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Non-Ureolytic Microbially Induced Calcium Carbonate Precipitation in Self-Healing Concrete: A Review

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Abstract. Non-ureolytic microbially induced calcium carbonate precipitation provides a possible route for concrete crack healing without using urea hydrolysis. It has attracted increasing interest in self-healing concrete research, especially in relation to environmental compatibility and sustainable healing strategies. This review outlines the biological cementation basis of MICP in cementitious materials and discusses the main non-ureolytic pathways, including oxidation of organic calcium salts, denitrification, and carbonic-anhydrase-mediated mineralization. Their mechanisms, engineering characteristics, and applications in self-healing concrete are examined. Existing studies suggest that these pathways can promote crack filling and improve resistance to substance transport, although the healing effect is still strongly influenced by bacterial survival, nutrient conditions, carrier protection, crack size, and local transport conditions. Several issues remain unresolved, including limited mineralization efficiency, insufficient system stability, and inconsistent evaluation methods. Further development of non-ureolytic MICP for self-healing concrete will depend on more suitable pathway design, more sustainable precursor selection, more effective carrier systems, and validation under practical service conditions.

Keywords: Self-healing concrete, non-ureolytic MICP, biomineralization, durability

1. Introduction

Concrete is the dominant construction material in modern infrastructure, yet its limited tensile resistance makes cracking unavoidable during service. Cracks generated by shrinkage, thermal stress, mechanical loading, and environmental actions create preferential pathways for water, chloride ions, carbon dioxide, and sulfate-bearing solutions. Once these aggressive agents penetrate the matrix, matrix degradation, and long-term durability loss are markedly accelerated [1,2].

To mitigate such deterioration, self-healing concrete has been proposed as a material-based damage management strategy that can restore part of the functionality of cracked concrete without repeated external intervention. De Belie et al. [1] reviewed the development of self-healing concrete and showed that existing approaches generally include autogenous healing, capsule-based systems, vascular networks, expansive or crystalline admixtures, and bacteria-based healing. Among these approaches, microbial self-healing has attracted particular interest because it promotes in situ calcium carbonate precipitation within cracks, thereby improving crack closure, transport resistance, and durability-related properties [1-5].

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Most early studies on bacteria-based self-healing concrete focused on ureolytic metabolism because of its rapid carbonate production and high mineralization efficiency. Nodehi et al. [2] systematically summarized bacteria-based self-healing concrete and highlighted that ureolytic systems remain the most extensively studied route in terms of biomineralization efficiency and durability improvement. However, ureolysis is accompanied by ammonia-related by-products, which raises concerns regarding environmental compatibility and long-term sustainability [6,7].

In response to these limitations, increasing attention has been directed toward non-ureolytic MICP. Instead of relying on urea hydrolysis, non-ureolytic mineralization can proceed through pathways such as the oxidation of organic calcium salts, denitrification, and carbonic-anhydrase-mediated carbon dioxide hydration. Justo-Reinoso et al. [6] reviewed aerobic non-ureolytic bacteria-based self-healing cementitious composites and emphasized the environmental advantages of this route. Wong et al. [1] further pointed out that non-ureolytic systems are attracting renewed interest because they may offer improved sustainability, broader chemical compatibility, and new possibilities for pathway-oriented healing design.

Although the concept of non-ureolytic MICP has become increasingly visible, the available literature is still fragmented across different mineralization pathways, bacterial systems, nutrient designs, and evaluation methods. A focused review is therefore needed to clarify the biological cementation basis of non-ureolytic MICP, its principal mineralization modes, its healing performance, and the major barriers that still constrain engineering application. On this basis, the present review discusses the fundamental requirements of MICP-based self-healing concrete, examines the mechanisms and characteristics of non-ureolytic pathways, and summarizes current challenges and future research directions.

2. Biological cementation basis of MICP-based self-healing concrete

2.1. Self-healing concrete and the position of MICP

Self-healing concrete refers to a class of cementitious materials capable of recovering part of their functionality after cracking through internal healing mechanisms. From a general perspective, self-healing strategies are commonly classified as autogenous or autonomous. Autogenous healing mainly arises from continued hydration, carbonation, and the natural filling of fine cracks. Autonomous healing, by contrast, depends on added healing agents or intentionally designed triggering systems incorporated into the material [1,2].

