

Analysis of the Mechanical Properties of Bioconcrete

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Abstract

This study evaluates the effect of incorporating the bacterium *Bacillus subtilis* together with calcium lactate on the mechanical properties and self-healing capacity of concrete. *B. subtilis* was supplied by a commercially available biological fungicide at a concentration of 1×10^9 colony-forming units (CFU)/mL, verified in the laboratory, and calcium lactate was used as the nutrient source that enables the autonomous precipitation of calcium carbonate (CaCO_3) inside cracks, a mechanism that extends service life and lowers maintenance costs. The work comprised material characterisation, mix design for a target strength of 210 kg/cm^2 (20.6 MPa), and the evaluation of compressive strength, static modulus of elasticity, self-healing capacity and the environmental conditions required for bacterial survival. Calcium lactate was dosed at 0.25%, 0.50% and 1.00% by weight of cement, and the bioproduct at 2.5%, 5.0% and 10.0% by volume of mixing water. The addition of 1% calcium lactate raised the 28-day compressive strength to 336.14 kg/cm^2 , 25.8% above the baseline concrete ($267.20 \pm 12.12 \text{ kg/cm}^2$) and 60% above the design value. When the bioproduct was introduced, compressive strength fell as the bacterial dose increased, although every bacterial mix still exceeded the design strength; the best-performing combination was 1% calcium lactate with 2.5% bioproduct ($289.27 \pm 6.90 \text{ kg/cm}^2$ at 28 days). The experimental modulus of elasticity, by contrast, fell progressively below the value predicted by the Ecuadorian Construction Code, with reductions of 28.1%, 32.5% and 47.4% at bioproduct doses of 2.5%, 5.0% and 10.0% respectively, indicating increased porosity and reduced stiffness. Cylinders containing 1% calcium lactate and 2.5% bioproduct that were cracked and then immersed in water showed complete crack closure after 21 days by a white deposit that SEM-EDS analysis associated with calcium carbonate. Bacterial activity was greatest at an alkaline pH of 9 to 11, temperatures of 20–25°C and constant moisture; specimens held at ambient humidity did not precipitate CaCO_3 . The results confirm that bioconcrete is a technically feasible self-healing alternative for regions such as Ecuador, where high seismic activity and humid conditions cause continuous damage to infrastructure, provided the bacterial dose is limited so that the accompanying loss of stiffness remains acceptable.

1. Introduction

In the field of civil engineering, concrete is the most widely used construction material because of its strength, versatility and low cost. (Mehta & Monteiro, 2006). One of its main drawbacks, however, is its susceptibility to the formation of cracks, which over time compromises the durability and the structural performance of buildings and infrastructure. (Jonkers & Schlangen, 2008).

Worldwide, cracks in concrete structures have been repaired by injecting epoxy resins, applying mortars and using waterproofing coatings. (Aci, 2011) procedures that require constant intervention and raise maintenance costs. For this reason, the use of bioconcrete represents an innovative solution for the repair of cracks in concrete, improving its durability and reducing maintenance costs. Bioconcrete is a material capable of self-repair through the precipitation of calcium carbonate (CaCO_3) induced by bacteria of the genus *Bacillus*. (De Muynck, De Belie, & Verstraete, 2010). When incorporated into the

concrete mix together with calcium lactate, *Bacillus* bacteria can induce the precipitation of calcium carbonate (CaCO_3) and thereby seal the cracks. (Jonkers & Schlangen, 2008). The crack-sealing mechanism through CaCO_3 precipitation occurs because the combination of the bacterial spores (for example, *Bacillus alkalinitrilicus*) and the calcium ions supplied by calcium lactate ($\text{Ca}(\text{C}_3\text{H}_5\text{O}_3)_2$) reacts with oxygen, and the precipitated calcium carbonate seals the crack. The process also releases water, which promotes bacterial activity and cement hydration. (Zhang, Suleiman, Marani, & Nehdi, 2017).

Recent studies have found that *Bacillus* bacteria can survive the setting process of concrete, as well as exposure to ultraviolet radiation and other external conditions. (Pérez, Sánchez, & Sánchez, 2023). Originally used in agriculture for pest control, these bacteria have demonstrated their ability to adapt to the alkaline environment of concrete, which makes them a viable biological agent for the self-repair of cracks. (Pérez et al., 2023). In countries such as Germany and the Netherlands, research on bioconcrete has tested different bacterial strains to improve the durability of the material and reduce repair costs. (Jonkers & Schlangen, 2008). Although its initial implementation is more expensive, the significant reduction in maintenance makes it an efficient and sustainable solution for infrastructure exposed to adverse conditions throughout its service life. (Rezende-Lopes, Tannous, & Rezende-Lopes, 2023).

Some studies focus on the self-healing capacity of concrete, consider the proportion of microorganisms and analyse moisture, temperature and pH as key factors in its formation. (Fahimizadeh et al., 2024).

In Ecuador, the implementation of bioconcrete is particularly relevant because of the high seismic activity of the region and its adverse environmental conditions, which result in the degradation of infrastructure (Alcívar, Pico, & Mera). Buildings, bridges and roads face constant damage to their mechanical properties, which causes the deterioration of the concrete and increases the need for more efficient and sustainable repair techniques. In this context, the application of bioconcrete seeks to validate its technical and economic feasibility by evaluating its compressive strength and its self-repair capacity under different proportions of microorganisms (Pérez et al., 2023).

Accordingly, the objective of this research is to analyse how the mechanical properties of concrete are affected when self-healing bacteria are incorporated into the mix to produce bioconcrete.

The specific objectives were: (i) to verify the concentration and purity of the commercial bioproduct used as the source of *B. subtilis* and to confirm its ability to precipitate calcium carbonate in vitro; (ii) to determine the calcium lactate content that maximises compressive strength; (iii) to quantify the effect of an increasing bacterial dose on both compressive strength and the static modulus of elasticity; and (iv) to verify crack closure and identify the chemical nature of the sealing product. The working hypothesis was that the incorporation of *B. subtilis* and calcium lactate confers self-healing capacity on the concrete without reducing its compressive strength below the design value of 210 kg/cm^2 .

2. Materials and Methods

The bacteria were obtained from a readily available bioproduct marketed as a biological fungicide whose active ingredient is the bacterium *Bacillus subtilis* at a concentration of 1×10^9 colony-forming units (CFU)/mL, a value that was verified in the laboratory.

All concrete specimens were produced with Portland cement type GU (general use), classified in accordance with NTE INEN 2380, and with fine and coarse aggregates drawn from a single local source and a single batch, so that the only variables between mixes were the calcium lactate content and the bioproduct content.

2.1. Microbiological tests

2.1.1. Quality control of the bioproduct

Two dilutions of the commercial bioproduct, 1:1000 and 1:10 000, were prepared and spread-plated onto three nutrient-agar Petri dishes, counting the number of CFU on each plate in accordance with standard NTE INEN 1205:2013, *Water. Determination of the total number of bacteria on plates* and verification that there was no contamination by other microorganisms. A Gram stain of the colonies was carried out, and the morphology of the bacteria was observed at the microscopic level, isolating one of the colonies on nutrient agar.

