Universidade Federal do Rio Grande do Sul
Centro de Biotecnologia
Programa de Pós-graduação em Biologia Celular e Molecular

DISSERTAÇÃO DE MESTRADO

ADVANCED TECHNIQUES IN SELF-HEALING CONCRETE: AUTOMATED CRACK ANALYSIS AND STARCH-BASED CAPSULES FOR BIOCONCRETE

João Vitor Bitencourt Borowski
Orientador: Profª. Drª. Marilene Henning Vainstein

Porto Alegre
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Dissertação apresentada ao Programa de Pós-graduação em Biologia Celular e Molecular da Universidade Federal do Rio Grande do Sul, como requisito parcial para a obtenção do título de **Mestre em Biologia Celular e Molecular**.

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Lista de abreviaturas, símbolos e unidades

ATCC	*American Type Culture Collection*
CO_2	Dióxido de Carbono
$Ca(NO_3)_2$	Nitrato de Cálcio
MICP	Precipitação de Carbonato de Cálcio Induzida Microbiologicamente
CAS	Calcium source
CBS	Carbonate source
NDT	Non destructive techniques
µM	Micrômetro
mm	Milímetro
cm	Centímetro
°C	Graus Celsius

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Resumo

A durabilidade das estruturas de concreto é frequentemente comprometida pela formação de fissuras, que servem como pontos de entrada para água, sais e outros agentes agressivos que aceleram a degradação estrutural. Os métodos tradicionais de reparo são reativos e exigem manutenção frequente, o que impulsiona a necessidade de soluções inovadoras, como o bioconcreto, que utiliza a precipitação de carbonato de cálcio induzida microbicamente (MICP) para selar fissuras de forma autônoma. Esta dissertação aborda desafios fundamentais na tecnologia do bioconcreto, desenvolvendo um pipeline automatizado de análise de fissuras e explorando a encapsulação à base de amido como uma estratégia para melhorar a sobrevivência bacteriana e a estabilidade de nutrientes em concreto autocurativo. A primeira parte desta pesquisa apresenta o pipeline automatizado "Ilhoa", projetado para padronizar e melhorar a precisão das avaliações de fissuras em concreto autocurativo. Ao integrar aquisição de dados automatizada, processamento de imagens e análise estatística, Ilhoa oferece uma avaliação abrangente da morfologia das fissuras, reduzindo o viés do operador e permitindo comparações mais confiáveis entre os tratamentos. A segunda parte do estudo investiga o uso de amido como um material de encapsulação biocompatível para incorporar bactérias e nutrientes na matriz de concreto. As cápsulas de amido protegem as células bacterianas do ambiente hostil do cimento, mantêm a viabilidade bacteriana e aumentam a eficiência do MICP. Por meio de experimentos de otimização, o estudo identifica as condições ideais para o MICP - pH 8,5, 1% de amido e 7% de nitrato de cálcio - demonstrando que Bacillus subtilis supera outras cepas na precipitação de carbonato de cálcio em condições alcalinas. As cápsulas de amido revestidas controlam efetivamente a expansão durante a hidratação, preservando a integridade estrutural do concreto e garantindo desempenho bacteriano consistente. Os achados combinados desta dissertação ressaltam o papel crítico de ferramentas de avaliação avançadas e estratégias de encapsulação sustentáveis no avanço da tecnologia do bioconcreto. Essas contribuições abrem caminho para o desenvolvimento de materiais de concreto autocurativo de próxima geração que aumentam a longevidade estrutural e promovem práticas de construção sustentáveis.

Abstract

The durability of concrete structures is often compromised by the formation of cracks, which serve as entry points for water, salts, and other aggressive agents that accelerate structural degradation. Traditional repair methods are reactive and require frequent maintenance, driving the need for innovative solutions such as bioconcrete, which leverages microbially induced calcium carbonate precipitation (MICP) to autonomously seal cracks. This dissertation addresses key challenges in bioconcrete technology by developing an automated crack analysis pipeline and exploring starch-based encapsulation as a strategy for enhancing bacterial survival and nutrient stability in self-healing concrete. The first part of this research presents the automated pipeline "Ilhoa", designed to standardize and improve the accuracy of crack evaluations in self-healing concrete. By integrating automated data acquisition, image processing, and statistical analysis, Ilhoa provides a comprehensive assessment of crack morphology, reducing operator bias and enabling more reliable comparisons across treatments. The second part of the study investigates the use of starch as a biocompatible encapsulation material for incorporating bacteria and nutrients into the concrete matrix. Starch capsules protect bacterial cells from the harsh cement environment, maintain bacterial viability, and enhance MICP efficiency. Through optimization experiments, the study identifies the optimal conditions for MICP - pH 8.5, 1% starch, and 7% calcium nitrate - demonstrating that Bacillus subtilis outperforms other strains in calcium carbonate precipitation under alkaline conditions. Coated starch capsules effectively control expansion during hydration, preserving the concrete's structural integrity and ensuring consistent bacterial performance. The combined findings of this dissertation underscore the critical role of advanced evaluation tools and sustainable encapsulation strategies in advancing bioconcrete technology. These contributions pave the way for developing next-generation self-healing concrete materials that enhance structural longevity and promote sustainable construction practices.

