sustainable concrete technology, *RSC Adv.* 5 (41) (2015) 32175–32182.
- [64] S. Ghosh, M. Biswas, B.D. Chattopadhyay, S. Mandal, Microbial activity on the microstructure of bacteria modified mortar, *Cem. Concr. Compos.* 31 (2) (2009) 93–98.
- [65] M. Biswas, S. Majumdar, T. Chowdhury, B. Chattopadhyay, S. Mandal, U. Halder, et al., Bioremediase a unique protein from a novel bacterium BKH1, ushering a new hope in concrete technology, *Enzyme Microb Technol* 46 (7) (2010) 581–587.
- [66] P. Padma Rao, S.S. Asadi, M. Ravindra Krishna, A. Suri Babu, S. Alla, An experimental investigation of bacteria impact on compressive strength of cement mortar and concrete, *Mater. Today Proc.* 43 (2020) 1949–1955, <https://doi.org/10.1016/j.matpr.2020.11.213> [Internet].
- [67] R. Chidara, R. Nagulagama, S. Yadav, Achievement of early compressive strength in concrete using *Sporosarcina pasteurii* bacteria as an admixture, *Adv. Civ. Eng.* 2014 (2014).
- [68] S. Bang, J. Galinat, V. Ramakrishnan, A. Et, Calcite precipitation induced by polyurethane-immobilized *Bacillus pasteurii*, *Enzym. Microb. Technol.* 28 (2001) 404–409.
- [69] Balam N. Hosseini, D. Mostofinejad, M. Eftekhari, Effects of bacterial remediation on compressive strength, water absorption, and chloride permeability of lightweight aggregate concrete, *Constr. Build. Mater.* 145 (2017) 107–116, <https://doi.org/10.1016/j.conbuildmat.2017.04.003> [Internet].
- [70] R. Siddique, K. Singh, P. Kunal, M. Singh, V. Corinaldesi, A. Rajor, Properties of bacterial rice husk ash concrete, *Constr. Build. Mater.* 121 (2016) 112–119, <https://doi.org/10.1016/j.conbuildmat.2016.05.146> [Internet].