Plates were incubated at $30 \pm 2^\circ\text{C}$ for 24 h before counting, and only plates bearing between 30 and 300 colonies were used for the calculation, as required by NTE INEN 1205. Counts were performed in triplicate for each dilution, and the result was expressed as the arithmetic mean of the three replicates.

2.1.2. Evaluation of the ability of the bacteria to produce calcium carbonate (CaCO_3)

A spread-plate culture was prepared at room temperature on a medium containing 80 g of calcium lactate, 4 g of yeast extract and 15 g of nutrient agar per litre of water (Shaheen et al., 2021), and incubated for 7, 14 and 28 days until a white halo was observed around the colonies.

2.1.3. Determination of the conditions governing bacterial activity

To identify the environmental conditions that control carbonate precipitation, parallel sets of the calcium-lactate medium and of cracked concrete specimens were held at pH values in the alkaline range of 9 to 11 and at temperatures between 20°C and 25°C , under two moisture regimes: continuous immersion in water, and exposure to the ambient conditions of Sangolquí, Ecuador (19°C and 55% relative humidity). The pH of the medium was measured with a calibrated benchtop pH meter, and the temperature and relative humidity were recorded daily throughout the test period. The presence or absence of a white precipitate was used as the response variable. This subsection has been added because the conditions reported in Section 3.1.7 of the Results were not previously described in the methodology.

2.2. Concrete mix design

The concrete was proportioned using the ACI 318 methodology (Aci, 2011). The material characterisation tests followed the Ecuadorian standards INEN 858:2010, *Aggregates. Determination of bulk density (unit weight) and voids*; INEN 696:2010, *Sieve analysis of fine and coarse aggregates*; INEN 862:2011, *Aggregates for concrete. Determination of total moisture content*; INEN 1578:2010, *Hydraulic-cement concrete. Determination of slump*; and INEN 857:2010, *Aggregates. Determination of density, relative density (specific gravity) and absorption*. For the tests carried out, concrete cylinders were prepared in accordance with ASTM C31 and INEN 1576, *Hydraulic-cement concrete. Making and curing test specimens in the field*, with a design strength of 210 kg/cm², a water/cement ratio of 0.49, a fine-aggregate density (Fine Agg.) of 2.57 kg/dm³ and a coarse-aggregate density (Coarse Agg.) of 2.11 kg/dm³.

Table 1

Proportions for 1000 dm³ of concrete with different percentages of calcium lactate (L) and of *Bacillus subtilis* at a concentration of 1×10^9 CFU/mL (S).

Mix	Cement (kg)	Fine Agg. (kg)	Coarse Agg. (kg)	Water (L)	S (L)	Calcium lactate, L (kg)
Baseline (BL)	662.55	1505.63	1179.00	416.70	–	–
0.25% L	662.55	1505.63	1179.00	416.70	–	1.66
0.50% L	662.55	1505.63	1179.00	416.70	–	3.31
1.00% L	662.55	1505.63	1179.00	416.70	–	6.63
2.5% S + 1% L	662.55	1505.63	1179.00	406.29	10.42	6.63
5.0% S + 1% L	662.55	1505.63	1179.00	395.87	20.84	6.63
10.0% S + 1% L	662.55	1505.63	1179.00	375.03	41.67	6.63

2.2.1. Baseline concrete

For the baseline concrete mixes, nine cylinders 20 cm high and 10 cm in diameter were prepared, requiring a concrete volume of 10.99 dm³ (Table 1).

2.2.2. Calcium lactate (L) dosage test in concrete cylinders

Three percentages of calcium lactate by weight of cement were evaluated: 0.25%, 0.50% and 1.00%, since a higher percentage may adversely affect the strength of the concrete (Irwan et al., 2021). Three

cylinders 20 cm high and 10 cm in diameter were prepared for each lactate dosage, which represented 4.72 dm³ of concrete (Table 1).

2.2.3. *Bacillus subtilis* (S) dosage test in concrete

Bacillus subtilis at a concentration of 1×10^9 CFU/mL was dosed at 2.50%, 5.00% and 10.00% relative to the amount of water in the concrete mix. For this procedure, eight cylinders were prepared for each bacterial dosage: five of them 20 cm high and 10 cm in diameter, and the remaining three 30 cm high and 15 cm in diameter, requiring a concrete volume of 23.8 dm³ (Table 1).

2.3. Mechanical tests

2.3.1. Curing tests

For the analysis of maximum strength, the concrete was held under two temperature and humidity conditions: the first at 20°C to 25°C and 100% humidity, and the second at the ambient temperature and humidity of Sangolquí, Ecuador (19°C and 55%), for 28 days, evaluating the strength of the cylinders under these humidity and temperature conditions (Fig. 1).

2.3.2. Compression tests

On the basis of the Ecuadorian standard INEN 1573:2010, *Hydraulic-cement concrete. Determination of the compressive strength of specimens* [16], tests were carried out on cylinders cured for 7, 14 and 28 days (Fig. 2), which had been kept at 20°C to 25°C and 100% humidity and at 19°C and 55% humidity (ambient conditions). A first group of 15 cylinders 30 cm high with varying calcium lactate content (0.25%, 0.50% and 1.00%) and a second group of 20 cm cylinders with varying *B. subtilis* concentration (2.50%, 5.00% and 10.00%) were tested (Irwan et al., 2021). Note: the specimen dimensions stated in this paragraph appear to be interchanged with respect to Sections 2.2.2 and 2.2.3, where the lactate cylinders are 20 cm high, and the bacterial series includes the 30 cm cylinders; the authors should confirm which series corresponds to each size.

2.3.3. Strength and modulus of elasticity

The strength test was carried out on two cylinders at 7 days, two cylinders at 14 days and two further cylinders at 28 days.

The modulus of elasticity was determined in accordance with standard ASTM C469-94 (American Society for Testing and Materials, 2023), using three replicate cylinders containing *B. subtilis* at the three concentrations (2.50%, 5.00% and 10.00%). The first two groups were used to analyse the failure stress, and the third group was used for the modulus of elasticity test, at 40% of the failure stress.

2.3.4. Crack generation and evaluation of self-healing

Cylinders produced with the optimum combination identified in the compression tests (1% calcium lactate and 2.5% bioproduct) were used to evaluate self-healing. After curing, cracks were induced in the specimens, and the cracked cylinders were then fully immersed in water at room temperature for 21 days, so that the *B. subtilis* population could precipitate calcium carbonate inside the crack. Crack closure was inspected visually at 7, 14 and 21 days of immersion, and companion specimens held under ambient conditions (19°C and 55% relative humidity) without immersion served as a control to establish the role of moisture.

