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Experimental Investigation of the Self Healing Potential of Bacteria for Sustainable Concrete Structures

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Report 23-04

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16. Abstract Although concrete is the most widely used building material in the world, its limited tensile strength makes cracking a common phenomenon in concrete elements. This study investigates the potential of autonomous self-healing as an eco-friendly and low-cost method to increase the durability of concrete. The crack-healing potential of different types of high-alkaline-tolerant bacteria or calcite-precipitation microorganisms is investigated. High-alkaline-tolerant bacteria and calcite-precipitation microorganisms were used to retrofit lab-fractured concrete samples. The samples healed with each of these bacteria groups were cast and tested under compressive load up to failure to measure the compressive strength of the concrete samples. The outcomes of experimental tests on concrete samples healed with biological processes demonstrate how this technique can be implemented when retrofitting durability-enhanced, eco-friendly concrete structures to improve the strength of durability of the material and ultimately improve the durability of many forms of concrete infrastructure.			
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ARS: Agricultural Research Service Culture Collection

ASTM: American Society for Testing and Materials

ATCC: American Type Culture Collection

MICP: Microbially Induced Calcium-carbonate Precipitation

NRRL: North Regional Research Laboratory

NH4-YE: Ammonium-Yeast

TYG: Tryptone-Yeast-Glucose

1. Introduction

The durability of infrastructures is defined by their ability to exceed expected design life while maintaining the desired mechanical properties without loss of serviceability. Most concrete structures have an expected service life of around 50 years. Several factors may affect their durability, such as temperature and moisture, mechanical loads, concrete permeability, and cracking. Thus, finding a way to preserve and extend the service life of concrete structures is a key task in civil engineering.

Cracking is the most common degradation phenomenon of concrete elements. Cracks may develop due to different causes, such as plastic shrinkage, expansion, settling, and overloading. Wide-open and widespread cracks increase the permeability of the concrete and facilitate the flow of potentially harmful liquids or gases. This may lead to degradation phenomena such as carbonatation, pitting, corrosion of the steel rebars, and so on.

Therefore, it is essential to limit the crack's width and diffusion. Different manual repair methods were proposed, including filling the cracks with a variety of expansive materials such as epoxy resins, polyurethane-based polymers, and latex emulsions. However, manual repairs can only be applied to visible and accessible cracks, while micro-cracks would remain unrepaired.

An innovative and relatively recent solution is self-healing (or self-repairing) concrete. Cracks may heal over time due to continued hydration of clinker minerals or carbonation of calcium hydroxide. Three self-healing general methods were studied so far:

- *Autogenous or natural self-healing*. Different natural processes can partially repair concrete cracks such as: (i) formation of calcium carbonate or calcium hydroxide, (ii) impurities, (iii) hydration of the unreacted cement, and (iv) the expansion of hydrated cementitious matrix.

- *Stimulated autogenous healing*. These methods are used when crack widths are constrained, stimulating continuous hydration or crystallization by providing a suitable addition such as mineral, crystalline admixtures, and superabsorbent polymers.

- *Autonomous self-healing*. Depends on integrating engineering modifications of the concrete matrix.

An innovative autonomous self-healing method using microbial activity has recently been introduced. Specific bacteria mixed with concrete have been seen to increase the effectiveness of self-healing. The bacteria are meant to activate and repair any damage without the need of manual intervention.[6]

Three main techniques have been studied for the optimization of microbially induced calcium carbonate precipitation (MICP), namely: (i) the direct method of incorporating the bacteria in the concrete mixture, (ii) encapsulation, and (iii) external application. The direct method is the most used and can significantly affect concrete strength.[11] In this method, the bacteria are usually applied within a protective material, including supplements and water, in the cement mix.[11,12,13] Encapsulation is preferable in internal applications due to its resulting evenness and uniformity in the mixture, as well as guaranteed bacterial survival. Yet, a high rate of encapsulation could affect the compressive strength of the concrete.[20] External application of self-healing microorganisms entails treating the concrete surface. This could be obtained by brushing or spraying the bacteria onto the surface after clearing loose particles and spraying a safe discharge medium.[21] The spray application has proven successful on different materials while biodeposition treatments have displayed promising results on cement-based materials.[21,22]

A positive impact on concrete's mechanical performance was seen when using green waste-derived biochar in cementitious mixes.[8,9] Wood waste biochar concrete has shown compressive strength of the same order as basic concrete.[9] In similar studies, concrete with added wood and sludge waste demonstrated improved ultimate strength, suggesting that good mechanical behavior could be obtained for biochar produced by different types of waste whilst proposing safe, green, eco-friendly, and low-cost admixtures.[8,9,10]

Although several techniques have been applied, different features still need to be investigated such as bacterial persistence, water absorption, strength reduction, and cost effectiveness/production.[19,20] Also, proper nutrients need to be provided to the bacteria throughout the concrete's lifespan (i.e., throughout the service life of the structure).[14]

Furthermore, in a concrete element, cracks of different lengths, widths, depths, and locations can be detected, depending on different factors. Early-age cracks caused by drying and shrinkage are generally smaller than loading-induced ones. Consequently, the type and number of cracks to be healed need to be identified a priori. For retrofitting intervention involving self-healing mechanisms, early-age cracks appear to be the ideal candidates since the cracking period is predictable and the average crack widths are generally smaller when compared to tensile-induced ones.[17,18]

This study evaluates the microbiological self-healing ability of Portland cement-based concrete at 28 days of curing. High-alkaline-tolerant bacteria and calcite-precipitation microorganisms were used to retrofit lab-fractured concrete. Different types of bacteria were studied in this report, namely:

- *Bacillus subtilis*. Known also as the *hay bacillus* or *grass bacillus*, this is a gram-positive bacterium commonly found in soil and the gastrointestinal tract of ruminants, humans, and marine sponges.