Introduction

Cracks and the Self-Healing

Cracks in concrete structures serve as entry points for harmful agents, such as water and salts, which accelerate material degradation and lead to the corrosion of reinforcement bars, compromising structural integrity (SCHLANGEN; JONKERS, 2008). To address these vulnerabilities, self-healing concrete has emerged as a promising solution, capable of autonomously repairing cracks and extending the lifespan of concrete infrastructures (DE BELIE et al., 2018; WANG et al., 2019). Self-healing mechanisms operate primarily through two pathways: autogenous and autonomous. Autogenous healing relies on the inherent properties of the concrete matrix, such as continued hydration and carbonation. At the same time, autonomous self-healing involves the deliberate incorporation of healing agents into the concrete mix (LUHAR; LUHAR; SHAIKH, 2022). These agents can be activated upon crack formation, initiating a healing process that can restore structural performance.

Bioconcrete

Among the autonomous self-healing techniques, bioconcrete stands out as one of the most innovative and sustainable approaches (NODEHI; OZBAKKALOGLU; GHOLAMPOUR, 2022; SEIFAN; SAMANI; BERENJIAN, 2016). Bioconcrete leverages the metabolic activities of microorganisms, primarily bacteria, to facilitate the self-healing process through microbially induced calcium carbonate precipitation (MICP) (Figure 1). In this process, bacteria metabolize nutrients, typically carbon and nitrogen sources, to produce carbonic acid. This acid subsequently reacts with available calcium ions to form calcium carbonate, which precipitates within the cracks, effectively sealing them. Bioconcrete enhances the durability of concrete structures and provides an environmentally friendly alternative to traditional repair methods, as it exploits natural biological processes for structural maintenance (CASTRO-ALONSO et al., 2019).

