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

Andrea Calabrese, PhD
Pitiporn Asvapathanagul, PhD
Nisarg N Patel
Nanubala Dhruvan
Austin Adams
Michael Hernandez
Douglas S Lopez-Cruz

CSUTC California State University Transportation Consortium

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Report 24-17

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16. Abstract Concrete is a critical component to so much of the modern construction industry. This material, well known for its versatility, robustness, longevity, and strength, is well-suited for a wide range of structural applications. Nonetheless, the widespread occurrence of cracks in concrete structures, primarily attributed to its limited tensile strength, shrinkage, and overstain, imposes a considerable economic and environmental challenge when it comes to retrofitting these fissures. This study tackles this problem by harnessing bacteria tolerant to high alkaline conditions to enable Microbially Induced Calcium Carbonate Precipitation (MICP) for the self-repair of concrete. This is achieved through an external application method, wherein bacteria are manually and externally applied to the cracks of the concrete surface. This report presents the results of testing three different bacterial species (Bacillus subtilis, Bacillus megaterium, and Sporosarcina pasteurii) to retrofit laboratory-manufactured cracks. The self-repaired groups underwent compressive load-to-failure testing and were compared to a control group (With Crack), revealing a notable increase in compressive strength ranging from 8.59% to 21.61%. The outcomes of the compressive strength tests illustrate the viability of implementing this technique for retrofitting concrete structures, showcasing its environmentally friendly nature and its ability to significantly enhance structural durability. This, in turn, has the potential to impact existing and future developments that incorporate concrete.			
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Mineta Transportation Institute
College of Business
San José State University
San José, CA 95192-0219

Tel: (408) 924-7560
Fax: (408) 924-7565
Email: mineta-institute@sjsu.edu

transweb.sjsu.edu/research/2331

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Executive Summary

Concrete, the backbone of the modern construction industry, is well known for its versatility, robustness, longevity, and strength. Concrete is a material that exhibits exceptional resistance to compression, rendering it well-suited for a wide range of structural applications. The material can be cast into molds or forms of various shapes and sizes, enabling the creation of a wide range of constructions, including buildings, bridges, highways, and dams.

However, concrete structures are susceptible to many factors that may deteriorate their integrity. One of the major issues usually found is the formation of cracks, which can lead to a reduction in structural integrity, increased permeability, and an increased chance of rebar corrosion. Innovative solutions must be introduced to mitigate this issue. In this respect, the development of *self-healing concrete*, as the name indicates, aims to provide concrete structures with an ability to repair or minimize cracks autonomously. The scope of this research work is that of testing the self-healing potential of bacteria for sustainable concrete structures through the application of the microbially induced carbonate precipitation (MICP) method. Three pure cultures are considered for this study, *Bacillus subtilis* (B-14596), *Bacillus megaterium* (B-350), and *Sporosarcina pasteurii* (ATCC Catalog no: 11859), which are laboratory-cultivated.

The experimental design included five distinct specimen groups: Control without Crack (Set-A); Control with Crack (Set-B); and three Cracked Specimen sets treated with different bacterial species (namely, *Bacillus subtilis* (Set-C), *Bacillus megaterium* (Set-D), and *Sporosarcina pasteurii* (Set-F)). Each group comprised 11 samples featuring a concrete grade of 40 MPa aimed at securing statistical reliability. The production of the concrete specimens entailed careful blending of a high-strength concrete mixture according to standards detailed below. To ensure uniformity in material qualities across all specimens, the mixing technique was standardized. The curing techniques adhered to ASTM requirements, guaranteeing the most favorable concrete hydration and strength enhancement.

The methodology section of this report describes the cultivation of bacteria, followed by concrete specimen preparation and curing. After curing, high-alkaline-tolerant bacteria were introduced three times into the cracks of the concrete samples. These self-healed specimens were tested in compression using a Humboldt Compression Machine (HCM-5000-iHA, HUMBOLDT) after 84 days from their manufacturing.

The findings showed a significant enhancement in compressive strength, ranging from 8.59% to 21.61%, in the self-repaired groups as compared to the control group (With Crack). *Bacillus subtilis* and *Bacillus megaterium* exhibited similar strength to the control specimens; however, *Sporosarcina pasteurii* surpassed them. The variations seen across the data set were consistently within an acceptable range, suggesting a high level of dependability.

The results demonstrate the effectiveness of using bacteria to initiate Microbially Induced Calcium Carbonate Precipitation (MICP) in concrete fissures, hence improving the long lastingness of concrete structures. The use of the external application technique offers a very promising strategy for upgrading pre-existing buildings in an ecologically conscious way by extending the lifespan of structures and their components, therefore making a significant contribution to the advancement of sustainable growth within the construction sector. This study emphasizes the possibility of using bio-based solutions to solve issues related to the durability of concrete. It also identifies areas for further research and implementation.

1. Introduction

The enduring strength of a structure relies heavily on the lasting quality of its construction materials, particularly concrete and steel. Typically, concrete structures are designed with a minimum lifespan of 50 years.[1] However, various factors, including environmental conditions, material overstrain, and concrete cracks can influence durability.[2] The presence of cracks in concrete facilitates the infiltration of detrimental elements, such as water, corrosive chemicals, and gases, into the structure. The infiltration of these elements adversely impacts the resilience of materials, leading to the degradation of the structure and ultimately reducing its lifespan.[3] Consequently, a pivotal objective in civil engineering involves identifying methods to preserve and enhance the longevity of structures.

