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

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Abstract

The gradual development of microcracks reduces concrete's durability. Their appearance reduces performance properties, including water resistance, frost resistance, and strength. One promising approach to lowering microcrack development is the use of spore-forming bacteria that can precipitate 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 that bacteria can 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, in microcracks with elevated CO_2 content, 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 hypothesizes that combining urease and carbonic anhydrase may yield better results in concrete stabilization. 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 concrete and other materials gained momentum at the beginning of the 2000s, driven by advances in biological and chemical healing agents, and expanded rapidly in the 2010s [1]. Reviews [2] and [3] indicate that self-healing concrete seals internal damage, such as cracks, without the need for external repair. 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 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 pavement [5] and cement concrete pavements [6] to improve intrinsic self-repair capacity. Encapsulated rejuvenators proved viable for asphalt repair, raising healing temperature and duration to achieve up to 80% healing efficiency [7]. A study developed self-healing concrete incorporating low-calcium fly ash and partial replacement with recycled aggregate, which reduced overall strength [7].

Accumulated data from self-healing concrete research now supports the application of machine learning techniques to forecast material properties [8], [9], [10], [11] and optimize designs, complemented by fractal analysis methods [12]. An attempt to develop cyber-physical modeling and prediction of self-healing processes dynamics in bioconcrete [13] seems interesting, but attempts to validate such models have not yet been made.

Bio-based bacterial self-healing concrete is an alternative to conventional concrete in the construction industry [14], [15], [16], [17], [18], [19]. The bibliometric overview of 2025 [20] covers 150 references. Ongoing research targets resilient bacterial strains and spores that endure concrete's alkaline environment, tackling challenges like production costs, long-term viability, and environmental impact, while genetic engineering and smart tech enhance their efficiency [21], [17], [18] .

Microbially induced calcium carbonate precipitation (MICP) has emerged as an essential biomineralization process with wide-ranging applications, including space exploration. The possibility of using local resources to construct extraterrestrial bases through microbial-induced carbonate precipitation on the Moon and Mars was reviewed [22] as an ambitious project and as one of the most unusual applications of biomineralization.

An experimental study [23] 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 identifying *Bacillus subtilis* [24] as the most effective bacterial strain, with a 3% concentration enhancing beam load-bearing capacity by 20.2%. *Bacillus cohnii, Bacillus halodurans,* and *Bacillus pseudofirmus* enhance their performance properties, including strength and service life, in bioconcrete [25].

Further development of self-healing concrete, also known as bioconcrete, requires intensive microbiological research before it can proceed [26]. Microorganisms from ancient unique structures [13] can serve as a basis for the search for rational bacterial strains for bioconcrete.

The review [27] aimed to reduce the cost of microbial growth media for microbially induced calcium carbonate precipitation, the utilization of plant extractives as enzyme substitutes in enzyme-induced carbonate precipitation, the substitution of urea with urine as a low-cost source of nitrogen, the exploration of affordable alternatives to calcium ions, and the valorization of ammonia or ammonium byproducts, and other pathways.

Due to the aggressive environment of concrete mixes, spore-forming bacteria that 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 [28]. 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 [29].

The use of genetic engineering methods to develop an actual self-healing biological agent appears promising. The bioremediase-like gene of a thermophilic anaerobic bacterium, BKH2 (GenBank accession no. KP231522), was thus transferred to the Bacillus strain to develop a self-healing agent [30]. The transposon mutagenesis method was employed to modify the genes of *B. halodurans* and create a mutant bacterial strain with higher efficiency of calcium carbonate productivity by catalyzing the combination of carbonate and calcium ion [31]

Although culturable bacterial strains with high urease activity already exist, carbonate formation can be improved by enhancing urease activity through the addition of accessory proteins or by using alternative urea-hydrolysis pathways. This work aimed to review bacterial metabolic pathways and enzymes used for biomineralization [32], as well as other promising enzymes that could accelerate existing pathways or exploit alternative principles 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 in which the crystal structure of the urea transporter protein (UT) was first determined [36], providing 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 example 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 that met inclusion criteria (e.g., empirical studies or reviews of 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 reviewed in [32], [33], [39], and [35]. 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 [36], [37], [43], and [39].

For the correct assembly of the urease complex, chaperones are used, namely the proteins UreD, UreE, UreF, and UreG [29],[40], [41]. These accessory proteins form a complex that facilitates Ni^{2+} insertion into the dinuclear active site of UreC, preventing the toxic release of free nickel in the cell. UreE acts as a metallochaperone, UreG as a GTPase, UreF as a regulator, and UreD (or UreH ortholog) as a scaffold [36], [42], [43]. 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 [41], [44], [45].

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 [36], [41], [44], [45], [46]. 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 preactivation 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, [41], [47], [48]. The article [45] 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. The research [49] confirms that UreH [UreD] and UreF form an initial complex with apo-urease before UreG joins. Early evidence for UreD-UreF-UreG-apourease complex formation, implying sequential assembly, was published in [50].

UreE, a nickel-specific metallochaperone, interacts with the preformed UreDFG-apo-urease complex to deliver Ni^{2+} ions, often as the final step before insertion. It binds Ni^{2+} at sites such as His-rich regions or C-terminal motifs and transfers it via protein-protein interactions, without being essential for complex formation but rather facilitating maturation [51], [52].

UreG, a SIMIBI-class GTPase, binds GTP, undergoes hydrolysis-induced conformational changes (e.g., switch I/II regions and dimer-monomer transitions), and couples this to Ni^{2+} release from UreE/UreG, channeling it through the accessory complex to UreC's dinuclear site. This coupling prevents premature Ni release and ensures fidelity [53].

The study [54] elucidates the structure and the regulatory mechanism of urease gene expression in *S. pasteurii* under different culture conditions were studied using transcriptome sequencing. The results revealed a urease biosynthesis *S. pasteurii* is determined by two operons. Operon-1 containing *ureA*, *ureB*, *ureC*, *ureE*, and *ureF* genes, and operon-2 containing *ureG* and *ureD* genes.

The study [55] investigated urease activity in a newly isolated strain, YX-3 of *Sphingobacterium thalpophilum*, which exhibits the ability to precipitate calcium carbonate. Genome sequencing revealed the presence of the structural genes *ureA*, *ureB*, and *ureC*, along with the auxiliary genes *ureD*, *ureE*, *ureF*, and *ureG*. Reverse Transcription Quantitative Polymerase Chain Reaction analysis showed that the addition of NiCl2 resulted in a significant up-regulation of *ureC* expression. His267, His294, and Gly325 in the UreC domain were further demonstrated to coordinate simultaneously with nickel ions and urea through homology modeling and molecular docking.

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 primary urease enzyme, *ureABC*. Still, these genes are fewer than in organisms with a complete genes set, indicating the presence of alternative pathways for urease maturation [40].

One of the bottlenecks in increasing urease activity is the rate of urea transport across the membrane. Several kinetic and physiological studies indicate that urease activity may be limited by the transport of urea across the cell membrane [56], [57].

