



USE OF NATURAL MINERALS AS PROTECTIVE BARRIERS OF BACTERIA FOR SELF-HEALING MORTAR

M.M. Tezer¹, Z.B. Bundur^{1*}

¹ Ozyegin University, Department of Civil Engineering, Cekmekoy, 34987, Istanbul

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

Abstract

Microcracks are one of the main reasons for decreasing service life of concrete structures. Recent research in the field of concrete materials suggested that it might be possible to develop a cement-based material that is capable of remediating microcracks by triggering biogenic calcium carbonate (CaCO₃). This paper summarizes the study undertaken to investigate the influence of a 2-phase biogenic self-healing agent on fresh and hardened properties of cement-based materials. To develop biogenic self-healing agent, *S. pasteurii* cells were immobilized to the locally available, lightweight and porous natural minerals such as bentonite, diatomaceous earth, and sepiolite. In addition, a portion of the minerals was saturated with a nutrient medium including urea and calcium acetate. Upon immobilization, minerals including cells and the nutrient medium were incorporated in the mortar mix. The mortar mix samples were evaluated in terms of fresh and hardened state performance such as workability, initial set and, compressive strength. Incorporation of cells, as well as the nutrient media with the minerals did not affect the initial setting of the mix negatively, however, a substantial decrease was observed in compressive strength of samples particularly prepared by minerals only including nutrient medium. However, depending on the practical application of the mortar, this two-phase immobilization system can be potentially used for self-healing.

Keywords: Self-healing; biomineralization, immobilization, compressive strength, performance

1 INTRODUCTION

Early age cracks are one of the primary problems that decrease the service life of the concrete structures. These cracks can create pathways for hazardous chemicals and excessive water and increasing permeability of concrete. Recent studies in the field showed that it might be possible to develop a smart cement-based material that is capable of remediating cracks by triggering microbial induced calcium carbonate precipitation (MICP) within the cracked regions [De Muynck, De Belie, and Verstraete 2010; Zhang et al. 2015; J. Wang et al. 2016]. MICP is a biochemical process in which microorganisms stimulate the formation of calcium carbonate (CaCO₃) [Mann 2001]. With this approach, tensile or flexural cracks as wide as 0.7 mm were remediated and the water permeability of mortar was reduced [J. Y. Wang, Belie, and Verstraete 2012; J. Y. Wang, Soens, et al. 2014; Henk M Jonkers and Schlangen 2007; Wiktor and Jonkers 2011].

Crack healing through MICP requires a suitable bacterial culture and the nutrients to support metabolic activity and create the proper environment for crack closure. The main challenge of the application is to find a microorganism that can tolerate highly alkaline conditions of cement paste, can survive the mixing process, and can remain viable with limited access to nutrients [Tiago, Chung, and Verissimo 2004]. An early

approach was simply incorporating endospores rather than vegetative cells (metabolically active) [H M Jonkers 2010]. These endospores were found to be viable up to 4 months without any protection. Then, concerns regarding the viability of the endospores within the restrictive and high pH environment of cement-based materials have led researchers to propose encapsulation for the endospores. The encapsulation methods consist of embedding the endospores in a protective covering, e.g. inorganic lightweight porous aggregates (LWAs) [Wiktor and Jonkers 2011], polymeric membrane [Bang et al. 2010; J. Wang et al. 2012], microcapsules [J. Y. Wang, Soens, et al. 2014], hydrogels [J. Y. Wang, Snoeck, et al. 2014] and natural minerals [J. Y. Wang, De Belie, and Verstraete 2012; Alazhari et al. 2018]. Amongst all these approaches, LWAs and hydrogels have shown the most promising developments regarding the viability. The methods were promising in terms of healing cracks at various ages of concrete, but to-date most of the studies revealed that cracks could be healed in samples as old as 28 days.

