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Role of fungal-mediated mineralization in biocementation of sand and its improved compressive strength

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ABSTRACT

The process of microbially induced calcite precipitation (MICP) is widely used recently in construction engineering in improving compressive strength, durability and self-healing of building materials and culture heritages. However, most of researches to date have concentrated on prokaryotic systems despite of associated limitation of urease-positive bacteria in biocementation. In the present study, we exploited the role of one urease-positive fungal strain *Penicillium chrysogenum* CS1 for the first time in biocementation of sand in column to produce sandstone of significant compressive strength. Further, the research provided understanding of the involved mechanisms and advantages of fungal-mediated production of calcite in cementing sand granules over same process using bacteria.

1. Introduction

All researches around the world widely report the biocementation process as 'microbially induced calcite precipitation' that could be preferably 'bacterially induced calcite precipitation' because of utilization of different bacteria mainly from *Bacillus* genera (Jimenez-Lopez et al., 2008; De Muynck et al., 2010; Achal et al., 2015; Daskalakis et al., 2015; Schwantes-Cezario et al., 2017). Microbes generally include group from bacteria and fungi; however, till so far, there is no extensive research on biocementation using fungi. Fungal-based calcite precipitation was recently reported for heavy metals remediation (Qian et al., 2017), and opens a way to utilize the same process in cementation to produce sandstone with improved engineering properties.

Advances in research in construction engineering demand need for novel fibers for the improvement in performance and durability of cementitious materials (Shirakawa et al., 2015; Simões et al., 2017). Due to non-environment friendly nature of cement, eco-friendly practices have been adopted to reduce the environmental impact (Fantilli and Chiaia, 2013). Bio-based fibers including vegetal fibers of bamboo and hemp were used to reinforce some cement-based composites, which provide advantages of being environmental friendly than synthetic fibers, and can enhance the toughness of cementitious mortars (Pacheco-Torgal and Jalali, 2011; Hamzaoui et al., 2014). In the same way, fungi have mycelial structures with biomass higher than bacteria, which could also serve as fiber in improving durability or strength of cementitious materials.

In order to show the efficiency of fungi in biocementation, the present research reports use of fungal mycelia that act as bio-based fiber in cementing sand in a column to form sandstone, for the first time. The urease-positive fungal strain *Penicillium chrysogenum* CS1 was used in the present study. Thus formed bio-based fiber was visualized under scanning electron microscopy (SEM). In addition, the biocementation process was confirmed by Fourier transform infrared spectroscopy (FTIR), where responsible functional chemical groups forming biominerals were in agreement with MICP in biosandstone, which were identified as calcite by X-ray diffraction (XRD). This is one of first few reports on the role of fungi in biocementation related research. The results of present study have important insights into the greater significance of urease-positive fungi in biocementation that can also be used to repair damaged culture heritages.

2. Materials and methods

2.1. Urease-positive fungal stain

Ureolytic fungal strain used in this study was isolated from cement sludge and identified as *Penicillium chrysogenum* CS1 based on molecular characteristics by amplifying 5′-end of the nuclear large subunit rRNA.

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Details are provided in Qian et al. (2017). In order to measure urease activity by CS1, fungal discs (d = 1 cm) grown on Malt Extract agar plates were transferred into 100 mL Modified Martin broth supplemented with 2% (w/v) urea and 40 mM $CaCl_2$ and grew in shaker at 27 °C for 11 days. Modified Martin Broth (per L) composed of 20 g of glucose, 5 g of peptone, 2 g of yeast extract, 1 g of K_2HPO_4 and 0.5 g of $MgSO_4$, pH 6.5. Supernatants were extracted using sterilized pipette at regular time interval to measure pH and urease activity. Urease activity was calculated by the amount of ammonia released from urea and expressed in terms of amount of enzyme required to hydrolyze one millimole of urea per minute (Fujita et al., 2017). The statistical analysis was performed using Origin 9.1 software program.

2.2. Biocementation in sand column

Polypropylene tubes (length = 14 cm; diameter = 4 cm) were used to prepare sandstones. In order to grow the fungal mycelia, *Penicillium chrysogenum* CS1 discs grown on malt extract agar plate were transferred on 50 ml Modified Martin Broth containing 2% urea and 40 mM $CaCl_2$, and grown at 25 °C for five days under shaking condition. The grown mycelium was macerated with the help of a tissue homogenizer. The macerated mycelia were mixed well with sterilized river sand, and the column was packed uniformly with it after placing gauze at the bottom of tube and positioned vertically with downward flow direction to avoid the generation of preferential flow paths. A control reaction in column was performed only with media (without fungal mycelia). All sand columns were fed continuously with Modified Martin Broth containing 2% urea and 40 mM $CaCl_2$ at an interval of 48 h. The experiments in all the sand columns were terminated after two weeks. Thus produced sandstones were dried at room temperature in air for 72 h and the compression testing was performed using a loading rate of 1 mm/min.

