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BIO-BASED ADMIXTURE FOR SELF-HEALING CEMENT-BASED MORTAR

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Abstract
Factors affecting the 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. Recently, interest in using biomineralization processes for self-healing applications in cement-based materials has gained broader attention in the field. Biomineralization is a biochemical process in which microorganisms stimulate the formation of minerals, and in this particular case calcium carbonate ($CaCO_3$). Due to the restricted environment of cement paste matrix, the main challenge is to find a microorganism that can tolerate the high alkaline conditions, can survive the mixing process, and can remain viable with limited access to nutrients and space to remediate cracks. This paper summarizes the results of a study undertaken to investigate the possible application of a bio-based admixture consisting *Sporosarcina pasteurii* (*S.pasteurii*) cells and urea-corn steep liquor (UCSL) medium to remediate flexural cracks in cement-based mortar. To develop biogenic self-healing agent, vegetative *S. pasteurii* cells were grown in UCSL medium and then mixed with cement and sand. Incorporation of cells, as well as the nutrient medium,,,did not affect the compressive negatively. Biogenic $CaCO_3$ crystals were observed inside the flexural cracks and precipitates were able to seal cracks as large as 0.2 mm and decrease the permeability of the material.

Keywords:
Biomineralization, self-healing, calcium carbonate, *Sporosarcina pasteurii*, mortar, corn steep liquor

1 INTRODUCTION

While concrete is one of the most consumed materials in the world, microcracks initiated due to internal stress causes a concern in the field applications. These microcracks not only provide pathways for chemicals and water to penetrate, it is hard and expensive to repair[1]. Concrete durability is closely related to permeability. To address these problems, recent researchers suggested that it might be possible to alter conventional concrete to achieve a smart cement-based material that is capable of remediating cracks by triggering self-healing of cracks via biomineralization [2–5]. Biomineralization is a bio-chemical process in which microorganisms induce mineral precipitation[6]. In this particular case, the microorganisms stimulate the formation of $CaCO_3$, which is also known as microbial-induced calcium carbonate precipitation (MICP).

Earlier studies in the literature showed that MICP could be used to improve soil properties [7,8]. Following these applications, MICP has been used in cement-based material to promote self-healing of cracks [9,10]. However, the main challenge of this application was to find the suitable microorganism and nutrient medium, which can tolerate a restricted environment of concrete. An early approach was the incorporation of *Bacillus pseudofirmus* and *Bacillus cohnii* endospores by suspending them in mixing water of mortar, in which microorganisms could remain viable for 4 months. Another possible method was to encapsulate the microorganisms prior to mixing them into the mortar [3,11,12]. 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[3] (LWAs), polymeric membrane [13,14], microcapsules[9] and hydrogels[15]. Amongst all these approaches, LWAs and hydrogels have shown the most promising developments regarding the viability.
Up-to-date, so-called self-healing applications in cement-based materials targeted to remediate cracks induced after 28-days of casting, which can be considered as an early age application. For early age cracks, such as shrinkage cracks during casting, vegetative cells could also provide a fast response to crack formation. Therefore, considering the time frame for crack formation, importance of metabolic state becomes more critical. With a proper microbial selection and nutrient medium, 2% of the initial

bacterial inoculum were found to be viable up to 11 months after mortar mixing [16]. These approaches were conducted by using urea-yeast extract (UYE) as a nutrient for bacterial growth, however using yeast extract (YE) caused a significant delay in initial setting time [16]. Beside this negative aspect for using YE in self-healing application, it is one of the most expensive ingredients of the nutrient medium. Almost 60% of UYE cost is related to yeast extract content of it [17]. As a cheaper alternative nutrient source for bacterial growth, corn steep liquor (CSL) can be used, which is as efficient as UYE in bacterial growth and biomineralization [18]. Moreover, use of CSL as a replacement for UYE showed some improvements in urease activity and calcite production of microorganism [18].

Having very limited knowledge regarding the use of CSL in biomineralization applications in cement-based materials in terms of its impact on fresh and hardened state of concrete, challenges researchers in this field to evaluate the use of CSL in self-healing applications. This study evaluates the use of CSL as an alternative carbon source for *S. pasteurii* cells incorporated within the cement-paste matrix. We examined the influence of CSL on bacterial growth and *in-vitro* $CaCO_3$ precipitation as well as the impact of cells and their nutrient medium on the setting, strength and chemical composition of cement-based mortar. Results of this study will provide a better understanding of the influence of vegetative *S. pasteurii* cells on cement-based properties and provide insight regarding their self-healing ability.