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 as a proton-dependent channel from the bacterium *Helicobacter pylori*. It helps bacteria survive in acidic conditions (pH 4-6) but is inactive in the alkaline environment of concrete mix (pH 11-14), so it has no practical value for bioconcrete pH [58], [59]. 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 [60], [61], [62]. Hereafter, these proteins are referred to as UT [70], since the bacterial channels of this class lack nomenclature; for example, the urea transporter Yut has been described in *Yersinia pseudotuberculosis* [61], [63]. Active systems for transporting urea across the membrane have also been identified in bacterial cells, for example, the UrtABC urea transporter [60], [63], which requires ATP for function and may reduce the cell's urease potential.

Functional characterization of bacterial UTs, such as ApUT from *Actinobacillus pleuropneumoniae*, demonstrates saturable urea flux that is inhibited by compounds such as phloretin, underscoring their potential to enhance intracellular urea availability.

Further research on UT structures has provided insights into permeation mechanisms, including cryo-EM structures of human and zebrafish UTs that reveal trimeric assemblies and selective urea-binding sites, which could inform the engineering of bacterial variants for bioconcrete applications [64], [65], [66]. Evolutionary studies suggest that UTs in vertebrates evolved to adapt to urea concentration gradients, a principle that might be leveraged in bacteria to optimize mineralization in concrete [67]. Molecular dynamics simulations of UTs indicate conformational changes during urea transport, facilitating efficient diffusion without energy input [68]. In colonic models, UT-B transporters mediate urea movement to support symbiotic bacteria, highlighting a role in nitrogen salvaging that parallels potential

enhancements in bioconcrete microbial systems [69]. Oligomeric structures of bacterial UTs, such as dimers or trimers, in lipid membranes ensure stability and high permeability, which is essential for rapid urease substrate delivery [70]. Comparative studies of urea analogs transport through UTs provide data on selectivity, aiding in the design of transporters that avoid off-target substrates in engineered strains [65].

No urea transporters were found in the genome of *S. pasteurii*, which is one of the most well-known bacteria used to produce bioconcrete. Apparently, there is some alternative pathway for urea deposition in *S. pasteurii*, which has yet to be investigated.

3.2 Carbonic anhydrase for CO_2 fixation

Carbonic anhydrase can serve as an additional way to increase the efficiency of biomineralization. This enzyme catalyzes the conversion of CO_2 to bicarbonate ion, which can contribute to increased urease activity. It has been shown that *S. pasteurii*, in addition to the complete urease operon *ureABCEFGD*, also contains carbonic anhydrase [71]. One study demonstrated that co-expression of carbonic anhydrase with urease increases urease activity compared to bacteria containing only one enzyme [72].

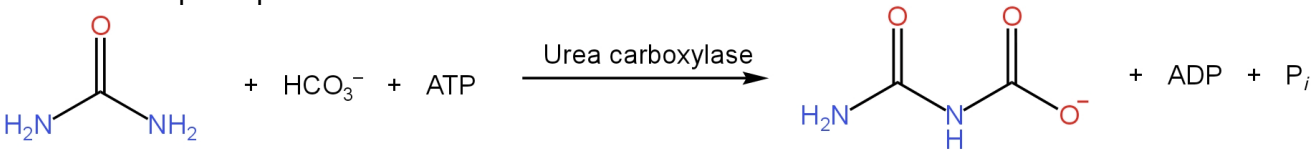
In addition to existing methods for converting urea into carbon dioxide, there are alternative ways to increase mineralization in concrete. One possibility is to express carbonic anhydrase on the cell membrane or on the outside of the cell. Then, the carbon dioxide escaping from the cell will be fixed as bicarbonate ions, increasing calcium carbonate formation [72], [56]. This expression could work even in the absence of urease, fixing atmospheric carbon dioxide and thereby reducing the carbon footprint.

Coupled effects with synthetic fibers, such as polypropylene and polyvinyl alcohol, alongside bacterial carbonic anhydrase, have shown improved self-healing efficiency in concrete, where the enzyme accelerates carbonate formation to seal cracks more rapidly [73]. Studies on biomineralization in other bacteria reveal that carbonic anhydrase isoforms differ in activity, with some extracellular forms optimizing CO_2 hydration in alkaline environments similar to those in concrete [74]. Genetic modifications to overexpress carbonic anhydrase in Bacillus species have led to enhanced calcite yields, supporting its integration into bioconcrete strains [75]. Furthermore, synergistic interactions between carbonic anhydrase and urease pathways have been modeled, predicting up to 30% increases in mineralization rates through co-expression [76].

3.3 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 [77], [78]. This pathway involves two sequential reactions.

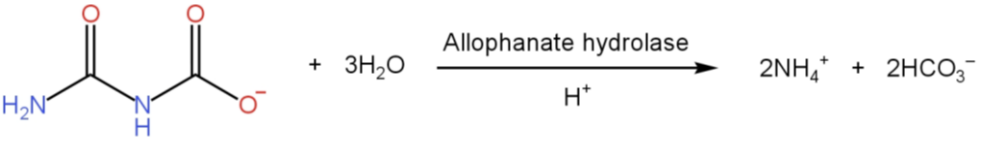
In the first step, urea carboxylase (UC; EC 6.3.4.6) catalyzes the ATP-dependent carboxylation of urea with the participation of the bicarbonate ion:



Urea Allophanate

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* [79]. Biochemical studies confirm that UC and allophanate hydrolase (AH) activities in urea amidolyase are functionally independent, allowing modular engineering in bacterial systems [80], [81]. Structural analyses of urea amidolyase reveal its bifunctional nature, with distinct domains for carboxylation and hydrolysis, providing insights for optimization in biomineralization[80].

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



Allophanate

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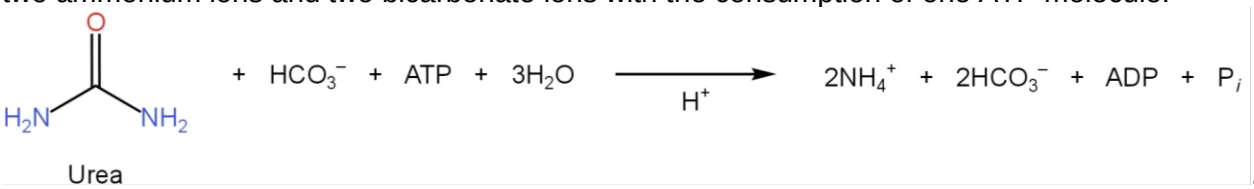
which was demonstrated for yeast urea idolase and confirmed for bacterial UC systems during biochemical characterization [82], [83] [84],.