- *Bacillus megaterium*. A rod-shaped, gram-positive bacterium which is one of the largest eubacteria in soil.

- *Pseudomonas stutzeri*. A gram-negative soil bacterium classified as *bacillus*, or rod-shaped.

- *Sporosarcina pasteurii*. A gram-positive bacterium with the ability to precipitate calcite and solidify sand given a calcium source and urea.

The different concrete samples healed with each of these bacteria groups were cast and tested under compressive load up to failure. The compressive strengths obtained for the healed samples were compared to the results obtained on non-healed samples. This was done to investigate the self-healing capacity of each of the four types of bacteria.

2. Methodology

This project consists of three key components:

i. *Bacteria.* As said, this study compared the healing potential of several types of bacteria. In particular, four bacteria genera were used: (i) *Bacillus subtilis* (B-14596), (ii) *Bacillus megaterium* (B-350), (iii) *Pseudomonas stutzeri* (B-2461), and (iv) *Sporosarcina pasteurii.*[24] Each bacteria group was grown via broth culture. Following the instructions provided by the Agricultural Research Service Culture Collection (ARS) at the North Regional Research Laboratory (NRRL), *B. subtilis*, *B. megaterium*, and *P. stutzeri* were grown in Tryptone-Yeast-Glucose broth (TYG broth), while *S. pasteurii* was grown in Ammonium-Yeast broth (NH4-YE broth) as instructed by the American Type Culture Collection (ATCC).[23,24]

ii. *Biochar.* The biomass within the biochar acts as an immobilizer for the bacteria. This enhances the bacteria's healing efficiency.[27] In this study, food waste was used as biomass. The food waste included meat, butter, bread, rice, fruits, and salad. Starting from the data proposed in different research studies (see Figure 1) we aimed to replicate, at a smaller scale, the different food types of municipal wastes.[26] Thus, this study proposes an eco-friendly and low-impact method to recycle a large amount of a common type of waste.

iii. *Concrete samples.* Concrete samples were tested under pure compression. A test protocol was designed to investigate the effects of bio-healing on the mechanical properties of the samples and to compare this response against that of samples without bio-healing.

The detailed descriptions of these three components are given in the following subsections.

2.1 Bacterial Media and Growth Conditions

Description of the cultures and their growth condition

B. subtilis (B-14596), *B. megaterium* (B-350), and *P. stutzeri* (B-2461) were provided by the Agricultural Research Service Culture Collection (ARS) at the North Regional Research Laboratory (NRRL) and were grown in the recommended TYG media, which includes 2 grams of Tryptone, 5 grams of yeast extract, 1 gram of dipotassium phosphate, and 1 gram of glucose within 1 L of deionized water. The TYG broth was subsequently autoclaved prior to downstream culturing.[23] *S. pasteurii* was obtained from the American Type Culture Collection (ATCC) and was grown in the suggested Ammonium-Yeast (NH_4-YE) broth, which was composed of 10 grams of ammonium sulfate, 20 grams of yeast extract, and 0.13 mol/L of Tris(hydroxymethyl)aminomethane (tris) buffer at pH 9.0 in 1 L of final volume.[24] Each

ingredient was separately sterilized by autoclaving prior to mixing. Table 1 displays the composition of TYG and NH_4-YE media.

Figure 1. Box and Whisker Plot of Percentage of Food Wasted by Group in Municipal Solid Wastes.[26]

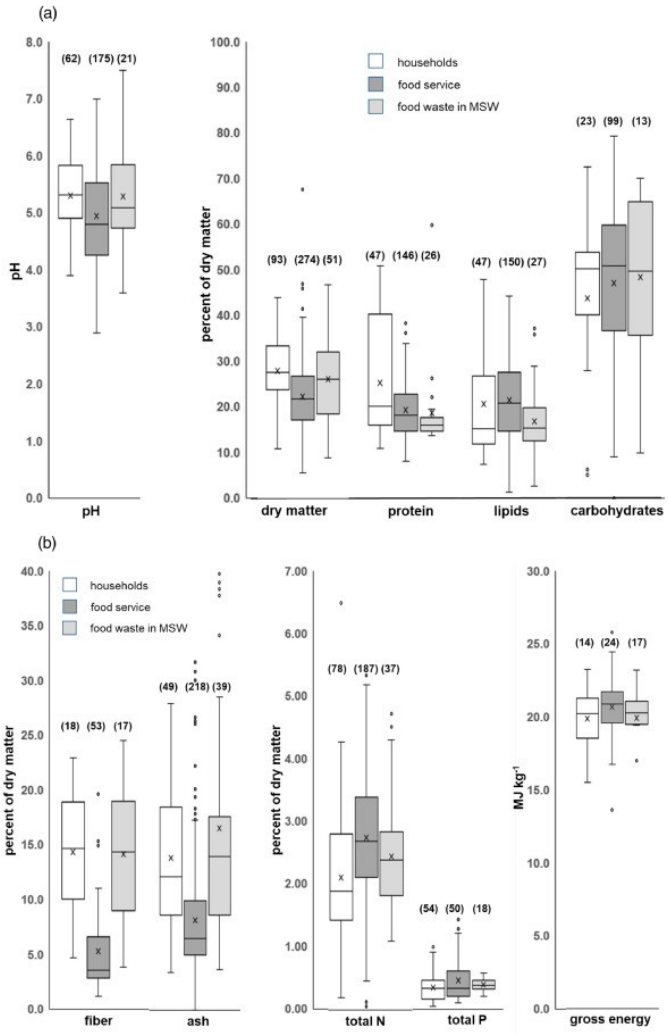


Table 1. Compositions of the two Broth Cultures Used For Bacteria Growth

Tryptone-Yeast-Glucose broth (1 L)	Ammonium-Yeast broth (1 L)
Tryptone (2 g)	Ammonium sulfate (10 g)
Yeast extract (5 g)	Yeast extract (20 g)
Dipotassium phosphate (1 g)	Tris buffer (0.13 M)
Glucose (1 g)	