2 MATERIALS

2.1 Microorganism growth:

Leibniz Institute- German Collection of Microorganisms and Cell Cultures: *S. pasteurii* (DSMZ 33) was used in this study. Urea-Corn Steep Liquor (UCSL) was used as the nutrient medium for bacterial growth. UCSL medium was obtained by adding tris base (0.13M), urea (10g) and corn steep liquor (15g) to a liter of DI water. CSL was provided in liquid form as a commercially available product from Sigma Aldrich and the chemical composition was not specified[19].

The media were adjusted to pH 9 by adding 0.1M HCl after the tris base was added to 1L DI water. Twelve-grams of agar per liter was added to the media when the solid medium was required. *S. pasteurii* cells were inoculated in 600 mL of UCSL medium and incubated aerobically with shaking conditions (180 rpm) at 30°C. Sample aliquots were taken from these media periodically and plated on agar plates. Bacterial growth curves were developed in terms of colony forming units (CFU/mL) vs. time. Growth experiments were conducted as triplicates.

2.2 Cement and aggregates:

CEM I 42.5R was used in this experiment[20]. For mortar samples, sand was used as fine aggregates. ASTM C128-15 Standard Test Method for Density, Relative Density (Specific Gravity) and Absorption of Fine Aggregate was used to determine the absorption coefficient of the sand [21] and ASTM C136-14 Standard Test Method for Sieve Analysis of Fine and Coarse Aggregates was used to determine the PSD of the sand [22]. Absorption capacity for the sand was found 0.67% and specific gravity was found as 2.6. Figure 1 shows the particle size distribution (PSD) for the sand and fineness modulus of the sand was 2.8.

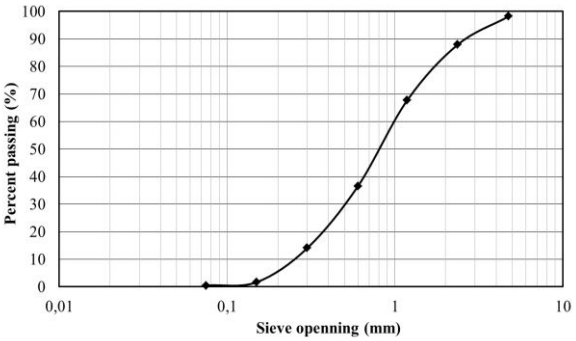

Figure 1: PSD for fine aggregate used in the mortar mix.

3 EXPERIMENTAL METHODS:

3.1 In-vitro $CaCO_3$ precipitation

To determine the influence of external $[Ca]^{+2}$ addition on MICP, 3 different compounds were used: Calcium nitrate tetra hydrate- $Ca(NO_3)_2 . 4 H_2O$ (28 g/L of nutrient medium), Calcium chloride- $CaCl_2$ (16 g/L of nutrient medium) and Calcium lactate $C_6H_{10}CaO_6 .5H_2O$ (35 g/L of nutrient medium). To induce precipitation, *S. pasteurii* cells were incubated in UCSL media and once the cells reach their exponential growth phase, $[Ca^{+2}]$ sources were added to media. After 24 hours of incubation at 30°C under shaking conditions, precipitates were collected by centrifuging (Nuve NF 800R, Ankara, Turkey) at 6300 x g for 15 minutes. To determine the crystal structure of biogenic $CaCO_3$ precipitated, a qualitative X-ray diffraction (XRD) analysis was conducted with BRUKER D8 Advance X-ray Diffractometer (Karlsruhe, Germany). In general, collected precipitates were kept in a drying chamber at 40°C for 24 hours prior to testing. Then, the samples were placed and compacted into a sample holder and analysis was conducted at angles from 10-90° 2θ at a step size of 0.02° 2θ.

3.2 Preparation of cement paste and mortar

For this study, 3 types of cement paste mixes were prepared: neat paste was prepared by mixing tap water and cement. The bacterial paste was prepared by replacing the water of the cement paste with the bacterial culture and nutrient paste was prepared by replacing the water of the cement paste with UCSL medium. The mass ratio of solution (water, UCSL media or bacterial culture) to cement (s/c) in the neat paste, bacterial paste and nutrient paste were kept at 0.45. Mortar samples were prepared by adding sand to the above-mentioned paste samples by 65% of mortar mix volume (0.45:1:3.65 by mass ratio).

3.3 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 [23]. 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.