Widespread annotation corrections indicate that many bacterial proteins labeled as ureases are actually urea carboxylases, expanding the known distribution of this pathway [81]. Prokaryotic urea carboxylase from sources like *Kluyvera intermedia* lacks the AH domain but retains high activity, suggesting potential for hybrid systems [83]. Single-particle cryo-EM of urea amidolyase shows hexameric organization, enhancing catalytic efficiency in microbial urea degradation [85]. Allophanate hydrolase from *O. sagaranensis* generates ammonia, adjacent to UC genes, forming an ATP-dependent alternative pathway [83]. Crystal structures of UC provide mechanistic insights into biotin-mediated carboxylation, a crucial step in the production of bicarbonate in bioconcrete[86]. Structures of AH from *Granulibacter bethesdensis* reveal substrate binding sites, aiding in understanding hydrolysis yields [87]. In cyanuric acid metabolism, AH functions independently of urease, highlighting the versatility of urea catabolism in bacteria [88]. Evolutionary analyses show that fungi exhibit fusion events, but bacterial forms remain separate, offering flexibility for genetic engineering [89].

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:



Thus, the amidolyase pathway is an ATP- and biotin-dependent alternative to urease hydrolysis of urea. This process is self-sustaining with a sufficient urea supply, as the bicarbonate ion required for urea carboxylase is generated by the hydrolysis of allophanate [85].

3.4 Survival of bacteria in concrete

One of the main problems is survival in concrete, starting with hydration of the concrete when the pH reaches 12-13. Survivability in concrete is also affected by the chemical composition and the tensile stress that occurs when mixing concrete. It has been shown that spore viability in concrete can be as low as 1% after 9 days of incorporation into concrete [90]. To increase the bacterial titer, preparations containing mainly bacterial spores in concrete are used. In addition, various carriers are used, such as diatomaceous earth [91],[92], expanded clay [93], perilite [94] and hydrogel [95]. Which can increase the lifespan of bacteria in concrete by an order of magnitude [95], [96] ,[97], [98]. Standard tests are used to compare the effectiveness of different self-healing concrete systems [99], [100].

The survival of bacteria can also be negatively affected by increased consumption of ATP, as in the amidolyase pathway, due to the poor environment. Bacteria may simply not have time to seal the cracks. To solve this problem, it may be necessary to increase the concentration of spores in the concrete, which will increase the cost.

For urease to work, nickel ions are required, which are toxic to bacteria. To reduce the toxicity of nickel, it can be added in the form of slightly soluble, bioavailable salts, which will reduce toxicity.

4 Conclusions

In this article, methods are proposed to increase urease activity in strains already used for bioconcrete production. Also, the possibility of developing strains containing the proposed genes in different combinations is explored to find an optimal 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. Recent breakthroughs in bacteria-powered self-healing concrete emphasize genetic modifications to enhance spore viability and calcite deposition, addressing challenges such as bacterial survival in harsh concrete matrices [30], [31].

The use of genetically engineered strains that produce urease, carbonic anhydrase, and other enzymes involved in the biomineralization process represents a promising approach to improving concrete durability. Combining these enzymes in a single strain can accelerate calcium carbonate precipitation and enhance the efficiency of microcrack repair. Based on existing research and the mechanisms described in this article, such as the urease and amidolyase pathways, these mechanisms may enable further improvements in bioconcrete properties. Advances in microbial self-healing concrete using engineered bacteria show up to 50% better crack closure rates, with synthetic biology tools

optimizing enzyme expression [21]. Characteristics of rubberized concrete with bacteria such as *S. pasteurii* demonstrate enhanced mechanical properties through genetic that increase output [21]. Genetically modified *B. subtilis* strains yield self-healing bioconcrete with improved compressive strength, as shown in mortar specimens [101]. Engineered microbial strategies in low-carbon concrete incorporate Bacillus-based encapsulation and genetic enhancements for alkaline resistance [102].

In the future, it is also necessary to explore the potential applications of these approaches to real building materials and their adaptation to optimal concrete conditions, such as alkaline pH. The development of effective genetically engineered strains that incorporate a complex of enzymes to enhance mineralization and restore concrete will reduce environmental impact. Bio-based bacterial concrete reviews highlight *Sporosarcina* spp. for sustainable infrastructure, with genetic engineering advancing crack-sealing technologies [103]. Tailored strains with improved calcite production via synthetic biology promise reduced environmental impact [75].

Thus, bioconcrete improved with urease, carbonic anhydrase, and amidolyase can play an essential role in creating new materials that are not only stronger and more durable but also more environmentally protective through biocarbonization and carbon dioxide fixation.

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6 Conflict of Interests

The authors declare no conflict of interest.

References

1 Vafaeva, Kh.M., Vatin, N.I. and Kordas, G. (2023) Self-Healing Building Materials: The Future of Construction. *AlfaBuild*, **29**, 2912–2912. https://doi.org/10.57728/ALF.29.12.

2 Amran, M., Onaizi, A.M., Fediuk, R., Vatin, N.I., Rashid, R.S.M., Abdelgader, H. and Ozbakkaloglu, T. (2022) Self-Healing Concrete as a Prospective Construction Material: A Review. *Materials*, **15**. https://doi.org/10.3390/ma15093214.

3 He, X., Amin, M.N., Khan, K., Ahmad, W., Althoey, F. and Vatin, N.I. (2022) Self-Healing Concrete: A Scientometric Analysis-Based Review of the Research Development and Scientific Mapping. *Case Studies in Construction Materials*, **17**. https://doi.org/10.1016/j.cscm.2022.e01521.

4 Beskopylny, A.N., Shcherban', E.M., Stel'makh, S.A., Shilov, A.A., Chernil'nik, A., El'shaeva, D. and Chistyakov, V.A. (2024) Analysis of the Current State of Research on Bio-Healing Concrete (Bioconcrete). *Materials*, MDPI AG, **17**, 4508. https://doi.org/10.3390/ma17184508.

5 Maksudovna, K. (2023) Engineering Polymer Systems with Self-Healing Functionality to Enhance Structural Longevity. *Alfabuild*, **29**, 2913–2913. https://doi.org/10.57728/ALF.29.13.

6 Soleimanbeigi, A., Hayati, P., Sobhani, J. and Saffarian, P. (2025) Synergistic Effects of Sporosarcina Ureae and Silica Fume on Self-Healing and Plastic Shrinkage Cracks in Bio-Green Concrete Pavement. *Results in Engineering*, Elsevier B.V., **26**. https://doi.org/10.1016/j.rineng.2025.105526.

7 Abadeen, A.Z.U., Hussain, A., Kumar, V.S., Murali, G., Vatin, N.I. and Riaz, H. (2022) Comprehensive Self-Healing Evaluation of Asphalt Concrete Containing Encapsulated Rejuvenator. *Materials*, **15**. https://doi.org/10.3390/ma15103672.

8 Algaifi, H.A., Bakar, S.A., Alyousef, R., Sam, A.R.M., Alqarni, A.S., Wan Ibrahim, M.H., Shahidan, S., Ibrahim, M. and Salami, B.A. (2021) Machine Learning and RSM Models for Prediction of Compressive Strength of Smart Bio-Concrete. *Smart Structures and Systems*, Techno-Press, **28**, 535–551. https://doi.org/10.12989/sss.2021.28.4.535.