However, in case of cracks occur before 7 days it might not be necessary to protect the cells in the mortar. Previously, Bundur et al. [Bundur et al. 2017] showed that vegetative *S. pasteurii* cells could survive in mortar up to 11 months when they were added to the mix without any encapsulations. These remaining cells were found to be effective in remediation of the microstructure

when internal microcracks [Liu et al. 2016] and flexural surface cracks [Amiri, Azima, and Bundur 2018]. In addition, limited viability and lack of O₂ would decrease the performance of CaCO₃ yield through all crack the depth. Instead, the precipitation was found to be limited to the crack mouth in microscale cracks [Amiri, Azima, and Bundur 2018]. However, considering the larger surface cracks, the amount of retained viable cells may not be able to precipitate sufficient biogenic CaCO₃ to seal the cracks. Thus, it is crucial to develop a simpler and natural protection system to improve the robustness of the bacterial cells against the restrictive environment.

Studies showed that among several alternatives such as diatomaceous earth (DE), metakaolin, zeolites and expanded clay could be suitable for protection of the bacteria based on their effects on compressive strength and setting, in particular DE was found to be effective in self-healing of cracks [Erşan et al. 2015; J. Y. Wang, De Belie, and Verstraete 2012].

Considering the natural resources, the list of these natural protective barriers could be extended. A correct choice of the protection barrier and application methodology are of crucial for further development of self-healing concrete. This study presents an investigation on the possible use of DE, bentonite (BT) and sepiolite (SP) as a protective barrier for bacterial cells by evaluating mortar properties such on initial setting time and compressive strength.

2 MATERIALS AND METHODS

2.1 Microorganism Selection and Growth

Leibniz Institute- German Collection of Microorganisms and Cell Cultures: S. pasteurii (DSMZ 33) cells were selected and used as a self-healing agent in cement-based materials. *S. pasteurii* were cells grown in a Urea-peptone-sodium acetate nutrient medium (UPSA) which includes tris base (0.13M), peptone (10 g), sodium acetate (10g) and urea (20 g) per liter of distilled (DI) water. The pH of the medium was adjusted to 9. Twelve grams (per liter of DI water) of agar was used when a solid medium was necessary for agar plates. Then, the cells were inoculated in 300 mL of above- mentioned UCSL medium and incubated aerobically with shaking conditions (175 rpm) at 30°C. Aliquots from bacterial culture were collected periodically for viable plate counts and counts were conducted by plating onto UPSA agar medium and incubating at 30 °C. Colony forming units (CFU) were counted after 48 hours. A correlation of bacterial growth (CFU/mL) vs. time was obtained. A Bacterial growth curve was generated as colony forming units (CFU/mL) vs. time (see Fig. 1) and this was used for further determination of cell concentration in the study. The *S. pasteurii* inoculum for mortar mixes was grown from freezer-stock in 300-mL batches until the stationary phase (10⁹ CFU/mL) was reached.

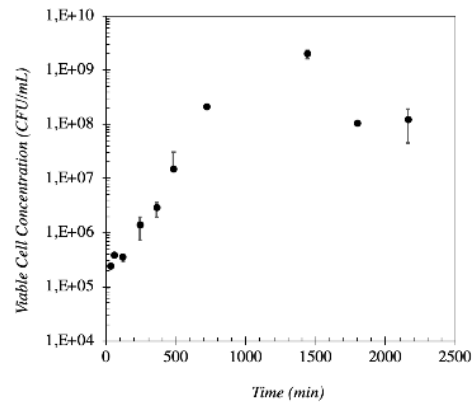


Fig. 112 : Representative growth profile for *S. pasteurii* (DSMZ 33). Data points average of triplicates of samples and error bars represent the one standard deviation.

2.2 Natural minerals for immobilization and protection procedure

Throughout the study 3 different protection barriers, namely DE (diatomaceous earth), BT (bentonite), and SP (sepiolite) were tested. These minerals were selected due to their porous structure and high absorption capacity. The absorption capacity of these minerals could be listed as 110%, 300 % and 80%, respectively. The particle size was ranging from 5-300 µm.