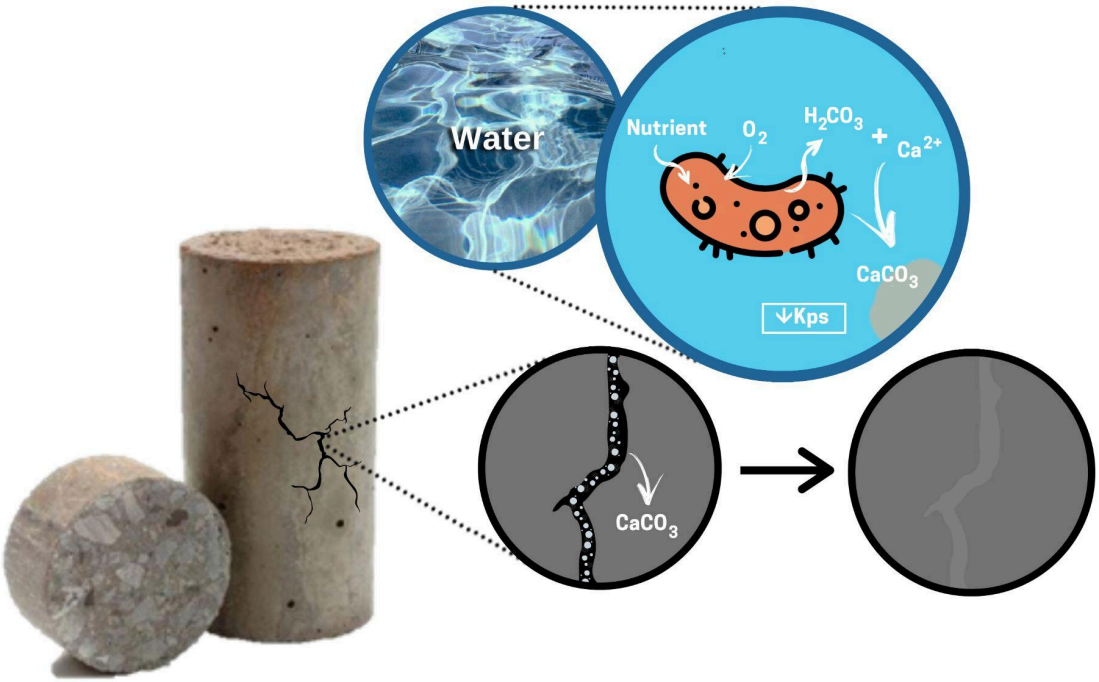


Figure 1: Illustrative scheme of the MICP process

Key factors governing MICP

MICP occurs naturally in the environment with bacteria in water and is reproducible in cementitious cracks (SEIFAN; BERENJIAN, 2019). In the MICP process, bacteria metabolize a carbonate source (CBS) - which provides energy for their growth - generating carbon dioxide as a byproduct. This CO_2 is converted into carbonic acid by the enzyme carbonic anhydrase and secreted. Upon contact with soluble calcium - from a calcium source (CAS) - the two molecules react to form calcium carbonate, which precipitates in crystal form due to its low solubility product (Ksp) (Figure 1). The availability and concentration of both CAS and CBS are crucial, as they directly influence the efficiency and extent of calcium carbonate precipitation. Adequate nutrient sources ensure optimal bacterial growth and metabolic activity, while sufficient calcium availability provides the necessary substrate for carbonate mineral formation (GARG; GARG; EDDY, 2022; PUNGRASMI et al., 2019; ZHANG et al., 2023). Here is the chemical reaction for calcium carbonate formation:

$$CBS + 2O_{2\,(aq)} + CAS \Leftarrow \overset{Carbonic\ Anhydrase}{\Rightarrow} H_2CO_{3\,(aq)} + Ca^{2+}_{(aq)} + \frac{1}{2}O_{2\,(aq)} \Leftarrow \overset{pH}{\underset{\Delta}{\Rightarrow}} CaCO_{3\,(s)} + H_2O_{(l)} \quad [1]$$

The pH level of the environment plays a significant role in MICP, as the solubility of calcium carbonate decreases with increasing pH, promoting precipitation (Figure 2). Additionally, water availability is essential for both bacterial activity and the MICP reaction, as it facilitates the transport of nutrients and calcium ions. Reaction time is also a critical factor; longer durations allow more extensive crystal formation, improving the overall effectiveness of the MICP process. Initial bacterial density affects the rate of calcium carbonate formation, as higher densities can enhance the reaction kinetics and increase the amount of precipitate produced (CASTRO-ALONSO et al., 2019; TANG et al., 2020).

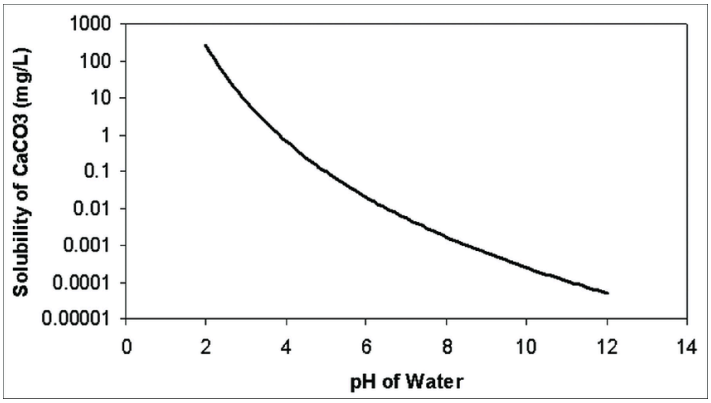


Figure 2: Solubility of calcium carbonate by pH. Adapted from (HART; COLSON; BURRIS, 2011).

Overall, these factors - availability of CAS and CBS, pH level, water availability, reaction time, and initial bacterial density - are pivotal in governing the efficiency and effectiveness of MICP in both natural and engineered systems.

State of the art

The development of bioconcrete has evolved rapidly in recent years, driven by the growing need for sustainable, low-maintenance construction materials (AYTEKIN; MARDANI; YAZICI, 2023; BESKOPYLNY et al., 2024). Current advancements focus on optimizing bacterial strains, nutrient sources, and incorporation methods to maximize the efficiency of MICP) (DORFAN et al., 2023; GOODCHILD-MICHELMAN et al., 2023; JIANG et al., 2020; MUTITU et al., 2019; PAN; GENCTURK, 2023; PUNGRASMI et al., 2019). Research has identified key bacterial species such as *Sporosarcina pasteurii* and

