Review Article
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Potential of Genetically Engineered Strains to Enhance Concrete Strength: A Review

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Abstract

The durability of concrete is limited by the gradual development of microcracks. Their appearance leads to a decrease in performance properties, affecting water resistance, frost resistance and strength. One promising approach to reducing the rate of microcrack development is the use of spore-forming bacteria capable of precipitating calcium carbonate in the environment. When water enters concrete cracks, bacterial spores germinate and initiate mineralization, facilitating partial restoration of the material's structure. The first experiments on producing bioconcrete were conducted in the mid-2000s and demonstrated the ability of bacteria to form calcite particles up to 100 μm in size and seal microcracks. Concrete has a limited capacity for self-healing through atmospheric carbonation, in which CO_2 reacts with calcium hydroxide to form calcite; however, this process is slow and unpredictable. In most biotechnological systems, $CaCO_3$ precipitation is enhanced by the enzymatic hydrolysis of urea, catalyzed by the enzyme urease. In addition to the urease pathway, alternative mechanisms for urea degradation to form carbonates are also known. For example, the amidolyase pathway, in which allophanate is formed from urea, and in a subsequent reaction, allophanate hydrolase decomposes it to ammonia and bicarbonate. Furthermore, with elevated CO_2 content in microcracks, bicarbonate can be produced by carbonic anhydrase. However, in the surface layers of concrete exposed to the atmosphere, this approach is apparently impossible due to the reverse reaction as the dehydration of bicarbonate to CO_2, which will escape into the atmosphere. This publication hypothesize that the combined use of urease and carbonic anhydrase may yield better results in concrete stabilization due to synergy. Both enzymatic reactions produce hydrocarbonates, but CO_2, the product of the first reaction, becomes the substrate for the second. The creation of new genetically engineered strains with genes encoding enzymatic systems based on the described pathways can increase the efficiency of mineralization and improve the performance properties of bioconcrete.

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1 Introduction

Systematic research into engineered self-healing concretes commenced in the early 1990s. The discipline accelerated during the 2000s through advances in biological and chemical healing agents, followed by rapid expansion of investigations starting in the 2010s [1]. Reviews [2] and [3] indicate that self-healing concrete seals internal damage such as cracks without external repairs. Review [4] examines autonomous self-healing technology based on biological mechanisms for crack closure. The review process encompassed four phases: examination of global prior research, ecological and economic evaluations, consideration of future directions and benefits, and final conclusions. This analysis incorporated 257 publications sourced from approximately 10 international databases [4].

A comparable technique applied encapsulated rejuvenators for in-situ healing of asphalt concrete cracks to improve intrinsic self-repair capacity [5]. Encapsulated rejuvenators proved viable for asphalt repair, raising healing temperature and duration to achieve up to 80% healing efficiency [6]. Study developed self-healing concrete incorporating low-calcium fly ash and partial replacement with recycled aggregate, which reduced overall strength [5]. Accumulated data from self-healing concrete research now supports application of machine learning techniques to forecast material properties [7] and optimize designs, complemented by fractal analysis methods [8].

Experimental study [9] evaluates how different calcite-to-sand ratios affect mechanical properties and microstructure in self-healing concrete, utilizing Hay Bacillus to trigger calcite formation. Results from identify *Bacillus subtilis* [10] as the most effective bacterial strain, with a 3% concentration enhancing beam load-bearing capacity by 20.2%. *Bacillus cohnii, Bacil-lus halodurans,* and *Bacillus pseudofirmus* enhances its performance properties in both strength and service life of bioconcrete [11].

Further development of self-healing concrete, also known as bioconcrete, requires microbiological testing before it can be carried out. Due to the aggressive environment of concrete mixes, spore-forming bacteria, which can survive these conditions, are most often used. The main property of these strains is their ability to initiate calcium carbonate mineralization in microcracks that form in concrete when water penetrates them. Bacteria with urease activity are primarily used for this purpose. Urease catalyzes the hydrolysis of urea to form carbon dioxide, which, after exiting the cell, promotes the precipitation of calcium carbonate in an aqueous environment [12].

Although culturable bacterial strains with high urease activity already exist, carbonate formation can be improved by enhancing this function through the addition of accessory proteins or by using alternative urea hydrolysis pathways. This work aimed to review the bacterial metabolic pathways and enzymes used for biomineralization, as well as other promising enzymes that could accelerate existing pathways or exploit a different principle of calcium carbonate precipitation.

2 Literature Search Methods

A systematic literature search was conducted to identify relevant studies on bioconcrete, bacterial biomineralization, and enzymatic pathways for calcium carbonate precipitation. The search encompassed multiple bibliographic databases, including Scopus, and PubMed to ensure comprehensive coverage of interdisciplinary fields such as biotechnology, materials science, and civil engineering. The time frame for the search was limited to publications from 1995 onward, marking the year when the crystal structure of the urea transporter protein (UT) was first determined [13], which laid foundational insights into urea transport mechanisms relevant to this review. The search was updated through December 2025 to incorporate the most recent advancements.