A prominent degradation feature commonly observed in concrete materials is cracking. Cracks may form due to various factors, such as shrinkage and excessive loading. The presence of extensive cracks amplifies the vulnerability of concrete elements to the penetration of harmful liquids or gases, leading to deterioration marked by steel bar corrosion, carbonation, and similar effects. The consequences of cracking in reinforced concrete extends beyond aesthetic concerns, adversely affecting reliability and stability.[3, 4]

Consequently, it is crucial to limit the extent of concrete cracks. Several methods for manually repairing concrete cracks have been proposed in literature, including the use of latex emulsions and epoxy resins to fill and expand within the fissures.[5]

However, certain cracks, particularly those with a maximum width of 0.1 mm, can be sealed through the hydration process of unhydrated cement.[6] It is worth noting that this process is unlikely to effectively seal larger cracks with precision.

Because of this, there has been a notable increase in research on the self-healing potential of concrete owing to its proven efficacy in repairing cracks.

1.1 Autonomous Self-Healing [8, 9]

Autogenous healing refers to the inherent ability of concrete to seal cracks when there is moisture and no tensile stress. This is mainly considered as a natural process where healing is achieved through two primary mechanisms:

a. cracks are eventually sealed through the formation of Calcium Carbonate (CaCO3) crystals or Calcium Hydroxide (Ca (OH)2); and7

b. the buildup of reaction products is a result of the ongoing hydration of unhydrated cement exposed to the surfaces of cracks.6

Autonomous healing involves integrating additives at different stages of manufacturing, incorporating both organic and inorganic compounds, as well as microbial compounds into the cracks.[9] Autonomous healing is achieved through various mechanisms, including:

- Biological healing: Recently explored biological methods involve introducing bacteria into concrete samples, demonstrating the potential to enhance concrete durability and facilitate crack healing.[10, 11]

- Encapsulation method: Encapsulation stands out as a widely employed and highly effective approach for crack treatment. This involves immobilizing bacteria within microcapsules, where they remain dormant within the concrete. Upon the occurrence of cracks in the concrete, these capsules rupture, contributing to the healing process.

- Vascular method: This method reflects the circulatory system of the human body, which uses blood to circulate nutrients to the body. With concrete, the vascular method is used because of its ability to circulate healing substances from external sources to the different points in the element.[11]

The primary goal of this research is to evaluate the effectiveness of the microbially induced carbonate precipitation method in concrete healing. This involves introducing different bacteria into cracked concrete samples and comparing the strength of treated samples to untreated and uncracked ones.

2. Methodology

2.1 Bacterial Pure Cultures, Growth Media, and Conditions

The bacteria used in this study were the *Bacillus subtilis* (B-14596), *Bacillus megaterium* (B-350), and *Sporosarcina pasteurii* (ATCC Catalog no: 11859). While *B. subtilis* and *B. megaterium* were obtained from the Agricultural Research Services Culture Collection (ARS), Northern Regional Research Laboratory (NRRL), United State Department of Agriculture (USDA) (https://nrrl.ncaur.usda.gov/), the *S. pasteurii* was purchased from American Type Culture Collection (ATCC).

B. subtilis and *B. megaterium* were grown using tryptone-yeast-glucose extract (TYG) broth, while *S. pasteurii* used an ammonium sulfate ($(NH_4)_2SO_4$)) and yeast extract (NH_4-YE) broth. To make 1 L of TYG broth, 5.0 g of tryptone, 5.0 g of yeast extract, 1.0 g of dipotassium phosphate (K_2HPO_4), and 1.0 g of glucose (D Glucose) were mixed into 1 L of distilled water in accordance with the ARS, NRRL, and USDA. The TYG broth was autoclaved for sterilization prior to culturing. The *B. subtilis* and *B. megaterium* were incubated at a temperature of 30° C for 24 hr or until growth was detected in an orbital shaker operated at 100 rpm (MAXQ4000, Thermo SCIENTIFIC, EN-3-111 Laboratory). *S. pasteurii* was cultured in a NH_4-YE broth with the same conditions mentioned earlier. The NH_4-YE broth consisted of 10.0 g of $(NH_4)_2SO_4$, 20.0 g of yeast extract, and 1.0 L of 0.13 M tris buffer with a pH of 9.0, and all ingredients were autoclaved separately. Consequently, each ingredient was mixed using sterilized glassware and a magnetic stirring bar.[12]

2.2 Cell Growth Measurement

Each collected pure culture was subjected to optical density measurement at a wavelength of 600 nm (OD_{600}) using a spectrometer (GENESYS 20: Thermo Fisher Scientific, USA) to verify cell growth. TYG and NH_4-YE broth were separately employed to set the spectrometer baseline according to each culture. Positive OD_{600} reading indicates bacteria growth. Once bacterial growth was confirmed, bacteria was prepared at a final concentration of $1.0\times10^8 \frac{cells}{mL}$, approximately OD_{600} at 1.0, using biocementation solution.[13, 16]

2.3 Specimen Geometry, Structure Sizing, and Experimental Design

Cylindrical concrete specimens with a diameter of 4 in (101.6 mm) and a height of 8 in (203.2 mm) were used in this experiment, equating to a volume of 100.48 in^3 (1,647,859 mm^3). Two different control groups were employed: one without cracks and another with a crack introduced. The crack was created using a triangular Teflon shim with dimensions of 2 in (50.8 mm) at the base, 4 in (101.6 mm) in height, and a thickness of 0.02 in (0.508 mm), resulting in a volume of 0.08 in^3 (1310.96 mm^3), equivalent to 1.31 mL for one sample.[20]

The five specimen groups are as follows:

- Set A: Control without Crack, from sample A1 to A11.
- Set B: Control with Crack; from sample B1 to B11
- Set C: Cracked Specimen treated with *B. subtilis*; from sample C1 to C11
- Set D: Cracked Specimen treated with *B. megaterium*; from sample D1 to D11.
- Set F: Cracked Specimen treated with *S. pasteurii*; from sample F1 to F11

2.4 Concrete specimen preparation and curing.

The concrete samples were prepared using a 90-lb (40.8 kg) QUIKRETE® high-strength concrete mixture.[17] The mixture was placed into a RYOBI 5-ft^3 (0.14-m^3) portable concrete mixer. Consequently, the mixer was started, and 0.58 gal. (2.2 L) of water was added to the mixture during the beginning of the mixing. The mixer was continuously operated for 2 mins and 30 secs before the remaining 0.58 gal (2.2 L) of water was poured into the mixer. The machine continued to mix for another 2 mins and 30 secs, for a total mixing time of 5 mins. The total volume of water used per 90-lb concrete batch was 1.16 gal (4.4 L).