The immobilization was achieved by simply submerging 22.5 g of minerals to either nutrient solution or bacterial culture. Herein, the nutrient solution includes calcium acetate instead of sodium acetate. The bacterial cells were grown in UPSA nutrient medium until they reach the desired concentration. Then, the cells were collected by centrifuging at 6300g for 10 minutes and washed by sterilized phosphate buffer solution (PBS) twice. Then, the cells were suspended in a sterilized PBS solution for immobilization.

A set of minerals were treated by only urea-peptone-calcium acetate (UPCA) nutrient medium for control. The second set of minerals was treated with only cell-PBS suspension. Finally, half of the third set (11.25 g) was treated with UPCA nutrient medium and the cells were immobilized to the other half. Immobilization was achieved by submerging the minerals in nutrient medium or the cell-PBS suspension for 24 hours under shaking conditions (175 rpm at 30°C). Then, any remaining solution was removed from the minerals and excess water on mineral surfaces was calculated. This value was then subtracted from the mixing water content.

The morphology of the minerals was evaluated before and after treatment by JEOL JIB-4501 Multi-Beam Focused Ion Beam-Scanning Electron Microscope (FIB-SEM) (Freising, Germany). Figure 2 summarizes the change in morphology of DE, BT and SP before and after nutrient treatment.

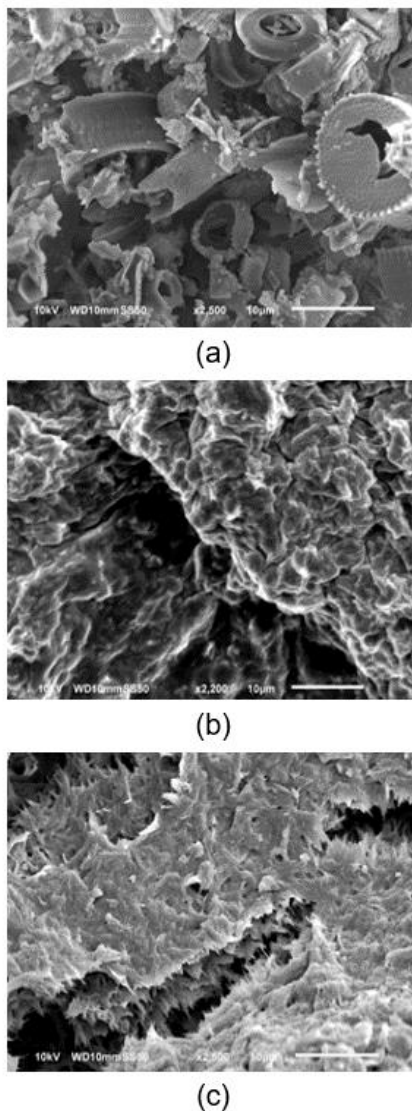


Fig. 113 : Morphology of minerals (a) Diatomaceous earth (DE); (b) Bentonite (BT), and (c) Sepiolite (SP)

2.3 Sample preparation

To evaluate the performance of bio-based additive on initial set and compressive strength, 7 different mixes were prepared in this study including control neat paste/mortar samples (see Table 1). All samples were prepared by CEM V/A 32.5N (including %25 slag + natural puzzolan) with a water to cement ratio (w/c) of 0.45. Mortar samples were prepared with adding standard sand according to norm EN 196-1 to the cement paste samples listed in Table 1 and the cement to sand ratio was kept 1:3.

For the remaining mixtures, natural minerals were added as 5% by weight of cement. Water content was corrected according to the excess moisture on the minerals.

The mixes were prepared according to ASTM C305-14 Standard Practice for Mechanical Mixing of Hydraulic Cement Pastes and Mortars of Plastic Consistency. Saturated minerals were added to cement content and mixed at a slower speed (140 ± 5 rpm) for 60s. Then, the mixing procedure was continued according to the abovementioned standard.

