A Review on Role of Enzymes and Microbes in Healing Cracks in Cementitious Materials

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Abstract Crack is the most common damage in concrete structures and it could compromise the serviceability and durability of concrete. The environmental concerns and sustainability issues associated with cement and concrete necessitate alternative better cracks maintenance and repair strategies. Self-healing concrete has the ability to heal itself, but only is able to heal small cracks that below 0.2 mm and the improvement of crack self-healing performance becomes hotpot of research. Enzyme-induced carbonate precipitation (EICP) and microbially induced calcium carbonate precipitation (MICP) have been widely explored and applied in the improvement of construction materials. The paper briefly documents the advantages and disadvantages of bacteria/fungi-based self-healing and EICP as well as their current and potential applications. For EICP and fungi-based calcite precipitation to heal, current observations reveal that these two techniques hold beneficial prospects for crack self-healing on cementitious composites. Meanwhile, bacteria-based calcite precipitation has been most investigated in self-healing concrete, and its laboratory studies have advanced understanding of bacterial self-healing concrete. Moreover, bacterial self-healing concrete was also brought into large-scale application but it remains various challenges.

Keywords Bacteria · Biomineralisation · Concrete · Cracks · Self-healing

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1 Cracks in Concrete

Urbanization drives national infrastructure constructions growth in which concrete is the most widely used construction material. Concrete is a versatile material with distinct advantages (Wang et al. 2016b). However, due to the extensive consumption, the negative impacts of concrete industries on environment become a source of worry. Statistically, the global cement production in 2018 up to 4.1 billion metric tons and most cement is used to make concrete, mortars, or stuccos (USGS 2019). As a consequence, cement industries produced 6% of global anthropogenic CO_2 emissions (Achal and Mukherjee 2015). The later problem is concrete waste which must be responsible for 67% of the construction and demolition wastes (Gencel et al. 2020). In China, the annual generation of construction and demolition wastes has reached 2 billion tons in 2017 (He et al. 2019) while the situation of creating concrete waste in other countries are also not optimistic (Akhtar and Sarmah 2018; Rao et al. 2007; Vieira et al. 2019). Thinking about the environmental issues from cement industries and large quantities of concrete waste including energy consumption, pollutant emissions and land occupation (Marzouk and Azab 2014), although it is not possible to eliminate cement and concrete in the near future, researchers have been engaging in repairing concrete damage that aims to reduce a continued increase in cement production and concrete waste.

The concrete's susceptibility to cracking is a constant concern. As the most common damage in concrete structures, crack could compromise the serviceability and durability of concrete and then accelerate construction wastes production. Cracks damage concrete structures by increasing the penetration of aggressive substances into concrete (Wiktor and Jonkers 2011). Exactly, in the modern concrete design codes, certain cracks are acceptable in special circumstances, but the critical crack widths generally should not exceed 0.30–0.40 mm (ACI 2001; BSI 1985; CEN 2004). Micro crack may not affect structural properties of constructions, however, crack may grow wider and consequently it facilitates the activities which cause structural problems. For example, Wang et al. (2016a) studied the effect of crack width on chloride diffusion of concrete. A marginal influence on chloride diffusivity when the concrete crack width is smaller than 100 μm, but the concrete diffusivity increases rapidly with the crack width when the crack width ranges from 100 to 400 μm. Liu and Weyers (1998) reported that steel corrosion in concrete increases with an increase in the chloride content. In the worst case, the corrosion of reinforcing steel bar in concrete leads to bridges collapse, highway failures, buildings damage, etc., which brings huge economic losses, and even endangers public safety (Daniyal and Akhtar 2019). These examples make clear that the effective crack repair technologies are highly desirable to restrict the development of early age small cracks and to repair large cracks as well in order to extend the serviceability of existing concrete structures.

When cracks are detected and easy to approach, they can be repaired manually (Issa and Debs 2007; Otsuki and Ryu 2001). However, when cracks are not visible or accessible, repair becomes difficult. In addition, long-term inspection and maintenance of concrete infrastructures require tremendous manpower and high costs.

