The performance evaluation of protection barriers in bacterial self-healing mortar

Ilgin Sandalci[1], *Ayca Gisu* Guldogan[1], *Zeynep* Basaran Bundur[1*]

[1]Ozyegin University, Department of Civil Engineering, Istanbul, Turkey

Abstract. The early age microcracking is a significant problem in concrete structures resulting in increased permeability and decreased durability. The previous work showed that *Sporasarcina pasteurii* cells immobilized on natural minerals such as bentonite, diatomaceous earth, sepiolite, and pumice effectively remediated early-age microcracks in the cementitious systems by triggering microbial-induced calcite precipitation (MICP). This promising approach can solve early-age shrinkage cracking in cementitious systems. Therefore, it is essential to assess the impact of self-healing additives on drying shrinkage. This study investigates the influence of mineral-based biological additives on the drying shrinkage capacity of cement-based mortar and the possible self-healing of cracks if any occur. To achieve this goal, the free shrinkage in control (containing only minerals) and bacterial (containing bio-based additive) samples were measured based on ASTM 596-18 norms. Moreover, the performance assessment of developed self-healing additives was done by determining compressive strength and initial setting time of bacterial self-healing mortar.

1 Introduction

The cement and concrete industry plays a vital role in the European economy. On the other hand, their production process significantly contributes to global warming and is responsible for 8% of global CO_2 emissions [1, 2]. With the recent Paris Agreement, the cement and concrete industry must develop more sustainable strategies to decrease CO_2 emissions to reach the goals of governments to keep global warming below 2˚C [3, 4]. To date, it is evident that eliminating cement from structural concrete is not feasible. Thus, it might be more efficient to extend the service life of structures which will decrease the demand to cement production and lessen the economic burden on the cement industry to implement breakthrough technologies. Extending the service life of concrete structures could be achieved by limiting crack formation and decreasing the material's permeability. Cracking is inevitable in concrete, because of its brittle nature and low tensile strength. These cracks eventually reduce the material's durability and decrease the life span of concrete structures [5].

Microbial-induced calcite precipitation (MICP) is a promising autonomous self-healing approach to remediate microcracks. Herein, the microorganisms trigger the formation of calcium carbonate ($CaCO_3$), consequently enabling the remediation of microcracks in cementitious materials [6]. There are several methodologies developed to inoculate the bacteria cells into the cement-based mortars, such as immobilization and encapsulation by using protective barriers (lightweight aggregates, nanomaterials, natural minerals, hydrogels, fibers) to enhance the viability of bacteria cells and the microbial-induced calcite precipitation [7-10]. As a consequence of developed bacterial-based self-healing technologies, it was seen that microcracks up to 1 mm were healed, and the permeability of the cementitious systems was decreased [11].

Previously, our studies showed that natural minerals such as sepiolite, diatomaceous earth, bentonite, and pumice could be used to immobilize bacterial cells to trigger self-healing in mortars [12, 13]. Herein, the biological self-healing additive effectively remediates flexural cracks in less than 21 days. Even though the scope of the studies mostly focuses on evaluating self-healing ability, it is still critical to assess the influence of these biological additives on the material performance. Throughout the literature, studies show that adding different types of self-healing agents generally increases the compressive strength of cementitious materials [14, 15].

Another performance parameter in assessing the quality of cement-based systems is drying shrinkage. In addition, shrinkage is a primary factor causing plastic and hardened concrete microcracks. Some of the minerals used to immobilize cells can enhance the shrinkage performance of cementitious materials due to their reactivity and autogenous self-healing property [16-18]. However, it is still essential to understand how minerals affect the shrinkage in cement-based materials when they are incorporated with bacterial cells. This study aims to investigate the effect of a biological self-healing additive obtained by immobilizing *S. pasteurii* cells on natural minerals on the performance of cement-

* Corresponding author: zeynep.basaran@ozyegin.edu.tr

based materials, considering strength, setting, and free drying shrinkage.