2.3.5. SEM-EDS characterisation of the healing product

The white material formed inside the cracks was recovered by scraping and analysed by scanning electron microscopy coupled with energy-dispersive X-ray spectroscopy (SEM-EDS) in order to identify its elemental composition. The recovered material was mounted on aluminium stubs with conductive carbon tape, sputter-coated to render it conductive, and examined at an accelerating voltage of 15 kV. Elemental spectra were acquired at the points where the deposit was most abundant, and the identification of calcium and oxygen, together with the morphology of the crystals, was used to confirm the formation of calcium carbonate.

2.4. Statistical analysis

For each mix and test age, the compressive strength is reported as the arithmetic mean of the available replicates together with the sample standard deviation. Because the number of replicates per condition was small and, for the lactate-only mixes, only one specimen was tested at each age, the differences between mixes are presented as descriptive results and no inferential test of significance was applied. Percentage variations are expressed with respect to the baseline concrete (BL) or to the design strength of 210 kg/cm², as indicated in each case, and reductions in the modulus of elasticity are expressed with respect to the theoretical value predicted by the NEC equation for the measured compressive strength of the same mix. Extending the programme to at least three replicates per condition would allow a one-way analysis of variance (ANOVA) with a post-hoc comparison to confirm that the differences observed here are statistically significant.

3. Results and Discussion

3.1. Results

Following the incorporation of calcium lactate and *B. subtilis* into the concrete, an impact on its mechanical and self-healing properties was observed (Sandbhor, Apte, Puranik, Asati, & Rai, 2025).

3.1.1. Quality control of the bioproduct

Culturing the bioproduct at dilutions of 1:1000 and 1:10 000 on nutrient agar allowed well-defined *B. subtilis* colonies to develop at both dilutions. The number of colony-forming units (CFU) was consistent

between replicates, with a mean of 1.13×10^9 CFU/mL for the 1:1000 dilution and 0.95×10^9 CFU/mL for the 1:10 000 dilution. The approximate concentration of 1×10^9 CFU/mL stated on the bioproduct label was therefore confirmed. No signs of contamination by fungi or other types of microorganisms were detected. Gram staining revealed the presence of Gram-positive bacteria with a uniformly distributed bacillary morphology (Fig. 3).

The bioproduct used was shown to contain exclusively *B. subtilis*, since no contamination was detected in the Petri dish culture. In addition, the Gram stain confirmed that all colonies presented a uniform microbial morphology, which demonstrates the purity of the culture.

The measured counts correspond to deviations of + 13% and – 5% with respect to the nominal value declared on the label, a range that is within the variability normally accepted for commercial biological formulations. The dose applied to the concrete mixes can therefore be taken as 1×10^9 CFU/mL.

3.1.2. Production of calcium carbonate (CaCO_3)

The isolated bacteria (*Bacillus subtilis*) were able to grow on the medium supplemented with calcium lactate, yeast extract and nutrient agar. From the third day of incubation at room temperature, visible deposits of an opaque white material were observed around the *Bacillus subtilis* colonies. These deposits became more intense over time, which suggests the formation of calcium carbonate (CaCO_3) as a product of bacterial activity (Fig. 4).

The formation of precipitates was consistent between replicates, which confirms the ability of the isolated *Bacillus subtilis* strain to induce the precipitation of calcium carbonate under laboratory conditions.

3.1.3. Mechanical tests

3.1.3.1. Compressive strength

Concrete with different lactate concentrations showed improved compressive strength values, withstanding a higher axial load without failure and reaching values above the design strength of 210 kg/cm² (20.6 N/mm²), as shown in Fig. 5.

The difference in strength between the concrete mix without calcium lactate (BL), which ranged from 162.77 kg/cm² (7 days) to 267.20 ± 12.12 kg/cm² (28 days), and the concrete with different proportions of calcium lactate (L) (0.25%, 0.50% and 1.00%) shows that the highest compressive strength is obtained by adding 1% lactate to the plain concrete mix (Table 2), with a strength ranging from 258.00 kg/cm² (7 days) to 336.14 kg/cm² (28 days), an increase of between 58% (7 days) and 25.8% (28 days).

Table 2
Compressive strength of concrete with different percentages of calcium lactate (L).

Cylinder	Calcium lactate (%)	Compressive strength (kg/cm ²)			
		Analysis	7 days	14 days	28 days
Baseline concrete (BL)	0	1	162.77	231.91	261.52
		2	–	227.68	277.57
		3	–	228.57	252.82
		4	–	208.64	276.89
		Mean	162.77	224.20	267.20
		Std. deviation	0	10.53	12.12
Concrete + L 0.25%	0.25	1	200.81	226.21	273.77
		Mean	200.81	226.21	273.77
Concrete + L 0.50%	0.50	1	221.73	272.91	296.42
		Mean	221.73	272.91	296.42
Concrete + L 1.00%	1.00	1	258.00	328.39	336.14
		Mean	258.00	328.39	336.14

Since the highest strength, 336.14 kg/cm², was obtained in the concrete containing 1% calcium lactate, it is evident that calcium lactate, the nutrient of the bacteria within the concrete, improves rather than impairs the compressive strength of the concrete.

For the compressive strength tests with the liquid bioproduct (Fig. 6), the concrete mix with 1% calcium lactate was used, and the bioproduct content was varied at 0%, 2.5%, 5% and 10% with respect to the amount of water. The results obtained (Table 3 and Fig. 6) show a decrease in compressive strength in inverse relation to the bacterial dose: the greater the proportion of *B. subtilis*, the lower the strength, which at the highest doses even falls below the value of the baseline mix (BL) that contains no *B. subtilis*, although all of the mixes remain above the design value of 210 kg/cm². The strength varies from 336.14 kg/cm² with 1% calcium lactate and no bioproduct down to 251.32 kg/cm² with 10% bioproduct, following the sequence L 1.0%; R = 336.14 kg/cm² > 2.5% S; R = 289.27 kg/cm² > BL 0%; R = 267.20 kg/cm² > 5.0% S; R = 257.28 kg/cm² > 10.0% S; R = 251.32 kg/cm² > design 210 kg/cm². Increasing the *B. subtilis* content therefore reduces the compressive strength progressively, even though every mix still exceeds the design strength; the smallest bioproduct proportion tested, 2.5%, is consequently the optimum.

Table 3

Compressive strength results for the baseline mix, the optimum calcium lactate content and *Bacillus subtilis* at different percentages.