9 Xia, Z. and Rüppel, U. (2024) Narrow Concrete Crack Repair with Bio-Concrete Agent and AI Based on Jetson Nano. *Proceedings of the 31st International Workshop on Intelligent Computing in Engineering, EG-ICE 2024*. https://www.scopus.com/pages/publications/85207215687?origin=resultslist.

10 Althoey, F., Amin, M.N., Khan, K., Usman, M.M., Khan, M.A., Javed, M.F., Sabri, M.M.S., Alrowais, R. and Maglad, A.M. (2022) Machine Learning Based Computational Approach for Crack Width Detection of Self-

Healing Concrete. *Case Studies in Construction Materials*, **17**, e01610. https://doi.org/10.1016/j.cscm.2022.e01610.

11 Šovljanski, O., Pezo, L., Milović, T., Pezo, M., Radenković, M., Mejić, L., Bulatović, V., Dramićanin, M. and Tomić, A. (2026) Bio-Intelligent Approach for Rapid Healing of Cracks via Artificial Neural Network-Optimized Urban Bacterial Consortium. *Journal of Building Engineering*, Elsevier Ltd, **117**. https://doi.org/10.1016/j.jobe.2025.114851.

12 Vafaeva, K.M., Júnior, J.F. de O. and Pizzo, H. da S. (2023) Fractal Aspects in the Technology of Self-Healing Materials. *AlfaBuild*, **29**, 2911–2911. https://doi.org/10.57728/ALF.29.11.

13 Kirsanova, T.A., Chistyakov, V.A., Beskopylny, A.N., Aramova, O.Y., Alliluyeva, E.V. and Bahari, A. (2025) Cyber-Physical Modeling and Prediction of Self-Healing Processes Dynamics in Bioconcrete. *Construction of Unique Buildings and Structures*, **119**, 11902–11902. https://doi.org/10.4123/CUBS.119.2.

14 Mitikie, B.B. and Elsaigh, W.A. (2025) Bio-Based Bacterial Concrete: A Sustainable Alternative in Construction. *Innovative Infrastructure Solutions*, Springer Science and Business Media Deutschland GmbH, **10**. https://doi.org/10.1007/s41062-025-02058-y.

15 Omoregie, A.I., Wong, C.S., Rajasekar, A., Ling, J.H., Laiche, A.B., Basri, H.F., Sivakumar, G. and Ouahbi, T. (2025) Bio-Based Solutions for Concrete Infrastructure: A Review of Microbial-Induced Carbonate Precipitation in Crack Healing. *Buildings*, Multidisciplinary Digital Publishing Institute (MDPI), **15**, Article 1052. https://doi.org/10.3390/buildings15071052.

16 Devaki, K.G. and Revathi, P. (2025) Effect of Microbial Calcite Precipitation on the Performance of Concrete Incorporating Different Bacterial Strains. *Journal of Environmental Nanotechnology*, Institute for Environmental Nanotechnology, **14**, 733–743. https://doi.org/10.13074/jent.2025.09.2531765.

17 Singh, S.K. and Mishra, S.S. (2025) Unlocking the Potential of Bacterial Self-Healing in Concrete: Applications, and Challenges for Sustainable Infrastructure. *Indian Chemical Engineer*, Taylor & Francis. https://doi.org/10.1080/00194506.2025.2524383.

18 Kirsanova, T.A., Chistyakov, V.A., Rahmani, H., Aramova, O.Y. and Alliluyeva, E.V. (2024) Factors Influencing the Mechanisms of Self-Healing of Cementing Materials. A Review. *Construction of Unique Buildings and Structures*, **113**, 11309–11309. https://doi.org/10.4123/CUBS.113.9.

19 Mirshahmohammad, M., Rahmani, H., Maleki-Kakelar, M. and Bahari, A. (2022) Effect of Sustained Service Loads on the Self-Healing and Corrosion of Bacterial Concretes. *Construction and Building Materials*, Elsevier Ltd, **322**. https://doi.org/10.1016/j.conbuildmat.2022.126423.

20 Ranjith, A. and Das, B.B. (2025) Self-Healing Bacterial Concrete: A Bibliometric Overview and State-of-the-Art Review on Fundamentals, Techniques and Future Perspectives. *Journal of Building Engineering*, Elsevier Ltd, **104**. https://doi.org/10.1016/j.jobe.2025.112324.

21 Elgendy, I.M., Elkaliny, N.E., Saleh, H.M., Darwish, G.O., Almostafa, M.M., Metwally, K., Yahya, G. and Mahmoud, Y.A.G. (2024) Bacteria-Powered Self-Healing Concrete: Breakthroughs, Challenges, and Future Prospects. *Journal of industrial microbiology & biotechnology*, J Ind Microbiol Biotechnol, **52**. https://doi.org/10.1093/jimb/kuae051.

22 Dikshit, R., Gupta, N., Dey, A., Viswanathan, K. and Kumar, A. (2022) Microbial Induced Calcite Precipitation Can Consolidate Martian and Lunar Regolith Simulants. *PLOS ONE*, Public Library of Science, **17**, e0266415. https://doi.org/10.1371/journal.pone.0266415.

23 Prasad, V., Sabri, M., Devi, S., Najm, H., Majeed, S. and Qaidi, S. (2023) Mechanical and Microstructural Properties of Self-Healing Concrete Based on Hay Bacillus. *Magazine of Civil Engineering*, **122**. https://doi.org/10.34910/MCE.122.4.

24 Salem, G.G., Galishnikova, V. V., Elroba, S.M., Vatin, N.I. and Kharun, M. (2022) Finite Element Analysis of Self-Healing Concrete Beams Using Bacteria. *Materials*, Multidisciplinary Digital Publishing Institute, **15**, 7506. https://doi.org/10.3390/ma15217506.

25 Jonkers, H.M. and Schlangen, E. (2008) Development of a Bacteria-Based Self Healing Concrete. *Proceedings of the International FIB Symposium 2008 - Tailor Made Concrete Structures: New Solutions for our Society*, CRC Press/Balkema, 109. https://doi.org/10.1201/9781439828410.ch72.

26 Alvarado-Mata, L.Y., Sánchez-Lozano, J.M., Cervantes-de la Torre, J.I., Álvarez-Valdez, A.R., Salinas-Mireles, J.P., Chávez Hita-Wong, A.Y., Castro-Alonso, M.J., Luévanos-Escareño, M.P., Narayanasamy, R. and Balagurusamy, N. (2025) Advances in Microbially Induced Carbonate Precipitation Metabolic Pathways and Regulatory Mechanisms for Bioconcrete Development. *Journal of Sustainable Cement-*

Based Materials, Taylor and Francis Ltd., **14**, 1987–2011. https://doi.org/10.1080/21650373.2025.2525559.