2 Materials and methods

2.1 Microorganism selection and growth

Sporosarcina pasteurii (S. pasteurii) from Leibniz institute – German collection of microorganisms and cell cultures (DSMZ 33) was selected as a self-healing agent in cement-based materials. The cells were aerobically incubated (IKA KS 4000) in a sterilized nutrient medium at pH=9 (UCSLS), which included 0.13 M Tris base, 20 g/L urea, 10 g/L corn steep liqueur (CSL) and 10 g/L sodium acetate per liter of distilled (DI) water. The nutrient medium was sterilized at 121˚C for 45 minutes (NUVE NC 40M, Istanbul, Turkey). The cells were grown in the sterilized nutrient medium with shaking conditions (175 rpm) at 30˚C until the stationary phase was obtained (10^9 Colony Forming Unit (CFU)/mL) [12]. After the incubation, the cells were collected from the culture by centrifuging at 8,000 g for 10 minutes. The cells were washed twice with PBS (Phosphate Buffer Solution, pH=9).

2.2 Material selection

Mortar samples were prepared with CEM I 42.5 R, and *S. pasteurii* cells were immobilized on bentonite, sepiolite, diatomaceous earth, and pumice. The bio-based additive was obtained by submerging the minerals in an aqueous solution, either PBS or UCSL-CA medium, for 24-h. The absorption capacity was defined in terms of the 24-hours absorption capacity for each protection mineral. This was determined by submerging the oven-dried minerals and aggregates in water for 24 hours and calculating the total absorbed water within the specified time frame. Particle size distributions of fine minerals were determined by a Mastersizer 200 particle analyzer with a Hydro MU 2000 (Malvern, Worcestershire, United Kingdom) wet dispersion unit. The average particle size and the 24-h absorption capacities of the minerals are summarized in Table 1.

Table 1. The characteristic of minerals and cement used in the mixture.

Sample	Particle Size Range	Water Absorption Capacity
Cement	5-70 µm	-
Bentonite	<50 µm	300%
Sepiolite	<110 µm	80%
Diatomaceous Earth	<50 µm	110%
Pumice	0-2 mm	45%

2.3 Preparation of biological self-healing additive

Half of the mineral was saturated with bacteria cells and PBS to develop a biological additive. The other half was a saturated nutrient medium, including calcium acetate, labeled UCSL-CA. UCSL-CA medium was prepared as explained in section 2.1, but herein, sodium acetate was replaced with 10 g/L calcium acetate per liter of distilled water. The immobilization was achieved by saturating half of the mineral (11.25 g) with 1 g of vegetative bacteria cells suspended in 45 mL PBS and saturating the other half (11.25 g) with a UCSL-CA nutrient medium.

Immobilization of minerals was achieved with shaking conditions (175 rpm) at 30˚C for 24 hours. Then slurry was removed from incubation and kept at 40˚C in an oven for an additional 12 hours. A saturated surface dry (SSD) biological additive was obtained by filtering the excess solutions through MN615 A Grade I filter paper and drying the mineral-cell compound in an oven at 40˚C.

2.4 Performance assessment of bacterial self-healing mortar

2.4.1 Compressive strength

Cement-based mortars containing biological additives and negative control were prepared using CEM I 42.5 R cement and standard sand, which complied with the norm EN-196-1, according to ASTM C305-14 standard [19]. The water-to-cement and sand-to-cement ratios were kept at 0.45 and 3, respectively. To maintain the same flowability of the mortars, polycarboxylate ether (PCE)-based superplasticizer (BASF) was used. Table 4 summarizes the mixture proportions of prepared samples. The amounts of minerals and sand were replaced depending on the used minerals' type and size. The mortar samples were cast in 5x5x5 cm cubes and kept in a humid environment at 21˚C for 24 h. Then the molds were removed, and the samples were cured in 100% humidity at 23˚C. The compressive strength was conducted according to ASTM C109/C109M-21 standard at 3, 7, 28, and 90 days after casting [20].