Actually, the phenomenon of self-healing in cementitious composites including concrete, has been known for many years which is mainly attributed to the further hydration of cement particles and carbonation of $Ca(OH)_2$. That's to say, cracks in cementitious composites would be repaired automatically without any human intervention. Compared to manual maintenance and repair of cementitious composites, self-healing repair is more efficient with respect to cost, accessibility or ongoing service requirement for infrastructures (Nasim et al. 2020; Van Tittelboom et al. 2012). Nevertheless, self-healing property inside cementitious composites succeeds in healing small cracks below 0.2 mm because of only small non-hydrated cement particles on the surfaces of crack (Jonkers 2011). Over the years, various concepts of self-healing cementitious composites have been developed with target on the improvement of crack self-healing performance by incorporating healing materials or adopting self-healing technologies (Choi et al. 2016; Hilloulin et al. 2015; Roig-Flores et al. 2015; Termkhajornkit et al. 2009). In particular, biological self-healing cementitious composites based on the calcium carbonate precipitation ($CaCO_3$) are environmentally friendly and have received much attention.

2 Biologically Based Self-healing Cementitious Composites

Biologically based self-healing cementitious composites can be divided into two main categories: the use of living microorganisms or free enzymes to induce $CaCO_3$ precipitation, namely microbially-induced carbonate precipitation (MICP) or enzyme-induced carbonate precipitation (EICP). Candidate MICP pathways include ureolysis, photosynthesis, denitrification, ammonification, sulfate reduction and methane oxidation (Achal et al. 2015; Baumgartner et al. 2006; Erşan et al. 2015; Krause et al. 2018; Reeburgh 2007). These different microbial metabolic pathways influence the rate of carbonate production, thereby effecting the rate of $CaCO_3$ precipitate (De Muynck et al. 2010b). To date, most extensively studied MICP is ureolysis by ureolytic bacteria and fungi. EICP method is similar to ureolytic MICP except that directly using a plant-derived urease enzyme instead of relying on microbial urease enzyme. MICP and EICP demonstrate their advantages for cracking repair. Presented herein is an overview on the application of EICP and ureolytic MICP in concrete, mainly for crack self-healing repair.

The basic premise behind ureolytic MICP and EICP is that urease enzyme catalyzes the hydrolysis of urea ($CO(NH_2)_2$) into ammonium (NH_4^+) and carbonate (CO_3^{2-}), either increasing pH. An extra source of Ca^{2+} ions react with the CO_3^{2-} leading to $CaCO_3$ precipitation which acts as a type of bio-cement to heals cracks (Eqs. 1–2). Urea hydrolysis can quickly produce a large amount of carbonate (Zhang et al. 2020) and create high alkaline environments for carbonate precipitation in shorter time, which can hardly be achieved under natural conditions (Reddy 2013). At the same time, however, a potential drawback of this reaction is that unwanted excessive ammonium production is released which would harm the mechanical integrity of cement structures (Lee and Park 2018) and bring many other environmental concerns

(Reddy 2013).