As summarized by De Belie et al. [1] autonomous approaches may include encapsulated polymers, hollow tubes, superabsorbent polymers, crystalline admixtures, and biological healing agents. Within this wider framework, MICP has a special position because it is based on biologically driven mineral formation rather than simple release of a sealing material. Calcium carbonate precipitation within cracks not only narrows crack openings, but may also densify the damaged region and improve durability-related transport behavior [2].

For this reason, MICP has attracted particular attention in durability oriented concrete design. Nguyen et al. [8] demonstrated that bacterial self-healing can help recover transport-related performance, including permeability-associated properties. Accordingly, MICP should be understood not merely as a crack-filling technique, but as a bio-mediated cementation process that can partially restore the protective function of the cementitious matrix. Table 1 summarizes the main self-healing approaches used in concrete and illustrates the distinctive role of MICP as a biologically induced mineralization strategy.

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The increasing interest in non-ureolytic MICP is closely linked to this broader view of self-healing. When microbial mineralization is intended to support durable and sustainable concrete systems, the metabolic pathway itself becomes an essential part of material design. This is also why recent reviews have increasingly separated non-ureolytic routes from the traditional ureolytic framework [6,7].

Table 1. Main self-healing strategies for concrete and the position of MICP

Strategy	Main principle	Main advantages	Main limitations	Ref.
Autogenous healing	Continued hydration of unhydrated cement particles, carbonation of $Ca(OH)_2$, and natural filling of fine cracks by hydration products	No additional healing agent required; simple material system	Effective mainly for very small cracks; strongly dependent on moisture and service conditions	[1,2]
Capsule- or carrier-based healing	Encapsulated polymers, bacterial spores, or other healing agents are released after cracking	Triggered healing response; potentially efficient local crack sealing	Additional phases may reduce workability or early-age properties; preparation can be complex	[1,3,9]
Crystalline or mineral-assisted healing	Crystalline admixtures, expansive components, or mineral-based additives promote crack filling and densification	Good compatibility with cementitious matrices; useful for permeability reduction	Healing efficiency depends on crack width and exposure conditions; repeatability may be limited	[1,2]
MICP-based healing	Microbial activity induces $CaCO_3$ precipitation inside cracks, leading to filling, densification, and recovery of transport resistance	Combines crack sealing with durability improvement; biologically driven mineralization is attractive for sustainable design	Sensitive to bacterial viability, carrier protection, nutrient delivery, and crack environment	[2,5,6]

2.2. Conditions required for effective MICP-based self-healing

The effectiveness of MICP-based self-healing depends on a chain of coupled processes rather than on bacterial addition alone. These processes include bacterial survival during mixing and hardening, reactivation after cracking, access to water and nutrients, production of carbonate species, calcium carbonate nucleation, and stable crystal growth within the crack region [2,6,10]. If any of these steps is interrupted, the expected healing effect is significantly reduced.

A prerequisite for successful healing is the selection of microorganisms that can tolerate the harsh cementitious environment. Fresh and hardened cement-based materials are characterized by high alkalinity, limited nutrient supply, low pore connectivity, and mechanical disturbance during mixing. Lee and Park [11] pointed out that these conditions are highly unfavorable for microbial survival, which explains why spore-forming bacteria are often preferred in self-healing systems. Their dormant form offers greater resistance to alkaline and nutrient-poor conditions before reactivation is triggered by crack formation and moisture ingress.

Bacterial protection through carrier systems is equally important. Wiktor and Jonkers [4] embedded bacteria and nutrients into expanded clay particles and quantified crack healing in bacteria-based concrete, demonstrating that porous carriers can provide both protection and storage. Wang et al. [3] developed a microencapsulation strategy using bacterial spores and showed that

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encapsulation could preserve biological activity during mixing and release healing agents upon cracking. Fahimizadeh et al. [12] further reported that non-ureolytic bacteria encapsulated in alginate hydrogel capsules were able to induce healing in cement paste and mortar. These studies indicate that carriers are not merely containers; they actively influence bacterial survival, nutrient localization, and healing activation.