2.4.2 Vicat needle test

The initial setting time of cement pastes containing biological additives and negative control pastes was determined with a modified ASTM C191-19 standard [21]. Herein, the test was conducted by using a constant w/c of 0.45. This was done to be consistent with the ratios used throughout the other analysis conducted in the study. Tests were performed based on triplicates of samples.

2.4.3 Free drying shrinkage

The influence of biological additives on drying shrinkage was evaluated based on ASTM C596-18 norms [22]. The samples containing 750 g cement to 1500 g standard sand were prepared, and the flow diameter was kept at 110±5 mm. The w/c was held at 0.45. The mortar samples were cast into 25x25x250 mm beam molds and kept in 100% relative humidity (RH) at 23˚C for 24 hours. Upon demolding, the samples were

submerged in lime-saturated water for 48 hours. At the end of 48 hours, the beams were removed from lime-saturated water and wiped with a towel. The initial weight and length measurements were recorded after the samples were dried. The length measurements of beams were carried out with a comparator based on ASTM C157/C157M-17 norms [23]. Following the first measurement, the samples were moved to the controlled climate chamber at 23˚C and 50% RH for 16 weeks. The length and weight of the samples were measured at 4, 7, 11, 18, 25 days, 8, and 16 weeks. The length changes of triplicates of samples were calculated based on ASTM C157/C157M-17 norms.

Table 2. Mix design of prepared mortars. C: only includes minerals, B: mineral was saturated with bacterial cells and PBS, BN: half of the mineral was saturated with bacterial cells and PBS, and the other half was saturated with UCSL-CA nutrient medium.

Sample Name	Cement (g)	Sand (g)	Mineral (g)
Control	450	1350	-
BTC	450	1350	22.5
DEC	450	1350	22.5
SEPC	450	1350	22.5
PUMC	450	1282.5	67.5
BTB	450	1350	22.5
DEB	450	1350	22.5
SEPB	450	1350	22.5
PUMB	450	1282.5	67.5
BTBN	450	1350	22.5
DEBN	450	1350	22.5
SEPBN	450	1350	22.5
PUMBN	450	1282.5	67.5

3 Results and discussion

3.1 Compressive strength of bio-based mortar

Figure 1 summarizes the compressive strength of C, B, and BN series specimens compared to the negative control cement-based mortar prepared without any minerals and bacteria. While the mineral addition had no distinct negative impact on the compressive strength, a decrease was observed in the compressive strength of B and BN series specimens compared to their counterpart C series samples. The maximum reduction in compressive strength was observed in samples containing pumice. The compressive strength decreased due to replacing normal-weight sand with saturated lightweight aggregate in the mix design. Moreover, the addition of bacteria into the mixture decreased the compressive strength at B series specimens, regardless of mineral type. This could be related to the ions on the cells from the decomposed growth culture, decreasing the rate of hardening in the mortar samples.

