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Two-Part Bio-Based Self-Healing Repair Agent for Cement-Based Mortar

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Abstract. *Factors affecting durability of concrete structures are generally associated with each other. Due to its brittle nature, concrete can crack under stress and these cracks are one of the main reasons for a decrease in service life in concrete structures. Therefore, it is crucial to detect and recover microcracks, then to repair them as they were developed to wider cracks. Recent research in the field of concrete materials suggested that it might be possible to develop a smart cement-based material that is capable of remediate cracks by triggering biogenic calcium carbonate ($CaCO_3$) precipitaton. This paper summarizes a study undertaken to investigate the self-healing efficiency of Sporosarcina pasteurii (S. pasteurii) cells immobilized on both diatomaceous earth and pumice, to remediate flexural cracks on mortar in early ages (28 days after mixing). To obtain a two-phase bio additive, half of the minerals were saturated with a nutrient medium consisting of urea, corn-steep liqueur(CSL) and calcium acetate and the cells with immobilized to the other half without nutrients. Screening of the healing process was done with ultrasonic pulse velocity (UPV) testing and stereomicroscopy. With this approach, the cracks on mortar surface were sealed and the water absorption capacity of the so-called self-healed mortar decreased compared to its counterpart cracked mortar samples.*

Keywords: *Self-Healing, Biomineralization, Diatomaceous Earth, Pumice, Mortar.*

1 Introduction

Concrete is the most widely used material in the construction industry due to its high compressive strength, durability and cost efficiency compared to the alternative building materials. Nevertheless, early age cracks occurring in concrete can decrease the strength of the materials and limit the service life of the concrete structures. These cracks can create pathways for hazardous chemicals and excessive water and increasing permeability of concrete. Conventional repair methods like, epoxy sealing or grouting are applied from the outside and expected to penetrate into the cracks. While this approach is feasible for repairing large cracks, penetration of the abovementioned sealants to the inner parts of small and deep cracks is very limited. Therefore, for narrow microcracks, there is a need for an alternative repair method by mostly means of self-healing.

Recent studies in the field showed that it might be possible to develop a bio-based self-healing system where bacterial cells are being used to remediate cracks by triggering microbial induced calcium carbonate precipitation (MICP) (De Muynck *et al*. 2010; Wang *et al*. 2016; Zhang *et al*. 2015). MICP is a biochemical process in which microorganisms stimulate the formation of calcium carbonate ($CaCO_3$) (Mann 2001) . With this approach, tensile or flexural cracks as wide as 0.7 mm was remediated and the water permeability of mortar was reduced (Jonkers and Schlangen 2007; Wang *et al.* 2012c, 2014b; 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 *et al.* 2004) . A simple approach by means of limiting the labor work in processing is adding the vegetative bacterial cells directly to the mix Previously, 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 in 7 day old samples (Amiri *et al.* 2018). However, limited viability and lack of O_2 decreased the performance of $CaCO_3$ yield through all crack the depth. Instead, the precipitation was found to be limited to the crack mouth in microscale cracks (Amiri *et al.* 2018). However, considering the larger surface cracks, the amount of retained viable cells may not be able to precipitate sufficient biogenic $CaCO_3$ 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.

Concerns regarding the viability of the cells and limited crack healing led researchers to propose various encapsulation methodologies, particularly for dormant state 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; Wang *et al*. 2012a), microcapsules (Wang *et al.* 2014b), hydrogels (Wang *et al.* 2014a) and natural minerals (Alazhari *et al.* 2018; Wang *et al.* 2012b). 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.

Instead of using synthetic encapsulation covering, a more sustainable approach could be achieved by using natural additives or natural lightweight aggregates. Throughout the literature, 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; Wang *et al*. 2012b). Nevertheless, 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 a comparative study on the possible use of a mineral additive (DE) and a porous lightweight aggregate (pumice) as a protective barrier for bacterial cells.

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* cells were grown in a Urea-corn steep liqueur (CSL)-sodium acetate nutrient medium (UCSL) which includes tris base (0.13M), CSL (15 g), sodium acetate (10g) and urea (20 g) per liter of distilled (DI) water. The pH of the medium was adjusted to 9. First, the nutrient medium was sterilized at 121°C for 45 minutes. The cells were incubated aerobically in sterilized liquid

medium with shaking conditions (175 rpm) at 30°C until the stationary phase (10^9 CFU/mL) was reached. Then, the cells were collected from the culture by centrifuging at 6300g for 15 min. The cells were washed twice by PBS (Phosphate buffered solution) and stored at 4°C until immobilizing.