The mixed concrete paste was subjected to the ASTM C31 for concrete making and curing protocols[18] using cylindrical molds with a diameter of 4 in (101.6mm) and a height of 8 in (203.2 mm). Further, 0.02-in (0.508-mm) Teflon sheets with a width of 2 in (50.8 mm) and a height of 4 in (101.6mm) were employed to induce a crack by inserting the film during concrete molding. Subsequently, the film was removed after 48 hrs. After specimen preparation, the concrete samples were submerged under water for curing purposes over a subsequent 28-day period. Prior to downstream testing, all specimens were demolded and dried for at least 6 hrs. A total of 55 samples were produced and tested.

Figure 1: Cylinder Concrete Samples

2.5 Biocementation solution and treatment.

Pure cultures were collected and centrifuged at 10,000 rpm for 10 mins using centrifugal equipment (International Clinical Centrifuge:33408M, International Equipment Co, EN-3-111 Laboratory). The supernatant was removed afterwards. Biocementation solutions were prepared as outlined in Table 3.1 and were employed to resuspend bacteria to yield specified cell abundance of $1.0 \times 10^8 \frac{cells}{mL}$. 1.29×10^8 bacterial cells with a volume of 1.29 mL introduced to each cracked specimen. The mixture of pure culture and biocementation solution was injected into a concrete fracture using a micropipette at day 0, 7, and 14. Consequently, all specimens were incubated at room temperature for the next 6 weeks after the last application.

Table 1 Chemical Compositions in Biocementation Solutions.[19, 23, 24]

Bacteria	Ingredients	Concentration per liter	Remarks
B. subtilis	Calcium Lactate	22.52 g.	Solution 1:
	Tap Water	1 L	Calcium Lactate Sol^{n}[19][21]
B. megaterium	50 mM Sodium Bicarbonate ($NaHCO_3$)	100 mL	Solution 2:
	100 mM Sodium Citrate	29.46 g.	Sodium Citrate Sol^{n}[23]
	25 mM Calcium Chloride ($CaCl_2$).	2.78 g.	
	Tap Water	0.9 L	
S. pasteurii	Urea	166.5 mL	Solution 3:
	Ammonium Chloride (NH_4Cl)	187 mL	Nutrient Broth Sol^{n} [24, 28]
	Sodium Bicarbonate ($NaHCO_3$)	25 mL	
	Nutrient broth	500 mL	
	Calcium Chloride ($CaCl_2$)	50 mL	
	Tap Water	71.5 mL	

2.6 Concrete Compression Test

The determination of the ultimate compression loading force involved the sequential application of ASTM C469 and ASTM C39 standards, utilizing the Humboldt Compression Machine (HCM-5000-iHA, HUMBOLDT, CSULB, California).[25, 26] The ASTM C469 test was initially conducted to establish baseline conditions, with load and displacement measured in pound-force and inches, respectively. Both loading force and displacement were set to zero before initiation. The default manufacturer-programmed values for the loading rate and preloading rate in ASTM C469 were maintained throughout.

Upon identifying the ultimate compression force (F_c) on the load and displacement plot generated by the machine during ASTM C469, the subsequent step involved applying the ASTM C39 procedure under identical settings. In ASTM C39, the preloading rate was adjusted to 35 $\frac{psi}{sec}$, and the loading rate was set to 35 $\frac{psi}{sec}$. The instrumentation operation continued until concrete failure occurred, either manually or automatically terminated.[20]

Figure 2: Humboldt HCM-500-iHA Compression Tester of the California State University Long Beach

2.7 Data Analysis and Visualization

Upon completion of the compressive tests, the force applied and sample deformation data recorded by the Humboldt Compression Machine were extracted as Excel files. These files were aggregated into a unified document to facilitate comprehensive data analysis. Utilizing the default formulas and settings in Microsoft Excel, the analysis, as detailed in Section 3 below, encompassed the computation of average compressive strengths, assessment of variation in these strengths, and elucidation of elastic responses across different sets.

3. Results

3.1 Average Compressive Strengths

The key parameter that was used to determine the efficacy of the experimental investigation was the average compressive strengths of the different sets, shown in Figure 3. This figure shows the average compressive strength, representing the set label, ranging between A, B, C, D, and F. As shown in the Figure 3, the average compressive strength of control Set A (no crack and no self-healing) was 4,411 ± 970 psi (mean ± standard deviation), while the average compressive strength of control Set B (cracked and no self-healing) was 3,990 ± 751 psi, which constitutes11.8% strength reduction between two control sample sets. The subsequent bacterial self-healing sets were *B. subtilis* (denoting C), *B. megaterium* (denoting D), and *S. pasteurii* (denoting F), demonstrating strength recovery, reaching strengths of 4,332 ± 632 psi, 4,365 ± 515 psi, 4,852 ± 480 psi, respectively. Figure 4 displays the relative average strength ($\frac{\sigma_{Set\ X}}{\sigma_{Set\ A}}$) compared to the control concrete specimens without cracks (Set A). The data demonstrates the noticeable reduction in strength of control Set B with no self-healing with a reduction ratio of 0.90, while the bacterial Sets C, D, and F demonstrated nearly equivalent or even exceeding strengths relative to Set A, with ratios of 0.98, 0.99, and 1.10, respectively. These results show a promising future for the use of bacteria to improve the mechanical properties of cracked concrete.