2.4 Evaluation of flowability

One of the drawbacks of using highly absorbent minerals is the decrease in workability of mortar samples. Thus, it is crucial to evaluate their impact on workability before further proceeding on self-healing evaluation. Fresh mortar samples were tested to evaluate the mortars flowability. Tests were conducted according to ASTM C1437-15 Standard Test Method for Flow of Hydraulic Cement Mortar and ASTM C230-14 Standard Specification for Flow Table for Use in Tests of Hydraulic Cement. The base plate and the conical mold were lubricated prior to the test for preventing the adhesion. Four readings were taken with a standard caliper. The flow percentage of the mortars was calculated by Eq. (1).

$$\text{Flow percentage, \%} = \left(\frac{A}{d_{base}} \right) \times 100 \quad (1)$$

in A being the average of four reading minus the base diameter of the cone.

2.5 Vicat needle test

A modified Vicat Needle test was conducted to determine the setting time of the cement pastes. For the test, cement paste samples were prepared according to a modified ASTM C191-13 Standard Test Methods for Time of Setting of Hydraulic Cement by Vicat Needle. Instead of determining the w/c ratio that will yield a "normal consistency" paste as suggested in the standard, the s/c ratios were kept constant at 0.45 to be consistent with the ratios used throughout the other analysis conducted in the study.

2.6 Compressive strength

For compressive strength testing, mortar samples were prepared as it was stated in Section 2.3 and then cast in $5 \times 5 \times 5$ cm cubes and kept in a humid environment at 21°C for 24 hours. Then the molds were removed, and the samples were further cured in humid room until testing (22°C). Compressive strength testing was conducted according to ASTM C109-13e1 Standard Test Method for Compressive Strength of Hydraulic Cement Mortars (Using 2-in. or [50-mm] Cube Specimens) at 3,7 and 28 on triplicates of samples.

Tab. 1: Summary of the mix design. DE: Diatomaceous Earth; BT: Bentonite; SP: Sepiolite; Nutrient medium: Urea-Peptide-Calcium Acetate (UPCA)

Sample Name	Abbreviation	Cement (g)	Water (g)	Nutrient medium (mL)	Mineral (g)	Bacterial cells + PBS (mL)
Control	CP	450	202.5	-	-	-
Nutrient-DE	DENP	450	150	87.5	22.5	-
Nutrient-BT	BTNP	450	150	87.5	22.5	-
Nutrient-SP	SPNP	450	150	87.5	22.5	-
Bacterial-DE	DEBP	450	150	-	22.5	87.5
Bacterial-BT	BTBP	450	150	-	22.5	87.5
Bacterial-SP	SPBP	450	150	-	22.5	87.5
2-phase-DE	DENBP	450	150	45	22.5	42.5
2-phase-BT	BTNBP	450	150	45	22.5	42.5
2-phase-SP	SPNBP	450	150	45	22.5	42.5

The test was conducted for all the mixes mentioned in Table 1 in Section 2.3. The initial setting of the cement paste samples was determined according to penetration depth of the Vicat needle. Analyses were conducted based on triplicates of samples.

3 RESULTS AND DISCUSSION

3.1 Flowability

Workability of the mixes was evaluated by mini-slump test. Table 2 summarizes the average flow table results for all cement paste samples.

Based on the absorption capacities and particle fineness, it was hypothesized that BT or DE would decrease the workability of the mixes. However, SP with having lowest absorption capacity significantly decreased the workability. Since the minerals were already saturated, the relative loss in mixing water due to absorption should be limited. Thus, the workability loss was mainly due to the shape and morphology of the minerals. Angular and fine SP particles resulted in lower workability. Further experiments and possible mix designs are being evaluated to understand the complex mechanism of sepiolite as a protection barrier for bacterial cells.