27 Yan, Z., Nakashima, K., Takano, C. and Kawasaki, S. (2025) Strategies for Cost-Optimized Biocement Production: A Comprehensive Review. *World Journal of Microbiology and Biotechnology*, Springer Science and Business Media B.V., **41**. https://doi.org/10.1007/s11274-025-04281-2.

28 Aramova, O.Y., Kornienko, I.V., Chistyakov, V.A., Alliluyeva, E.V. and Kirsanova, T.A. (2024) Properties of the Urease Enzyme as a Component of Self-Healing Concrete. A Review. *Construction of Unique Buildings and Structures*, **113**, 11310–11310. https://doi.org/10.4123/CUBS.113.10.

29 Javeed, Y., Goh, Y., Mo, K.H., Yap, S.P. and Leo, B.F. (2024) Microbial Self-Healing in Concrete: A Comprehensive Exploration of Bacterial Viability, Implementation Techniques, and Mechanical Properties. *Journal of Materials Research and Technology*, Elsevier Editora Ltda, **29**, 2376–2395. https://doi.org/10.1016/j.jmrt.2024.01.261.

30 Sarkar, M., Adak, D., Tamang, A., Chattopadhyay, B. and Mandal, S. (2015) Genetically-Enriched Microbe-Facilitated Self-Healing Concrete-a Sustainable Material for a New Generation of Construction Technology. *RSC Advances*, Royal Society of Chemistry, **5**, 105363–105371. https://doi.org/10.1039/c5ra20858k.

31 Zhang, Z., Weng, Y., Ding, Y. and Qian, S. (2019) Use of Genetically Modified Bacteria to Repair Cracks in Concrete. *Materials*, MDPI AG, **12**. https://doi.org/10.3390/ma122333912.

32 Achal, V. and Mukherjee, A. (2015) A Review of Microbial Precipitation for Sustainable Construction. *Construction and Building Materials*, Elsevier, **93**, 1224–1235. https://doi.org/10.1016/j.conbuildmat.2015.04.051.

33 Krajewska, B. (2018) Urease-Aided Calcium Carbonate Mineralization for Engineering Applications: A Review. *Journal of Advanced Research*, Elsevier, **13**, 59–67. https://doi.org/10.1016/J.JARE.2017.10.009.

34 Zhu, T. and Dittrich, M. (2016) Carbonate Precipitation through Microbial Activities in Natural Environment, and Their Potential in Biotechnology: A Review. *Frontiers in Bioengineering and Biotechnology*, Frontiers Media S.A., **4**, 179892. https://doi.org/10.3389/FBIOE.2016.00004/FULL.

35 Anbu, P., Kang, C.H., Shin, Y.J. and So, J.S. (2016) Formations of Calcium Carbonate Minerals by Bacteria and Its Multiple Applications. *SpringerPlus 2016 5:1*, SpringerOpen, **5**, 250-. https://doi.org/10.1186/S40064-016-1869-2.

36 Farrugia, M.A., Macomber, L. and Hausinger, R.P. (2013) Biosynthesis of the Urease Metallocenter. *Journal of Biological Chemistry*, Elsevier, **288**, 13178–13185. https://doi.org/10.1074/jbc.R112.446526.

37 Carter, E.L., Boer, J.L., Farrugia, M.A., Flugga, N., Towns, C.L. and Hausinger, R.P. (2011) Function of UreB in Klebsiella Aerogenes Urease. *Biochemistry*, American Chemical Society, **50**, 9296–9308. https://doi.org/10.1021/BI2011064.

38 Jabri, E., Carr, M.B., Hausinger, R.P. and Karplus, P.A. (1995) The Crystal Structure of Urease from Klebsiella Aerogenes. *Science*, **268**, 998–1004. https://doi.org/10.1126/SCIENCE.7754395.

39 Ma, Z., Chen, M., Lu, J., Liu, S. and Ma, Y. (2025) Exploration of Urease-Aided Calcium Carbonate Mineralization by Enzyme Analyses of Neobacillus Mesonae Strain NS-6 . *Microbiology Spectrum*, American Society for Microbiology, **13**. https://doi.org/10.1128/SPECTRUM.01891-24;JOURNAL:JOURNAL:SPECTRUM;WGROUP:STRING:PUBLICATION.

40 Kim, J.K., Mulrooney, S.B. and Hausinger, R.P. (2005) Biosynthesis of Active Bacillus Subtilis Urease in the Absence of Known Urease Accessory Proteins. *Journal of bacteriology*, J Bacteriol, **187**, 7150–7154. https://doi.org/10.1128/JB.187.20.7150-7154.2005.

41 Fong, Y.H., Wong, H.C., Yuen, M.H., Lau, P.H., Chen, Y.W. and Wong, K.B. (2013) Structure of UreG/UreF/UreH Complex Reveals How Urease Accessory Proteins Facilitate Maturation of Helicobacter Pylori Urease. *PLOS Biology*, Public Library of Science, **11**, e1001678. https://doi.org/10.1371/journal.pbio.1001678.

42 Yuen, M.H., Fong, Y.H., Nim, Y.S., Lau, P.H., Wong, K.B. and Fletterick, R.J. (2017) Structural Insights into How GTP-Dependent Conformational Changes in a Metallochaperone UreG Facilitate Urease Maturation. *Proceedings of the National Academy of Sciences of the United States of America*, National Academy of Sciences, **114**, E10890–E10898. https://doi.org/10.1073/PNAS.1712658114;PAGEGROUP:STRING:PUBLICATION.

43 Zambelli, B., Cremades, N., Neyroz, P., Turano, P., Uversky, V.N. and Ciurli, S. (2011) Insights in the (Un)Structural Organization of Bacillus Pasteurii UreG, an Intrinsically Disordered GTPase Enzyme. *Molecular BioSystems*, The Royal Society of Chemistry, **8**, 220–228. https://doi.org/10.1039/C1MB05227F.

44 Yang, X., Li, H., Lai, T.P. and Sun, H. (2015) UreE-UreG Complex Facilitates Nickel Transfer and Preactivates GTPase of UreG in Helicobacter Pylori. *Journal of Biological Chemistry*, American Society for Biochemistry and Molecular Biology Inc., **290**, 12474–12485. https://doi.org/10.1074/jbc.M114.632364.

45 Nim, Y.S. and Wong, K.B. (2019) The Maturation Pathway of Nickel Urease. *Inorganics 2019, Vol. 7, Page 85*, Multidisciplinary Digital Publishing Institute, **7**, 85. https://doi.org/10.3390/INORGANICS7070085.

46 Merloni, A., Dobrovolska, O., Zambelli, B., Agostini, F., Bazzani, M., Musiani, F. and Ciurli, S. (2014) Molecular Landscape of the Interaction between the Urease Accessory Proteins UreE and UreG. *Biochimica et biophysica acta*, Biochim Biophys Acta, **1844**, 1662–1674. https://doi.org/10.1016/j.bbapap.2014.06.016.