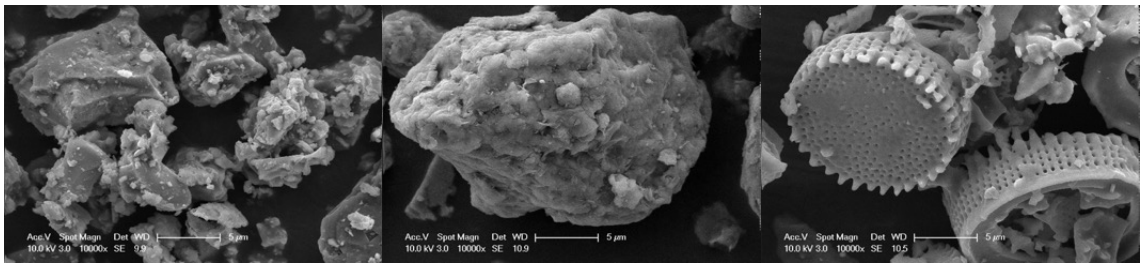
2.2 Material Composition

DE (Diatomaceous Earth), and pumice were used to immobilize bacterial cells. DE was provided regionally from a source in Turkey. DE particles used in the experiments were raw materials without any further treatment and having irregular shapes. A Mastersizer 2000 particle size analyzer with a Hydro MU 2000 (Malvern, Worcestershire, United Kingdom) wet dispersion unit was used to measure the particle size distributions of cement and DE. To prevent hydration of the cement particles, the cement particles were dispersed in ethanol. The average particle sizes of DE and cement were determined as 19.20 and 23.2 μm, respectively. The particle size was ranging from 0.375 to 90 μm for DE and it was ranging from 5 to 90 μm for OPC. DE was used as an addition to the mix by 5% of cement weight. The absorption capacity of DE was found to be almost 100% by its weight. Pumice was used as aggregate replacement such that 5% of the sand used in mortar mixes. The particle size of light weight pumice aggregate was in between 0.3 to 2.5 mm, while the absorption capacity was 45% by its weight.

Besides the additions or replacements, mortar samples were prepared using OPC CEM I 42.5R and standard sand accordingly to the norm EN 196-1. A polycarbooxylate superplasticizer was used to maintain the same workability criterion for all samples. The water to cement ratio (w/c) was kept at 0.45.

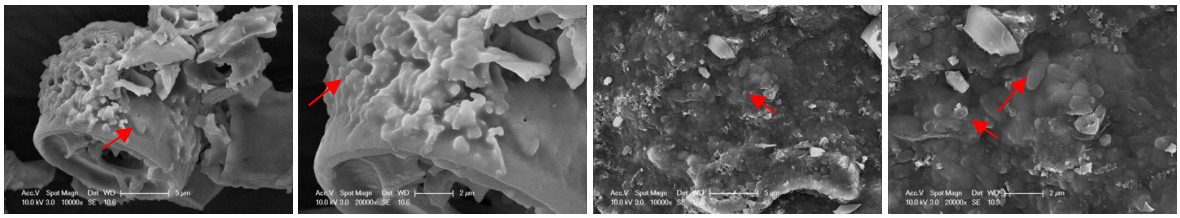
2.3 Immobilization Procedure

The immobilization was achieved by simply submerging the DE or pumice to a bacterial suspension. To immobilize the cells on DE, 2 grams of viable *S. pasteurii* cells were collected as it was stated in Section 2.1, and they were resuspended in a sterilized 50 mL PBS solution. Then, 22.5 g of DE was added to the suspension. Sixty-seven grams of pumice was added to the 6 grams of cell suspension in 80 mL PBS. Immobilization for both materials was achieved with shaking conditions (175 rpm) at 30°C for 24 hours. Then, the slurry was removed from incubation and kept at 40°C in an oved for an additional 12 hours. Excess PBS content on DE and pumice was calculated by subtracting the known weight of DE/pumice, bacterial cells and theoretical absorbed PBS from the final weight of oven dry slurry. This value was subtracted from the mixing water content. To ensure the immobilization was achieved, the morphology of the DE and pumice was evaluated before and after treatment by a FEI-Philips XL30 Environmental Scanning Electron Microscope with Field Emission Gun (FEG). Figure 1 and 2 show the SEM images obtained before and after immobilization procedure, respectively.



