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Use of corn-steep liquor as an alternative carbon source for biomineralization in cement-based materials and its impact on performance

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HIGHLIGHTS

- *In-vitro* microbial induced calcium carbonate precipitation was evaluated.
- Two different nutrient media, Urea-Yeast Extract and Urea CSL were compared.
- Use of CSL instead of yeast extract improved the problems related to initial set.
- Performance of *so called* self-mortar was improved by using microorganisms.

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ABSTRACT

Early age microcracks are generally the primary cause for a decrease in service life of cement-based structures. Recent studies suggested that it might be possible to develop a smart cement-based material that could self-heal microcracks. The use of microbial induced calcium carbonate precipitation (MICP) in cement-based materials is a novel approach to trigger self-healing and it has become an interesting field of research. MICP is a biochemical process where calcium carbonate ($CaCO_3$) precipitation is obtained via metabolic pathways for microorganism and MICP via urea hydrolysis is the most common approach used in cement-based materials. Through the literature the most commonly used nutrient media for urea hydrolysis was composed of yeast extract and urea. However, use of yeast extract as a carbon source not only resulted with a severe retardation of initial setting and it increases the cost of the application. This study investigates the suitability of corn steep liquor (CSL) as an alternative replacement of yeast extract. CSL was found to be a suitable alternative for MICP applications without compromising bacterial growth, ability to promote $CaCO_3$ precipitation. In addition, use of a nutrient medium including CSL and urea did not have such an adverse effect on initial set and compressive strength as compared to a urea and yeast extract medium.

1. Introduction

Even though concrete is one of the most used materials in the construction industry; microcracks induced due to internal stress creates a concern in the field applications of the material. These microcracks not only provide pathways for chemicals and water to penetrate, they are hard to repair. While common repair methods are sufficient to remediate macrocracks, the healing agents might not be able to fully penetrate through microcracks. Recent researches suggested that it might be possible to develop a smart self-healing cement-based material that can remediate microcracks by triggering biomineralization [1–5]. Biomineralization is a series of complex bio-chemical reactions 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) [6].

Earlier studies in the literature have shown that MICP can be used to bind non-cohesive sand particles and improve their properties under shear [7,8]. Following the applications in soil mechanics, MICP has been used in cement-based materials to remediate surface microcracks [9–12]. However, the nature of cement-based materials is much more complex compared to soil materials, thus the main challenge is to find a microorganism that can tolerate these highly alkaline conditions and survive the mixing

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process, and can remain viable with limited access to nutrients. In particular, alkaliphilic and endospore-forming microorganisms can tolerate the stresses induced within the cement-based materials. An early approach was the incorporation of *Bacillus pseudofirmus* and *Bacillus cohnii* endospores by suspending them in mixing water of mortar [3]. These endospores were found to be viable up to 4 months without any protection; however, these endospores with their nutrient sources reduced the compressive strength of mortar [3]. One of the possible reasons to observe this strength decrease due to the addition of endospores can be explained by the degradation of proteins by pH and induce the formation of air bubbles [13]. Then, concerns regarding the use 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 immobilizing the bacterial endospores in a protective covering, such as inorganic lightweight porous aggregates (LWAs) [10], polymeric membranes [14,15], hydrogels [16] and microcapsules [12]. Wiktor and Jonkers [10] used lightweight inorganic expanded clay particles to encapsulate *Bacillus alkalinitriculus* endospores and their nutrient source, calcium lactate. With this approach, the researchers could extend the viability of the bacteria; however, use of LWAs decreased the compressive strength of the material.

Wang et al. [16] proposed a biocompatible hydrogel encapsulation for *Bacillus sphaericus* endospores to induce self-healing in cement-based mortars. It has been shown that these hydrogels were able to keep the endospores viable *within* the cement paste matrix and the microorganisms were able to self-heal cracks as large as 0.5 mm within 7 days. However, the strength recovery of the cracked mortar samples was not investigated in this work. On a related note, Wang et al. [15] conducted a series of tests to determine the self-healing ability of *B. sphaericus* endospores encapsulated micro silica gel and polyurethane membranes when they were introduced through glass tubes embedded in mortar. The results showed that polyurethane membranes showed a higher self-healing efficiency compared to silica gels in terms of strength recovery and reduction in permeability [15].