Cylinder	Calcium lactate, L (%)	Bioproduct, S (%)	Compressive strength (kg/cm ²)			
			Analysis	7 days	14 days	28 days
BL	0	0	1	162.77	231.91	261.52
			2	–	227.68	277.57
			3	–	228.57	252.82
			4	–	208.64	276.89
			Mean	162.77	224.20	267.20
			Std. deviation	0	10.53	12.12
L 1.0%	1.0	0	1	258.00	328.39	336.14
			Mean	258.00	328.39	336.14
L1% – 2.5% S	1.0	2.5	1	229.58	274.01	284.39
			2	224.87	270.01	294.15
			Mean	227.23	272.01	289.27
			Std. deviation	3.33	2.83	6.90
L1% – 5.0% S	1.0	5.0	1	201.63	221.29	282.54
			2	199.54	241.97	232.01
			Mean	200.59	231.63	257.28
			Std. deviation	1.48	14.62	35.73
L1% – 10.0% S	1.0	10.0	1	141.61	219.62	253.20
			2	187.05	236.48	249.43
			Mean	164.33	228.05	251.32
			Std. deviation	32.13	11.92	2.67

The compressive strength results for the calcium lactate and bioproduct mixes shown in Fig. 6 indicate that the best result corresponds to the combination L1% – 2.5% S, with values ranging from $227.23 \pm 3.33 \text{ kg/cm}^2$ at 7 days to $289.27 \pm 6.90 \text{ kg/cm}^2$ at 28 days. This shows that the inclusion of calcium lactate and *B. subtilis* improves the compressive strength of the concrete. (Sandbhor et al., 2025), reaching an increase of up to 60% relative to the reference value of 210 kg/cm^2 when only calcium lactate is included. When both the bacteria and the lactate were included in the mix, the 28-day strength increased by between 19% and 37%, the strength being higher the lower the bacterial content of the mix.

3.1.4. Modulus of elasticity

The experimental modulus of elasticity of the bioconcrete with different proportions of calcium lactate and *B. subtilis* was compared with the theoretical value obtained from the equation given in the Ecuadorian Construction Code (NEC):

$$E_c = 4700 \sqrt{f'_c} \quad (1)$$

where f'_c is the compressive strength of the concrete in MPa. A variation was found between the experimental and the theoretical modulus of elasticity (Table 4).

Table 4

Modulus of elasticity of cylinders with 1% calcium lactate and different percentages of bioproduct.

Cylinder	Strength (kg/cm ²)	Strength (MPa)	Experimental E_c (MPa)	Theoretical $E_c =$ $4700 \sqrt{f'_c}$ (MPa)	Reduction (%)
1% calcium lactate – 2.5% bioproduct	289.27	28.36	17 986.19	25 029.43	28.1
1% calcium lactate – 5% bioproduct	257.28	25.23	15 925.25	23 607.85	32.5
1% calcium lactate – 10% bioproduct	251.32	24.64	12 263.34	23 330.19	47.4

The inclusion of *B. subtilis* in the concrete cylinders produced a progressive decrease in the modulus of elasticity as the percentage of bacteria in the mix increased: a reduction of 28.1% in the mix with 1% calcium lactate and 2.5% bioproduct, 32.5% in the mix with 1% calcium lactate and 5% bioproduct, and 47.4% in the mix with 1% calcium lactate and 10% bioproduct. The decrease is greater in the mix with the higher bioproduct content, as shown in Fig. 7.

Note for the authors: the original manuscript reported reductions of 17.2%, 26.7% and 43.6%. Recalculating each reduction as $(E_{\text{theoretical}} - E_{\text{experimental}})/E_{\text{theoretical}}$ from the values listed in Table 4 gives 28.1%, 32.5% and 47.4%, respectively. The originally reported percentages are internally

consistent with a single reference modulus of approximately 21 725 MPa rather than with the mix-specific theoretical values in Table 4; please confirm which reference was intended before submission.

Expressed as a proportion of the code-predicted stiffness, the bioconcrete retains 71.9%, 67.5% and 52.6% of the theoretical modulus at bioproduct doses of 2.5%, 5.0% and 10.0% respectively. The loss of stiffness is therefore not proportional to the change in strength: at 2.5% bioproduct, the compressive strength is still 8.3% above that of the baseline concrete, while the modulus is already almost 30% below the code prediction. This decoupling is the practically significant finding of the study, because deflections, cracking, and the distribution of internal forces in statically indeterminate structures are governed by stiffness rather than by strength.

3.1.5. Self-healing of cracks

Concrete cylinders with 1% calcium lactate and 2.5% bioproduct (the optimum concentration) were taken, cracks were induced in them, and they were immersed in water for 21 days so that the *Bacillus subtilis* bacteria would produce calcium carbonate. (De Muynck et al., 2010), the chemical compound that acts as the sealant.

After 21 days, a white calcium carbonate (CaCO_3) sealant produced by the *Bacillus subtilis* bacteria was observed in the cracks, as shown in Fig. 8. This filling material was analysed by scanning electron microscopy (SEM-EDS) (Sandbhor et al., 2025).

3.1.6. Elemental characterisation of the sealing product

The material sealing the cracks was collected by scraping and analysed by scanning electron microscopy with energy-dispersive spectroscopy (SEM-EDS), identifying the presence of calcium, oxygen and carbon in the crack samples and confirming the formation of calcium carbonate (CaCO_3) as a result of bacterial activity (Dixon et al., 1991) (Fig. 9).

The EDS spectrum reproduced in Fig. 9 shows labelled peaks for calcium and oxygen, together with a silicon peak attributable to the surrounding cement matrix. Because carbon is a light element and is also present in the conductive coating usually applied to non-conductive samples, its quantification by EDS is not reliable; the identification of CaCO_3 would therefore be strengthened either by labelling the carbon peak explicitly in the spectrum or by adding a complementary technique such as X-ray diffraction (XRD) or FTIR, which would also establish which polymorph calcite, vaterite or aragonite was formed.

3.1.7. Conditions for bacterial activity

The *Bacillus subtilis* bacteria showed greater activity in the concrete samples under conditions of alkaline pH (9 to 11), at temperatures between 20°C and 25°C and in the presence of constant moisture. (Villarreal-Delgado et al., 2018) This favoured the precipitation of calcium carbonate (CaCO_3) and contributed to the self-healing capacity of the concrete. These results confirm that *Bacillus subtilis* is

able to survive in the concrete environment and to produce mineral compounds that promote the self-healing of cracks.

The samples that were not subjected to constant moisture did not precipitate calcium carbonate (CaCO_3). Moisture is therefore important in the self-healing process, which shows that it is a key factor for bacterial activity. (Jonkers & Schlangen, 2008).

3.2. Discussion of results

The results of this study showed that the addition of calcium lactate and *Bacillus subtilis* to concrete contributes significantly to improving its mechanical properties and its self-healing capacity, promoting the precipitation of calcium carbonate and increasing the durability of the material, although this improvement applies to compressive strength and crack sealing and not to stiffness, which was reduced in every bacterial mix tested. (Van Tittelboom & De Belie, 2013; Villarreal-Delgado et al., 2018; Wiktor & Jonkers, 2011).

The increase in compressive strength with the addition of 1% calcium lactate suggests that this compound performs well both as an additive to the concrete and as food for the bacteria, favouring a greater precipitation of calcium carbonate (CaCO_3). The incorporation of the bioproduct, however, despite the fact that it contains the bacteria, caused a decrease in strength, which indicates the need to find an optimal balance between the amount of calcium lactate and the bacterial concentration in order to maximise mechanical strength without impairing the self-repair capacity of the concrete. (Seifan, Samani, & Berenjian, 2016; Shaheen, Jalil, Adnan, & Arsalan Khushnood, 2021; Tziviloglou, Wiktor, Jonkers, & Schlangen, 2016).