Figure 3. Average Compressive Strengths of the Different Sets

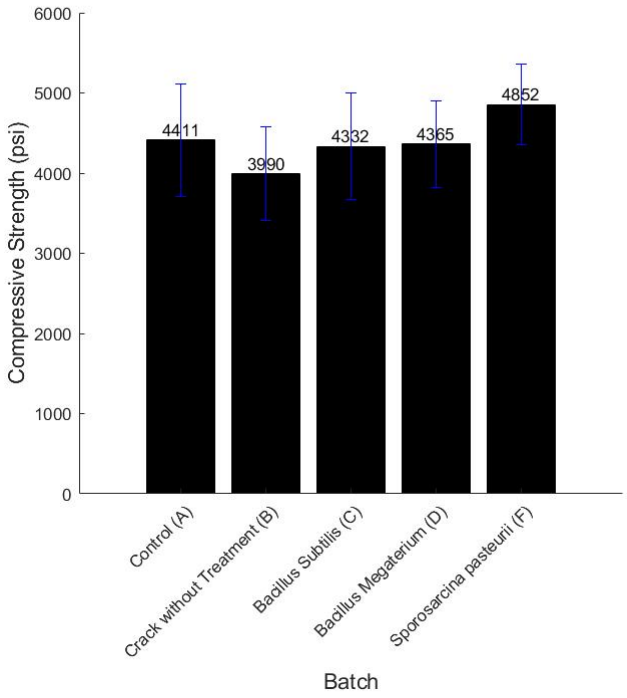
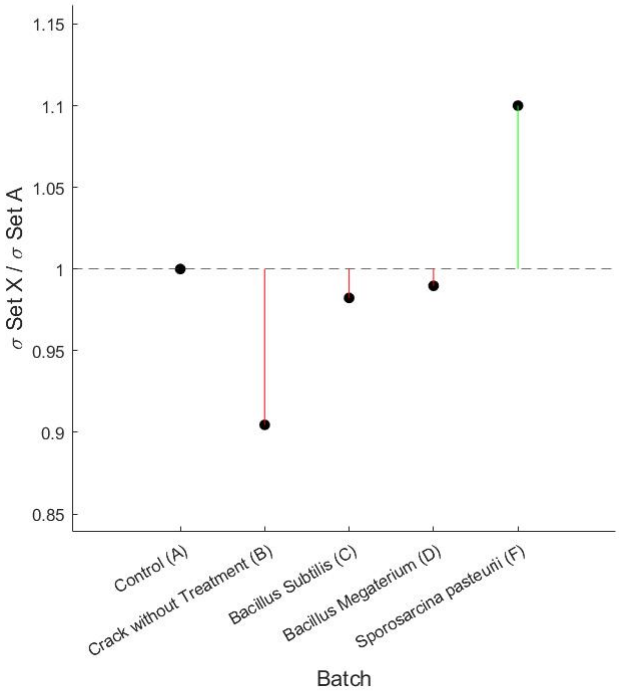


Figure 4. Percentage Variation of the Average Compressive Strengths of the Generic Set, Relative to Set A



3.2 Coefficient of Variation of the Different Sets and Modulus of Elasticity

The Coefficient of Variation of the Different Sets is an important parameter in determining the reliability of the results. As shown in Figure 5, Sets A through F were calculated at 15.9%, 14.6%, 15.3%, 12.3%, and 10.3%, respectively. The variational results show consistency across the entire data set across different groups. The coefficients of variation are relatively low and acceptable considering the sample size of 11 samples for each set and the variable and potentially inconsistent nature of concrete.

The comparison of modulus of elasticity (MOE) values among the concrete batches reveals significant variations in stiffness, providing valuable insights for quality control and engineering design applications. In Figure 6, the MOE values calculated for Sets A through F were 307.0 ksi, 217.6 ksi, 250.0 ksi, 206.2 ksi, and 307.5 ksi, respectively. From the obtained results, batch F exhibits the highest MOE at 307.5 ksi, indicating its superior stiffness compared to the other batches. Conversely, Set D displays the lowest MOE at 206.2 ksi, suggesting relatively lower stiffness compared to the rest.