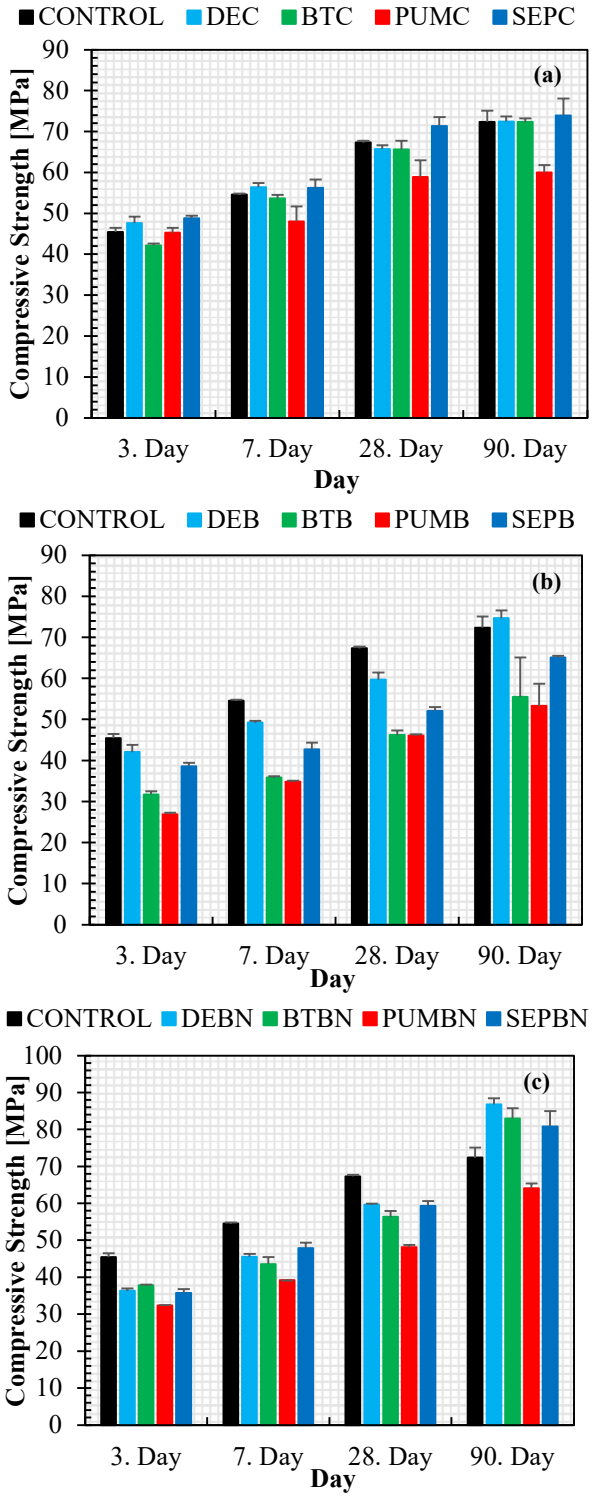

Fig. 1. Compressive strength test results. (a) C series (b) B series (c) BN series.

Although adding diatomaceous earth and sepiolite partially increased the compressive strength for the C series specimens, adding bacteria and nutrient medium into the mixture decreased the compressive strength. However, while this negative impact of incorporating

the cells and the nutrient medium was distinctively observed in 3-day and 7-day compressive strengths, the impact was less pronounced at 28 days. Even the increase was observed in the 90-day compressive strength of the DEBN, SEPBN, and BTBN samples. Accordingly, the bacteria addition was only effective on early-stage compressive strength, which was less than 30%.

According to the compressive strength test results, the biological additive prepared with the fine mineral has a lower impact on the compressive strength than that observed in samples containing lightweight aggregate. Even though adding bacteria into the mixture negatively affected the early-stage compressive strength, this effect was reduced in time. When the 28-day compressive strength results were evaluated, the BN series biological additive developed with fine minerals caused a reduction of less than 20% in the compressive strength. Further, there was an increase in the 90-day compressive strength compared to the control sample. Therefore, it was concluded that the biological additive has no significant impact on the hardened performance in the long term.

3.2 Influence of developed biological additive on setting time

The impact of biological additives on the fresh state properties of mortar was examined in terms of initial setting time. Previous studies showed that the addition of bacteria without any encapsulation increased the workability and the initial setting time significantly. This was attributed to the high sugar content in yeast extract used in the mixture [24]. Using CSL instead of yeast extract relatively reduced the negative impact on the initial setting time [25] . Within the scope of this study, the nutrient medium was only used in a minimal amount for the BN series of samples. Figure 2 shows the initial setting times of 13 mixtures. Regardless of mineral type, the initial settings times of samples containing only minerals decreased by 20% compared to the control sample. It can be related to the reactivity of minerals which affects the reaction rate and the water-holding properties of minerals retaining mix water, such as bentonite.