$$CO(NH_2)_2 + 2H_2O \rightarrow 2NH_4^+ + CO_3^{2-} \quad (1)$$

$$CO_3^{2+} + Ca^{2+} \leftrightarrow CaCO_3 \quad (2)$$

2.1 EICP for Potential Application of Self-healing Cementitious Composites

So far in soil improvement, EICP technique has been widely investigated. Almajed et al. (2020) investigated the influence of soil type (Ottawa 20/30, filtration, Alrasheed, and Al-Nafud) on the strength of the treated soil by using different stabilization techniques including EICP alone, ordinary Portland cement (OPC) alone or lime alone, and hybrid method (a combination of 10% OPC with an EICP cementing solution). A commercially available urease enzyme with a urease activity of round 1500U/g was extracted from jack bean meal and served as catalyst in this study. Finally, an important result was drawn that EICP without the addition of cement was able to stabilize sands successfully and EICP mixing with cement OPC in hybrid treatment methods had a negative effect on the performance of the treated soils. Additionally, EICP had satisfactory performance in fugitive dust control (Hamdan and Kavazanjian 2016; Song et al. 2020). Noteworthy, free urease enzyme is usually on a small size and water soluble, and bacterial cultures contain the big size of media ingredients and microbes. Thereby free enzyme would not bioplug the transport paths of cementing solutions in the pores of soil ahead like bacterial cultures. Ureolysis reaction could always continue, and ultimately EICP achieves more uniform cementation than MICP (Kavazanjian and Hamdan 2015).

As mentioned above, EICP eliminates the need to use microbes, thereby this process does not require extra cultivation for microbial growth, which would greatly reduce the cost of cultivation, storage and transportation, etc. in potential filed application. What's more, urease is a ubiquitous enzyme that is found in many microorganisms and virtually all plants (Das et al. 2002) and the most studied urease enzyme is extracted from *jack-bean* and *soybean.* However, current commercially available urease enzyme is expensive because of its low yield with high purity, which is one of the main barriers to the field applications of EICP for engineering purposes (Almajed et al. 2018). With the purpose of lowering the cost of EICP, Tirkolaei et al. (2020) compared units of enzyme per 1 g of raw material for crude and purified extracts from jack beans, jack bean meal, soybeans, and watermelon seeds, and evaluated the efficacy of crude urease extracts from jack beans for biocementation of a granular soil. The results suggested that the jack bean crude extract showed the highest unit yield than the other plant sources. Meanwhile, the crude extract and the less purified commercially available enzyme were actually more effective than commercially available highly purified urease enzymes at enhancing soil strength.

In EICP, ureolysis begins immediately upon contact of the enzyme with urea and thus, enables faster precipitation of carbonates, which would make EICP technique an attractive option for the maintenance and repair of infrastructures where requires ongoing service or rapid repair. Well actually, urease enzyme has been investigated to induce carbonate precipitation for cracks repair in cementitious composites. Zulfikar et al. (2021) discussed the effect of soybean as the catalyst for crack healing in concrete. The results showed that after the 4th injection, 18.3% of the crack surface area of 0.316 mm in the sample was covered by calcite, which was able to reduce the concrete permeability value by 95.43%. Dakhane et al. (2018) studied the crack healing of mortars using an EICP solution which contains jack bean urease activity of 200 units/g. All notched beams had similar crack-mouth opening displacement (CMOD), crack tip opening displacements (CTOD) and crack lengths, which were approximately 0.032 mm, 0.020 mm and 12 mm, respectively. After EICP treatments, a flexural strength enhancement of approximately 33% on the notched beams were observed. EICP-treated samples also exhibited significantly lower crack extension rate compared to the control mortar. Overall, outcomes from these previous studies reveal that EICP treatment holds beneficial prospects for crack self-healing on cementitious composites.

2.2 MICP for Crack Self-healing Cementitious Composites

Compared to EICP, MICP technique has been more extensively applied to repair cracks, especially ureolytic bacteria based repair. MICP certainly has its upsides. One of the critical factors that affect microbial calcium carbonate precipitation is the availability of the nucleation sites (Seifan et al. 2016). The microbial cell walls act as nucleation sites for $CaCO_3$ precipitation. In general, to make microbes-based self-healing concrete, the microbial spores alongside nutrients are added to concrete during the mixing process. When cracking occurs, the dormant microbes (bacteria or fungi) would be activated by crack ingress water and oxygen, etc., and then precipitate $CaCO_3$ to heal the cracks. When cracks are healed, microbes will become dormant again and be ready to start a new cycle of self-healing when cracks form again (Jin et al. 2018; Menon et al. 2019). Spores (dormant microbes) can survive in nature for long periods (Luo et al. 2018; Setlow 1994), which would likely contribute to the sustainability of microbe-based self-healing concrete. Research on self-healing cementitious composites usually refers to bacteria and fungi are neglected. Recently, it has become apparent to outline the potential and the important roles of fungi as biorepair agents in concrete structures.