(a) (b) (c)

Figure 1. Morphology of (a) cement (b) pumice and (c) diatomaceous earth before immobilization. Scale: 5 µm.



(a) (b) (c) (d)

Figure 2. Immobilization of bacterial cells on (a), (b) diatomaceous earth (c), (d) pumice. Scale: 2 and 5 µm.

2.3 Experimental Planning

2.3.1 Preparation of mortar samples

Mortar samples were prepared by ASTM C305-14. Samples were made with a water- to-cement ratio of 0.45 and a sand-to-cement ratio of 3. The mortar samples were then cast into 40 x 40 x 160 mm molds and kept in humid environment at 21°C for 24 h. Then the molds were removed, and the samples were further cured in moist environment until testing (22°C). To provide flexural resistance during crack initiation, 12-mm micro synthetic fibers were added to mortar (4 g/m^3 of mortar). A polycarboxylate ether (PCE)-based superplasticizer (BASF) was used to maintain the required workability of mortars in terms of flow table diameter based on ASTM C1437-15. To quantify the workability, a flow table test was conducted based on ASTM C1437-15 (ASTM International 2015). The workability of pastes was adjusted using PCE superplasticizer when necessary. PCE was added into mortar mixes until the desired flow reached (±10% of the control neat mortar). Average flow values were calculated from triplicates of samples from 5 batches (15 samples in total). Table 1 summarizes the composition and flow behavior of different mixes used in this study.

Table 1. Summary of mixing ingredients and flow (%). PBS: Phosphate buffes solution; SP: Superplasticizer; DE: Diatomaceous earth; Pum: Pumice. C: Only includes air dry minerals; Bac: all mineral content was saturated with bacterial cells and PBS; 2P: Two-phase bio additive containing bacterial cells and nutrients. Flow values were given average ± standard deviation.

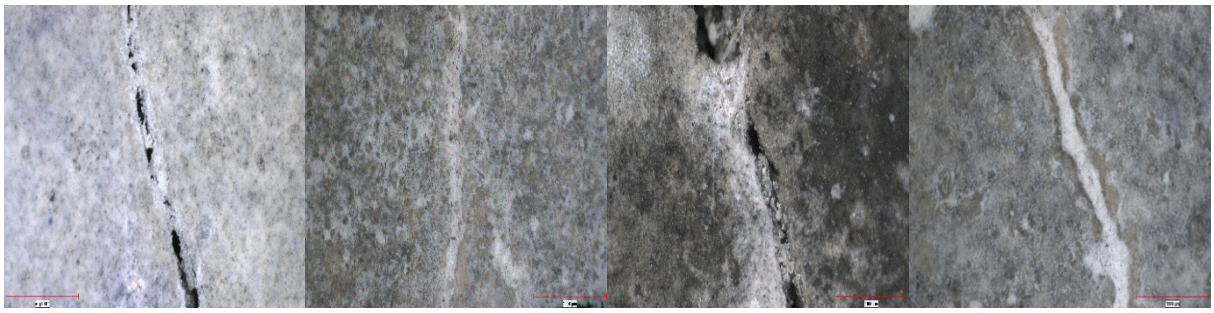
Sample	*Cement*	*Sand*	*S. pasteurii cells*	*Mineral content*	*PBS*	*Nutrient solution*	*SP*	*Flow (%)*
Kontrol	450 g	1350 g	-	-	-	-	3 g	21 ± 0.4
DE-C	450 g	1350 g	-	22.5 g	-	-	4.5 g	30 ± 0.4
Pum-C	450 g	1282.5 g	-	67.5 g	-	-		15 ± 0.4
DE-Bac	450 g	1350 g	2 g	22.5 g	90 g	-	3 g	36 ± 0.4
Pum-Bac	450 g	1282.5 g	6 g	67.5 g	90 g	-		25 ± 0.4
DE-2P	450 g	1350 g	2 g	22.5 g	45 g	45 g	1.5 g	34 ± 0.4
Pum-2P	450 g	1282.5 g	6 g	67.5 g	45 g	45 g		32 ± 0.4