3.4 Compressive strength testing

Mortar samples were prepared to examine the effect of microorganism on compressive strength. Five different series of mortar specimens were prepared by adding crush sand (Section 2.2) to cement pastes. The sand: cement: solution ratio was kept as 3:1:0.45. Mortar samples were prepared by ASTM C305-14 and the standard was modified by replacing the water content with UCSL medium or bacterial culture.

The mortar samples were cast in 5 x 5 x 5 cm^3 and kept in a humid environment at 22ºC for 24 hours. Then the molds were removed and the samples were further cured in UCSL medium until testing (22ºC). 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) [24] at 3,7,28 and 90 days on triplicates of samples.

3.5 Self-healing of mortar samples

Mortar samples were prepared as it was stated in section 3.4, to examine the effect of microorganism on self-healing of cracks. After 28 days, specimens were taken out from the solution and flexural cracks were induced in each beam by loading the samples in a 3-point bending test based on ASTM C348. After unloading the crack width was recorded within a range of 0.2 to 0.3 mm. Afterwards the cracked beams were submerged in UCSL medium for further curing. To analyze the impact of calcium source in self-healing mortar samples were also prepared by adding $Ca(NO_3)_2$ to the mix. The samples periodically were taken out from curing media for visualization of crack filling by light microscopy analysis (HIROX-USA, Inc KH7700 DIGITAL MICROSCOPE). The cracks were viewed under specific magnification.

4 RESULT AND DISCUSSION

4.1 *In-vitro* $CaCO_3$ precipitation

The possible use of corn steep corn steep liquor was evaluated by replacing 2% yeast extract (w/w ratio of DI water) content in *S. pasteurii* nutrient medium with 1.5% (w/w ratio of DI water) corn steep liquor. As mentioned in 2.1 Then, *S. pasteurii* was grown in 600 mL of the medium at 30°C. Figure 2 represents the growth profile for *S. pasteurii* cells in UCSL and UYE media averaged from triplicates of samples.

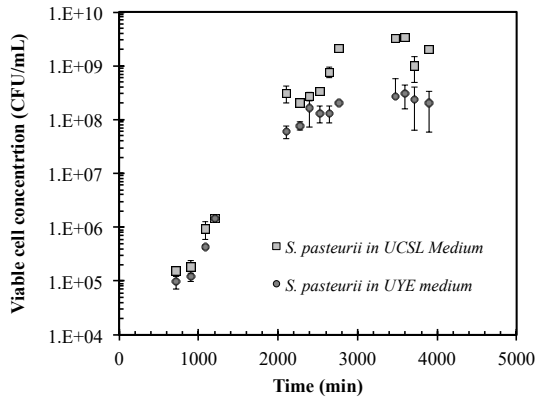

Figure 2: Representative growth profile for S. pasteurii (DSMZ 33) averaged from triplicates of viable plate counts (cell concentration vs. time) in UCSL media (pH 9) at 30°C.

Comparing to UYE medium[25], there was a substantial bacterial growth in UCSL media such that the growth profile is similar. This might indicate that corn steep liquor can be a potential replacement for UYE, since UCLS can provide the required carbon, nitrogen, amino acids and vitamins to stimulate the bacterial growth [26]. Figure 3 shows the X-Ray diffractograms for in-vitro biogenic $CaCO_3$ precipitation obtained in UCSL. Use of CaCl2 as a $[Ca^{+2}]$ source induced both biogenic calcite and vaterite precipitation in UCSL medium (pH 9). However, only vaterite precipitates were obtained from UCSL medium having $Ca(NO_3)_2$ as a calcium source. Incorporation of calcium lactate in a bacterial culture grown in UCSL medium resulted with mostly calcite crystals. According to Ogino et al.[27], calcite precipitation can be due to calcium lactate being less soluble compound comparing to other calcium sources which can interfere with the morphology of $CaCO_3$ by affecting $[Ca^{+2}]$.microorganisms were inoculated with their growth medium, the impact of nutrients to initial setting of cement paste was also evaluated.