Culture procedures and measurement

A sterilized pipette was used to transfer 35 mL of autoclaved broth into a 50 mL conical tube in a sterilized work environment. *B. subtilis*, *B. megaterium*, and *P. stutzeri* were transferred into tubes filled with TYG broth, while *S. pasteurii* was transferred into tubes of NH_4-YE broth using a sterilized inoculating loop. All types of bacteria grew aerobically at 28˚C in an orbital incubator shaker for 24 hours or until growth was observed. The control TYG and NH_4-YE broths without bacterial cultures were also placed in the incubator for contamination verification.

A spectrophotometer was used to measure the optical density at 600 nm ($O.D._{600}$) of the bacterial growth to confirm the growth of the bacteria in each vial. The $O.D._{600}$ of 1 was maintained to standardize each bacterial cell number at each concrete manufacturer batch.

2.2 Biochar

Biochar is charcoal made from biomass. In this study, the biomass was provided by food waste. For this research, food near the expiration date was bought and transformed into charcoal as it was not possible to obtain food waste from the municipality. Nevertheless, the food for the biochar was mixed following the composition described in Figure 1. For the study, 20 L of biochar was produced. The food was cut up into pieces and put into a dehydrator to remove all moisture. After at least 24 hours, the food was removed from the dehydrator and checked to see if all moisture had been removed.

The dried food was then wrapped in aluminum foil and placed into a furnace at 300˚C for 1 hour. Through this process, the food waste undergoes pyrolysis and turns into biochar. Finally, the resulting biochar was mixed in a large container.

2.3 Concrete

Description of the concrete samples

The manufacturing of the concrete samples is herein described. To manufacture the samples, the 90-lb (40.8 kg) QUIKRETE® ready-to-use concrete mix was used. This is a pre-blended mixture of cement and aggregates for general structural uses, requiring only the addition of water. The concrete was mixed in a Portable Concrete Mixer, with a 5 cu. ft. (0.14 m^3) drum capacity, paired with a 1/2 HP motor, and a tilting/pivoting drum. As per the manufacturers' recommendations, 3.96 L of water was gradually added to the concrete mix, which was mixed for 5 minutes. More specifically, the first half of the water was poured in at the beginning of the process, and the remaining portion was poured halfway through the mixing process.

For samples C1 to C11, biochar was added with the water, during the mixing of the samples. The volume of the biochar was 5% of that of the wet concrete.

The concrete mixture was cast into 4" x 8" cylinder molds (101.6 mm x 203.2 mm) to obtain samples of the required dimensions. The ASTM C31 – Making and Curing Concrete Test Specimens in the Field procedures were employed for this scope.[25]

Following ASTM C511 requirements, after an initial curing, all the concrete samples were positioned in curing tanks filled with water saturated with calcium hydroxide. All the tanks were located inside a curing room with a temperature within the 73.5˚F, +/- 3.5˚F range. During the initial curing, the cylinders were stored in a curing room and were not exposed to sunlight or heating for 48 hours. For all samples, curing in water lasted 28 days.

A total of 77 samples were manufactured and tested (see Table 2). The samples were subdivided into 7 sets of 11 samples each:

Set A, *Concrete samples to failure*, samples A1 to A11. This is the control set and consists of concrete samples tested up to failure after 56 days of curing. No self-healing procedures were applied to this set.

Set B, *No self-healing*, samples B1 to B11. These concrete samples are cracked after 28 days of curing and are tested to failure after an additional 28 days. These samples were not sprayed with bacteria after cracking (no self-healing) and have been used to investigate the influence of cracking on the ultimate response of concrete.

Set C, *Self-healing with biochar (no bacteria)*, samples C1 to C11. The samples of this set are cracked after 28 days of curing and tested to failure after an additional 28 days. Biochar was added to concrete during the mixing of the samples. The volume of biochar was 5% of that of wet concrete. After cracking, these samples were not treated with bacteria.

Set D, *Self-healing with B. subtilis*, samples D1 to D11. This set of samples is cracked after 28 days of curing and tested to failure after an additional 28 days; the samples were treated with *B. subtilis* after cracking.

Set E, *Self-healing with B. megaterium*, samples E1 to E11. This set of samples is cracked after 28 days of curing and tested to failure after an additional 28 days; the samples were treated with *B. megaterium* after cracking.

Set F, *Self-healing with P. stutzeri*, samples to F1 to F11. This set of samples is cracked after 28 days of curing and tested to failure after an additional 28 days; the samples were treated with *P. stutzeri* after cracking.

Set G, *Self-healing with S. pasteurii*, samples G1 to G11. This set of samples is cracked after 28 days of curing and tested to failure after an additional 28 days; the samples were treated with *S. pasteurii* applied after cracking.

Table 2. Description of the Samples Manufactured for this Study

Set Name	Sample Names	Cracking at 28 days	Self healing protocol
Concrete samples to failure	A1 to A11	Not implemented	Not implemented
No self-healing	B1 to B11	Not implemented	Not implemented
Self-healing with biochar (no bacteria)	C1 to C11	implemented	Just biochar
Self-healing with *B. subtilis*	D1 to D11	implemented	implemented
Self-healing with *B. megaterium*	E1 to E11	implemented	implemented
Self-healing with *P. stutzeri*	F1 to F11	implemented	implemented
Self-healing with *S. pasteurii*	G1 to G11	implemented	implemented

Testing procedures

After curing, concrete samples were ready to be tested. Compression tests were performed at the College of Engineering of the California State University, Long Beach, using the Humboldt HCM-500-iHA Compression Testing machine (Figure 2a). This machine can apply a maximum vertical force of 2,224 kN, while the maximum piston stroke is equal to 63.5 mm. Figure 2b depicts concrete samples after being cracked.