47 Tsang, K.L. and Wong, K.B. (2022) Moving Nickel along the Hydrogenase–Urease Maturation Pathway. *Metallomics*, Oxford Academic, **14**. https://doi.org/10.1093/MTOMCS/MFAC003.

48 Lam, R., Romanov, V., Johns, K., Battaile, K.P., Wu-Brown, J., Guthrie, J.L., Hausinger, R.P., Pai, E.F. and Chirgadze, N.Y. (2010) Crystal Structure of a Truncated Urease Accessory Protein UreF from Helicobacter Pylori. *Proteins: Structure, Function and Bioinformatics*, **78**, 2839–2848. https://doi.org/10.1002/prot.22802.

49 Fong, Y.H., Wong, H.C., Chuck, C.P., Chen, Y.W., Sun, H. and Wong, K.B. (2011) Assembly of Preactivation Complex for Urease Maturation in Helicobacter Pylori: Crystal Structure of UreF-UreH Protein Complex. *Journal of Biological Chemistry*, **286**, 43241–43249. https://doi.org/10.1074/jbc.M111.296830.

50 Soriano, A. and Hausinger, R.P. (1999) GTP-Dependent Activation of Urease Apoprotein in Complex with the UreD, UreF, and UreG Accessory Proteins. *Proceedings of the National Academy of Sciences of the United States of America*, **96**, 11140–11144. https://doi.org/10.1073/pnas.96.20.11140.

51 Musiani, F., Zambelli, B., Bazzani, M., Mazzei, L. and Ciurli, S. (2015) Nickel-Responsive Transcriptional Regulators. *Metallomics*, Royal Society of Chemistry, **7**, 1305–1318. https://doi.org/10.1039/c5mt00072f.

52 Ciurli, S., Safarov, N., Miletti, S., Dikiy, A., Christensen, S.K., Kornetzky, K., Bryant, D.A., Vandenberghe, I., Devreese, B., Samyn, B., Remaut, H. and Van Beeumen, J. (2002) Molecular Characterization of Bacillus Pasteurii UreE, a Metal-Binding Chaperone for the Assembly of the Urease Active Site. *Journal of Biological Inorganic Chemistry*, Springer Verlag, **7**, 623–631. https://doi.org/10.1007/s00775-002-0341-7.

53 Zhang, X., Zhao, S., He, Y., Zheng, N., Yan, X. and Wang, J. (2020) Substitution of Residues in UreG to Investigate UreE Interactions and Nickel Binding in a Predominant Urease Gene Cluster from the Ruminal Metagenome. *International Journal of Biological Macromolecules*, Elsevier B.V., **161**, 1591–1601. https://doi.org/10.1016/j.ijbiomac.2020.07.260.

54 Pei, D., Liu, Z. and Hu, B. (2025) A Novel Urease Gene Structure of Sporosarcina Pasteurii with Double Operons. *Molecular Genetics and Genomics*, Springer Science and Business Media Deutschland GmbH, **300**. https://doi.org/10.1007/s00438-025-02236-8.

55 Zhang, S., Liu, S., Chen, M., Lu, J. and Ma, Y. (2024) Characterization of Urease Active Calcite-Producing Strain YX-3 Combined with the Whole Genome. *Environmental Research*, Academic Press Inc., **262**. https://doi.org/10.1016/j.envres.2024.119855.

56 Hammes, F., Boon, N., De Villiers, J., Verstraete, W. and Siciliano, S.D. (2003) Strain-Specific Ureolytic Microbial Calcium Carbonate Precipitation. *Applied and Environmental Microbiology*, American Society for Microbiology, **69**, 4901–4909. https://doi.org/10.1128/AEM.69.8.4901-4909.2003.

57 Sridhar, S., Bhatt, N. and Suraishkumar, G.K. (2021) Mechanistic Insights into Ureolysis Mediated Calcite Precipitation. *Biochemical Engineering Journal*, Elsevier, **176**, 108214. https://doi.org/10.1016/j.bej.2021.108214.

58 Mobley, H.L.T., Island, M.D. and Hausinger, R.P. (1995) Molecular Biology of Microbial Ureases. *Microbiological Reviews*, American Society for Microbiology, **59**, 451–480. https://doi.org/10.1128/mr.59.3.451-480.1995.

59 Weeks, D.L., Eskandari, S., Scott, D.R. and Sachs, G. (2000) A H+-Gated Urea Channel: The Link Between Helicobacter Pylori Urease and Gastric Colonization. *Science*, American Association for the Advancement of Science, **287**, 482–485. https://doi.org/10.1126/science.287.5452.482.

60 Wang, C., Zhu, W. jing, Ding, H. tao, Liu, N. hua, Cao, H. yan, Suo, C. lei, Liu, Z. kun, Zhang, Y., Sun, M. ling, Fu, H. hui, Li, C. yang, Chen, X. lan, Zhang, Y.Z. and Wang, P. (2023) Structural and Molecular Basis for Urea Recognition by Prochlorococcus. *Journal of Biological Chemistry*, American Society for Biochemistry and Molecular Biology Inc., **299**. https://doi.org/10.1016/j.jbc.2023.104958.

61 Sebbane, F., Bury-Moné, S., Cailliau, K., Browaeys-Poly, E., De Reuse, H. and Simonet, M. (2002) The Yersinia Pseudotuberculosis Yut Protein, a New Type of Urea Transporter Homologous to Eukaryotic Channels and Functionally Interchangeable in Vitro with the Helicobacter Pylori UreI Protein. *Molecular Microbiology*, John Wiley & Sons, Ltd, **45**, 1165–1174. https://doi.org/10.1046/j.1365-2958.2002.03096.x.

62 Levin, E.J., Quick, M. and Zhou, M. (2009) Crystal Structure of a Bacterial Homologue of the Kidney Urea Transporter. *Nature 2009 462:7274*, Nature Publishing Group, **462**, 757–761. https://doi.org/10.1038/nature08558.

63 Sachs, G., Kraut, J.A., Wen, Y., Feng, J. and Scott, D.R. (2006) Urea Transport in Bacteria: Acid Acclimation by Gastric Helicobacter Spp. *Journal of Membrane Biology*, **212**, Article 71-82. https://doi.org/10.1007/s00232-006-0867-7.

64 Huang, S.M., Huang, Z.Z., Liu, L., Xiong, M.Y., Zhang, C., Cai, B.Y., Wang, M.W., Cai, K., Jia, Y.L., Wang, J. Le, Zhang, M.H., Xie, Y.H., Li, M., Zhang, H., Weng, C.H., Wen, X., Li, Z., Sun, Y., Yi, F., Yang, Z., Xiao, P., Yang, F., Yu, X., Tie, L., Yang, B.X. and Sun, J.P. (2024) Structural Insights into the Mechanisms of Urea Permeation and Distinct Inhibition Modes of Urea Transporters. *Nature Communications* , Nature Research, **15**. https://doi.org/10.1038/s41467-024-54305-y.