3.2 Initial setting time

The results of the Vicat needle test are summarized in Figure 3. Different compositions of cement paste samples including bacteria and minerals were evaluated compared to neat paste control cement paste (CP). The initial set of CP sample was recorded as 4.5 hours, which was expected due to the puzzolan content in CEM V/A cement. Since there was a severe retardation in samples prepared with DE (DENP) compared to CP an additional set of samples was also prepared by adding the nutrients to the mix water without any mineral or bacterial cells (NCP).

Interestingly, the retardation in the initial set in NCP sample was even higher compared to DENP.

Previously, it was found that use of a carbohydrate-based carbon source causes this retardation [Basaran Bundur, Kirisits, and Ferron 2015]. Use of urea-yeast extract medium resulted with almost tripled initial setting time [Amiri and Bundur 2018]. However, the nutrient medium in this study consists of urea-peptide and calcium acetate. Williams et al. [Williams, Kirisits, and Ferron 2016] showed that incorporation of peptone-sodium acetate significantly decreased the delay in initial setting compared to the use of only yeast extract. The authors showed that incorporation of yeast extracted extended the initial setting time by 10 hours compared to the control neat paste.

Tab. 2: Flow table test % for different cement paste mixes. The values are representing the average % flow of triplicates of samples calculated based on Equation (1). Error represents the standard deviation.

Sample Name	Flow %
Control	23.7 ± 1.8
Nutrient-DE	59.4 ± 2.7
Nutrient-BT	13.1 ± 0.1
Nutrient-SP	2.5 ± 0.9
Bacterial-DE	30.6 ± 2.7
Bacterial-BT	18.1 ± 0.9
Bacterial-SP	0.6 ± 0.9
2-phase-DE	36.8 ± 4.4
2-phase-BT	5.0 ± 1.8
2-phase-SP	0.0 ± 0.0

Incorporation of peptone-calcium acetate instead of yeast extract resulted in 2.5 hours of delay compared to neat paste. Herein, use of UPCA medium still resulted in a shorter initial setting time compared to the use of yeast extract [Amiri and Bundur 2018], but it still increased the initial setting by 4.5 hours. This is significantly higher than what was found when corn steep liquor (CSL) was used in the mixes [Amiri and Bundur 2018]. Since the delay in the initial set was attributed to the composition of the carbon source, these relative changes could be due to regional differences in

compositions of chemicals purchased. However, the trend of the relevant data shows that CSL could be better option in terms of their influence on setting time. Incorporation of minerals with the nutrient medium resulted with a decrease in initial setting time compared to NCP sample. A similar trend was also obtained when minerals were incorporated with bacterial culture. This is due to the different mixing procedure of samples. Such that the self-healing agents were incorporation by submerging the nutrients to 87.5 mL of solution and the minerals would absorb most of this solution. Whereas NCP sample was prepared by adding the nutrients to 202.5 mL of tap water, thus the amount of peptone incorporated was almost 3 times higher than what was added in DENP, SPNP and BTNP samples. It should also be noted that BTBP, DEBP and SPBP samples do not include any nutrients. Thus, the delay in the initial setting time was directly attributed to the nutrient medium rather than presence of cells.

3.3 Compressive strength

Figure 4 summarizes the compressive strength test results for mortar samples at 3,7 and 28 days after mixing. Incorporation of minerals which were saturated with UPCA nutrient medium (see DENM, BTNM and SPNM samples in Figure 4) negatively impacted in compressive strength regardless of age and type of the mineral used. This could be correlated with the delay in the hardening process due to retardation in initial set of these samples.