2.2.1 Potential of Fungal-Based Calcite Precipitation for Crack Self-healing

Fungi are ubiquitous in nature and exert great influence on fundamental biogeochemical processes. Notably, fungi are involved in mineral formation through precipitation of organic and inorganic secondary minerals and through nucleation and deposition of crystalline material on and within cell walls, such as oxalatesand carbonates (Gadd 2007). Many fungi can grow over a very wider pH range than many heterotrophic bacteria (Gadd 2008), and often between pH 2 and 11 (Magan 2007). As mentioned earlier, available nucleation sites affect calcium carbonate production. Chitin, the structurally important component of the fungal cell walls, is capable of binding Ca^{2+} ions and forming a substrate that could considerably reduce the required activation energies for nuclei formation (Menon et al. 2019), on which calcite will readily nucleate (Gadd 2008). Moreover, compared to yeast and bacteria, fungi have mycelial structures with higher biomass (with higher amounts of extracellular enzymes and greater surface area) (see in Fig. 1) and fungal mycelium can offer the benefit of stronger mechanical resistance conferring the benefit of growth in deeper/tougher areas.

To date, the feasibility of self-healing cementitious composites with fungi via ureolytic MICP has been investigated in limited studies. Fang et al. (2018) exploited the role of one urease-positive fungal strain *Penicillium chrysogenum* CS1 in calcite precipitation leading to cementation in sand column and confirmed the enhancement of compressive strength of building material by fungal-based calcite precipitation. Fungi have the ability to resist high alkalinity, which is essential for the application of self-healing concrete. Luo et al. (2018) identified that *Trichoderma reesei* (ATCC13631) spores germinated into hyphal mycelium and grew equally well in the environment of concrete with the drastic pH increase from 6.5 to 13.0. In addition, after 28 days of curing, the matrix pore diameter sizes of cured specimens decreased to less than 0.1 μm and the porosity decreased approximately 35% compared to that of one-day cured specimens. Zhang et al. (2021), *Fusarium oxysporum* was able

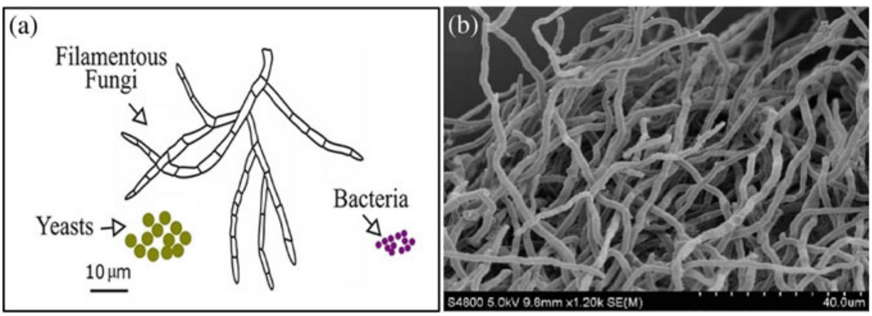

Fig. 1 **a** The main morphological and size differences between filamentous fungi, yeast, and bacteria (Jin et al. 2018); **b** Fungal mycelia grown in a medium free of urea and $CaCl_2$ (Qian et al. 2017)

to germinate spores and develop mycelium on mortar surface despite of the high pH value. And *Fusarium oxysporum* was also able to precipitate calcium minerals. Furthermore, 5% microcapsules by volume that contain fungal spores and nutrition medium was used to heal concrete cracks of 150 μm in width and 1 mm in depth across a concrete surface. Results showed that concrete surface covering with fungi mycelium showed a larger average contact angle and such larger contact would lead to higher water repellency and consequently would help reduce water infiltration into concrete through the cracks. The hydrophobicity of fungi mycelium is another advantage over bacteria when improving the durability of concrete.