The protocols used for the compression tests are described as follows.

Test protocol to determine the compressive strength of concrete

The testing procedure described in ASTM C39 was used to determine the compressive strength of concrete.[29] The curing time for each sample was 56 days. Sample Sets B to G were cracked after 28 days of curing before testing them to failure. The procedure used to create cracks in the samples is described below.

Test protocol to crack concrete samples (cracking at 28 days)

After an initial curing of 28 days, the samples of Sets C, D, E, F, and G were tested up to a compressive load equal to 75% of the average compressive strength of the samples of Set A. For this scope, the same loading rate used to determine the compressive strength of concrete was adopted up to 15.9 MPa, to then unload the samples. It is worth mentioning that while this testing protocol is sufficient to induce cracks in the samples, the pattern, width, and extension of these cracks will vary between samples. The procedure was deemed valid for the scope of this work as it allows for the rapid creation of cracks that would correspond to those present in highly strained concrete elements.

Test Protocol for self-healing

Immediately after cracking, the samples of Sets D, E, F, and G were set aside in a different non-leak tray. The samples were then poured with 50 mL of solution (Table 2):

Set D: Bacillus subtilis.

Set E: Bacillus megaterium.

Set F: Pseudomonas stutzeri.

Set G: Sporosarcina pasteurii.

These samples were then covered using a cling film and placed on a shelf in a curing room. The bacteria were three times: the first time after 24 hours from cracking, the second time after 48 hours from cracking, and the third time 72 hours after cracking, each time using 50 ml of the broth cultures described in Table 1.

Figure 2. (a) Humboldt HCM-500-iHA Compression Tester of the California State University Long Beach, (b) Concrete Samples after Cracking Test

(a) (b)

3. Results

3.1 Average Compressive Strengths

The average compressive strength was assumed as a key parameter of the experimental tests. The average compressive strengths of the concrete samples of each set are shown in Figure 3. In this figure, $f_{cm}^{Set\ X}$ indicates the compressive strength of the variable Set X, with X varying from A to G.

As can be seen, the average compressive strength of the samples with no pre-cracking and self-healing (i.e., $f_{cm}^{Set\ A}$) was equal to 21.2 MPa. This value was assumed as the reference strength for the remaining sets. In Figure 3, $f_{cm}^{Set\ A}$ is compared with each $f_{cm}^{Set\ X}$ using green or red arrows, to indicate an increase or a decrease, respectively of the average compressive strength of the generic set.

Loading the concrete samples up to 75% of $f_{cm}^{Set\ A}$, unloading, and then reloading up to failure, reduces the average compressive strength of the set ($f_{cm}^{Set\ B}$) to 18.9 MPa (red arrow). Results on this set of samples' compressive strength demonstrated how cracking plays a key role in reducing the capacity of the concrete samples. As compressive strength is the most relevant property of the concrete, methods to prevent degradation of this parameter, such as the self-healing proposed in this study, are of great interest.

Set C samples are cracked after 28 days of curing and tested to failure after an additional 28 days. The average compressive strength of this set of specimens ($f_{cm}^{Set\ C}$) was equal to 12.0 MPa. This is the lowest value among all this study's sets of samples. As a result, biochar alone was found to have no healing properties on cracked concrete specimens.

Bacterial self-healing was applied to the last four sets, i.e., Sets D, E, F, and G. The average compressive strengths of these sets were found to be 22.5 MPa ($f_{cm}^{Set\ D}$), 15.6 MPa ($f_{cm}^{Set\ E}$), 21.7 MPa ($f_{cm}^{Set\ F}$), and 24.0 MPa ($f_{cm}^{Set\ G}$), respectively.

In Figure 3, the generic set's average compressive strength is compared to that of the control set (i.e., $f_{cm}^{Set\ X} / f_{cm}^{Set\ A}$). In this figure, the percentage increments ($f_{cm}^{Set\ X} / f_{cm}^{Set\ A} - 1 \geq 1$, green markers) or reductions ($f_{cm}^{Set\ X} / f_{cm}^{Set\ A} - 1 < 1$, red markers) obtained for the different sets are also shown and plotted on a secondary vertical axis.

From Set C to Set G, three out of four treatments with bacteria contributed to incrementing the average compressive strength of the concrete samples: (i) self-healing with *Bacillus subtilis* (Set D), (ii) with *Pseudomonas stutzeri* (Set F), and (iii) with *Sporosarcina pasteurii* (Set G), corresponding to +6.11%, +2.49%, and +13.5% of the strength of Set A, respectively. These results are encouraging, demonstrating how these low-cost and eco-friendly treatments can be a valid method to improve the mechanical properties of the concrete.

For the remaining sets, the average compressive strength reduces compared to the control set with a -43.1% reduction in Set C and -26.2% in Set E.