Keywords and phrases were selected based on core concepts from the field, including "bioconcrete," "self-healing concrete," "calcium carbonate precipitation," "urease," "urea amidolyase," "carbonic anhydrase," "Bacillus subtilis," "Sporosarcina pasteurii," "bacterial biomineralization," "genetically engineered bacteria," "microcrack repair," and "enzymatic mineralization." Boolean operators and advanced search techniques were employed to refine results. For example, in Scopus, queries such as

TITLE-ABS-KEY ((bioconcrete OR "self-healing concrete" AND bacteria AND (urease OR amidolyase OR "carbonic anhydrase")) AND PUBYEAR > 1994

were used to target studies on bacterial enhancements in concrete. Another query focused on alternative pathways:

TITLE-ABS-KEY ((urease OR "urea hydrolysis" OR amidolyase OR "allophanate hydrolase" OR "carbonic anhydrase") AND "calcium carbonate" AND (concrete OR cement)) AND PUBYEAR > 1994.

The initial search yielded approximately 1,200 unique records after deduplication using tools like Zotero. These were screened based on title and abstract relevance, with full-text reviews conducted for 250 articles meeting inclusion criteria (e.g., empirical studies or reviews on bacterial enzymatic systems for concrete applications). To map research connections and identify clusters of related work, the bibliographic data (including keywords, citations, and authors) were analyzed and visualized using VOSviewer software (version 1.6.20). This tool generated network maps of keyword co-occurrence and citation networks, highlighting thematic clusters such as urease-dominated biomineralization and emerging amidolyase pathways, thereby illustrating the evolution and interconnections within the field.

3 Literature Review

3.1 Genes encoding urease and accessory proteins

The primary method for producing calcite is the release of carbon dioxide in an aqueous environment during the enzymatic breakdown of urea by urease, as it reviewed in [14], [15], [16] and [17]. Bacterial urease is a nickel-containing enzyme consisting of three subunits: UreA, UreB, and UreC. UreC contains a catalytic center containing two ions Ni^{2+}, without which the enzyme is inactive [18], [19], and [20].

For the correct assembly of the urease complex, chaperones are used, namely the proteins UreD, UreE, UreF and UreG, which ensure the delivery of nickel ions to the catalytic center of UreC [12,21,22]. These accessory proteins form a complex to facilitate Ni^{2+} insertion into the dinuclear active site in UreC, preventing toxic free nickel release in the cell. UreE acts as a metallochaperone, UreG as a GTPase, UreF as a regulator, and UreD (or UreH ortholog) as a scaffold [18], [23], [24]. The assembly starts with UreD (or UreH) binding apo-urease, followed by sequential recruitment of UreF and UreG (sometimes as a preformed complex), with UreE interacting last for nickel handover [25], [26], [27]. This assembling creates a supercomplex for activation.

UreG's GTP hydrolysis induces a conformational shift (e.g., dimer dissociation), enabling Ni^{2+} release from UreE-bound complexes and transfer through a tunnel-like path in the accessory complex to UreC. This transfer couples energy from hydrolysis to metal insertion [27], [25], [26], [18].

It is believed that UreD first binds to apo-urease and forms an initial complex, which is subsequently joined by UreF, UreG, and the nickel-binding metallochaperone UreE, which carries nickel ions [12,22]. During urease maturation, UreG hydrolyzes GTP and changes into a different conformational form, thereby catalyzing the transfer of nickel ions from UreE to the active site of urease, located in UreC. [12,28]. The maturation process involves sequential or modular assembly of accessory proteins onto apo-urease (the inactive form lacking nickel), starting with UreD (or UreH) as a scaffold that induces conformational changes, followed by UreF recruitment to form UreDF, and then UreG to complete the UreDFG pre-activation complex. This order is evidenced by affinity pull-downs, cross-linking, and structural analyses showing UreD's initial interaction prepares the enzyme for subsequent bindings [25], [29], [30]. The research [31] confirms UreH [UreD] and UreF form initial complex with apo-urease before UreG joins. The article [27] states that UreD and UreF can form a UreFD complex, which can then recruit UreG to form a UreGFD complex and interactions start with UreD-apo-urease. Early evidence for UreD-UreF-UreG-apourease complex formation, implying sequential assembly, was published in [32].

However, strains used in practice that can synthesize urease may lack chaperone genes *ureD*, *ureE*, *ureF*, *ureG* and *ureD*. For example, in *B. subtilis*, the genome contains only the genes for the main enzyme, urease *ureABC*, but it is lower than in organisms with a full set of genes, which indicates the presence of alternative pathways for urease maturation [22].

3.2 Urea transport

One of the bottlenecks in increasing urease activity is the rate of urea transport across the membrane. No urea transporters were found in the genome of *Sporosarcina pasterii*, which is one of the most well-known bacteria used to produce bioconcrete [33]. Several kinetic and physiological studies indicate that urease activity may be limited by the transport of urea across the cell membrane [34,35].