The initial setting time of the B series showed relatively longer setting times (a maximum of 35% increase) compared to their counterpart C series samples. For instance, the initial setting times of BTC and BTB samples were determined at 337 and 446 min., respectively. Although the addition of bacteria increased the initial setting time of the sample, including bentonite, by 30%, the initial setting time of the BTB sample increased by 7% compared to the control sample. While the incorporation of cells increased the initial setting time, using the nutrient medium in BN series samples did not yield a significant increase in setting compared to their counterpart B series samples. This indicates that bacterial cells are the predominant parameter affecting the setting time rather than using nutrients. It should be noted that the influence of the nutrient medium was only seen when the bacterial cells were incorporated with their growth culture, so the nutrient medium was decomposed [24, 26]. Herein, the bacterial cells can carry ions from their growth culture (like sugars) and delay the initial set. The BN series of samples contain fresh nutrients and the bacterial cells collected from their growth culture. Our previous studies showed that incorporating nutrients containing yeast extract or corn steep liquor delays the initial setting time [24, 26]. Adding the nutrient medium with the minerals reduces their negative impact on the initial setting time.

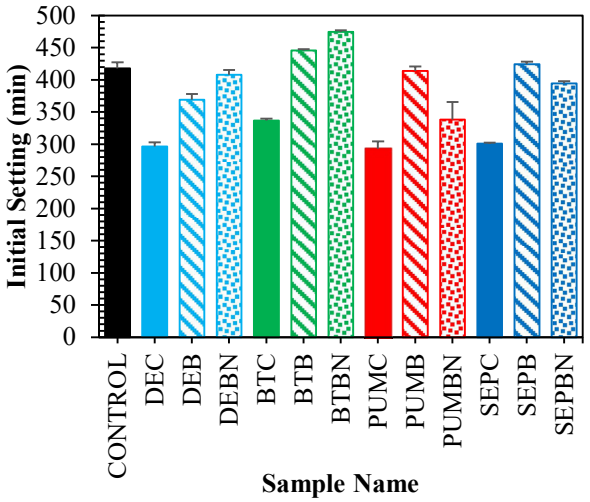

Fig. 2. Vicat Needle test results. C: only includes minerals, BN: half of the mineral was saturated with bacteria.

3.3 Influence of biological additive on drying shrinkage

The effect of biological additives on free drying shrinkage was evaluated at a relative humidity of 50% and 23°C. Both C and BN series specimens were cast to assess the impact of incorporating bacteria and minerals on the shrinkage of cement-based mortars separately.

Figure 3 represents the drying % shrinkage in mortar samples. It was seen that the addition of unsaturated minerals increased the % strain due to drying shrinkage compared to the control sample. The impact of mineral addition on the drying shrinkage can be due to the higher water absorption capacities of minerals which entraps the water content during mixing. Another possible explanation could be the pozzolanic reactivity of minerals [18]. Previous studies evaluating the effects of clays on the performance of concrete showed that the addition of montmorillonite by 4% of the weight of cement showed similar behaviour and resulted in a higher increased drying shrinkage % [27]. The values obtained with montmorillonite were identical to the bentonite used in this study. Montmorillonite and bentonite are very similar and have a high absorption capacity. A higher absorption rate of these clays can increase the drying shrinkage when used in cement-based systems. The literature demonstrated that adding 2% clay could increase the drying shrinkage due to the high-water adsorption of clays [28]. Other minerals used in this study, such as diatomaceous earth and sepiolite, could be classified as clays or clayey minerals. Therefore, they interact with water [29-31]. The higher

water absorption capacities of bentonite, diatomaceous earth, sepiolite, and pumice used in this study were determined as 300%, 110%, 80%, and 45%, respectively [13].