Figure 3. Average Compressive Strengths of the Different Set

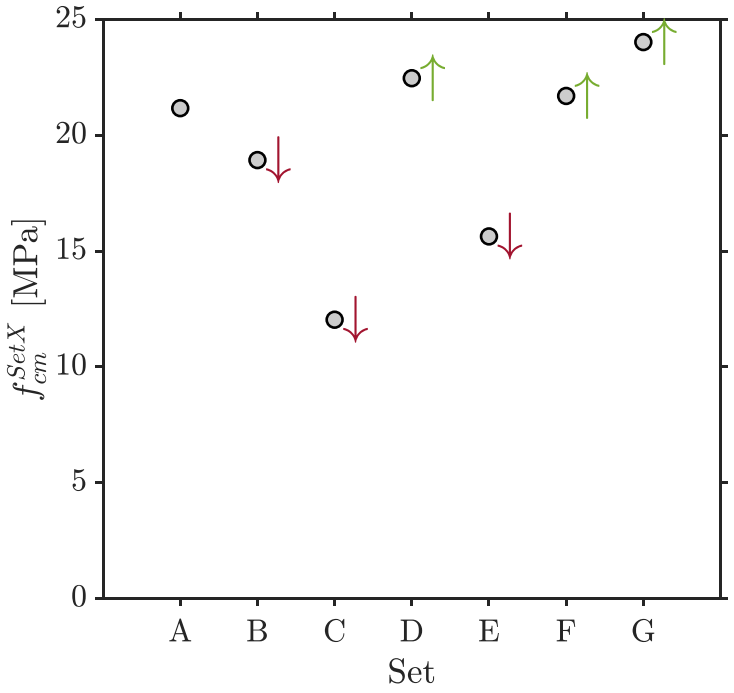
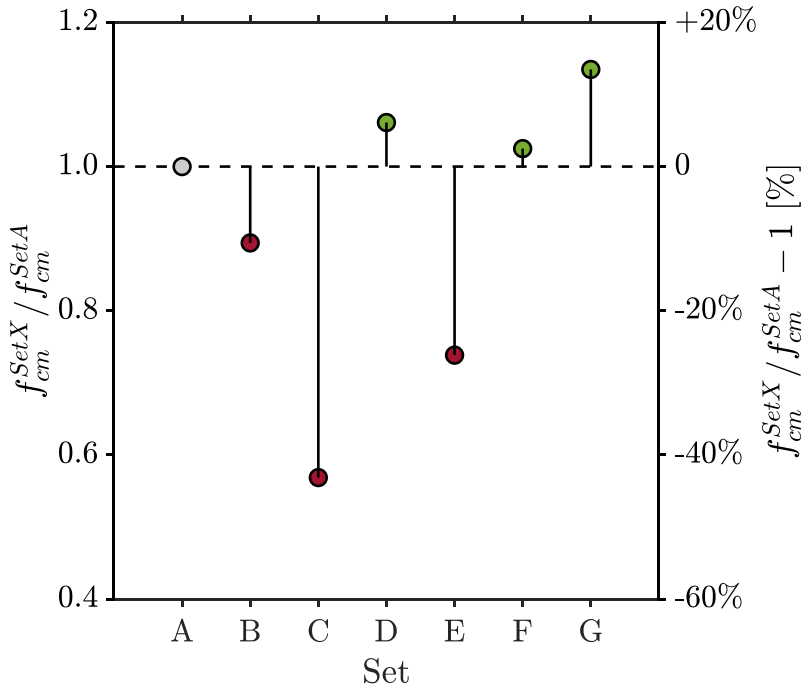


Figure 4. Percentage Variation of the Average Compressive Strengths of the Generic Set, Relative to Set A



3.2 Variation of the Average Compressive Strengths

Figure 5 shows the average compressive strengths of the different sets with the corresponding standard deviations, calculated as:

$$\sigma_{Set\,X} = \sqrt{\frac{\sum_{i=1}^{n}(x_i - \bar{x})^2}{n-1}}$$

In Figure 5, the green and the red markers show how the generic $f_{cm}^{Set\,X}$ may be increased or decreased with respect to Set A. The numerical values of these quantities are also reported in the same figure.

The standard deviations of Sets B, C, D, E, F, and G were found to be equal to: 7.53 MPa, 3.62 MPa, 10.1 MPa, 8.32 MPa, 7.40 MPa, and 9.54 MPa, respectively.

Comparing the maximum values of compressive strengths (green markers of Figure 5) of Sets D ($f_{c,max}^{Set\,D}$),E ($f_{c,max}^{Set\,E}$),F ($f_{c,max}^{Set\,F}$), and G ($f_{c,max}^{Set\,G}$) with $f_{cm}^{Set\,A}$, shows how all the self-healing treatments can increase the average compressive strength of the control samples. However, larger standard deviation values were obtained for these sets compared as to Set A.

Figure 6 shows the coefficient of variation (c.o.v.) of each set of tested samples, obtained as:

$$\sigma^{*}_{Set\ X} = \frac{\sigma_{Set\ X}}{f^{Set\ X}_{cm}}$$

As can be seen, for samples treated with bacteria a minimum dispersion of the order of 30% is obtained for the sets. This dispersion may depend on the crack patterns of the concrete samples. As each specimen was cracked, treated, and then tested up to failure, the first loading protocol causes non-uniform cracks in the different specimens that have an impact on the mechanical properties of the samples and the effectiveness of the self-healing treatments. In future research, the variability induced by the cracking protocol should be addressed. To obtain uniform responses, independent of the failure mode of the specimen, it is necessary to control the cracks' formation upstream. A simple method could consist of using plastic or metallic inserts embedded inside the cylinder samples prior to casting. These shims could be removed after curing and would allow to test samples with same crack width, pattern, and dimensions.

Regarding the average compressive strength, the following *efficacy parameter* can be used to estimate the effectiveness of the treatment:

$$\psi_{Set\ X} = \frac{f^{Set\ X}_{cm} / f^{Set\ A}_{cm} - 1}{\sigma_{Set\ X}}$$

This parameter simultaneously considers the increase in compressive strength due to the self-healing treatment and the dispersion obtained for each set. Thus, larger values of $\psi_{Set\ X}$ correspond to more effective treatments, while negative values represent treatments that produce no improvements. The values of $\psi_{Set\ X}$ are shown in Table 3, where a summary of the main parameters discussed so far are also reported. As can be seen, self-healing with *Sporosarcina pasteurii* (Set G) appears to be the most effective treatment.