The nutrient system and calcium source are equally important for efficient MICP in cement-based materials. In non-ureolytic systems, organic calcium salts such as calcium lactate and calcium acetate are widely studied because they can function both as metabolizable substrates and as calcium containing precursors. Tziviloglou et al. [13] compared different nutrients used in biogenic healing agents and showed that nutrient choice has a direct effect on bacterial metabolism and healing performance. Xu et al. [14] also demonstrated that non-ureolytic bacterial carbonate precipitation can be effectively induced on cementitious materials, underlining the importance of matching nutrient chemistry with the relevant microbial metabolic pathway.

The crack environment itself further controls healing performance. Khan et al. [10] reviewed the main factors affecting bacteria-based self-healing and pointed out that crack width, crack age, moisture supply, oxygen access, and curing conditions all have strong effects on healing behavior. Wong et al. [7] reached a similar conclusion and emphasized that future system design should optimize bacteria, carriers, nutrients, and environmental compatibility in a coordinated manner. This issue is particularly important for non-ureolytic systems, whose mineralization routes are often more sensitive to local transport conditions than ureolytic ones.

3. Non-ureolytic MICP pathways: mechanisms and characteristics

3.1. Mechanistic difference between ureolytic and non-ureolytic routes

Ureolytic MICP is based on urease-catalyzed hydrolysis of urea, which rapidly generates carbonate species and increases local alkalinity. Owing to this kinetic advantage, ureolytic systems have historically dominated bacteria-based self-healing research. Nodehi et al. [2] summarized numerous studies showing that ureolytic mineralization can significantly enhance crack sealing and durability-related performance.

Non-ureolytic MICP differs from this route at the metabolic level. Carbonate generation is achieved without urea hydrolysis and instead proceeds through alternative biochemical or enzymatic pathways. Justo-Reinoso et al. [6] reviewed the state of aerobic non-ureolytic systems and clarified that this family of methods includes the oxidation of organic calcium salts and related metabolic routes. Wong et al. [7] further noted that non-ureolytic systems are increasingly valued because they avoid ammonia-related by-products and offer greater flexibility in precursor design.

3.2. Main non-ureolytic mineralization modes

3.2.1. Oxidation of organic calcium salts

The aerobic oxidation of organic calcium salts is among the most representative non-ureolytic pathways in self-healing concrete. In this route, bacteria metabolize compounds such as calcium lactate or calcium acetate, releasing carbon dioxide that is subsequently converted into bicarbonate and carbonate species, which then combine with calcium to precipitate calcium carbonate.

Wiktor and Jonkers [4] quantified crack healing in bacteria-based concrete and showed that bacteria embedded in lightweight expanded clay particles could effectively promote crack closure.

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Xu and Yao [15] further demonstrated, through multiscale mechanical assessment, that non-ureolytic bacteria-based healing agents can improve the recovery of self-healing concrete. Xu et al. [14] also reported that non-ureolytic bacterial carbonate precipitation can be applied as a surface treatment for cementitious materials, suggesting that this route is useful not only within cracks but also at exposed cement-based surfaces.

Tziviloglou et al. [13] showed that nutrient selection affects both metabolic activity and healing efficiency. Rossi et al. [16] investigated a precursor derived from organic waste streams and demonstrated the feasibility of integrating bacteria-based self-healing with resource-recovery concepts. Tan et al. [17] proposed an aerobic non-ureolytic system without an externally added calcium precursor, indicating that the cementitious matrix itself may provide enough calcium under suitable conditions.

Overall, these studies suggest that mineralization driven by organic calcium salts is not only technically achievable, but also compatible with low-chloride and potentially waste-derived healing systems, which increases its appeal for sustainable concrete applications.

3.2.2. Denitrification-driven mineralization

Denitrification represents another important non-ureolytic route because it allows bacterial metabolism to continue under oxygen-limited conditions. This feature is especially relevant for internal crack zones, deep crack segments, or moisture rich regions where oxygen diffusion is restricted.

Erşan et al. [18] developed a self-protected nitrate-reducing culture for intrinsic crack repair in concrete and showed that denitrification-based systems can operate without the strong oxygen dependence associated with aerobic pathways. Their results demonstrated that nitrate-reducing enrichment cultures can be incorporated into mortar and can still promote crack repair effectively. This finding is significant because it broadens the applicability of non-ureolytic MICP beyond cracks directly exposed to air.