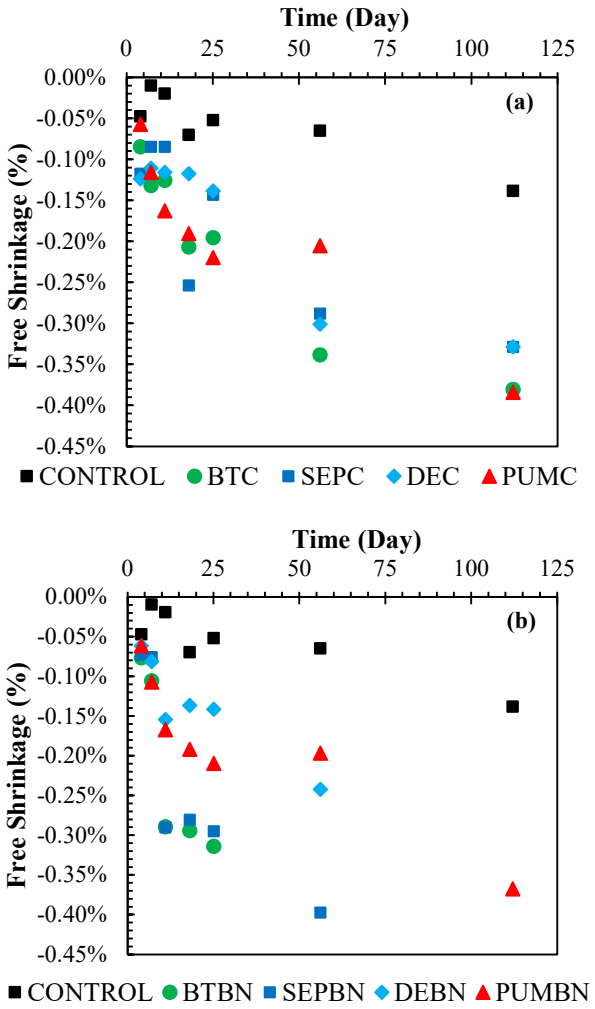

Fig. 3. Free shrinkage by time. (a) C series (b) BN series.

The impact of incorporating bacteria on shrinkage showed different results regarding mineral type. While adding bacteria increased the shrinkage of samples, including sepiolite and bentonite, the shrinkage of samples, including diatomaceous earth, decreased. Compressive strength results could explain the decrease in shrinkage of the DEBN sample. A 20% increase was observed in the 90-day compressive strength of the DEBN sample compared to the control sample. On the other hand, even though a similar trend in compressive strength was observed in SEPBN and BTBN, the addition of biological additives increased the shrinkage of these samples. Therefore, it was understood that the only factor that affected shrinkage was not compressive strength. An additional evaluation must be done to understand how the characteristics of minerals affect the water interaction and shrinkage of the material.

4 Conclusion

This study investigated the effects of biological self-healing additives on the performance of cement-based materials. The performance assessment was done regarding compressive strength, the initial setting, and free drying shrinkage. The biological additive did not significantly impact the initial setting time of cement-based mortar. The addition of bacteria cells into the mixture caused a decrease in the 3- and 7- days compressive strengths compared to the control sample. However, the biological additive, including mineral powders, increased late-term compressive strength.

Adding minerals increased the free drying shrinkage 2.5 times compared to the control sample. To understand the impact of biological additives on the drying shrinkage of cementitious materials more clearly, it is required to determine the pozzolanic reactivity and the chemical component of minerals. Moreover, the drying shrinkage results suggested that a high rate of cracks would occur in a restrained slab. Therefore, mesoscale experiments should be conducted to detect the problems in practice.

This research was conducted with financial assistance from the Scientific and Technical Research Council (TUBITAK) of Turkey Project: MAG-118M327.

References

[1] K. L. Scrivener, V. M. John, and E. M. Gartner, "Eco-efficient cements: Potential economically viable solutions for a low-CO2 cement-based materials industry," *Cem Concr Res*, vol. 114, pp. 2–26, Dec. 2018, doi: 10.1016/J.CEMCONRES.2018.03.015.