Therefore, to increase urease activity, urea transporters should be used. Bacteria have several types of transport systems for pumping urea into the cell. These include UreI – a proton-dependent channel from the bacterium *Helicobacter pylori*. It helps bacteria survive in acidic conditions with pH 4-6

and is inactive in the alkaline environment of concrete mix with pH 11-14, so it has no practical value for bioconcrete pH [36,37]. More promising proteins for creating strains with increased urease activity are membrane transporters of the UT (urea transporter) family, which facilitate the diffusion of urea through the cell membrane without energy expenditure [38–40]. Hereafter, the refering to these proteins as UT, since the bacterial channels of this class do not have a unified nomenclature; for example, the urea transporter Yut has been described in *Yersinia pseudotuberculosis* [39]. Active systems for transporting urea across the membrane have also been discovered in bacterial cells, for example, the UrtABC urea transporter [41,42], which requires ATP to function and may can reduce the urease potential of the cell.

3.3 Carbonic anhydrase for CO_2 fixation

In addition to the transporter, carbonic anhydrase, which catalyzes the conversion of CO_2 to bicarbonate ion, can contribute to increased urease activity. It has been shown that *S. pasterii*, in addition to the complete urease operon *ureABCEFGD*, also contains carbonic anhydrase. One study demonstrated that co-expression of carbonic anhydrase with urease increases urease activity compared to bacteria containing only one enzyme[43].

In addition to existing methods for converting urea into carbon dioxide, there are alternative ways to increase mineralization in concrete. One possibility is to express carbonic anhydrase on the cell membrane or on the outside of the cell. Then, the carbon dioxide escaping from the cell will be fixed as bicarbonate ions, increasing calcium carbonate formation [33,43]. This could possibly work even in the absence of urease, fixing carbon dioxide from the atmosphere, thereby reducing the carbon footprint.

3.4 Amidolyase pathway of urea catabolism

In addition to nickel-dependent urease, which breaks down urea into two ammonium ions and one bicarbonate ion, an amidolyase pathway for urea catabolism has been described in several microorganisms [44], [45]. This pathway involves two sequential reactions. In the first step, urea carboxylase (UC; EC 6.3.4.6) catalyzes the ATP-dependent carboxylation of urea with the participation of the bicarbonate ion:

$$\text{urea} + \text{HCO}_3^- + \text{ATP} \xrightarrow{urea\ carboxylase} \text{allophanate} + \text{ADP} + \text{P}_i.$$

Urea carboxylases belong to the family of biotin-dependent carboxylases, with biotin covalently bound within the enzyme; the first bacterial UC was characterized in *Oleomonas sagaranensis* [46].

In the second step, allophanate hydrolase (AH; EC 3.5.1.54) hydrolyzes allophanate to form two ammonium ions and two bicarbonate ions:

$$\text{carbonic anhydrase} + 3\text{H}_2\text{O} \xrightarrow{\text{allophanate hydrolase}} 2\,\text{NH}_4^+ + 2\,\text{HCO}_3^-,$$

which was shown for yeast urea-amidolase and confirmed for bacterial UC systems during biochemical characterization [47–49]. In summary, the amidolyase pathway converts one urea molecule and one bicarbonate ion into two ammonium ions and two bicarbonate ions with the consumption of one ATP molecule:

$$\text{urea} + \text{HCO}_3^- + \text{ATP} + 3\text{H}_2\text{O} \rightarrow 2\,\text{NH}_4^+ + 2\,\text{HCO}_3^- + \text{ADP} + \text{P}_i.$$

Thus, the amidolyase pathway is an ATP- and biotin-dependent alternative to urease hydrolysis of urea. This process is self-sustaining with sufficient urea supply, as the bicarbonate ion required for urea carboxylase is obtained from the hydrolysis of allophanate.

4 Conclusions

In this article, the methods are proposed for increasing urease activity in strains already in use for bioconcrete production. Also, the possibility of developing strains containing the proposed genes in different combinations is explored to find the best possible gene combination for increasing carbonate production. A combination of enzymes such as urease and carbonic anhydrase or the amidolyase pathway in combination with carbonic anhydrase may be particularly promising. Expressing carbonic anhydrase externally to fix the resulting carbon dioxide was also proposed to enhance mineralization.

The use of genetically engineered strains producing urease, carbonic anhydrase, and other enzymes associated with the biomineralization process represents a promising approach for improving the durability of concrete. Combining these enzymes in a single strain can accelerate calcium carbonate precipitation and improve the efficiency of microcrack repair. Based on existing research and the mechanisms described in this article, such as the urease pathway and the amidolyase pathway, may enable further improvements in the properties of bioconcrete.

In the future, it is also necessary to explore the potential application of these approaches to real building materials, as well as their adaptation to optimal concrete conditions, such as alkaline pH. The creation of effective genetically engineered strains with a complex of enzymes for enhanced mineralization and restoration of concrete will contribute to the development of environmentally friendly and sustainable construction technologies.

Thus, bioconcrete improved with urease, carbonic anhydrase, and amidolyase can play an important role in the creation of new materials that are not only stronger and more durable, but also more environmentally friendly due to biocarbonation and carbon dioxide fixation.

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6 Conflict of Interests

The authors declare no conflict of interest.

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