Figure 5. Coefficient of Variation of the Different Sets

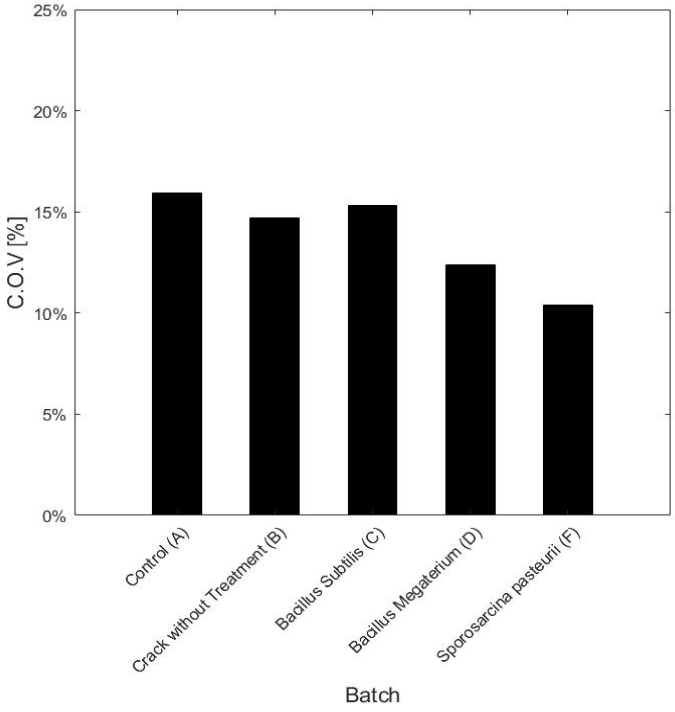
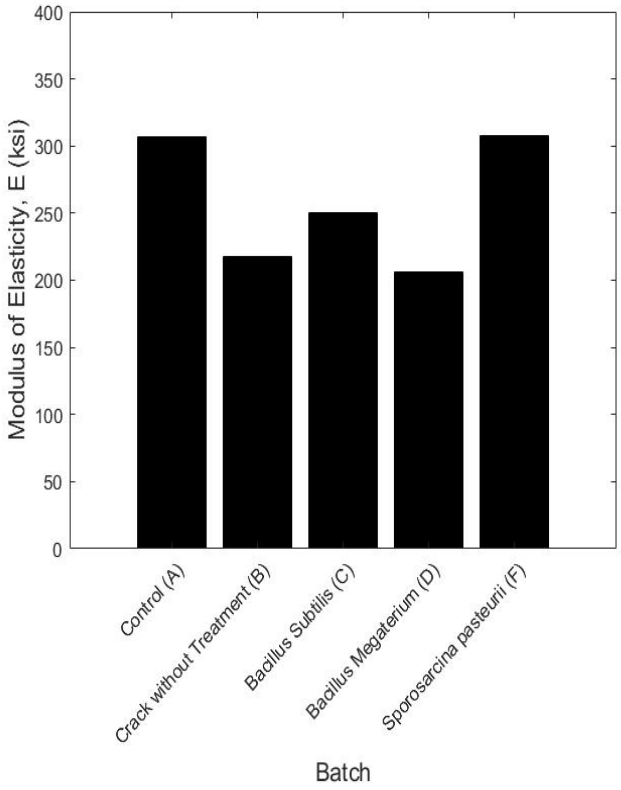


Figure 6. Moduli of Elasticity of the Different Sets



4. Conclusions

This study investigated the technology of microbiologically induced calcium carbonate precipitation (MICP) for concrete's self-healing potential in the aftermath of cracking. Concrete samples were manufactured following ASTM procedures, and sets that required cracking were produced using a Teflon shim to produce a consistent laboratory manufactured crack. Three different kinds of high alkaline-tolerant bacteria including *Bacillus subtilis*, *Bacillus megaterium,* and *Sporosarcina pasteurii* were used to retrofit lab-fractured concrete samples. Bacteria were grown in compliant growth media and provided biocementation solutions upon being added to concrete samples. All samples were tested in compressive load to failure using a Humboldt Compression Machine, and data was downloaded as CVS files. The results from the self-healed samples were compared to control groups using Microsoft Excel, and these results were visualized using MATLAB.

The average compressive strengths of the different sets with their respective variations were calculated. The results demonstrate successful bacterial self-healing with each kind of bacteria. *Bacillus subtilis* and *Bacillus megaterium* were almost of equivalent strength to the control Set A, whereas *Sporosarcina pasteurii* was able to exceed the control. The results are promising and show that each of these bacteria can be used to increase the compressive strength of cracked concrete samples using the external application method.

This study verifies the efficacy of using bacteria to induce MICP in concrete cracks, improving the durability of concrete structures. The external application method, in which bacteria is applied manually to concrete surface cracks, can be used to retrofit existing structures in an environmentally friendly way, promoting sustainable development for the future.

Endnotes

1. Czarnecki L, Geryło R, Kuczyński K. "Concrete Repair Durability." *Materials* (Basel). 2020 Oct 13; 13(20): 4535. doi: 10.3390/ma13204535. PMID: 33066087; PMCID: PMC7600629.

2. S.W. Tang, Y. Yao, C. Andrade, Z.J. Li, "Recent durability studies on concrete structure "Cement and Concrete Research, vol. 78, n. A, pp 143–154, 2015.

3. Amran M, Onaizi AM, Fediuk R, Vatin NI, Muhammad Rashid RS, Abdelgader H, Ozbakkaloglu T. "Self-Healing Concrete as a Prospective Construction Material: A Review". Materials (Basel). 2022 Apr 29; 15(9): 3214. doi: 10.3390/ma15093214. PMID: 35591554; PMCID: PMC9106089.

4. K. Li, L. Li, "Crack-altered durability properties and performance of structural concrete" Cement and Concrete Research, vol. 124, n. 105811, 2019.

5. Si-Yao Guo, Xu Zhang, Ji-Zhou Chen, Ben Mou, Huai-Shuai Shang, Pan Wang, Lihai Zhang, Jie Ren, "Mechanical and interface bonding properties of epoxy resin reinforced Portland cement repairing mortar." *Construction and Building Materials* 264 (2020) 120715.

6. Ryulri Kim, Ukyong Woo, Myoungsu Shin, Eunjong Ahn, Hajin Choi, "Evaluation of self-healing in concrete using linear and nonlinear resonance spectroscopy", Construction and Building Materials, Volume 335, 2022, 127492, ISSN 0950-0618

7. Joshi S, Goyal S, Mukherjee A, Reddy MS. "Microbial healing of cracks in concrete: a review". J Ind Microbiol Biotechnol. 2017 Nov; 44(11): 1511–1525. doi: 10.1007/s10295-017-1978-0. Epub 2017 Sep 12. PMID: 28900729.

8. M. Rajczakowska, K. Habermehl-Cwirzen, H. Hedlund e A. Cwirzen, "Autogenous Self-Healing: A Better Solution for Concrete", Journal of Materials in Civil Engineering, vol. 31, n. 9, 2019.

9. Zabanoot, M. S. S. (2020, November 11). "Review of autogenous and autonomous self-healing concrete technologies for marine environments". WIT Transactions on the Built Environment. https://doi.org/10.2495/hpsm200041.