Figure 5. Average Compressive Strengths of the Different Sets with Standard Deviations

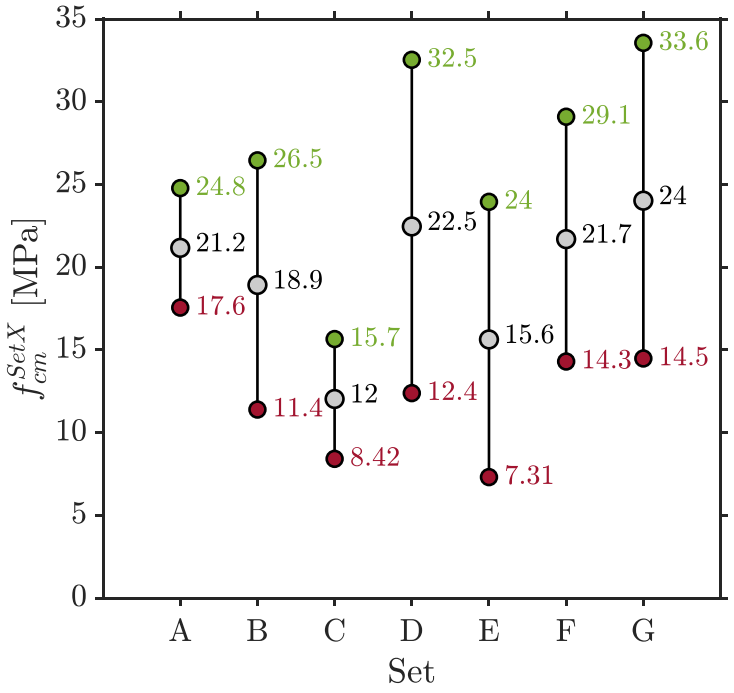
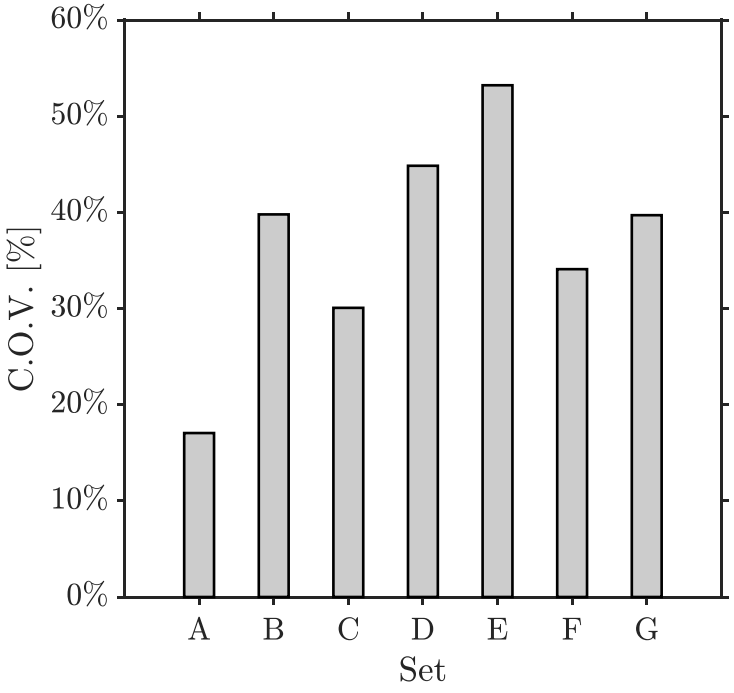


Figure 6. Coefficient of Variation off Each Set



3.3 Elastic, Ultimate Response, and Ductility

The elastic limits of the concrete samples are herein calculated according to Fib Model Code, where the Serviceability Limit State (SLS) is identified within a compressive stress as $f_{cy,m}^{Set\ X} = 0.4 \cdot f_{cm}^{Set\ X}$.[28] Figure 7 shows the average values of these limits for each set, with the corresponding elastic compressive strain $\varepsilon_{cy,m}^{Set\ X}$ (gray markers). In the same figure, the average values of the peak ($f_{cm}^{Set\ X} - \varepsilon_{cm}^{Set\ X}$, black markers) and ultimate ($f_{cu,m}^{Set\ X} - \varepsilon_{cu,m}^{Set\ X}$, red markers) compressive stresses—strains are plotted for a direct comparison.

Table 3. Summary of the Main Parameter Related to the Average Compressive Strengths

Set	f_{cm} [MPa]	σ [Mpa]	C.O.V. [%]	$f_{cm}^{Set\ X} / f_{cm}^{Set\ A}$ [%]	ψ
A	21.2	3.61	17.1%	0.0%	0.000
B	18.9	7.53	39.8%	-10.59%	-0.014
C	12.0	3.62	30.1%	-43.1%	-0.119
D	22.5	10.08	44.8%	6.11%	0.006
E	15.6	8.32	53.2%	-26.2%	-0.031
F	21.7	7.40	34.1%	2.49%	0.003
G	24.0	9.54	39.7%	13.5%	0.014

Starting from the values at the elastic limit and at the peak, the reduced modulus of elasticity $E_c^{Set\ X} = f_{cy,m}^{Set\ X} / \varepsilon_{cy,m}^{Set\ X}$ and the modulus of elasticity $E_{cm}^{Set\ X} = f_{cm}^{Set\ X} / \varepsilon_{cm}^{Set\ X}$ can also be calculated.[28] These values are plotted in Figure 8.