From an engineering standpoint, denitrification-based mineralization is particularly valuable when aerobic activity cannot be maintained easily. At the same time, the pathway introduces additional variables, such as nitrate supply, redox environment, and control of metabolic by-products. Denitrification should therefore not be treated simply as an alternative copy of aerobic healing, but rather as a route suited to specific crack environments within the material.

3.2.3. Carbonic-anhydrase-mediated mineralization

A further non-ureolytic pathway involves carbonic anhydrase, an enzyme that accelerates the hydration of carbon dioxide and thus promotes the formation of bicarbonate species in the aqueous environment of cracks. Qian et al. [19] showed that carbonic-anhydrase-producing microorganisms can promote the self-healing of early-age cracks in cement-based materials through biomineralization. Their study also revealed that crack width and cracking age strongly affect healing performance, which indicates that the efficiency of this route depends heavily on transport conditions and crack accessibility.

The importance of this pathway lies in its direct connection with carbon dioxide conversion. Unlike mineralization routes driven by urea hydrolysis, carbonic-anhydrase-mediated healing relies on enhanced carbon dioxide hydration followed by mineral deposition. This gives the pathway a distinct low-carbon and environmentally favorable character, although its performance still remains sensitive to mass transfer and internal crack geometry.

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3.3. Engineering significance of non-ureolytic pathways

The engineering significance of non-ureolytic MICP lies mainly in the possibility of pathway-specific healing design. Aerobic oxidation of organic calcium salts is more suitable for cracks where oxygen is readily available, whereas denitrification becomes more advantageous in internal or moisture-rich crack regions where oxygen diffusion is limited. Carbonic-anhydrase-mediated mineralization is particularly attractive when the objective is to link healing with carbon dioxide conversion.

Justo-Reinoso et al. [6] emphasized the environmental advantages of aerobic non-ureolytic systems over ureolytic ones, while Fahimizadeh et al [9]. highlighted the wider multifunctional and sustainable potential of non-ureolytic self-healing systems. Wong et al. [7] further argued that the future of microbial self-healing concrete lies in better integration of bacterial pathways with service conditions and material design.

At the same time, current studies make it clear that non-ureolytic pathways are not universally superior. Their mineralization rates are often slower, and their performance is more strongly affected by local transport conditions, nutrient supply, and bacterial survival. The practical value of non-ureolytic MICP should therefore be understood not as a straightforward replacement for ureolysis, but as an expansion of the design options available for biologically driven self-healing systems.

4. Conclusions and future perspectives

4.1. Conclusions

Non-ureolytic MICP has emerged as an important direction in self-healing concrete because it enables biologically induced mineralization without relying on urea hydrolysis. Compared with conventional ureolytic systems, its main advantage lies in the potential for lower environmental impact and broader compatibility with sustainable healing-agent design. Existing studies have shown that non-ureolytic pathways, including the oxidation of organic calcium salts, denitrification, and carbonic-anhydrase-mediated mineralization, can promote crack sealing and contribute to the recovery of transport resistance in cementitious materials.

At the same time, the current body of research indicates that non-ureolytic MICP is still at a developing stage. Its healing efficiency is strongly influenced by bacterial viability, nutrient supply, crack geometry, and mass transport conditions within the cementitious matrix. As a result, its performance remains less stable and less predictable than that of more mature ureolytic systems. The value of non-ureolytic MICP therefore lies not in simply replacing ureolytic approaches, but in broadening the design space of bio-based self-healing concrete.

4.2. Future perspectives

Future research should focus on improving the practicality and stability of non-ureolytic self-healing systems. One important direction is the development of more robust microorganisms and pathway-specific healing strategies that can function effectively under realistic crack environments, especially in deeper or oxygen-limited regions. Another promising direction is the design of more sustainable healing agents, including chloride-free nutrients, waste-derived precursors, and systems with reduced dependence on externally added calcium sources.

Further progress also requires more consistent evaluation methods. A clearer framework for comparing healing efficiency, transport recovery, and long-term durability would help identify the

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true engineering potential of different non-ureolytic pathways. In the long term, the advancement of non-ureolytic MICP will depend on closer integration among microbiology, cement chemistry, carrier design, and structural durability assessment. With such progress, non-ureolytic MICP is expected to become a more competitive and sustainable strategy for self-healing concrete.

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