Bacillus subtilis as leading candidates due to their robust metabolic pathways and ability to precipitate calcium carbonate in alkaline conditions (MA et al., 2020; YAMASAMIT et al., 2023). However, numerous bacterial strains remain unexplored and have been systematically prospected and evaluated for their potential in bioconcrete applications (MONDAL; (DEY) GHOSH, 2019; ZHANG et al., 2019, 2015, 2016). Innovations in delivery systems, including encapsulation within hydrogels, alginate beads, and porous carriers, have improved bacterial viability and activity within the concrete matrix, ensuring effective crack healing (BABY; PALANISAMY; ARJUN, 2023; PUNGRASMI et al., 2019; SHEN et al., 2021). Additionally, the integration of metabolic enhancers and tailored nutrient formulations has further refined MICP efficiency, allowing for targeted applications in various environmental conditions. Recent studies have also explored genetic modification and synthetic biology approaches to engineer bacteria with enhanced calcite-producing capabilities, pushing the boundaries of bioconcrete performance (DORFAN et al., 2023; GOODCHILD-MICHELMAN et al., 2023). These advancements underscore the potential of bioconcrete as a transformative technology in the construction industry, combining the principles of biotechnology and materials science to create self-healing concrete systems that meet the demands of modern infrastructure.

Current Challenges

Bioconcrete research is advancing rapidly, yet several key challenges must be addressed to realize its potential fully (LEE; PARK, 2018). One of the foremost challenges is ensuring the survival and activity of bacteria within the concrete matrix. Bacterial cells must endure the harsh conditions of concrete production and curing and remain viable over time to contribute to the self-healing process effectively. The encapsulation of bacteria and nutrients is critical to overcoming this issue (TANG; XU, 2021; ZHANG et al., 2021). However, selecting appropriate materials and techniques for encapsulation is complex. The encapsulation must protect the bacteria from the alkaline environment of the cement while allowing for their gradual release and activity.

Integrating these encapsulated bacteria and nutrients with the cement matrix presents another significant hurdle (ZHANG et al., 2021). The interaction between the encapsulation materials and the cement can affect the concrete's mechanical properties

and the self-healing process's overall effectiveness (MUTITU et al., 2019; TANG; XU, 2021). Thus, optimizing the integration process is essential to balance concrete strength and self-healing capabilities.

Control and optimization of the bioconcrete formulation are also crucial (ZHANG et al., 2016). This process involves fine-tuning factors such as bacterial density, nutrient availability, and environmental conditions to ensure optimal performance (TAN et al., 2023). Effective control mechanisms must be established to monitor and adjust these variables throughout the concrete's life cycle.

An accurate assessment of the self-healing process is vital to evaluate the effectiveness of bioconcrete (KHAN et al., 2023). Analyzing the entire crack rather than just isolated points over time is essential. Comprehensive analysis allows for a more detailed understanding of how self-healing mechanisms function across the full extent of a crack, providing insights into the overall healing efficacy and ensuring that any variability in healing response is accounted for (KHAN et al., 2023; PAYAB; ABBASINA; KHANZADI, 2018; VERMA; BHADAURIA; AKHTAR, 2013; YANG, 2024). This holistic approach accurately assesses bioconcrete’s real-world performance and facilitates necessary adjustments to enhance its self-healing capabilities.

Cost and scalability further complicate the development of bioconcrete. Incorporating bacteria and encapsulation materials can be expensive, and scaling up production to meet construction industry demands poses additional financial and logistical challenges (RYU et al., 2020). Addressing these issues requires innovative solutions to reduce costs while maintaining the high performance and reliability of the bioconcrete.

While bioconcrete holds promising potential for revolutionizing construction materials, overcoming these challenges is crucial. Advances in bacterial survival, encapsulation techniques, integration with cement matrices, accurate assessment methods, and cost-effective production will be key to unlocking the full benefits of bioconcrete technology.

Proposed work

Starch as a Potential Capsule for Bioconcrete

Starch, an organic polymer produced by plants primarily for energy storage, exhibits several advantageous properties for use as a capsule material in bioconcrete applications. Its characteristics include optimized volume occupancy or compaction, low reactivity with other compounds, minimal water solvation requirements, and high viscosity (TESTER; KARKALAS; QI, 2004). Additionally, starch's impact on osmolarity is notably low, four times less than glucose (JONKERS; SCHLANGEN, 2008).