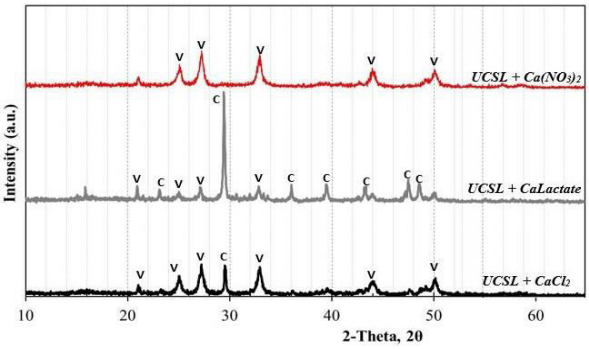

Figure 3: X-ray diffractograms of in-vitro biogenic CaCO3 induced by S. pasteurii grown in UCSL medium with 3 different calcium sources

4.2 Initial setting of cement-paste

Figure 4 represents modified Vicat needle test results. The different initial setting times were evaluated by comparing them with the reference *Neat paste* sample having an initial setting time of 5 hours. Since the microorganisms were inoculated with their growth medium, the impact of nutrients on initial setting of cement paste was also evaluated.

Previously Bundur et al. [28] showed that addition of UYE medium to cement paste significantly extend the induction period up to 14 hours. Even though, using UCSL also retarded the initial set for 7 hours, occurred delay in UCSL cement paste sample was significantly less than UYE cement paste. It should be mentioned, delay in initial setting time of samples containing viable bacterial cells can be due to use the same nutrient medium which microorganisms were grown in, to cast cement paste samples. This process can change the media due to metabolic activity of cells and influence the ionic concentration of the medium.

4.3 Compressive strength of mortar

Figure 5 shows the influence of *S. pasteurii* and nutrient media on compressive strength of mortar. *Nutrient mortar* samples exhibited a higher strength than the *Neat mortar* samples. Incorporation of S. pasteurii cells in *bacterial mortar* resulted with lower compressive strength than the *nutrient* sample, and this decrease was more pronounced by an increase in age of samples. However, using bacterial cells in

bacterial mortar samples resulted with increasing the compressive strength compared to the *Neat mortar*.

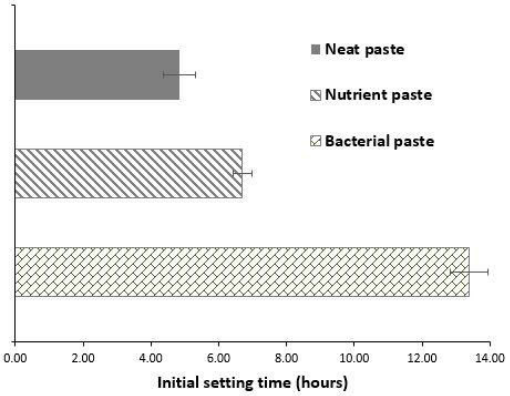

Figure 4: Modified ASTM C191-13 initial setting times of different cement paste mixes.

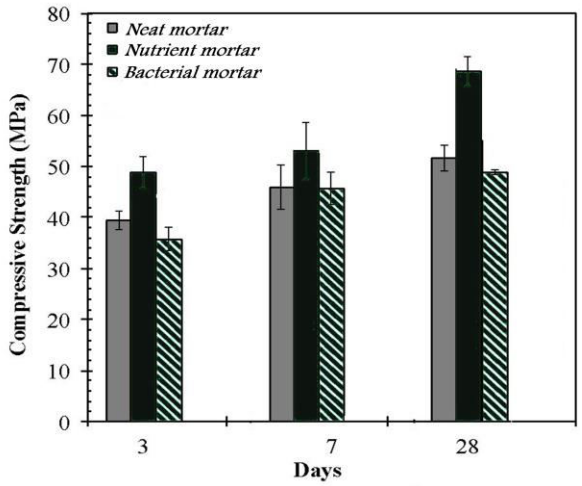

Figure 5: Influence of CSL medium and bacterial culture on the compressive strength of mortar. s/c of 0.45 was used.

Due to less carbohydrates in CSL and high amount of ammonia which increases the pH [26], nutrient mortar samples resulted with high compressive strength at 3-day age. Addition of *S. pasteurii* cells in bacterial mortar resulted with a lower compressive strength of bacterial samples comparing to nutrient samples, but did not result with a strength decrease relative to neat mortar. This can be due to composition and by-products of consumed UCSL growth medium. The proteins in CSL will degrade in nutrient mortar due to high pH and microorganism metabolic activity and can be resulted with a higher degree of protein decomposition[29].

4.4 Self-healing of mortar samples

The development of crack healing process can be observed by light microscopic images at different time intervals. Figure 6,7 and 8. are presenting visual crack remediation after 55 days of submersion in nutrient medium for three type of mortar beams.