Part of this decrease is also attributable to the mix design itself: the bioproduct was added as a liquid replacing an equal volume of mixing water, so that at the 10% dose 41.67 L of the 416.70 L of water were substituted by a commercial formulation whose full composition, carriers, surfactants and organic residues, is not declared by the manufacturer. Determining the solids and organic content of the bioproduct, or replacing it with a washed spore suspension of known concentration, would make it possible to separate the effect of the bacteria themselves from the effect of the carrier liquid.

The addition of *Bacillus subtilis* to concrete can improve compressive strength by between 10% and 20% (Jonkers & Schlangen, 2008) depending on the curing conditions, a trend that is also evident in this research with the addition of calcium lactate. The reduction observed as the bioproduct concentration increased could, however, be related to excessive biological activity and to a possible alteration of cement hydration. (Ahmed et al., 2021; Othman, Irwan, Alshali, & Anneza, 2016).

The ability of *Bacillus subtilis* to survive in a concrete alkaline environment and to produce calcium carbonate (CaCO_3) under the curing regime applied here not only seals the cracks but also improves the durability of the concrete by preventing the penetration of aggressive agents such as water and chlorides into the crack. (Jonkers, 2011).

In the tests carried out, complete recovery of the cracks occurred within 21 days, which lies within the range of 14 to 28 days reported in other studies. (Khaliq & Ehsan, 2016; Zhang et al., 2017). This demonstrates that bioconcrete with *Bacillus subtilis* has significant potential for applications in structures exposed to adverse environmental conditions, such as areas of high seismic activity or extreme climates.

Conditions of alkaline pH, moderate temperature and constant moisture are essential for the survival and activity of the bacteria and are characteristic of fresh concrete, which is therefore a suitable environment for the proliferation of *Bacillus subtilis*. In addition, the inclusion of calcium lactate not only provides nutrients for the bacteria but also helps to maintain a suitable pH of between 9 and 11, which favours microbial activity.

The experimental results show that the incorporation of calcium lactate and *B. subtilis* adversely affects the modulus of elasticity, significantly reducing the stiffness of the material in comparison with the theoretical values based on compressive strength. This effect becomes more pronounced as the bioproduct content increases: the higher the concentration of bioproduct, the lower the stiffness of the concrete, which has direct consequences for structural behaviour.

The incorporation of bacteria into concrete can reduce the modulus of elasticity by 30% to 40% (Tziviloglou et al., 2016) owing to changes in porosity and in the distribution of the hydration products. In this study, a reduction of up to 47.4% was found at the highest bioproduct doses, which indicates that this phenomenon could be linked to an increase in porosity and a lower density of the concrete mix. (Caicedo, Velandia, Pimiento, & Bueno, 2023; Seifan et al., 2016). The modulus of elasticity of conventional concrete according to the Ecuadorian Construction Code (NEC) and to international references such as ACI 318 ranges between 24 000 MPa and 25 000 MPa for concrete with a compressive strength of approximately 25 to 28 MPa (255 to 285 kg/cm²), which is the range of strengths reached by the mixes tested here (De La Construcción, 2014). The mixes with the highest bioproduct content show a significant decrease of up to almost 50% below this standard.

These results suggest that, although bacterial and chemical additives favour the self-repair process of concrete, they can reduce its mechanical properties, in particular its modulus of elasticity. This reduction could be associated with changes in the microstructure of the concrete, such as a lower density and an increase in porosity, produced by the interaction between the additives and the concrete mix design.

Practical implications. From a design standpoint, it is the loss of stiffness, rather than any loss of strength, that governs the use of this material. A reduction of 28% in the modulus of elasticity at the optimum dose of 2.5% bioproduct increases short-term deflections by a comparable proportion for a given load and section, lengthens the fundamental period of a structure and therefore alters its seismic demand, a consideration of particular importance in Ecuador, where the design of reinforced-concrete structures relies on the modulus of elasticity given by the NEC. In its present formulation, bioconcrete is therefore best suited to non-structural or secondary elements, to protective layers, and to elements in which crack sealing and impermeability rather than stiffness govern the design, such as slabs on grade,

linings, kerbs and water-retaining surfaces. Where it is used in structural members, the modulus of elasticity should be measured experimentally rather than inferred from the code equation.

Limitations of the study. Several limitations should be borne in mind when interpreting these results. The number of replicates was small for the lactate-only mixes; a single specimen was tested at each age, so the differences reported are descriptive rather than statistically demonstrated; the standard deviation of 35.73 kg/cm^2 recorded for the 1% lactate 5.0% bioproduct mix at 28 days illustrates the resulting scatter. The bacteria were added in a commercial liquid formulation rather than as encapsulated spores, which limits both their long-term survival within the hardened matrix and the reproducibility of the dose. Self-healing was evaluated on cracks induced after curing and was assessed only visually, without measurement of crack width or of the recovery of strength and water-tightness, so functional rather than merely visual recovery remains to be quantified.(Cai et al., 2020). Finally, all self-healing tests were conducted under continuous immersion, which is the most favourable condition for bacterial activity and is not representative of most exposed structures.

Future work. Future research should encapsulate the spores in a protective carrier; expanded clay, hydrogels and halloysite nanotubes have all been proposed for this purpose. (Cai et al., 2020; Fahimizadeh et al., 2024) so that they are protected during mixing and released only when a crack admits water. The experimental programme should be extended to include water absorption and permeability measurements before and after healing, the recovery of compressive strength after cracking, the healing of cracks of controlled and measured width, and repeated cracking–healing cycles in order to establish how many times the mechanism can operate. Characterisation of the precipitate by X-ray diffraction, together with mercury intrusion porosimetry of the hardened matrix, would confirm the polymorph formed and quantify the increase in porosity that is proposed here as the cause of the loss of stiffness.

4. Conclusions

The addition of *Bacillus subtilis* and 1% calcium lactate to concrete enables the formation of calcium carbonate (CaCO_3), which promotes the self-repair of cracks. Samples maintained under constant moisture achieved complete crack recovery within 21 days, confirming the feasibility of this technology under controlled curing conditions. Incorporation of 1% calcium lactate improved compressive strength by 25.8% relative to conventional concrete; however, increasing the bioproduct content adversely affected strength, underscoring the need to balance bacterial dosage and calcium lactate concentration in order to maximise mechanical performance without compromising durability. Experimental results further showed that the modulus of elasticity of the bioconcrete declined as bioproduct concentration rose, reaching a reduction of 47.4% compared with the theoretical value calculated according to the NEC and ACI 318. This reduction is attributed to greater microstructural porosity arising from bacterial activity and calcium carbonate precipitation. Among the formulations tested, the optimum mix is 1% calcium lactate combined with 2.5% bioproduct. It delivers a 28-day compressive strength of $289.27 \pm 6.90 \text{ kg/cm}^2$, 8.3% above the baseline concrete and 37.7% above the design value, while still demonstrating

effective crack sealing. Because this mix retains only about 72% of the modulus of elasticity predicted by the NEC, the modulus must be determined experimentally whenever bioconcrete is used in structural elements. At present the material is therefore better suited to applications in which durability and impermeability, rather than stiffness, govern the design.