[2] "Technology Roadmap: Low-Carbon Transition in the Cement Industry," *IEA, CSI & WBCSD*, 2018. https://www.wbcsd.org/Sector-Projects/Cement-Sustainability-Initiative/Resources/Technology-Roadmap-Low-Carbon-Transition-in-the-Cement-Industry (accessed Jul. 13, 2022).

[3] "The Paris Agreement | UNFCCC," *United Nations*, 2015.

[4] "The European Green Deal," *European Comission*, 2019.

[5] W. Zhang, Q. Zheng, A. Ashour, and B. Han, "Self-healing cement concrete composites for resilient infrastructures: A review," *Compos B Eng*, vol. 189, p. 107892, May 2020, doi: 10.1016/J.COMPOSITESB.2020.107892.

[6] S. Mann, "Biomineralization: principles and concepts in bioinorganic materials chemistry," 2001.

[7] J. Wang, K. van Tittelboom, N. de Belie, and W. Verstraete, "Use of silica gel or polyurethane immobilized bacteria for self-healing concrete," *Constr Build Mater*, vol. 26, no. 1, pp. 532–540, Jan. 2012, doi: 10.1016/J.CONBUILDMAT.2011.06.054.

[8] S. S. Bang, J. J. Lippert, U. Yerra, S. Mulukutla, and V. Ramakrishnan, "Microbial calcite, a bio-based smart nanomaterial in concrete remediation," *http://dx.doi.org/10.1080/19475411003593451*, vol. 1, no. 1, pp. 28–39, 2010, doi: 10.1080/19475411003593451.

[9] J. Y. Wang, N. de Belie, and W. Verstraete, "Diatomaceous earth as a protective vehicle for bacteria applied for self-healing concrete," *J Ind Microbiol Biotechnol*, vol. 39, no. 4, pp. 567–577, Apr. 2012, doi: 10.1007/S10295-011-1037-1.

[10] H. Singh and R. Gupta, "Cellulose fiber as bacteria-carrier in mortar: Self-healing quantification using UPV," *Journal of Building Engineering*, vol. 28, p. 101090, Mar. 2020, doi: 10.1016/J.JOBE.2019.101090.

[11] L. Jiang, G. Jia, C. Jiang, and Z. Li, "Sugar-coated expanded perlite as a bacterial carrier for crack-healing concrete applications," *Constr Build Mater*, vol. 232, p. 117222, Jan. 2020, doi: 10.1016/J.CONBUILDMAT.2019.117222.

[12] M. T. Mustafa and B. B. Zeynep, "Use of Natural Minerals to Immobilize Bacterial Cells for Remediating Cracks in Cement-Based Materials," *Journal of Materials in Civil Engineering*, vol. 34, no. 3, p. 04021461, Mar. 2022, doi: 10.1061/(ASCE)MT.1943-5533.0004098.

[13] M. M. Tezer, "Development of two-phase biological self-healing agents for cement-based mortar," 2020.

[14] N. De Belie, J. Wang, Z. B. Bundur, and K. Paine, "Bacteria-based concrete," *Eco-efficient Repair and Rehabilitation of Concrete Infrastructures*, pp. 531–567, Jan. 2018, doi: 10.1016/B978-0-08-102181-1.00019-8.

[15] D. Yang, G. Xu, Y. Duan, and S. Dong, "Self-healing cement composites based on bleaching earth immobilized bacteria," *J Clean Prod*, vol. 358, p. 132045, Jul. 2022, doi: 10.1016/J.JCLEPRO.2022.132045.

[16] K. Liu *et al.*, "Effects of Pumice-Based Porous Material on Hydration Characteristics and Persistent Shrinkage of Ultra-High Performance Concrete (UHPC)," *Materials 2019, Vol. 12, Page 11*, vol. 12, no. 1, p. 11, Dec. 2018, doi: 10.3390/MA12010011.

[17] Q. Wang, J. Zhang, and J. C. M. Ho, "Zeolite to improve strength-shrinkage performance of high-strength engineered cementitious composite," *Constr Build Mater*, vol. 234, p. 117335, Feb. 2020, doi: 10.1016/J.CONBUILDMAT.2019.117335.