As a capsule material, starch offers compelling benefits. Its natural origin and biodegradability align well with sustainable construction practices, making it an environmentally friendly choice (QAMRUZZAMAN; AHMED; MONDAL, 2022; SABERI RISEH et al., 2023). Starch forms polymeric structures that effectively encapsulate bacteria and nutrients, creating a protective barrier against the harsh conditions of the cement matrix. This encapsulation is crucial for maintaining bacterial viability during the mixing and curing processes, which is essential for the efficacy of microbial-induced calcite precipitation (MICP) (BABY; PALANISAMY; ARJUN, 2023; PUNGRASMI et al., 2019; SHEN et al., 2021).

Moreover, starch’s ability to absorb and retain water supports bacterial activity by ensuring adequate moisture availability, a key requirement for MICP. Starch also serves as a carbon source for bacteria, enhancing its utility by promoting efficient carbonate production (JONKERS; SCHLANGEN, 2008; ZHANG et al., 2019). Its cost-effectiveness and widespread availability further solidify its practicality for large-scale applications. Combining these protective, functional, and economic advantages positions starch as a valuable material in developing self-healing concrete technologies.

Different organisms produce amylase, an enzyme that facilitates starch metabolism and generates higher amounts of carbonate compared to lactate and urea (Table 1). Starch is composed of approximately one-quarter amylose and three-quarters amylopectin (TESTER; KARKALAS; QI, 2004). The primary difference between these components lies

in their glycosidic bonds: amylopectin contains both α-1,4 and α-1,6-glycosidic bonds, while amylose contains only α-1,4 bonds (Figure 3). Amylase hydrolyzes α-1,4-glycosidic bonds but does not entirely degrade starch. Isomaltase (α-1,6-glycosidase) can degrade amylopectin by cleaving α-1,6 bonds. *Bacillus subtilis* and *Bacillus velezensis* strains used in this study possess the necessary enzymes for starch degradation ("EO946_RS15605 alpha-glucosidase [Bacillus subtilis subsp. spizizenii ATCC 6633 = JCM 2499] - Gene - NCBI," [n.d.]; "KSO_RS17620 alpha-glucosidase [Bacillus amyloliquefaciens IT-45] - Gene - NCBI," [n.d.]). Amylase activity is influenced by pH, with optimal performance observed around a pH range of 7 to 8 (Figure 4).

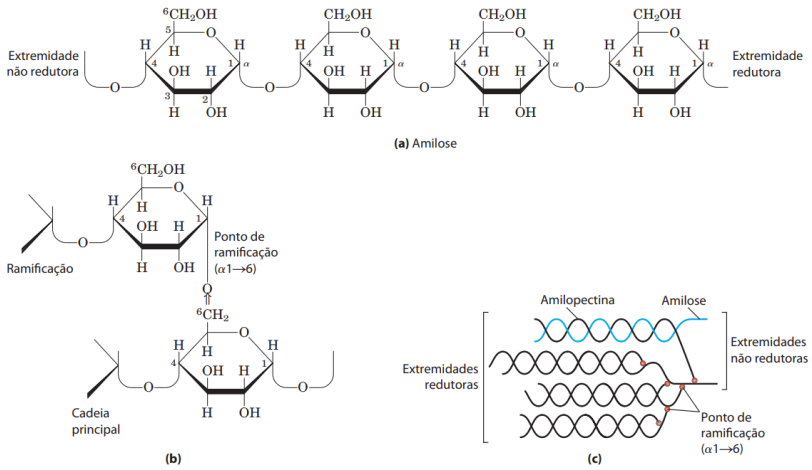


Figure 3: Structure of Amylose and Amylopectin. (a) Amylose is depicted as a linear polymer composed of D-glucose residues linked by α-1,4 glycosidic bonds, with individual chains potentially containing thousands of glucose units. Amylopectin features similar linkages but includes branching points. Glycogen, resembling amylopectin structurally, is more highly branched. (b) Illustrates the α-1,6 branching points found in glycogen and amylopectin. (c) Shows the clustering of amylose and amylopectin within starch granules. Amylopectin strands (black) form double helix structures with other amylopectin or amylose strands (blue), with frequent α-1,6 branching points (red). During starch mobilization for energy, glucose residues at the non-reducing ends of the outermost branches are enzymatically cleaved. Glycogen's structure is similar but features more extensive branching and compactness. Adapted from (LEHNINGER; NELSON; COX, 2014).