Declarations

Ethical approval

Not applicable.

Consent to participate

Not applicable.

Consent to publish

Not applicable.

Author Contribution

P.G.G. and K.F.V. conceived and designed the study, performed the concrete mix design and mechanical tests, and wrote the main manuscript text. J.J.A., D.C., J.D., M.P. and R.D. prepared the specimens, conducted compressive strength and modulus of elasticity tests, and analysed the experimental data. F.F. and S.G. carried out the microbiological characterisation of the bioproduct, evaluated calcium carbonate precipitation, and performed the SEM-EDS analysis of the healing product. All authors reviewed and approved the final manuscript.

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Data availability

All data generated or analysed during this study are included in this published article.

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Figures



Figure 1

Curing pool.



Figure 2

Compression test.

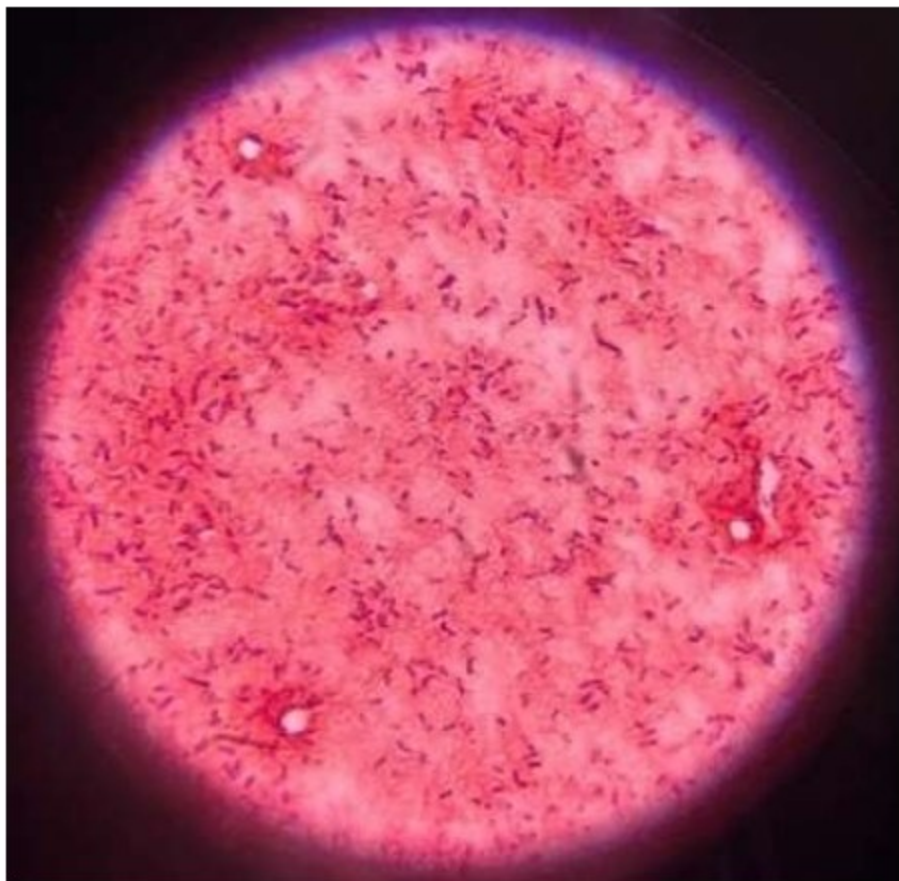


Figure 3

Gram stain of the bacterial isolate, 100×.

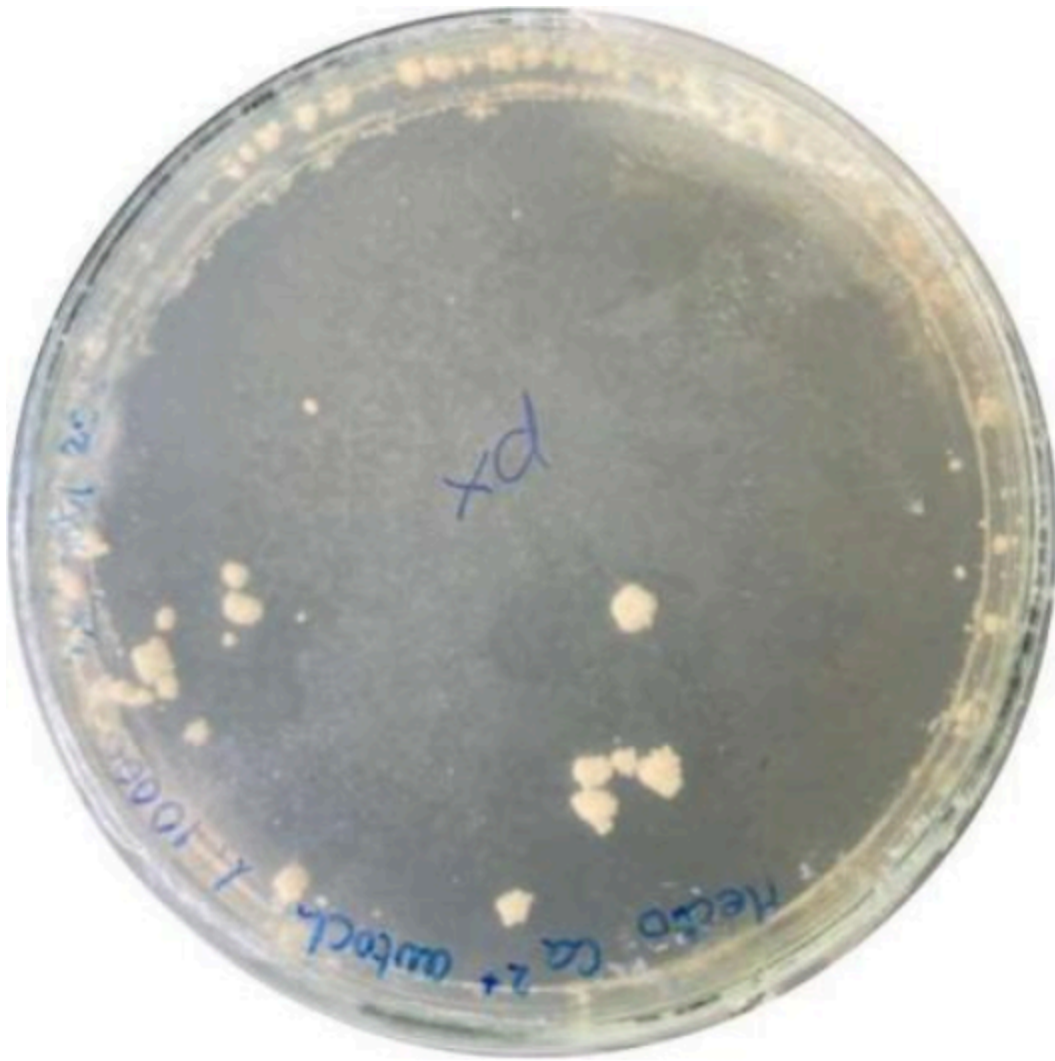


Figure 4

Precipitation of calcium carbonate in the Petri dish.