10. H. M. Jonkers, "Self Healing Concrete: A Biological Approach" Springer Series in Materials Science vol. 100, pp. 195-204, 200.

11. S A Susanto, D Hardjito and A Antoni "Review of autonomous self-healing cementitious material" S A Susanto et al 2021 IOP Conf. Ser.: Earth Environ. Sci. 907 012006.

12. "Agricultural Research Service (NRRL) Culture Collection Peoria, IL NRRL Medium No. 1." n.d. Accessed February 4, 2024.https://nrrl.ncaur.usda.gov/cgi bin/usda/medium/1/nrrl_medium_1.pdf.

13. Lin, Hai, Muhannad T Suleiman, Derick G Brown, and Edward Kavazanjian. 2016. "Mechanical Behavior of Sands Treated by Microbially Induced Carbonate Precipitation." *Journal of Geotechnical and Geoenvironmental Engineering* 142 (2). https://doi.org/10.1061/(asce)gt.1943-5606.0001383.

14. Omoregie, Armstrong Ighodalo, Ghazaleh Khoshdelnezamiha, Nurnajwani Senian, Dominic Ek Leong Ong, and Peter Morin Nissom. 2017. "Experimental Optimisation of Various Cultural Conditions on Urease Activity for Isolated Sporosarcina Pasteurii Strains and Evaluation of Their Biocement Potentials." *Ecological Engineering* 109 (December): 65–75. https://doi.org/10.1016/j.ecoleng.2017.09.012.

15. Chen Bingcheng, Weiwei Sun, Xichen Sun, Chong Cui, Jianzhong Lai, Yang Wang, and Jun Feng. 2021. "Crack Sealing Evaluation of Self-Healing Mortar with Sporosarcina Pasteurii: Influence of Bacterial Concentration and Air-Entraining Agent" 107 (August): 100–111. https://doi.org/10.1016/j.procbio.2021.05.001.

16. Yatish Reddy, P.V., B. Ramesh, and L. Prem Kumar. 2020. "Influence of Bacteria in Self-Healing of Concrete – a Review." Materials Today: Proceedings, August. https://doi.org/10.1016/j.matpr.2020.07.233.

17. "Cement and Concrete Products | QUIKRETE: Cement and Concrete Products." n.d. www.quikrete.com. https://www.quikrete.com/pdfs/data_sheet-concrete%20mix%201101.pdf.

18. "This International Standard Was Developed in Accordance with Internationally Recognized Principles on Standardization Established in the Decision on Principles for the Development of International Standards, Guides and Recommendations Issued by the World Trade Organization Technical Barriers to Trade (TBT) Committee. Standard Practice for Making and Curing Concrete Test Specimens in the Field." n.d. https://doi.org/10.1520/C0031_C0031M-19.

19. Feng, Jun, Bingcheng Chen, Weiwei Sun, and Yang Wang. 2021. "Microbial Induced Calcium Carbonate Precipitation Study Using Bacillus Subtilis with Application to Self-Healing Concrete Preparation and Characterization." *Construction and Building Materials* 280 (April): 122460. https://doi.org/10.1016/j.conbuildmat.2021.122460.

20. Pitiporn Asvapathanagul, Simone Galano, Andrea Calabrese, Mehran Rahmani, Maggie Ly, Daniela Flores, Michael Hernandez, and Nicholas Banuelos. 2023. "Experimental Investigation of the Self-Healing Potential of Bacteria for Sustainable Concrete Structures." San Jose State University Scholar Works (San Jose State University), May. https://doi.org/10.31979/mti.2023.2239.

21. Su, Yilin, Chunxiang Qian, Yafeng Rui, and Jianhang Feng. 2021. "Exploring the Coupled Mechanism of Fibers and Bacteria on Self-Healing Concrete from Bacterial Extracellular Polymeric Substances (EPS)." *Cement and Concrete Composites* 116 (February): 103896. https://doi.org/10.1016/j.cemconcomp.2020.103896.

22. Safiuddin, Mohammed, Shaik Ihtheshaam, Rizwan Abdul Kareem, and Shalam. 2021. "A Study on Self-Healing Concrete." *Materials Today: Proceedings*, November. https://doi.org/10.1016/j.matpr.2021.11.023.

23. Smitha, Muraleedharan Pillai, Dhanaraj Suji, Mercy Shanthi, and Adeyemi Adesina. 2022. "Application of Bacterial Biomass in Biocementation Process to Enhance the Mechanical and Durability Properties of Concrete." *Cleaner Materials* 3 (March): 100050. https://doi.org/10.1016/j.clema.2022.100050.

24. Mortensen, B.M., M.J. Haber, J.T. DeJong, L.F. Caslake, and D.C. Nelson. 2011. "Effects of Environmental Factors on Microbial Induced Calcium Carbonate Precipitation." *Journal of Applied Microbiology* 111 (2): 338–49.https://doi.org/10.1111/j.1365-2672.2011.05065.x.

25. "Standard Test Method for Compressive Strength of Cylindrical Concrete Specimens." n.d. Accessed February 4, 2024. https://cdn.standards.iteh.ai/samples/1504/c51b44b19f354c549e5d988fa24cebc1/ASTM-C39-C39M-01.pdf.

26. "Standard Test Method for Static Modulus of Elasticity and Poisson's Ratio of Concrete in Compression." n.d. Www.astm.org. Accessed February 4, 2024. https://www.astm.org/standards/c469.

27. Gerges, Nagib N., Camille A. Issa, and Samer Fawaz. 2015. "Effect of Construction Joints on the Splitting Tensile Strength of Concrete." *Case Studies in Construction Materials* 3 (December): 83–91. https://doi.org/10.1016/j.cscm.2015.07.001.