Cracks were fully filled with calcium carbonate in bacterial mortar samples while there was visually no precipitation in neat and nutrient mortar samples. These results suggested that non-encapsulated microorganism could be able to seal the cracks as large as 0.3 mm at early ages (i.e. 7 days). Even though similar visual crack closure was observed when $Ca(NO_3)_2$ were incorporated, the amount of CaCO3 precipitated might be influenced.

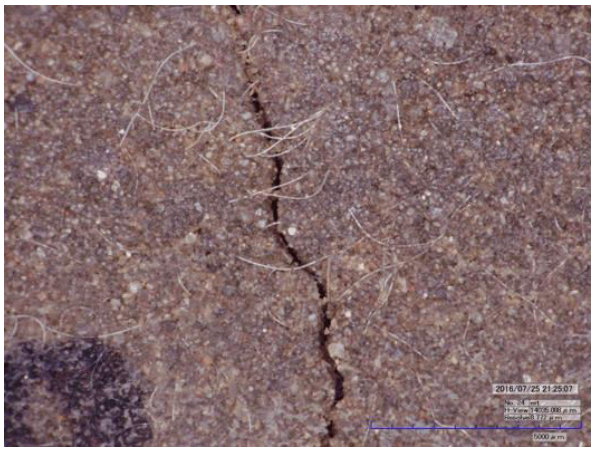

Figure 6: Neat mortar beam after 55 days of curing process.

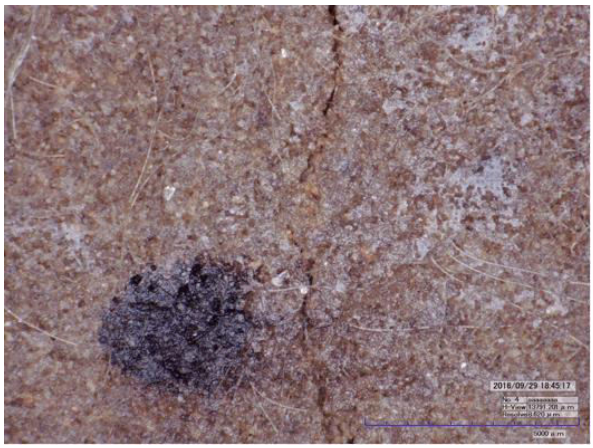

Figure 7: Nutrient mortar beam after 55 days of curing process.

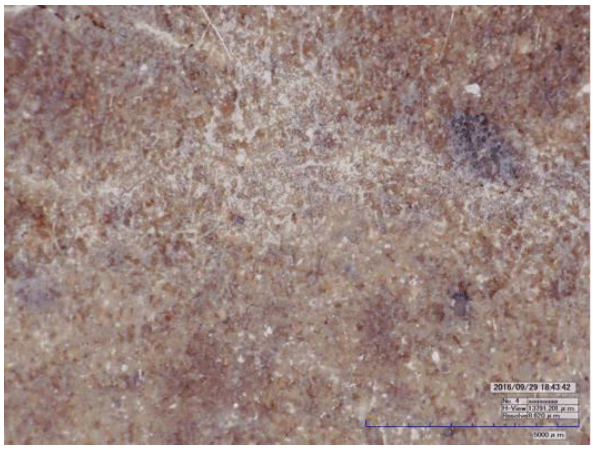

Figure 8: Bacterial mortar beam after 55 days of curing process. Cracks are filled with biogenic calcium carbonate precipitation.

While from a quantitative point of view, even though the cracks were closed there was not significant improvement in flexural strength in bacterial mortar samples. Further studies are being conducted to evaluate the impact of self-healing on permeability.

5 CONCLUSION

It can be concluded tha the use of waste material CSL did not have any negative impact on bacterial growth and supported the idea of using inexpensive waste material as a replacement for yeast extract. Incorporation of UCSL medium delayed the initial setting time of cement paste, which could be related to the carbohydrates in the nutrient medium. Moreover, this delay was found to be more pronounced when microorganisms were introduced to the mix. This might be due to the by-products of the growth process. The compressive strength of bacterial mortar samples was similar to neat mortar, whereas it was less than nutrient mortar samples. Thus, by-products of consumed UCSL growth medium not only affected the setting but also decreased the strength compared to a sample when fresh UCSL was incorporated. At last but not the least, microorganisms were able to seal flexural cracks as large as 0.3 mm but there was not any mechanical strength regain due to cracks sealing. Further studies are being conducted to evaluate the impacts of self-healing on permeability and durability.

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