65 Levin, E.J. and Zhou, M. (2014) Structure of Urea Transporters. *Sub-Cellular Biochemistry*, Springer New York, **73**, 65–78. https://doi.org/10.1007/978-94-017-9343-8_5.

66 Xiong, M., Huang, S., Sun, J. and Yang, B. (2025) Protein Structures of Urea Transporters. Subcellular Biochemistry, Springer Science and Business Media B.V., 19–43. https://doi.org/10.1007/978-981-96-6898-4_2.

67 Ma, L., Pang, A.P., Luo, Y., Lu, X. and Lin, F. (2020) Beneficial Factors for Biomineralization by Ureolytic Bacterium Sporosarcina Pasteurii. *Microbial Cell Factories*, BioMed Central Ltd., **19**. https://doi.org/10.1186/s12934-020-1281-z.

68 McNulty, R., Ulmschneider, J.P., Luecke, H. and Ulmschneider, M.B. (2013) Mechanisms of Molecular Transport through the Urea Channel of Helicobacter Pylori. *Nature Communications 2013 4:1*, Nature Publishing Group, **4**, 2900-. https://doi.org/10.1038/ncomms3900.

69 Collins, D., Winter, D.C., Hogan, A.M., Schirmer, L., Baird, A.W. and Stewart, G.S. (2009) Differential Protein Abundance and Function of UT-B Urea Transporters in Human Colon. *American Journal of Physiology - Gastrointestinal and Liver Physiology*, **298**, G345. https://doi.org/10.1152/AJPGI.00405.2009.

70 Raunser, S., Mathai, J.C., Abeyrathne, P.D., Rice, A.J., Zeidel, M.L. and Walz, T. (2009) Oligomeric Structure and Functional Characterization of the Urea Transporter from Actinobacillus Pleuropneunomiae. *Journal of molecular biology*, Academic Press, **387**, 619. https://doi.org/10.1016/J.JMB.2009.02.005.

71 Chen, L., Feng, Y., Hu, X., Zhao, Y., Liu, J., Wu, M., Li, X., Ha, M., Qu, Y. and Wang, Q. (2025) CO2 Mineralization Induced by Non-Urea Heterotrophic Microorganisms: Investigation of CO2 Sequestration and Mineralization Mechanisms. *Environmental Research*, Academic Press, **285**, 122446. https://doi.org/10.1016/j.envres.2025.122446.

72 Dhami, N.K., Reddy, M.S. and Mukherjee, A. (2014) Synergistic Role of Bacterial Urease and Carbonic Anhydrase in Carbonate Mineralization. *Applied Biochemistry and Biotechnology 2014 172:5*, Springer, **172**, 2552–2561. https://doi.org/10.1007/s12010-013-0694-0.

73 Feng, J., Su, Y. and Qian, C. (2019) Coupled Effect of PP Fiber, PVA Fiber and Bacteria on Self-Healing Efficiency of Early-Age Cracks in Concrete. *Construction and Building Materials*, Elsevier, **228**, 116810. https://doi.org/10.1016/J.CONBUILDMAT.2019.116810.

74 Bose, H. and Satyanarayana, T. (2017) Microbial Carbonic Anhydrases in Biomimetic Carbon Sequestration for Mitigating Global Warming: Prospects and Perspectives. *Frontiers in Microbiology*, Frontiers Media S.A., **8**, 277014. https://doi.org/10.3389/FMICB.2017.01615/FULL.

75 Gilmour, K.A., Ghimire, P.S., Wright, J., Haystead, J., Dade-Robertson, M., Zhang, M. and James, P. (2024) Microbially Induced Calcium Carbonate Precipitation through CO2 Sequestration via an Engineered

Bacillus Subtilis. *Microbial Cell Factories*, BioMed Central Ltd, **23**, 168. https://doi.org/10.1186/S12934-024-02437-7.

76 Niu, L., Duan, Y. ting, Xu, X., Wu, X. yan, Wang, Z. and Zheng, C. li. (2025) Double Enzyme Enhanced Microbial Induction of Carbonate Precipitation (E-MICP) to Improve Mechanical Properties of Sandy Soil and Its Biocementation Mechanism. *Journal of Environmental Chemical Engineering*, Elsevier, **13**, 115349. https://doi.org/10.1016/J.JECE.2025.115349.

77 Lavrov, K. V., Shemyakina, A.O., Grechishnikova, E.G., Novikov, A.D., Kalinina, T.I. and Yanenko, A.S. (2019) In Vivo Metal Selectivity of Metal-Dependent Biosynthesis of Cobalt-Type Nitrile Hydratase in Rhodococcus Bacteria: A New Look at the Nitrile Hydratase Maturation Mechanism? *Metallomics*, Oxford Academic, **11**, 1162–1171. https://doi.org/10.1039/C8MT00129D.

78 Novikov, A.D., Kalinina, T.I., Lavrov, K. V and Yanenko, A.S. (2025) Genetic Control of Urea Assimilation Pathways in Rhodococcus Rhodochrous M8. *Biotechnology*, **41**, 113–115. https://doi.org/10.56304/S0234275825050114.

79 Hausinger, R.P. (2004) Metabolic Versatility of Prokaryotes for Urea Decomposition. *Journal of bacteriology*, J Bacteriol, **186**, 2520–2522. https://doi.org/10.1128/JB.186.9.2520-2522.2004.

80 Zhao, J., Zhu, L., Fan, C., Wu, Y. and Xiang, S. (2018) Structure and Function of Urea Amidolyase. *Bioscience reports*, Biosci Rep, **38**. https://doi.org/10.1042/BSR20171617.

81 Schneider, N.O., Tassoulas, L.J., Zeng, D., Laseke, A.J., Reiter, N.J., Wackett, L.P. and Maurice, M.S. (2020) Solving the Conundrum: Widespread Proteins Annotated for Urea Metabolism in Bacteria Are Carboxyguanidine Deiminases Mediating Nitrogen Assimilation from Guanidine. *Biochemistry*, American Chemical Society, **59**, 3258–3270. https://doi.org/10.1021/acs.biochem.0c00537.

82 Lin, Y., Boese, C.J. and St. Maurice, M. (2016) The Urea Carboxylase and Allophanate Hydrolase Activities of Urea Amidolyase Are Functionally Independent. *Protein Science*, Blackwell Publishing Ltd, **25**, 1812–1824. https://doi.org/10.1002/pro.2990.

83 Kanamori, T., Kanou, N., Kusakabe, S., Atomi, H. and Imanaka, T. (2005) Allophanate Hydrolase of Oleomonas Sagaranensis Involved in an ATP-Dependent Degradation Pathway Specific to Urea. *FEMS Microbiology Letters*, Oxford Academic, **245**, 61–65. https://doi.org/10.1016/j.femsle.2005.02.023.