28. Stocks-Fischer, Shannon, Johnna K. Galinat, and Sookie S. Bang. 1999. "Microbiological Precipitation of CaCO3." Soil Biology and Biochemistry 31 (11): 1563–71. https://doi.org/10.1016/S0038-0717(99)00082-6.

Bibliography

Amran, M., Onaizi, A. M., Fediuk, R., Vatin, N. I., Muhammad Rashid, R. S., Abdelgader, H., & Ozbakkaloglu, T. (2022). "Self-Healing Concrete as a Prospective Construction Material: A Review." *Materials (Basel)* 15(9), 3214. doi:10.3390/ma15093214.

"Agricultural Research Service (NRRL) Culture Collection Peoria, IL NRRL Medium No. 1." n.d. Accessed February 4, 2024.

"Cement and Concrete Products | QUIKRETE: Cement and Concrete Products." n.d. www.quikrete.com.

Chen Bingcheng, Weiwei Sun, Xichen Sun, Chong Cui, Jianzhong Lai, Yang Wang, and Jun Feng. 2021. "Crack Sealing Evaluation of Self-Healing Mortar with Sporosarcina Pasteurii: Influence of Bacterial Concentration and Air-Entraining Agent" 107 (August): 100–111.

Czarnecki, L., Geryło, R., & Kuczyński, K. (2020). "Concrete Repair Durability." *Materials (Basel)* 13(20), 4535. doi:10.3390/ma13204535

Feng, J., Chen, B., Sun, W., & Wang, Y. (2021). Microbial Induced Calcium Carbonate Precipitation Study Using Bacillus Subtilis with Application to Self-Healing Concrete Preparation and Characterization. *Construction and Building Materials* 280, 122460. doi:10.1016/j.conbuildmat.2021.122460

Gerges, Nagib N., Camille A. Issa, and Samer Fawaz. 2015. "Effect of Construction Joints on the Splitting Tensile Strength of Concrete." Case Studies in Construction Materials 3 (December): 83–91. https://doi.org/10.1016/j.cscm.2015.07.001.

Guo, S. Y., Zhang, X., Chen, J. Z., Mou, B., Shang, H. S., Wang, P., Zhang, L., & Ren, J. (2020). Mechanical and Interface Bonding Properties of Epoxy Resin Reinforced Portland Cement Repairing Mortar. Construction and Building Materials, 264, 120715.

Jonkers, H. M. (2009). Self-Healing Concrete: A Biological Approach. Springer Series in Materials Science, 100, 195–204.

Joshi, S., Goyal, S., Mukherjee, A., & Reddy, M. S. (2017). Microbial Healing of Cracks in Concrete: A Review. Journal of Industrial Microbiology and Biotechnology, 44(11), 1511–1525. doi:10.1007/s10295-017-1978-0.

Kim, R., Woo, U., Shin, M., Ahn, E., & Choi, H. (2022). Evaluation of self-healing in concrete using linear and nonlinear resonance spectroscopy. Construction and Building Materials, 335, 127492.

Li, K., & Li, L. (2019). Crack-altered durability properties and performance of structural concrete. Cement and Concrete Research, 124, 105811.

Lin, Hai, Muhannad T Suleiman, Derick G Brown, and Edward Kavazanjian. 2016. "Mechanical Behavior of Sands Treated by Microbially Induced Carbonate Precipitation." Journal of Geotechnical and Geoenvironmental Engineering 142 (2).

M. Rajczakowska, K. Habermehl-Cwirzen, H. Hedlund e A. Cwirzen, "Autogenous Self-Healing: A Better Solution for Concrete", Journal of Materials in Civil Engineering, vol. 31, n. 9, 2019.

Mortensen, B. M., Haber, M. J., DeJong, J. T., Caslake, L. F., & Nelson, D. C. (2011). Effects of Environmental Factors on Microbial Induced Calcium Carbonate Precipitation. Journal of Applied Microbiology, 111(2), 338–349. doi:10.1111/j.1365-2672.2011.05065.x

Omoregie, A. I., Khoshdelnezamiha, G., Senian, N., Ong, D. E. L., & Nissom, P. M. (2017). Experimental Optimisation of Various Cultural Conditions on Urease Activity for Isolated Sporosarcina Pasteurii Strains and Evaluation of Their Biocement Potentials. Ecological Engineering, 109, 65–75. doi:10.1016/j.ecoleng.2017.09.012

Pitiporn Asvapathanagul, Simone Galano, Andrea Calabrese, Mehran Rahmani, Maggie Ly, Daniela Flores, Michael Hernandez, and Nicholas Banuelos. (2023). Experimental Investigation of the Self-Healing Potential of Bacteria for Sustainable Concrete Structures. San Jose State University Scholar Works (San Jose State University), May. doi:10.31979/mti.2023.2239

S.W. Tang, Y. Yao, C. Andrade, Z.J. Li. (2015). Recent durability studies on concrete structure. Cement and Concrete Research, 78(A), 143–154.

Safiuddin, Mohammed, Shaik Ihtheshaam, Rizwan Abdul Kareem, and Shalam. (2021). A Study on Self-Healing Concrete. Materials Today: Proceedings, November. doi:10.1016/j.matpr.2021.11.023

Si-Yao Guo, Xu Zhang , Ji-Zhou Chen , Ben Mou , Huai-Shuai Shang, Pan Wang, Lihai Zhang , Jie Ren. (2020). Mechanical and interface bonding properties of epoxy resin reinforced Portland cement repairing mortar. Construction and Building Materials, 264, 120715.

Smitha, Muraleedharan Pillai, Dhanaraj Suji, Mercy Shanthi, and Adeyemi Adesina. (2022). Application of Bacterial Biomass in Biocementation Process to Enhance the Mechanical and Durability Properties of Concrete. Cleaner Materials, 3, 100050. doi:10.1016/j.clema.2022.100050

S.A. Susanto, D. Hardjito and A. Antoni. (2021). Review of autonomous self-healing cementitious material. IOP Conference Series: Earth and Environmental Science, 907, 012006.