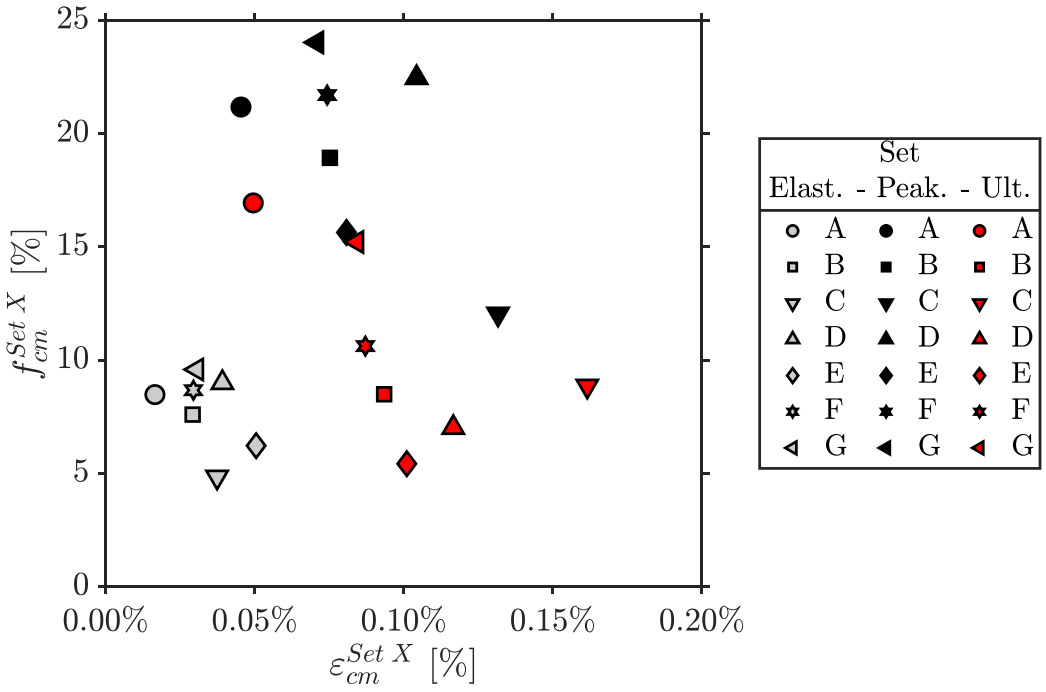
As expected, being loaded up to first cracking and then reloaded up to failure, the samples of Sets from B to G show greater vertical deformations compared to the control Set A (Figure 7). As a result, decreasing values of the elastic moduli of the same sets were also obtained (Figure 8).

As Section 3.2 shows, treatments with *Bacillus subtilis* (Set D), with *Pseudomonas stutzeri* (Set F), and with *Sporosarcina pasteurii* (Set G), increase the average compressive strength; the same treatments increase the limit elastic stress as well (Figure 7, gray markers). As for the ultimate responses, all sets return values of the stresses lower than Set A, but larger than the ultimate compressive strains (Figure 7, red markers). The ultimate compressive strain, a key parameter in building materials as it is related to ductility, is defined as:

$$\mu_{Set\ X} = \frac{\varepsilon_{cu,m}^{Set\ X}}{\varepsilon_{cy,m}^{Set\ X}}$$

The average values of the ductility are shown in Figure 9 for each set. In this figure, green markers represent sets of samples with average ductility greater than Set A, while red markers stand for reductions in ductility compared to Set A. The percentage increases/decreases of the ductility of the generic set compared to the control set are plotted in Figure 9 in the secondary axis. As shown, self-healing with *Bacillus subtilis* (Set D) and with *Sporosarcina pasteurii* (Set G) led to large increases of the average ductility of the concrete samples, equals to +25.8% and +49.6%, respectively; for the remaining self-healing treatments, slight reductions of the same parameter are obtained, corresponding to -2.85% for Set E and -3.04% for Set F.

Figure 7. Elastic, Peak, and Ultimate Stresses Vs. Strain of the Concrete Samples



Combining the results obtained in Section 3.1 on the average compressive strengths with the results of this section on the concrete's ductility, the best self-healing procedure appears to be the one with *Sporosarcina pasteurii*. For this set of samples, a significant increase in ductility and a good improvement of the compressive strength were obtained.