2.3.2 Crack formation, curing and evaluation of crack healing

At the end of the 28th day of mixing, the samples were removed from the curing environment and cracked under flexural loading using a servo hydraulic displacement-controlled device (0.05 mm/sec). The sample was unloaded once the crack was formed visually, which corresponded to 50 ± 3% of the ultimate flexural strength of samples. Upon unloading, the remaining crack width ranged from 0.30 mm to 0.35 mm. A set (3 samples) of control samples were not cracked for further analysis. Once the cracks were formed a set of cracked samples were cured in water and another set was cured in nutrient medium. Curing process was done by submerging the samples into curing solutions for 2-days and then, subsequently leaving them at ambient conditions for 2 days. This 2- day period curing process was applied until at least 90% crack sealing was observed. To investigate the self-healing in cement-based mortar, cracked beams were periodically observed under NIKON Inc. SMZ745T Stereomicroscope and analyzed with CLEMAX visual analysis system. Air dried samples were analyzed weekly for $CaCO_3$ crystal formation within cracks. Complementary quantification was done by Ultrasonic Pulse Velocity (UPV) test.

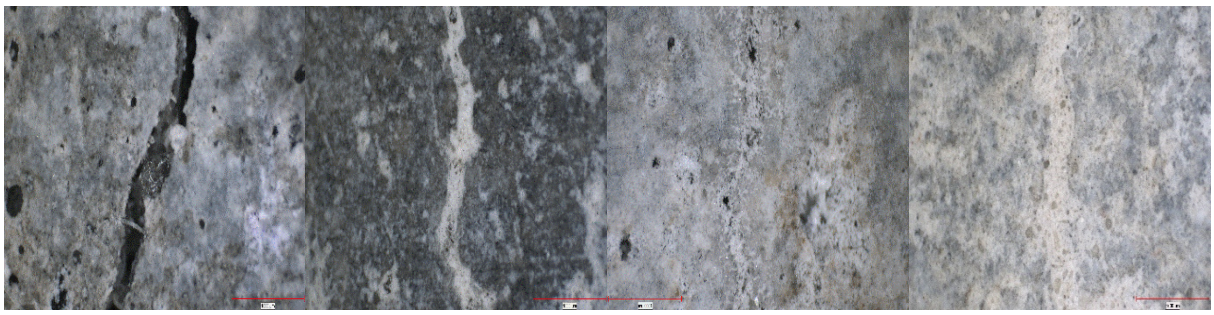
3 Results and Discussion

Upon 14 to 28 days of curing, the cracks were visually sealed in specimens containing bacterial cells (*DE-Bac; Pum-Bac; DE-2P and Pum-2P*) when additional nutrients were provided with curing. There was not any visual crack closure or any crystal precipitation in control samples without any bacterial cells regardless of the sample age and type of curing (within the 28-day observation period). From each set, triplicates of samples were cast and the specimens in the same sets showed similar crack- filling trend. Since the visual crack healing was almost the same for each set, one representative image of series with 28-day old samples containing bacterial cells is shown in Figure 3 and Figure 4.



(a) (b) (c) (d)

Figure 3. Stereomicroscopy images of the cracks (0.3 to 0.4 μm) in 28-day old specimens containing diatomaceous earth (a) DE-Bac after 28-days of water curing (b) DE-Bac after 28-days nutrient curing (c) DE-2P after 14 days of water curing and (d) DE-2P after 14 days nutrient curing.



(a) (b) (c) (d)

Figure 4. Stereomicroscopy images of the cracks (0.3 to 0.4 μm) in 28-day old specimens containing pumice (a) Pum-Bac after 28-days of water curing (c) Pum-Bac after 28-days of nutrient curing (c) Pum-2P after 14-days of water curing and (d) Pum-2P after 14-days of nutrient curing.