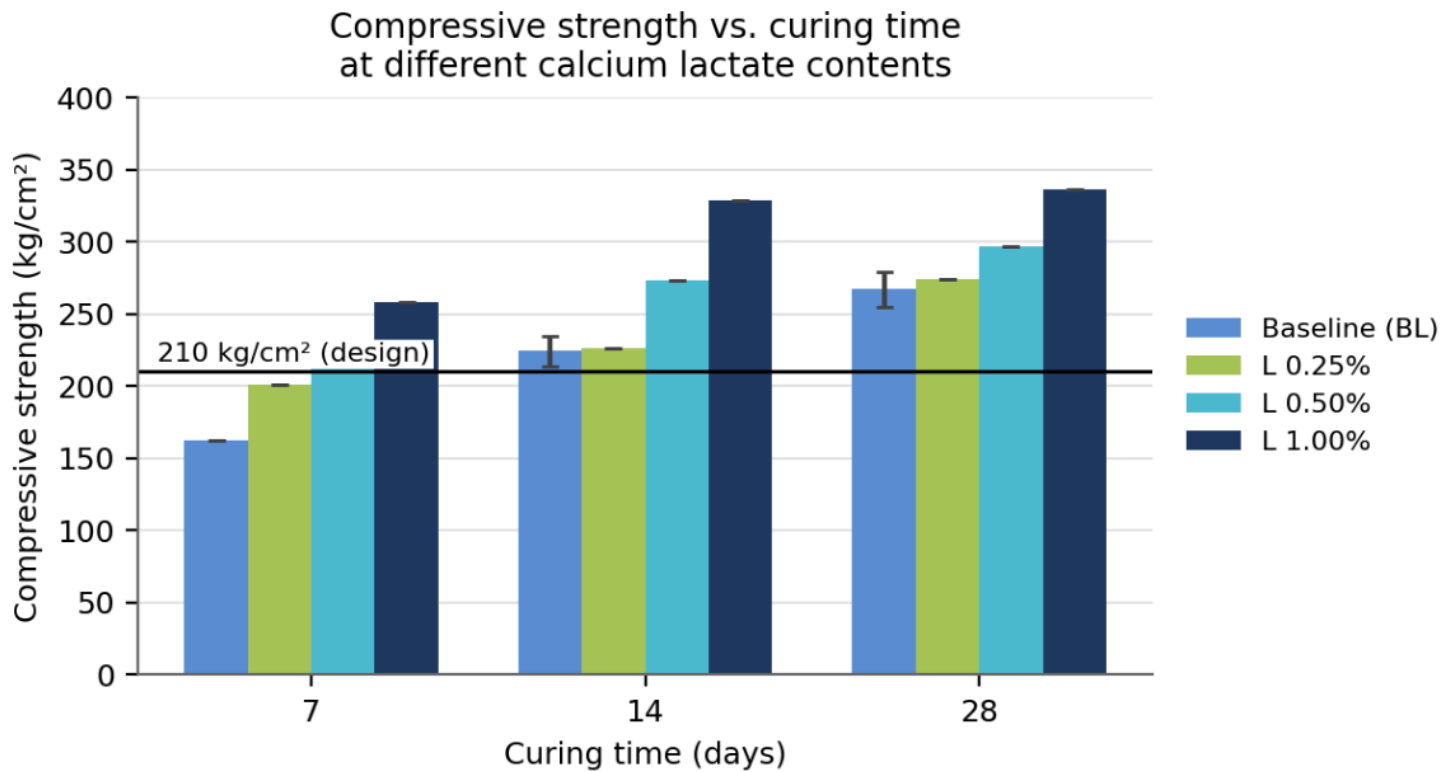


Figure 5

Compressive strength vs. time — baseline concrete with different calcium lactate concentrations.