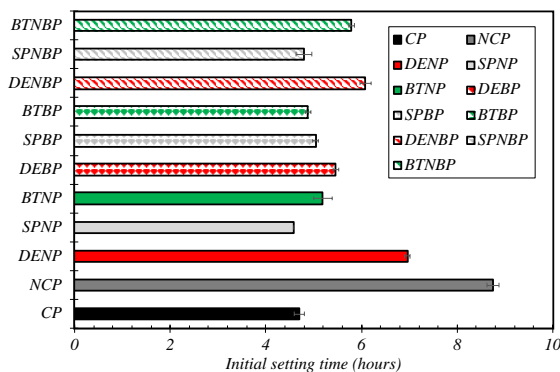


Fig. 3: Modified ASTM C191-13 initial setting times of different cement paste mixes. The bars are representing the average initial setting time values based on triplicates of cement paste samples and error bars represent the one standard deviation.

Incorporation of minerals saturated with bacterial cells with PBS slightly increased the strength compared to samples prepared with minerals saturated with UPCA nutrient medium. However, the compressive strength of these bacterial mortar samples (DEBM, SPBM and BTBM) was still lower than neat control mortar (CM). Thus, it could be concluded that incorporation of UPCA medium not only increases the initial setting time but also decreases the compressive strength. Even though the performance of samples prepared with UPCA medium showed superior performance compared to those previously reported samples prepared with urea-yeast extract samples, it was still lower than samples prepared with urea-CSL medium [Amiri and Bundur 2018]. This could also be due to the incorporation of peptone. Previously, Jonkers et al. [H M Jonkers 2010] found that incorporation of peptone by 1% of cement weight significantly reduced compressive strength.

Herein peptone was added by only 0.2% of the cement weight, thus its impact might be less severe than what was observed previously by Jonkers et al. [H M Jonkers 2010].

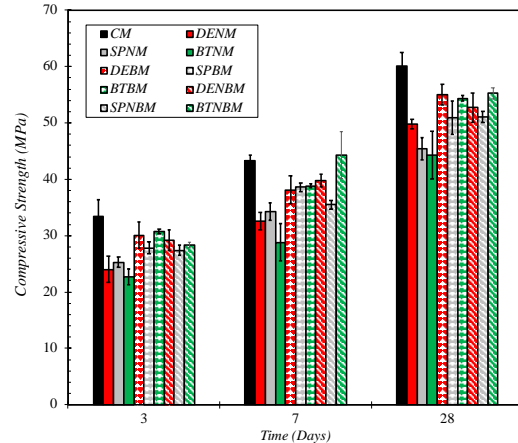


Fig. 4: Influence of minerals and bacterial culture on the compressive strength of mortar. An s/c of 0.45 was used. Bars show the average compressive strength (based on triplicate mortar samples), and error bars represent one standard deviation. M: stands for mortar samples prepared by adding standard sand to cement paste samples summarized in Table 1. Cement to sand ratio was kept at 1:3.

Comparing the strength values obtained at various dates, it was found that the decrease in strength was more pronounced in which SP was used in immobilization. Previously, it was shown that the addition of DE by 5% of cement weight had a minor influence on both 7- and 28-days compressive strength [Erşan et al. 2015]. Similar results were also obtained in this study where there was a slight decrease in the compressive strength when bacterial cells were incorporated with DE. BT is a mineral generally used in agriculture due to its high absorption coefficient and previously found a decreasing effect in compressive strength of concrete [Targan et al. 2008]. Interestingly, here in the 2-phase additive prepared with BT showed the highest performance at 7 and 28 days. The type of BT used here is not only highly absorbent and expansive, thus it might entrap additional water phase during mixing resulting with a higher strength.

4 CONCLUSIONS

Three different minerals, diatomaceous earth (DE), bentonite (BT) and sepiolite (SP) were used as a protection barrier for *S. pasteurii* cells. This paper summarizes the influence of this 2-phase self-healing agent on initial set and compressive strength. The results revealed that incorporation of bacterial culture (without nutrients) showed a better performance in terms of setting and strength. The compressive strength of samples prepared by 2-phase self-healing agents were on par with the control mix without significantly increasing the initial set. The negative impact of the bio-based self-healing component was attributed to the incorporation of peptone. Further studies should involve use of different alternatives like corn steep liquor as an alternative carbon source that could be used instead of peptone. The viability of cells and their as self-healing agents concrete should be also investigated.