Stocks-Fischer, Shannon, Johnna K. Galinat, and Sookie S. Bang. 1999. "Microbiological Precipitation of CaCO3." Soil Biology and Biochemistry 31 (11): 1563–71. https://doi.org/10.1016/S0038-0717(99)00082-6.

Su, Yilin, Chunxiang Qian, Yafeng Rui, and Jianhang Feng. 2021. "Exploring the Coupled Mechanism of Fibers and Bacteria on Self-Healing Concrete from Bacterial Extracellular Polymeric Substances (EPS)." *Cement and Concrete Composites* 116 (February): 103896.

"Standard Test Method for Compressive Strength of Cylindrical Concrete Specimens." n.d. Accessed February 4, 2024.

"Standard Test Method for Static Modulus of Elasticity and Poisson's Ratio of Concrete in Compression." n.d. Www.astm.org. Accessed February 4, 2024.

"This International Standard Was Developed in Accordance with Internationally Recognized Principles on Standardization Established in the Decision on Principles for the Development of International Standards, Guides and Recommendations Issued by the World Trade Organization Technical Barriers to Trade (TBT) Committee. Standard Practice for Making and Curing Concrete Test Specimens in the Field." n.d.

Yatish Reddy, P.V., B. Ramesh, and L. Prem Kumar. (2020). Influence of Bacteria in Self-Healing of Concrete - a Review. Materials Today: Proceedings, August. doi:10.1016/j.matpr.2020.07.233

Zabanoot, M. S. S. (2020). Review of autogenous and autonomous self-healing concrete technologies for marine environments. WIT Transactions on the Built Environment. doi:10.2495/hpsm200041

About the Authors

Andrea Calabrese, PhD, ING, CENG, MICE

Dr. Calabrese joined the California State University, Long Beach's (CSULB) Civil Engineering and Construction Engineering Management (CECEM) Department as an Assistant Professor in Fall 2017. He obtained a PhD in Construction Engineering with an emphasis in Structural Engineering in 2013. He was a visiting research fellow at the Pacific Earthquake Engineering Research Center (PEER) from 2010–2012 along with having been a postdoctoral researcher of the ReLUIS Consortium at the Italian Network of University Laboratories in Earthquake Engineering from 2013–2014. Dr. Calabrese has worked as a Structural Engineer at Foster & Partners (London) and other firms in Italy for seven years. He has been a registered engineer in Italy since 2009 and a Chartered Engineer (CEng) and Full Member of the Institution of Civil Engineers (MICE) in the UK since 2017. Dr. Calabrese's current research interests are in the fields of experimental testing, structural dynamics, base isolation, vibration engineering, and the development of novel low-cost devices for the seismic protection of buildings. He has carried out numerous large-scale experimental studies of base isolation systems and energy absorbing devices on the shaking table at the Department of Structural Engineering at the University of Naples in Italy. This work has been instrumental in developing low-cost seismic isolation systems using recycled rubber and flexible reinforcements for the seismic protection of buildings in developing regions. His responsibilities for this research included conceptualization of the work, data analysis, and final editing of the report.

Pitiporn Asvapathanagul, PhD, PE

Dr. Pitiporn Asvapathanagul is a faculty member in the CECEM Department at 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 the accomplishments of the self-healing concrete project.

Nisarg N Patel, Research Assistant

Nisarg N Patel is a former Teaching assistant and present Graduate Research Assistant at CSULB's CECEM Department. He is pursuing an MS in Civil Engineering with a specialization in structural engineering. Nisarg played a pivotal role in various facets of the project, including but not limited to culturing bacteria, preparing food for bacteria, manufacturing concrete test samples, inoculating bacteria into the concrete, and conducting tests on the concrete samples under the

supervision of Dr. Asvapathanagul. Additionally, he made significant contributions to drafting the report and meticulously editing its final version.

Nanubala Dhruvan, Graduate Student

Nanubala Dhruvan is a Graduate Student in the CECEM Department at CSULB pursuing an MS in Civil Engineering, specializing in Structural Engineering. Dhruvan played an important part in various aspects of the project, including manufacturing the concrete test samples, injecting the bacteria into the concrete, and performing tests on the concrete samples under the guidance of Dr. Asvapathanagul. In addition, his contribution also included developing the report's outline in different phases and also revising it extensively before submission.

Austin Adams, Graduate Student

Austin Adams is a Graduate Student in the CECEM Department in CSULB pursuing an MS in Civil Engineering, specializing in Structural Engineering. He assisted in the casting and testing of samples, preparing food for the bacteria, and contributing to drafting project reports.

Michael Hernandez, Research Assistant

Michael Hernandez is an undergraduate research assistant in CSULB's CECEM Department where he is pursuing a degree in Mechanical Engineering. Michael actively participated in the project's implementation. His responsibilities encompassed aiding in the manufacturing and testing of samples, as well as extracting results from scratch files. He also contributed to writing the draft and editing the final version of the report.

Douglas Lopez-Cruz, Research Assistant

At the time that this report was written, Douglas Lopez-Cruz was a Civil Engineering undergraduate student in his senior year and a research assistant under the supervision of Dr. Asvapathanagul at CSULB. Douglas was tasked with producing comprehensive summaries of scholarly journals and articles about *Bacillus subtilis* (B-14596); his literature reviews assisted in the preliminary selection process of the bacterium and foods outlined within this report. Throughout the project, he was responsible for and participated in the casting and uncasting of samples; plating, landing, and feeding of bacterium; testing samples; and advising how to improve the quality of the samples produced. He also contributed and participated in the editorial process of this report.

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