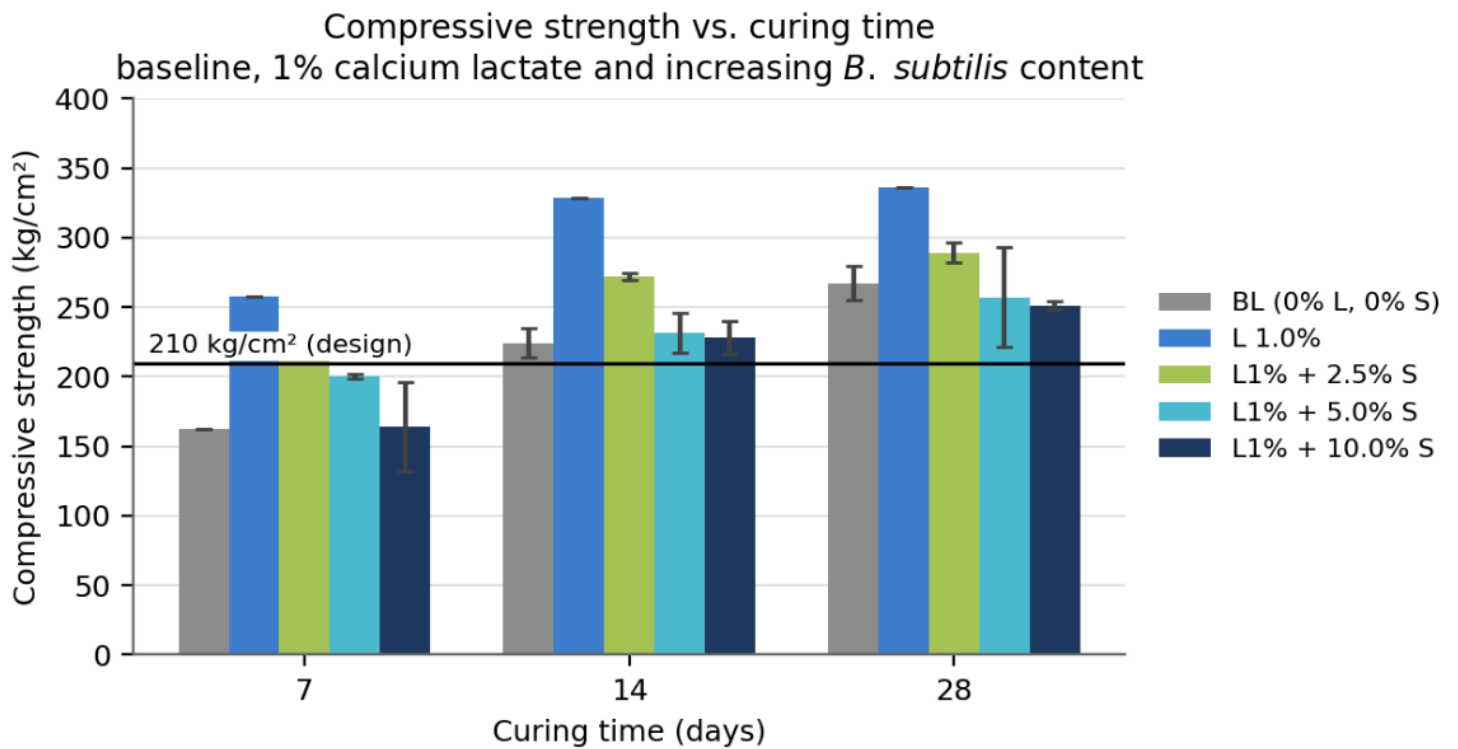


Figure 6

Compressive strength vs. time: baseline, 1% calcium lactate and different dosages of *Bacillus subtilis*.

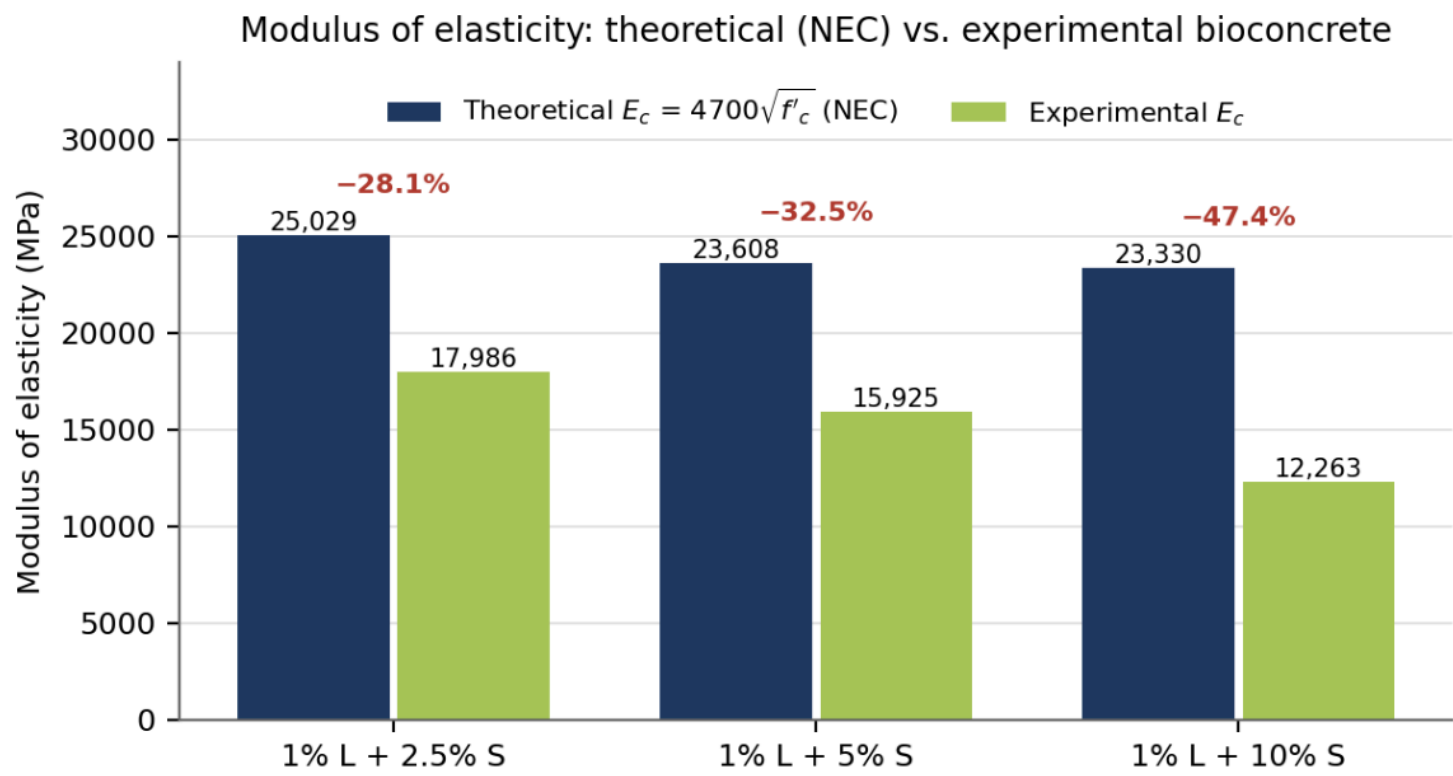


Figure 7

Theoretical modulus of elasticity vs. bioconcrete.



Figure 8

Concrete cylinders self-repaired with calcium carbonate (CaCO_3).

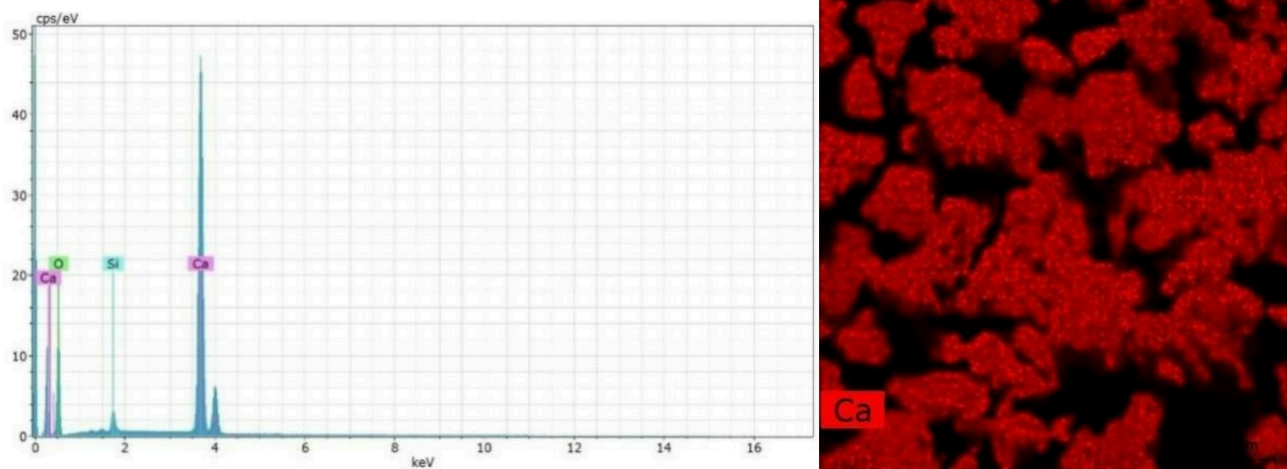


Figure 9

Characterisation of the calcium carbonate (CaCO_3): EDS spectrum of the crack deposit (left) and calcium elemental distribution map of the same area (right).