2.2.2 Bacteria-Based Calcite Precipitation for Crack Self-healing

The typical ureolytic bacteria induced carbonate precipitation and bacterial self healing are depicted in Figs. 2 and 3, respectively. Bacteria produce urease enzyme and act as nucleation sites leading to calcium carbonate precipitation which eventually heal cracks. The mechanical performance recoveries of bacteria-based self-healing cementitious composites have been confirmed in many studies. Wiktor and Jonkers (2011) reported that after 100 days submersion in water, width of completely healed cracks up to 0.46 mm in bacterial concrete but only up to 0.18 mm-wide cracks in control specimens. Nguyen et al. (2019) confirmed that the concrete samples incorporating *Bacillus subtilis* 5265T showed significant self-healing and higher precipitation products. The widths of the pre-cracks were controlled between 80 and 400 μm. After 23 days of immersion in water, approximately 60% crack surfaces of bacteria-based concrete were closed; at 44 days, 400 μm crack surface width was completely filled. This is explained by the formation of microbial carbonate precipitations. In addition, compared to other control concrete, bacteria concrete exhibited better resistance to capillary suction, water absorption and total porosity and achieved decrease in chloride ions diffusion and gas permeability with a relative reduction of about 70% at 210 days.

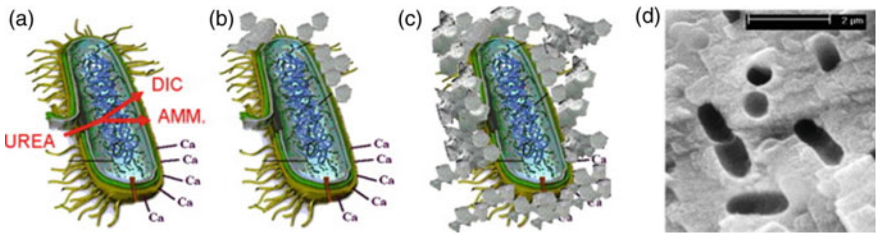

Fig. 2 Simplified representation of the events occurring during the ureolytic induced carbonate precipitation: (a) Microorganisms convert substrate urea into DIC and AMM, and release to the environment, (b) The presence of calcium ion causes the supersaturation condition and precipitation of calcium carbonate on the microbial cell wall, (c) The whole microbial cell becomes encapsulated by calcium carbonate precipitate, and (d) Shows the imprints of microbial cells involved in carbonate precipitation. DIC: Dissolved inorganic carbon; AMM: Ammonium (De Muynck et al. 2010a)

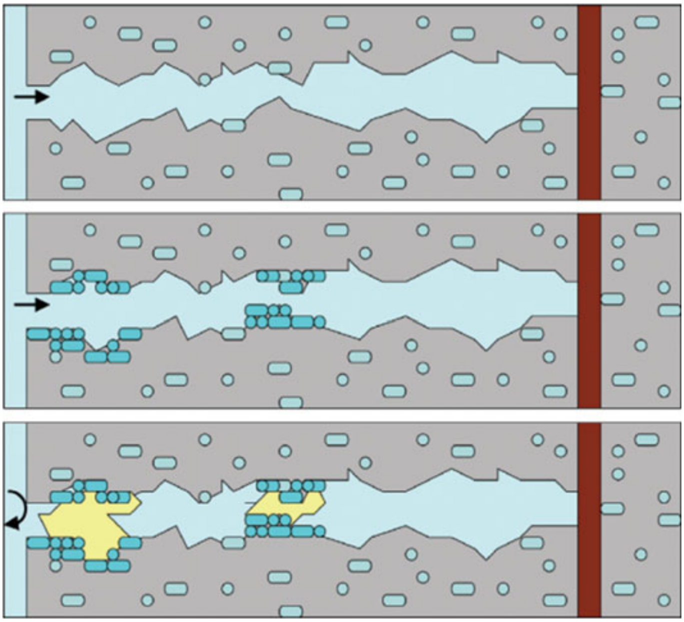

Fig. 3 Scenario of crack-healing by concrete-immobilized bacteria (Jonkers 2007)