2.3. SEM analysis

In order to examine MICP in sand column from both fungal-based and control, samples were collected and mounted on aluminum stubs prior to observation under SEM. The samples on stubs were sputter coated with gold to increase the conductivity and reduce the charge of the specimen. SEM was performed with an S4800 Scanning Electron Microscope (Hitachi). The detector, accelerating voltage and spot size were secondary electron detector, 5–15 kV and 3.0, respectively.

2.4. FTIR and XRD analyses

In order to know the functional chemical groups involved in MICP in fungal sand column, Fourier transform infrared spectroscopy (FTIR) was carried out with ground sand column samples using a Thermo Nicolet Nexus 600 Spectrometer. Samples were mixed with potassium bromide in a ratio of 1:100 and compacted to provide a smooth test surface prior to testing.

X-ray diffraction (XRD) was conducted with ground sample prepared from fungal sand column (and compared with control) using PANalytical X'Pert PRO using a Cu Kα radiation nickel foil filter. The high-speed linear detector was used and diffraction patterns were recorded from 2°2θ to 80°2θ at the velocity of 2°2θ/min.

3. Results and discussion

3.1. pH and urease activity by fungal stain

The fungal strain, CS1, used in this study was identified as *Penicillium chrysogenum*. Its DNA sequence is deposited in GenBank with accession number KY818294.

The pH of calcifying Modified Martin broth was monitored to know the potential of *P. chrysogenum* CS1 in growing at higher pH, raising

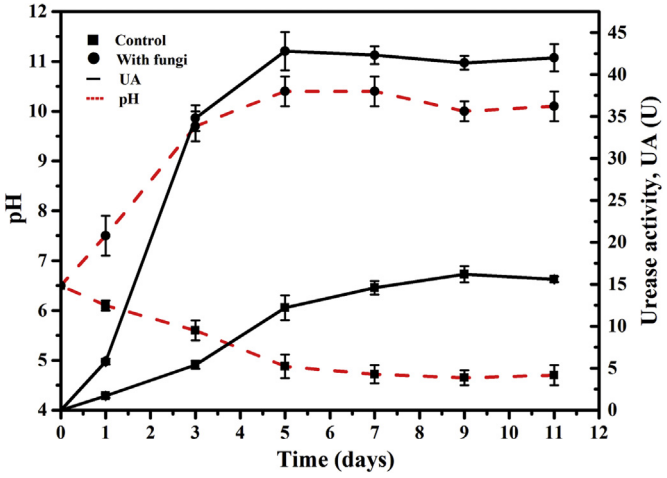

Fig. 1. Changes of pH and urease activity by *P. chrysogenum* CS1 in Modified Martin broth (control) and calcifying Modified Martin broth (with fungi).

alkalinity and thus calcite precipitation ability of fungal strain. pH values increased up to 10.4 on fifth day with no difference on day seven (Fig. 1), consistent with our previous study (Qian et al., 2017). On other hand, the pH in Modified Martin broth in the absence of urea and calcium source decreased from 6.5 to 4.71 as a result of heterotrophic respiration that led to an increase in pCO_2 and organic acids excretion during fungal growth (Bowen et al., 2007; Bindschedler et al., 2016). In calcification media where necessary substrates are provided to accelerate cementation, even excretion of organic acids, especially oxalic acid, by fungal filaments can re-precipitate secondary calcium minerals in the calcium carbonate rich environments (Verrecchia, 2000; Pinzari et al., 2010). Biodegradation of oxalate by fungal activity can also result in transformation into CO_3^{2-} leading to calcite precipitation in the pore interior (Burford et al., 2006).

The increased pH in calcification media showed the potential of *P. chrysogenum* CS1 in cementation work in building materials. There are many ammonia fungi in nature of alkaliphilic behavior such as *Paecillomyces lilacimus* and *Chrysosporium* spp., which can grow well when pH value is between 7.5 and 11.0 (Magan, 2017).

The fungal urease activity kept increasing till 7 days and decreased little during 11 days in calcification media and reached with maximum amount of 42.8 U; on other hand, urease was significantly lower in Modified Martin broth (Fig. 1). The data also showed better and constant urease activity by fungi compared to other reports on bacterially mediated calcite precipitation (Achal et al., 2009; Li et al., 2018). It is noteworthy the urease production by CS1 in calcifying Modified Martin broth was significantly higher than in same media containing heavy metals (Qian et al., 2017). The production of carbon dioxide resulting from both oxalate oxidation and fungal respiration during urease activity can enhance CO_3^{2-} concentration in the local environment and thus favor more precipitation of calcite (Luo et al., 2018).

3.2. Compressive strength

The compressive strength values of sand column prepared with fungal mycelial cells were higher than those of control sand column. In fact after removing specimens from control tubes, it was broken completely and formed a sand pile due to absence of any cementing material to bind sand granules. The compressive strength of bio-sandstone formed after cementing sand granules by fungal mycelia reached up to 1800 Kpa. The bio-sandstone was clearly visible as solid structure (Fig. 2).

Compared to the control group, the significant improvement in compressive strength of fungal sand column and thus, produced biosandstone can be attributed to deposition of microbially induced calcite