84 Whitney, P.A. and Cooper, T.G. (1972) Urea Carboxylase and Allophanate Hydrolase: Two Components of a Multienzyme Complex in Saccharomycescerevisiae. *Biochemical and Biophysical Research Communications*, Academic Press, **49**, 45–51. https://doi.org/10.1016/0006-291X(72)90007-1.

85 Liu, Y., Yuan, B., Peng, L., Zhao, J., Cheng, B., Huang, Y., Zheng, X., Zhou, Y., Xiang, S., Zhu, L. and Wu, Y. (2020) Single-particle Analysis of Urea Amidolyase Reveals Its Molecular Mechanism. *Protein Science : A Publication of the Protein Society*, Blackwell Publishing Ltd, **29**, 1242. https://doi.org/10.1002/PRO.3847.

86 Fan, C., Chou, C.Y., Tong, L. and Xiang, S. (2012) Crystal Structure of Urea Carboxylase Provides Insights into the Carboxyltransfer Reaction. *Journal of Biological Chemistry*, Elsevier, **287**, 9389–9398. https://doi.org/10.1074/jbc.M111.319475.

87 Lin, Y. and St. Maurice, M. (2013) The Structure of Allophanate Hydrolase from Granulibacter Bethesdensis Provides Insights into Substrate Specificity in the Amidase Signature Family. *Biochemistry*, **52**, Article 690-700. https://doi.org/10.1021/bi301242m.

88 Cheng, G., Shapir, N., Sadowsky, M.J. and Wackett, L.P. (2005) Allophanate Hydrolase, Not Urease, Functions in Bacterial Cyanuric Acid Metabolism. *Applied and Environmental Microbiology*, **71**, 4437. https://doi.org/10.1128/AEM.71.8.4437-4445.2005.

89 Strope, P.K., Nickerson, K.W., Harris, S.D. and Moriyama, E.N. (2011) Molecular Evolution of Urea Amidolyase and Urea Carboxylase in Fungi. *BMC Evolutionary Biology*, **11**, 80. https://doi.org/10.1186/1471-2148-11-80.

90 Jonkers, H.M., Thijssen, A., Muyzer, G., Copuroglu, O. and Schlangen, E. (2010) Application of Bacteria as Self-Healing Agent for the Development of Sustainable Concrete. *Ecological Engineering*, Elsevier, **36**, 230–235. https://doi.org/10.1016/j.ecoleng.2008.12.036.

91 Wang, J.Y., De Belie, N. and Verstraete, W. (2012) Diatomaceous Earth as a Protective Vehicle for Bacteria Applied for Self-Healing Concrete. *Journal of Industrial Microbiology and Biotechnology*, **39**, 567–577. https://doi.org/10.1007/s10295-011-1037-1.

92 Wiktor, V. and Jonkers, H.M. (2022) Diatomite and Na-X Zeolite as Carriers for Bacteria in Self-Healing Cementitious Mortars. *Construction and Building Materials*, Elsevier, **343**, 128103. https://doi.org/10.1016/j.cemconcomp.2011.03.012.

93 Lucas, S.S., Moxham, C., Tziviloglou, E. and Jonkers, H. (2018) Study of Self-Healing Properties in Concrete with Bacteria Encapsulated in Expanded Clay. *Science and Technology of Materials*, No longer published by Elsevier, **30**, 93–98. https://doi.org/10.1016/j.stmat.2018.11.006.

94 Zhang, J., Liu, Y., Feng, T., Zhou, M., Zhao, L., Zhou, A. and Li, Z. (2017) Immobilizing Bacteria in Expanded Perlite for the Crack Self-Healing in Concrete. *Construction and Building Materials*, Elsevier, **148**, 610–617. https://doi.org/10.1016/j.conbuildmat.2017.05.021.

95 Fahimizadeh, M., Abeyratne, A.D., Mae, L.S., Raman Singh, R.K. and Pasbakhsh, P. (2020) Biological Self-Healing of Cement Paste and Mortar by Non-Ureolytic Bacteria Encapsulated in Alginate Hydrogel Capsules. *Materials 2020, Vol. 13, Page 3711*, Multidisciplinary Digital Publishing Institute, **13**, 3711. https://doi.org/10.3390/MA13173711.

96 Alazhari, M., Sharma, T., Heath, A., Cooper, R. and Paine, K. (2018) Application of Expanded Perlite Encapsulated Bacteria and Growth Media for Self-Healing Concrete. *Construction and Building Materials*, Elsevier Ltd, **160**, 610–619. https://doi.org/10.1016/j.conbuildmat.2017.11.086.

97 Jakubovskis, R., Jankutė, A., Guobužaitė, S., Boris, R. and Urbonavičius, J. (2021) Prolonging Bacterial Viability in Biological Concrete: Coated Expanded Clay Particles. *Materials 2021, Vol. 14,* Multidisciplinary Digital Publishing Institute, **14**. https://doi.org/10.3390/ma14112719.

98 Teshome, H.M. and Battula, V.S. (2025) Using Bio-Based Self-Healing Agent for Durability and Environmentally Friendly Concrete Development. *International Journal of Concrete Structures and Materials 2025 19:1*, SpringerOpen, **19**, 102-. https://doi.org/10.1186/s40069-025-00823-y.

99 Jiang, L., Wu, M., Du, F., Chen, D., Xiao, L., Chen, W., Du, W. and Ding, Q. (2024) State-of-the-Art Review of Microcapsule Self-Repairing Concrete: Principles, Applications, Test Methods, Prospects. *Polymers 2024, Vol. 16, Page 3165*, Multidisciplinary Digital Publishing Institute, **16**, 3165. https://doi.org/10.3390/polym16223165.

100 Jiang, L., Wu, M., Du, F., Chen, D., Xiao, L., Chen, W., Du, W. and Ding, Q. (2024) State-of-the-Art Review of Microcapsule Self-Repairing Concrete: Principles, Applications, Test Methods, Prospects. *Polymers*, Polymers (Basel), **16**. https://doi.org/10.3390/polym16223165.

101 Nath Priyom, S., Moinul Islam, M., Shumi, W. and Raozan Pahartali Rd, H. (2021) The Utilization of Bacillus Subtilis Bacteria to Improve the Mechanical Properties of Concrete. *Journal of the Civil Engineering Forum*, Universitas Gadjah Mada, **7**, 97–108. https://doi.org/10.22146/JCEF.60216.

102 Xu, J., Wang, X. and Wang, B. (2018) Biochemical Process of Ureolysis-Based Microbial CaCO3 Precipitation and Its Application in Self-Healing Concrete. *Applied microbiology and biotechnology*, Appl Microbiol Biotechnol, **102**, 3121–3132. https://doi.org/10.1007/S00253-018-8779-X.

103 Bhadiyadra, K., Jong, S.C., Ong, D.E.L. and Doh, J.H. (2024) Trends and Opportunities for Greener and More Efficient Microbially Induced Calcite Precipitation Pathways: A Strategic Review. *Geotechnical Research*, ICE Publishing, **11**, 161–185. https://doi.org/10.1680/jgere.24.00039.