[18] C. Wu and S. Kou, "Effects of high-calcium sepiolite on the rheological behaviour and mechanical strength of cement pastes and mortars," *Constr Build Mater*, vol. 196, pp. 105–114, Jan. 2019, doi: 10.1016/J.CONBUILDMAT.2018.11.130.

[19] "ASTM C305-14, Standard Practice for Mechanical Mixing of Hydraulic Cement Pastes and Mortars of Plastic Consistency," *ASTM International*. 2014.

[20] "ASTM C109/C109M-21, Standard Test Method for Compressive Strength of Hydraulic Cement Mortars (Using 2-in. or [50 mm] Cube Specimens)," *ASTM International*. 2021.

[21] "ASTM C191-19, Standard Test Methods for Time of Setting of Hydraulic Cement by Vicat Needle," *ASTM International*. 2019.

[22] "ASTM C596-18, Standard Test Method for Drying Shrinkage of Mortar Containing Hydraulic Cement," *ASTM International*. 2018.

[23] "ASTM C157/C157M-17, Standard Test Method for Length Change of Hardened Hydraulic-Cement Mortar and Concrete," *ASTM International*. 2017.

[24] A. Amiri and Z. B. Bundur, "Use of corn-steep liquor as an alternative carbon source for biomineralization in cement-based materials and its impact on performance," *Constr Build Mater*, vol. 165, pp. 655–662, Mar. 2018, doi: 10.1016/j.conbuildmat.2018.01.070.

[25] A. Amiri, M. Azima, and Z. B. Bundur, "Crack remediation in mortar via biomineralization: Effects of chemical admixtures on biogenic calcium carbonate," *Constr Build Mater*, vol. 190, pp. 317–325, Nov. 2018, doi: 10.1016/J.CONBUILDMAT.2018.09.083.

[26] Z. Basaran Bundur, M. J. Kirisits, and R. D. Ferron, "Biomineralized cement-based materials: Impact of inoculating vegetative bacterial cells on hydration and strength," *Cem Concr Res*, vol. 67, pp. 237–245, 2015, doi: 10.1016/j.cemconres.2014.10.002.

[27] J. K. Norvell, J. G. Stewart, M. C. Juenger, and D. W. Fowler, "Influence of Clays and Clay-Sized Particles on Concrete Performance," *Journal of Materials in Civil Engineering*, vol. 19, no. 12, pp. 1053–1059, Dec. 2007, doi: 10.1061/(ASCE)0899-1561(2007)19:12(1053).

[28] X. Gao, S. Kawashima, X. Liu, and S. P. Shah, "Influence of clays on the shrinkage and cracking tendency of SCC," *Cem Concr Compos*, vol. 34, no. 4, pp. 478–485, Apr. 2012, doi: 10.1016/J.CEMCONCOMP.2012.01.002.

[29] J. A. Carmona, P. Ramírez, L. A. Trujillo-Cayado, A. Caro, and J. Muñoz, "Rheological and microstructural properties of sepiolite gels. Influence of the addition of ionic surfactants," *Journal of Industrial and Engineering Chemistry*, vol. 59, pp. 1–7, Mar. 2018, doi: 10.1016/J.JIEC.2017.09.030.

[30] J. Pinilla Melo, A. Sepulcre Aguilar, and F. Hernández Olivares, "Rheological properties of aerated cement pastes with fly ash, metakaolin and sepiolite additions," *Constr Build Mater*, vol. 65, pp. 566–573, Aug. 2014, doi: 10.1016/J.CONBUILDMAT.2014.05.034.

[31] H. Yang *et al.*, "Effects of bentonite on pore structure and permeability of cement mortar," *Constr Build Mater*, vol. 224, pp. 276–283, Nov. 2019, doi: 10.1016/J.CONBUILDMAT.2019.07.073.