Up until now, most researches were focused on small lab-scale bacterial self-healing and a few case studies can be found to demonstrate the self-healing efficiency in an in situ real life structure. Van Mullem et al. (2020) added bacterial healing agent (MUC^+) together with nutrients to a concrete mix and then bacterial concrete was cast into a roof slab for an inspection pit as well as accompanying lab specimens. After loading of specimens, the crack width was wider than 300 μm. Results showed that comparing the crack closure results of the different exposure conditions, the best results were obtained for W/D cycles, followed by water submersion and moist environment (>90% RH). When the specimens subjected to W/D cycles, the maximum mean crack widths for which a closure of 100% was measured were: 61 μm (top), 72 μm (side), and 245 μm (bottom). After healing, the capillary water uptake of the cracked specimens decreased slightly. After 27 weeks of submersion, the sealing efficiency (SE) of five samples is at least equal to 90% and even higher than 98.5%, resulting in an average SE of 96.7%.

Due to all the challenges ahead, application of self-healing in the field is rather limited. Bacteria are expected to precipitate calcium carbonate and they are usually introduced directly into concrete and mortars during the mixing process or have been immobilized into carrier materials along with nutrients. As mentioned earlier, spores (dormant microbes) can survive in nature for long periods. However, when bacterial spores were directly added to the concrete mixture, the high alkaline environment or other harsh conditions in concrete matrix affect the survival of bacteria. As a result, their survival time shorten to one-two months (Jonkers et al. 2010). In addition, with continuous decrease in pore size diameter due to ongoing cement hydration, bacteria cannot sustain life when directly embedded in concrete matrix and then appears limited life span. Therefore, protective carriers have been developed to encapsulate bacteria with their nutrients when they are added to cementitious structures. However, there are still some factors that should be take into account, such as biocompatible, mechanical strength and other mechanical properties of carriers, or unwanted loss of concrete properties (Lee and Park 2018). On the other hand, bacterial self-healing concrete is distinguished by its potential for environmental friendliness, but excessive ammonium is released in ureolytic MICP process. Non-ureolytic bacteria have been explored in cementitious composites self-healing to eliminate this drawback. Fahimizadeh et al. (2020) applied *Bacillus pseudofirmus*, an alkalophilic aerobe non-ureolytic bacterium, for self-healing of cement paste and mortar. CaAlg capsule was used as protective carrier to immobilize bacterial spores for the survival of the bacteria inside the cement environment. After 56 days of exposure to wet–dry conditions, the capsules successfully healed small cracks in the cement paste and mortars with a flexural strength recovery of 39.6% in cement mortars and 32.5% in cement paste. Overall, although laboratory studies have advanced understanding of bacterial self-healing concrete, field assessments are needed and some important aspects such as environmental factors, cost and biological factors, will be taken into account.

3 Conclusion

Compared to conventional technologies for cracks repair, biogenic $CaCO_3$ has the distinct advantages of environment friendliness. This paper has reviewed that the use of planted-derived urease enzyme and urease producing microbes (namely fungi and bacteria) in healing of cracks in cementitious composites. Planted-derived urease enzyme and fungi can potentially act as a self-healing agent in cementitious composites and they have their own advantages. EICP eliminates the need to use microbes and enables faster precipitation of carbonates. Fungi have mycelial structures with higher biomass (with higher amounts of extracellular enzymes and greater surface area). However, a few case studies can be used to identify the efficiency of EICP and fungal-based calcite precipitation for crack self-healing. Therefore, further research work is needed to obtain consistent repair characteristics. Of the three, bacteria have been applied to repair cracks, both in the laboratory and in the field, but its field application cases are limited. Field assessments are lacking as well and some important aspects must be taken into account in future research.

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