5 ACKNOWLEDGMENTS

This research was conducted by financial assistance of the Scientific and Technical Research Council (TUBITAK) of Turkey Project: MAG-118M327. SEM and XRD analysis were conducted in FENS laboratory facilities of Sabancı University, Istanbul. The authors graciously acknowledge Prof. Mehmet Ali Gulgun for allowing the authors access to his laboratory facilities. The authors also acknowledge undergraduate research assistants Pozhhan Mokhtari for his help in SEM and XRD analysis.

6 REFERENCES

- [Alazhari 2018] Alazhari M., Sharma T., Heath A., Cooper, et. al. "Application of Expanded Perlite Encapsulated Bacteria and Growth Media for Self-Healing Concrete." *Construction and Building Materials*, 160. 2018. The Authors: 610–19. doi:10.1016/j.conbuildmat.2017.11.086.
- [Amiri 2018] Amiri A., Azima M., and Bundur Z.B. "Crack Remediation in Mortar via Biomineralization: Effects of Chemical Admixtures on Biogenic Calcium Carbonate", 190: 317–25. 2018. doi:10.1016/j.conbuildmat.2018.09.083.
- [Amiri 2018] Amiri A. and Bundur Z.B. "Use of Corn-Steep Liquor as an Alternative Carbon Source for Biomineralization in Cement-Based Materials and Its Impact on Performance." *Construction and Building Materials* 2018. 165: 655–62. doi:10.1016/j.conbuildmat.2018.01.070.
- [Bang 2010] Bang S.S., Lippert J.J., Yerra U., Mulukutla S., et.al. "Microbial Calcite, a Bio-Based Smart Nanomaterial in Concrete Remediation." *International Journal of Smart and Nano Materials*. 2010. 1 (1): 28–39. doi:10.1080/19475411003593451.
- [Bundur 2017] Bundur Z.B., Bae S., Kirisits M.J. and Ferron R.D. "Biomineralization in Self-Healing Cement-Based Materials: Investigating the Temporal Evolution of Microbial Metabolic State and Material Porosity." *Journal of Materials in Civil Engineering* 29 (6). 2010. American Society of Civil Engineers. doi:10.1061/(ASCE)MT.1943-5533.0001838.
- [Bundur 2015] Bundur Z.B., Kirisits M.J. and Ferron R.D. "Biomineralized Cement-Based Materials: Impact of Inoculating Vegetative Bacterial Cells on Hydration and Strength." *Cement and Concrete Research* 67 (January). 2015. Elsevier Ltd: 237–45. doi:10.1016/j.cemconres.2014.10.002.
- [Erşan 2015] Erşan Y.Ç., Silva F.B.D., Boon N., Verstraete W., et. al. "Screening of Bacteria and Concrete Compatible Protection Materials." *Construction and Building Materials* 88. 2015. 196–203. doi:10.1016/j.conbuildmat.2015.04.027.
- [Jonkers 2010] Jonkers H.M. "Application of Bacteria as Self-Healing Agent for the Development of Sustainable Concrete." 2010. *Ecol. Eng.* 36: 230.
- [Jonkers 2007] Jonkers H.M. and Schlangen E. "Crack Repair By Concrete-Immobilized Bacteria." *Civil Engineering*. 2010. no. April: 1–7.
- [Liu 2016] Liu S., Bundur Z.B., Zhu J. and Ferron R.D. "Evaluation of Self-Healing of Internal Cracks in Biomimetic Mortar Using Coda Wave Interferometry." *Cement and Concrete Research* 83. 2016. doi:10.1016/j.cemconres.2016.01.006.
- [Mann 2001] Mann S. *Biomineralization: Principles and Concepts in Bioinorganic Materials Chemistry*. 2001. New York: Oxford.