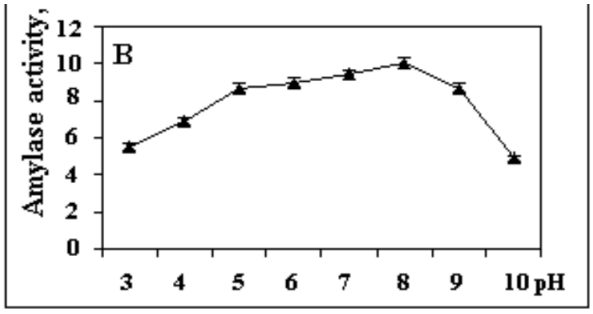


Figure 4: Effect of pH on amylase activity produced by *Bacillus* spp. Adapted from (SHAMALA; VIJAYENDRA; JOSHI, 2012).

Table 1: Carbon dioxide yields by type of carbonate source (CBS). Includes molecular formulas, molecular weights, CO_2 yields from metabolic processes, and the moles of carbonate produced from 300 g of each CBS.

Calcium Lactate	Urea	Starch
$C_6H_{20}CaO_{11}$	**CH_4N_2O**	**$(C_6H_{10}O_5)_n$**
308 g/mol	60 g/mol	162 g/mol*
3 CO_2 /mol	1 CO_2 /mol	6 CO_2 /mol*
For 300 g of this molecule, the maximum number of moles obtained is:		
3 CO_3^{2-}	5 CO_3^{2-}	11 CO_3^{2-}

*Values calculated for a monomer (glucose).

Calcium Nitrate as a Calcium Source

Calcium nitrate, with the chemical formula $Ca(NO_3)_2$, is an inorganic compound known for its high solubility in water, reaching 1290 g/L at 20°C. Similar to calcium lactate, calcium nitrate contains both calcium and nitrate components. Various bacteria, particularly those involved in the nitrogen cycle, can reduce nitrate to nitrite or nitrogen gas. For instance, *Bacillus subtilis* ATCC 6633, a soil isolate, and *Bacillus velezensis* are known nitrate reducers. Nitrate has already been explored in conjunction with urea for microbial-induced calcite precipitation (MICP) and has demonstrated promising results (XU et al., 2015; ZHU et al., 2019).

Selected Bacteria

Bacillus subtilis was selected for this study due to its extensive use in bio-self-healing applications, attributed to its osmotolerance and robust growth in alkaline conditions (FENG et al., 2021; JENA et al., 2020; MAKSIMOVA; ELISEEVA; MAKSIMOV, 2024; MUTITU et al., 2019; NAIN et al., 2019; YAMASAMIT et al., 2023). *Bacillus velezensis*, which is phylogenetically closely related to *B. subtilis*, was also chosen because both species can metabolize starch and nitrate effectively.

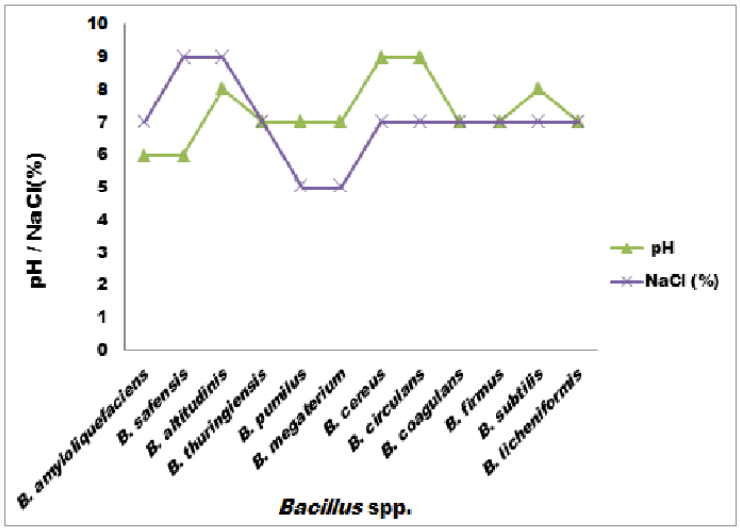


Figure 5: Maximum growth pH and halotolerance of *Bacillus* spp. strains. Adapted from (GLOVER; MADILO; TERLABIE, 2018).

Incorporation into Cementitious Matrix

Following bacterial selection, the next challenge lies in their effective incorporation into the cementitious matrix. Immobilization techniques are critical to protecting the bacteria and ensuring their longevity and activity in harsh concrete conditions. Standard methods include adsorption onto porous carriers and encapsulation within polymeric matrices, which serve as delivery systems. However, these carriers are typically inert in the MICP process, acting as protective agents for the bacteria. To optimize the integration and enhance self-healing efficiency, this study investigates the use of starch - a biodegradable polymeric carbon source - as an innovative delivery system. Starch offers multiple advantages, including low cost, availability, and compatibility with bacterial metabolism, particularly for *Bacillus* spp known to produce amylases that can utilize starch as a nutrient source. Chapter two focuses on the development and evaluation of starch-based delivery systems for bioconcrete applications.