Up-to-date, most of the 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 cement-based materials. In such a case, it might not be necessary to encapsulate the microorganisms. Studies have shown that with a proper microbial selection and nutrient medium, 2% of the initial bacterial inoculum remained as viable 11 months after mortar mixing [17,18]. The inoculated *Sporosarcina pasteurii (S. pasteurii)* cells were able to precipitate $CaCO_3$ within the cement paste and remediate internal microcracks [19,20]. However, incorporation of urea-yeast extract (UYE) medium significantly delayed the initial set of the cement-paste and decreased the compressive strength of the material. Even though the addition of *S. pasteurii* cells improved the compressive strength, they induced a significant delay in the initial setting time, which was more pronounced with increasing cell concentration [19]. Besides the fact that yeast extract induced a negative impact on setting and strength, it is one of the most expensive ingredients of UYE medium. Almost 60% of the total operating cost of UYE medium is due to use of yeast extract [21]. Thus, it is crucial to replace yeast extract with a cheaper alternative, which will also provide the same efficiency in bacterial growth and bio-mineralization reactions. Corn steep liquor (CSL), as being a waste product of corn industry, can be a cheap and a sustainable alternative for yeast extract. Achal et al. [22] showed a significant reduction in total cost by using CSL as a carbon source for *S. pasteurii.* Moreover, it was found that use of CSL in the nutrient medium improved the urease activity and calcite production of *S. pasteurii* cells [22,23]. However, there is a very limited information regarding the use of CSL for biomineralization applications in cement-based materials in terms of its impacts on setting, strength and chemical composition of cement-based materials.

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 *S. pasteurii* cells on cement-based properties and provide insight regarding the role of different carbon sources on development of a smart self-healing mortar.

2. Material and methods

2.1. Microorganism growth

Leibniz Institute- German Collection of Microorganisms and Cell Cultures: *S. pasteurii* (DSMZ 33) was used in this study. Two different nutrient media used in this study are UYE medium and Urea-Corn Steep Liquor (UCSL) medium.

UYE medium was composed of Tris base (15 g), urea (10 g) and yeast extract (20 g) per liter of distilled (DI) water. UCSL medium was obtained by adding Tris base (15 g), urea (10 g) and CSL (15 g) 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 by the manufacturer [24]. The media were adjusted to pH 9 by adding 0.1 M HCl after the Tris base was added to 1 L of 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 UYE medium or UCSL medium separately and incubated aerobically with shaking conditions (180 rpm) at 30 °C. Sample aliquots were taken from these media periodically and plated on agar plates. Samples for viable plate counts were serially diluted (10^0–10^{-7}); and the cell concentration was obtained by viable plate counts and represented as colony forming units (CFU/mL). Bacterial growth curves were developed in terms of CFU/mL vs. time. Growth experiments for both media were conducted as triplicates.

2.2. Cement and aggregates

Mortar samples with Ordinary Portland Cement (CEM I 42.5R) and crushed sand. 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 [25] and ASTM C136-14 Standard Test Method for Sieve Analysis of Fine and Coarse Aggregates was used to determine the particle size distribution (PSD) of the sand [26]. Absorption capacity for the sand was found 0.67% and specific gravity was found as 2.6 and fineness modulus of the sand was calculated as 2.8.

2.3. Experimental methods

2.3.1. In-vitro $CaCO_3$ precipitation

To induce *in vitro* $CaCO_3$ precipitation via MICP, the microorganisms require carbonate ($[CO_3]^{-2}$) and calcium ($[Ca]^{+2}$). In terms of reaction mechanisms, 1 mol of urea added in nutrient medium produces 1 mol of $[CO3]^{-2}$, which can react with 1 mol of $[Ca]^{+2}$ to form 1 mol $CaCO_3$. To determine the influence of external $[Ca]^{+2}$ addition on MICP, two different compounds, Calcium nitrate tetra hydrate- Ca $(NO_3)_2$. 4 H_2O (28 g/L of nutrient medium) and calcium chloride- $CaCl_2$ (16 g/L of nutrient medium), were used. To induce precipitation, *S. pasteurii* cells were incubated in both