Figure 8. Moduli of Elasticity of the Different Sets

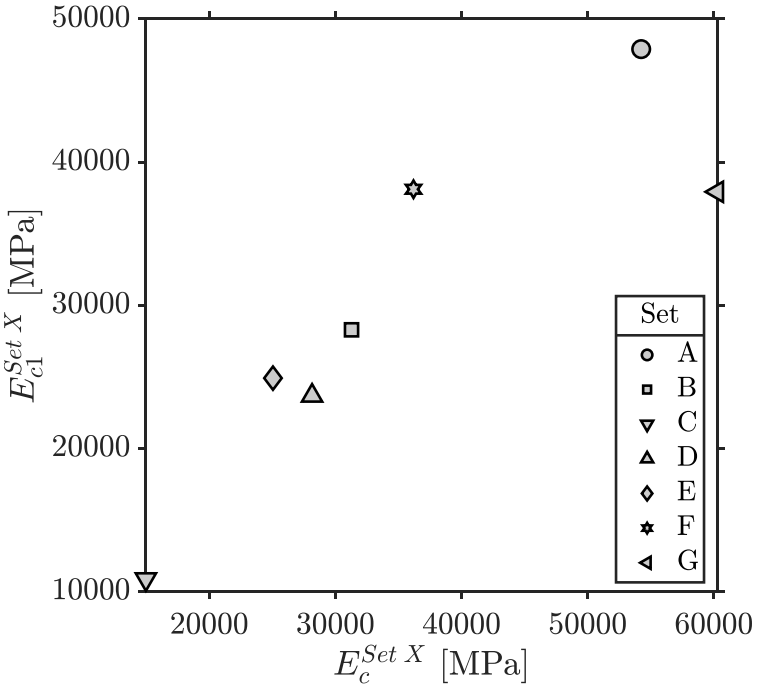
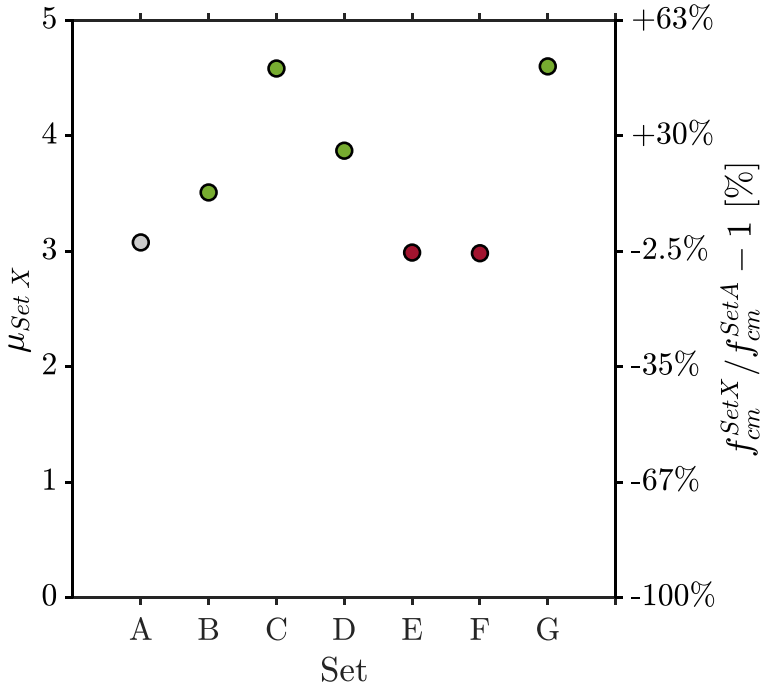


Figure 9. Average Ductility Values of the Concrete Sets



4. Conclusions

Durability of concrete structures is a significant topic in civil engineering. A significant research effort has been focused on the degradation phenomena of concrete, highlighting how these phenomena are significantly dictated by cracking of the material. The need to extend the durability of concrete infrastructures led to the development of several methods for improving the mechanical properties of cracked concrete.

This research work studied a low-cost and eco-friendly method to improve the durability of concrete using self-healing bacteria. Experimental compression tests were conducted on 7 sets of 11 concrete samples. The first two sets included samples with no treatments and were used as control samples; the remaining sets consisted of concrete samples treated with:

- Biochar (and no bacteria)
- Bacillus subtilis.
- Bacillus megaterium.
- Pseudomonas stutzeri.
- Sporosarcina pasteurii

The average compressive strength of each set was measured. The strength of the samples subjected to the self-healing procedures were compared to the compressive strength of the control set. Results showed how self-healing procedures with *Bacillus subtilis*, *Pseudomonas stutzeri*, and with *Sporosarcina pasteurii* increased the average compressive strength of the concrete samples, while the remaining self-healing procedure with *Bacillus megaterium* did not prove effective in increasing the mechanical properties of the material.

The standard deviation and the coefficient of variation of the compressive strengths were also reported for each tested set. These parameters showed how a slight dispersion resulted in computing the compressive strength of the single sample of the generic set as a result of the different failure modes observed during the experimental tests.

Finally, a discussion on the elastic and ultimate responses, as well as on the ductility of the concrete sets, was reported, showing how all the self-healing treatments increase the ultimate compressive strain of the samples, reducing the elastic moduli at the same time. The ductility of the concrete appeared to be improved by self-healing with *Bacillus megaterium* and *Sporosarcina pasteurii*, while *Bacillus subtilis* and *Sporosarcina pasteurii* played a minor role on this parameter.

The results of this work can contribute to the diffusion of sustainable concrete structures with the ability to fix its cracks autogenously or autonomously.

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Dr. Pitiporn Asvapathanagul is a faculty member in Civil Engineering and Construction Engineering Management (CECEM) Department at California State University, Long Beach (CSULB). Her area of competence is Environmental Engineering, primarily bioremediation, biology wastewater treatment, and molecular biology. Examples of her research are bacterial community dynamics in activated sludge, nutrient removal of biological water reclamation processes, biofilms on aeration diffuser membranes, microplastic removal technologies, etc. Dr. Asvapathanagul is also a registered Professional Engineer (PE) in the state of California. Dr. Asvapathanagul employed her expertise in microbiology to advance accomplishments of the self-healing concrete project.

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Dr. Simone Galano is a postdoctoral researcher at the University of Naples Federico II. He gained his PhD in Structural, Geotechnical, and Seismic Risk Engineering at the University of Naples Federico II in 2022. Since 2019, he has worked with the CSULB's CECEM Department as a visiting researcher. Dr. Galano's research activities are in the fields of seismic isolation with elastomeric devices, prestressed concrete structures, and the durability of existing prestressed concrete bridges. He authored many research studies including experimental tests and numerical analyses of fiber reinforced elastomeric isolators with natural, recycled, and reclaimed rubber, and reduced-scale post-tensioned concrete bridge girders.

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Nicholas Banuelos is an undergraduate student at the CSULB studying Environmental Science and minoring in Biology and Geography. His responsibilities included assisting in the manufacturing and testing of samples.

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