Quantification Methods

The efficacy of self-healing must be rigorously quantified to validate the performance improvements observed. However, most papers rely on non-optimal evaluation methods that depend on the observation of a single or few points as representative of the whole. A standardized approach to crack quantification that considers all data points in the evaluation is essential for reliable comparisons between treatments and conditions. This dissertation's first chapter describes the development of an automated pipeline for standardized crack quantification, providing a robust methodology for assessing the self-healing performance of bioconcrete. This pipeline integrates image analysis techniques to measure crack closure and evaluate the overall effectiveness of the incorporated biogenic healing agents.

General objective

This research aims to advance the understanding of bioconcrete, optimize bacterial incorporation strategies, and establish reliable quantification methods to support the development of next-generation self-healing concrete materials.

Specific objectives

- Create an automated crack quantification pipeline (Chapter 1)
- Develop a new delivery system for bacteria and nutrients incorporation (Chapter 2)

Chapter 1

Chapter 2

General Discussion

The research presented in this dissertation highlights significant advancements in developing and evaluating self-healing concrete, particularly focusing on integrating automated crack analysis and encapsulating bacteria using starch-based capsules. Together, these studies offer a comprehensive approach to enhancing the efficiency, precision, and sustainability of bioconcrete technologies, addressing critical challenges in construction materials.

The emergence of self-healing concrete, especially bioconcrete, represents a transformative shift in addressing the inherent vulnerabilities of concrete structures (CAPPELLESSO et al., 2023; WANG et al., 2019). Cracks, which serve as entry points for aggressive agents like water and salts, accelerate structural degradation, undermining the integrity and longevity of infrastructures. Traditional repair methods are often reactive and labor-intensive, necessitating repeated maintenance interventions. In contrast, bioconcrete leverages the natural metabolic activities of bacteria to precipitate calcium carbonate, effectively sealing cracks and restoring the material's structural properties (LUHAR; LUHAR; SHAIKH, 2022; NODEHI; OZBAKKALOGLU; GHOLAMPOUR, 2022). However, despite the promising potential of this technology, integrating bacterial cells and nutrients into the cement matrix poses significant challenges, including bacterial survival during concrete production and nutrient interference with cement setting (JONKERS; SCHLANGEN, 2008; LEE; PARK, 2018).

The first part of this dissertation addressed the critical need for standardized and precise evaluation methods for self-healing concrete, focusing on developing an automated crack analysis pipeline named Ilhoa. Crack evaluation is a complex and crucial aspect of assessing the performance of self-healing concrete, yet current methodologies often rely on manual, point-based assessments that are prone to operator bias and inconsistency (KHAN et al., 2023; PAYAB; ABBASINA; KHANZADI, 2018; VERMA; BHADAURIA; AKHTAR, 2013; YANG, 2024). By automating the process, Ilhoa ensures consistent, detailed crack profiles that accurately represent the self-healing dynamics across entire crack surfaces rather than isolated points. The comprehensive assessment of crack morphology provides deeper insights into the initiation, propagation, and healing

processes, highlighting the importance of a holistic evaluation approach. Automated data acquisition and image processing reduce human error, streamline data generation, and enable more frequent analysis throughout the healing process, offering a scalable solution that meets the demands of large-scale structural assessments.

The comparison between automated and manual methodologies revealed that automated approaches reduce variability and operator dependence and capture more intricate details of crack profiles, providing a more accurate representation of the self-healing phenomenon. The integration of Python-based analysis further enhanced the evaluation process by generating quantitative data and enabling comprehensive statistical analyses, thereby offering a robust framework for understanding the self-healing efficacy of various treatments. This approach underscores the need for advanced evaluation tools in bioconcrete, as accurate assessments are critical to optimizing material performance and validating the effectiveness of self-healing agents (KHAN et al., 2023; PAYAB; ABBASINA; KHANZADI, 2018).

The second part of this dissertation explored the use of starch-based capsules as an innovative encapsulation strategy for incorporating bacteria and nutrients into self-healing concrete. The encapsulation of bacteria is vital for protecting cells from the harsh conditions of concrete, such as high alkalinity and mechanical stresses, which can compromise bacterial viability (BABY; PALANISAMY; ARJUN, 2023; PUNGRASMI et al., 2019; SHEN et al., 2021). Starch, an organic polymer known for its biodegradability and low reactivity, was selected as the encapsulating material due to its ability to form protective polymeric structures that effectively trap bacteria and nutrients while serving as a carbon source (QAMRUZZAMAN; AHMED; MONDAL, 2022; SABERI RISEH et al., 2023). This dual functionality supports bacterial activity and enhances calcium carbonate precipitation, a critical process in MICP.