- [Muynck 2010] Muynck W.D., Belie N.D. and Verstraete W. "Microbial Carbonate Precipitation in Construction Materials: A Review." *Ecological Engineering* 36 2010. : 118–36. doi:10.1016/j.ecoleng.2009.02.006.
- [Targan 2008] Targan S., Olugun A., Erdogan Y. and Sevinc V. "Effects of Supplementary Cementing Materials on the Setting Time and Early Strength of Concrete." *Cement and Concrete Research* 7 (82). 2008. : 1551–58.
- [Tiago 2004] Tiago I., Chung A.P. and Verissimo A. "Bacterial Diversity in a Nonsaline Alkaline Environment: Heterotrophic Aerobic Populations." *Applied and Environmental Microbiology* 70 (12) 2004. : 7378–87. doi:10.1128/AEM.70.12.7378-7387.2004.
- [Wang 2012] Wang J.Y., Belie N.D. and Verstraete W. "Diatomaceous Earth as a Protective Vehicle for Bacteria Applied for Self-Healing Concrete." *Journal of Industrial Microbiology and Biotechnology* 39 (4) 2012. : 567–77. doi:10.1007/s10295-011-1037-1.
- [Wang 2014] Wang J.Y., Snoeck D., Vlierberghe S.V., Verstraete W. and Belie N.D. "Application of Hydrogel Encapsulated Carbonate Precipitating Bacteria for Approaching a Realistic Self-Healing in Concrete." *Construction and Building Materials* 68 (October). 2014. Elsevier Ltd: 110–19. doi:10.1016/j.conbuildmat.2014.06.018.
- [Wang 2014] Wang J.Y., Soens H., Verstraete W. and Belie N.D. "Self-Healing Concrete by Use of Microencapsulated Bacterial Spores." *Cement and Concrete Research* 56 (February). 2014. Elsevier Ltd: 139–52. doi:10.1016/j.cemconres.2013.11.009.
- [Wang 2012] Wang J.Y., Belie N.D. and Verstraete W. "Diatomaceous Earth as a Protective Vehicle for Bacteria Applied for Self-Healing Concrete." *Journal of Industrial Microbiology & Biotechnology* 39 (4) 2012. : 567–77. doi:10.1007/s10295-011-1037-1.
- [Wang 2016] Wang J.Y., Ersan Y.C., Boon N. and Belie N.D. "Application of Microorganisms in Concrete: A Promising Sustainable Strategy to Improve Concrete Durability." *Applied Microbiology and Biotechnology*. 2016. doi:10.1007/s00253-016-7370-6.
- [Wang 2012] Wang J.Y., Tittelboom K.V., Belie N.D. and Verstraete W. "Use of Silica Gel or Polyurethane Immobilized Bacteria for Self-Healing Concrete." *Construction and Building Materials* 26 (1). 2012. Elsevier Ltd: 532–40. doi:10.1016/j.conbuildmat.2011.06.054.
- [Wiktor 2011] Wiktor V. and Jonkers H.M. "Quantification of Crack-Healing in Novel Bacteria-Based Self-Healing Concrete." *Cement and Concrete Composites* 33 2011. : 763–70. doi:10.1016/j.cemconcomp.2011.03.012.
- [Williams 2016] Williams S.L., Kirisits M.J. and Ferron R.D. "Optimization of Growth Medium for *Sporosarcina Pasteurii* in Bio-Based Cement Pastes to Mitigate Delay in Hydration Kinetics." *Journal of Industrial Microbiology & Biotechnology*, 2016. 1–9. doi:10.1007/s10295-015-1726-2.
- [Zhang 2015] Zhang B., Bundur Z.B., Mondal P. and Ferron R.D. "Use of Biomineralisation in Developing Smart Concrete Inspired by Nature." *International Journal of Materials and Structural Integrity* 9 (1–3). 2015. doi:10.1504/IJMSI.2015.071109.