Based on the visual crack evaluation, both DE and pumice was found to be effective in terms of immobilizing the bacterial cells and trigger self-healing. The possible mechanism of these minerals could be attributed to their relatively high capacity to absorb and hold bacterial cells on the surfaces. In addition, it could also be noted that additional nutrients as urea and [Ca^{+2}] source should be provided either during the mixing or in the curing solution. DE was already known to be a good mineral agent to immobilize and protect the cells from high pH environment of cement paste. This could be attributed to its relatively higher absorption capacity and high specific surface area which could enable a more homogenous. Since DE is also a microbiological formation, it could also provide a more suitable microenvironment for the bacterial cells compared to cementitious environment and thus bacteria could still decompose urea (Wang *et al*. 2012c). Even though, both minerals were effective in terms of remediating the cracks, however pumice was slightly more efficient compared to DE in terms of crack closure, particularly in samples containing 2-phase mineral additives. This could be directly related to the number of bacterial cells added to the mix. Since pumice have larger particle size, it was used as a sand replacement which was 3 times more than DE addition. Relatedly, the amount of bacterial cells and nutrient solution was also tripled. This might lead to a more efficient crack healing in pumice containing samples compared to DE modified samples.

UPV test results also confirmed that healing was occurred in cracks. Figure 5 shows the UPV test results for mortar samples cured in different conditions after crack initiation. There was a full crack closure in Pum-2P and DE-2P samples in 14 days, thus the evaluation of the sample was further done by water absorption test. Similar to the visual inspection, healing was observed in almost all samples containing bacterial cells. In particular, for samples containing DE, a higher self-healing was only observed in *DE-Bac* and *DE*-2P samples when they were cured in nutrient medium. However, there was also a change in UPV reading in DE-Bac and DE-2P samples cured in water. This might indicate that there might be an internal crack healing process occurred in the samples rather than crack mouth sealing, which might not be detected via visual inspection. Similar results were also obtained with *Pum-Bac* sample in which a higher UPV reading was observed without any visual crack sealing. Further investigations are being done in terms of quantifying self-healing through water absorption.

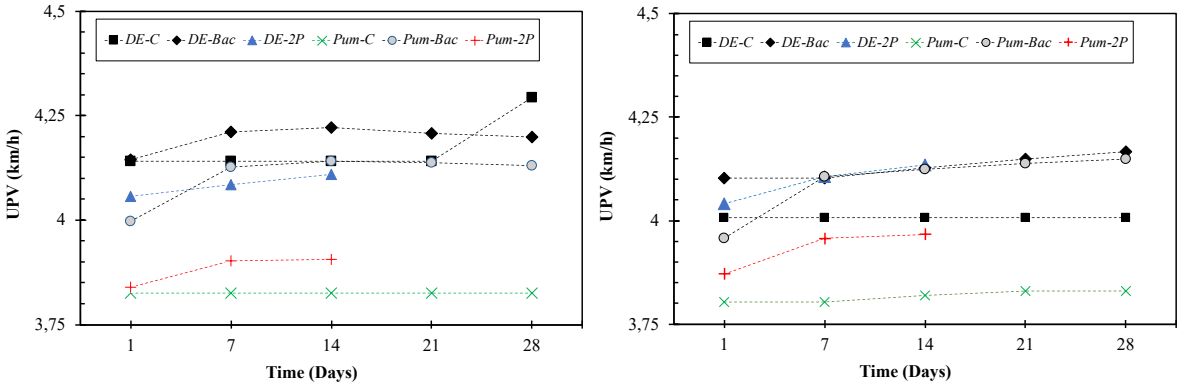


Figure 5. UPV record after crack healing (a) 28-days of water curing (b) 28-days of nutrient curing

4 Conclusion

This study was undertaken to investigate the possible use of diatomaceous earth and pumice as an immobilization barrier for bacterial cells to trigger crack remediation in mortar. Cracks with an average width of 0.4 mm in 28-day old mortar specimens were almost completely filled by bio-based precipitate depending on the curing regime. Results showed that cells require additional nutrient source as urea and calcium acetate either in the mix or as curing regardless of type of the immobilization barrier. Cracks were sealed even in sample including relatively smaller dosage of nutrients and bacterial cells in presence of moisture. Moreover, the duration of crack healing was approximately 21 days, which was almost half of the duration to remediate the cracks when cells were directly incorporated to the mix. Further investigation has to be done to characterize the precipitate and evaluate the influence of crack sealing on permeability.

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