The study demonstrated that starch encapsulation significantly improves bacterial survival and nutrient stability during the concrete curing process, addressing one of the major challenges in bioconcrete technology. The optimized encapsulation conditions, identified through fractional factorial and central composite designs, were critical to enhancing MICP performance. Key parameters, such as pH, starch concentration, calcium nitrate levels, and bacterial density, were systematically evaluated, identifying

optimal conditions—pH 8.5, 1% starch, and 7% calcium nitrate—that maximized calcium carbonate yields. The superior performance of *B. subtilis*, particularly under alkaline conditions, aligned with the literature for its potential as a robust candidate for bioconcrete applications, achieving significant calcium carbonate precipitation that effectively contributes to crack closure (FENG et al., 2021; JENA et al., 2020; MAKSIMOVA; ELISEEVA; MAKSIMOV, 2024; MUTITU et al., 2019; NAIN et al., 2019; YAMASAMIT et al., 2023).

Moreover, the research highlighted the importance of controlling capsule expansion during hydration to maintain the structural stability of the concrete matrix. Coated starch capsules significantly reduce unwanted expansion compared to uncoated capsules, preserving the matrix's integrity and ensuring consistent bacterial viability. This control over capsule behavior is crucial, as excessive expansion can lead to structural weaknesses and compromise the overall performance of the self-healing system (MUTITU et al., 2019; TANG; XU, 2021; ZHANG et al., 2021). The findings underscore the necessity of optimizing encapsulation techniques to balance bacterial protection with minimal impact on concrete properties.

The integration of starch-based encapsulation enhances the performance of bioconcrete but also aligns with sustainable construction practices (QAMRUZZAMAN; AHMED; MONDAL, 2022; RYU et al., 2020). Starch's biodegradability and natural origin make it an environmentally friendly choice, supporting the development of greener and more resilient construction materials (QAMRUZZAMAN; AHMED; MONDAL, 2022; SABERI RISEH et al., 2023). This research contributes to a growing body of evidence that encapsulation strategies can significantly improve the functionality and reliability of bioconcrete, paving the way for scalable and cost-effective solutions in the field (RYU et al., 2020).

Overall, the findings of this dissertation emphasize the critical role of advanced methodologies and innovative materials in advancing bioconcrete technology. The automated crack analysis pipeline provides a robust framework for accurately assessing self-healing efficacy. At the same time, starch-based encapsulation offers a practical and sustainable approach to enhancing bacterial incorporation in concrete. Together, these advancements address key challenges in the field, including bacterial survival, effective

integration with the cement matrix, and the need for precise evaluation techniques. As the demand for durable and self-repairing infrastructure continues to grow, the insights gained from this research will be instrumental in guiding the development of next-generation bioconcrete formulations that meet the needs of modern engineering.

Perspectives

Incorporation of starch-based capsules in cementitious matrix:
Evaluation of self-healing through both microscopy and ultrasound techniques. Microscopy allows for high-resolution analysis of crack closure, while ultrasound provides non-destructive testing for internal healing and matrix density variations.

Machine learning applied to microscopy datasets:
Development of machine learning models for analyzing microscopy datasets, enabling accurate predictions of self-healing progress. Data augmentation techniques will allow cross-comparisons of self-healing behavior across different samples and conditions.

Screening of environmental bacteria for bioconcrete applications:
Exploration of environmental bacteria with potential for bioconcrete applications, focusing on alkalitolerance, halotolerance, and metabolic capabilities. Strains will be evaluated for their ability to perform microbially induced calcium carbonate precipitation (MICP), with the goal of identifying new candidates for enhanced self-healing.

Evaluation of the synergistic effect of oxalate and carbonate in calcium precipitation in bioconcrete:
Investigating the combined role of oxalate and carbonate in calcium precipitation processes within bioconcrete formulations. The synergistic effects of these ions could lead to improved precipitation efficiency and crack healing performance.

Hallmark genes for bioconcrete applications:
Creation of a manually curated database containing sequences of experimentally validated proteins and genes relevant to bioconcrete applications. This resource will be used for annotation purposes, aiding in the identification of new microbial strains involved in bioconcrete processes.

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