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Cracks Repairing and Resistance to Water Penetration Properties of Microbial Self-Healing Cement

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ABSTRACT

This study focuses on applying microbial self-healing cement in repairing cracks in cement-based materials and enhancing its resistance to water penetration performance. Traditional cement is susceptible to environmental influences, leading to the formation of microcracks and a reduction in durability. This research used *Bacillus pseudofirmus* to prepare microcapsules through sodium alginate gelation technology. We mixed microcapsules into the cement. The results indicate that the microbial self-healing cement, with a 1% self-healing agent added, increased its resistance to water penetration ability by 29.2% after 28 days. This improvement rose to 39.3% after 84 days. Additionally, we used the embedded needle method to make mortar blocks through microcracks, mimicking the cracks found in real cement. The self-healing effect of the microcapsules was especially noticeable for cracks under 0.3 mm in diameter, compared to the commonly used commercial crystallization penetration technology. This is attributed to the crystalline bodies formed by the self-healing agent in the microcapsules blocking the cracks and preventing water penetration. This study provides an environmentally friendly solution for the repair of cracks in cement-based materials using microbial self-healing technology and lays the foundation for improving the repair efficiency and durability and exploring stability and reliability in the future.

Practical Application: This study investigated the application of microbial self-healing cement in repairing cracks in cement-based materials and enhancing its resistance to water penetration properties. Cement, a material widely used in infrastructure, has low tensile strength and often forms microcracks. These microcracks reducing the durability of cement and posing risks to the economy and safety. Adding 1% self-healing agent to microbial self-healing cement significantly increases the resistance to water penetration pressure of the mortar blocks. Compared to the standard specimens, the resistance to water penetration ability increased by 29.2% at 28 days and further increased to 39.3% at 84 days. Microbial self-healing cement could effectively restore the resistance to water penetration performance of the mortar blocks after repairing cracks. The repairing results are significantly better than the methods of mixing or applying cement crystalline materials.

1 | Introduction

Cement, a material widely used in infrastructure, has low tensile strength and is inherently brittle [1]. It is also easily affected by external loads and the environment, which can lead to the formation of microcracks. These microcracks provide flow paths, reducing the durability of cement and posing risks to the economy and safety. Therefore, finding a method to effectively mitigate the issue of cement cracks is very important.

Traditional cement repair methods include structural reinforcement, surface treatment, grouting, and filling [2–4]. However, these methods are time-consuming and labor-intensive, and they are mostly suitable for cracks, that are larger than 0.3 mm. (Code for quality acceptance of concrete structures construction, GB50204-2015 [5].) Repairing microcracks and internal cracks is quite difficult with these methods. To overcome the limitations of traditional repair methods, self-healing technology for cement has become a hot research topic in recent years. This technology includes electrochemical self-healing [6], shape memory alloy (SMA) self-healing [7, 8], self-healing with built-in repair agents [9, 10], and microbial self-healing [11]. Electrochemical self-healing mainly relies on electrochemical reactions, including processes such as anodic corrosion, cathodic protection, and electrodeposition. By controlling the composition of the electrolyte solution and conditions such as voltage and current, precise repair of the material can be achieved. Applying a weak direct current between the reinforcing steel in the concrete structure (cathode) and the electrodes located in seawater (anode) allows the electrolyte material to precipitate onto the electrode surface, thus repairing the cracks in the cement [12]. Electrochemical self-healing needs to be carried out in a specific electrolyte solution and requires electrical energy from an external power source. Electrochemical self-repair can only repair a small area of damage, and the preparation process to form a conductive circuit is complicated to construct. SMAs have shape memory effect (SME), super elasticity (PE), high damping properties, electrical resistance property (ER), and so on. Unique physical and mechanical properties are used in intelligent concrete materials. The application of SMA in cement repair is greatly hindered by the fact that the material is expensive and tends to soften at high temperatures [8, 13]. Currently, embedded repair agents include hollow core optical fibers, microcapsules, fibers, etc. Compared with ordinary non-additive concrete, the embedded repair agent concrete has been significantly improved in terms of corrosion resistance, seismicity, and durability. The compatibility between the built-in repair agent and cement is poor, and the self-repair performance is affected by many factors, and the mechanism of action is not clear [9].

Many microorganisms in nature can complete the mineralization and crystallization of calcium carbonate outside the cell body, one of the most stable minerals in nature, and its unparalleled superiority as a crack repair material for cementitious materials [14]. Microbial self-healing cement is a process that uses compatible materials to encapsulate and uniformly mix bacteria, nutrients, and calcium sources into the cement. As microcracks form, oxygen and moisture penetrate the cement along the cracks, activating dormant bacterial spores and generating CO_2 through their growth and metabolism, which induces calcium carbonate mineralization deposition under alkaline conditions to repair the cracks [15]. Electrochemical self-healing is limited to reinforced concrete, has solution requirements, and requires a large anode plate [16]. The pre-embedded amount of SMA self-healing is limited, the cost is high and there are certain ambient temperature requirements [17]. There is an uneven distribution of internal and external self-healing agents, which greatly affects the mechanical properties of the concrete [18, 19]. Microbial self-healing has the advantages of green environmental protection, intelligent repair mechanism, multiple repairs, stable nature of deposited minerals, and good compatibility with the matrix. In addition, microorganisms are compatible with cement and can be uniformly distributed in the concrete to achieve "point repair". Mineral deposition is caused by the metabolism of microorganisms, which is in line with the development trend of green and low-carbon society. Therefore, microbial self-repair has a superior application prospect in concrete crack repair.

The microbial bacteria used in concrete self-healing agents must be able to withstand highly alkaline environments and survive for long periods. *Bacillus pseudofirmus* is tolerant of the harsh alkaline environment of cement and can metabolize organic matter to release CO_2 under the right conditions [20]. This carbon dioxide reacts with calcium ions to form calcium carbonate, which is commonly used for concrete [21], cement [22], and soil enhancement [23]. However, in practical applications, many bacterial spores are crushed during the aging process of cement, which reduces the self-healing ability of the cement. To effectively protect the bacterial spores, they can be encapsulated and stabilized before being added to the cement. Several protective carriers for bacterial spores have been developed, such as expanded perlite [24], hydrogels [25], and sodium alginate [26]. Encapsulation of spores in a polymer matrix is a promising technique for application, not only to improve spore survival in cement but also to facilitate isolation and recovery [26–28], Significantly improving the self-healing properties of cement.

The study aimed to tackle leakage problems due to cracks in cement using microbial self-healing cement. Spores of *B. pseudofirmus* were embedded and then incorporated into the cement. Pre-made cracks were used to mimic the conditions of cracks in actual cement. After a set curing period, the maximum resistance to water penetration pressure of the microbial self-healing cement was measured. This was compared with conventional repair methods to assess its resistance to water penetration capabilities. It provides a solid foundation for future research and the application of microbial self-healing cement. Microbial self-healing cement has shown great potential in mitigating concrete cracking due to its environmental friendliness, intelligent repair, durability, corrosion resistance, and low cost, significantly extending the service life of concrete.

2 | Experimental Materials and Methods

2.1 | Specimen Preparation Materials and Methods

Experimental materials: Yeast extract was purchased from OXOID. Peptone and beef extract powder were purchased from Beijing Aoboxing Bio-Tech. Co., Ltd. Sodium alginate was purchased from Sinopharm Chemical Reagent Co., Ltd. Cement

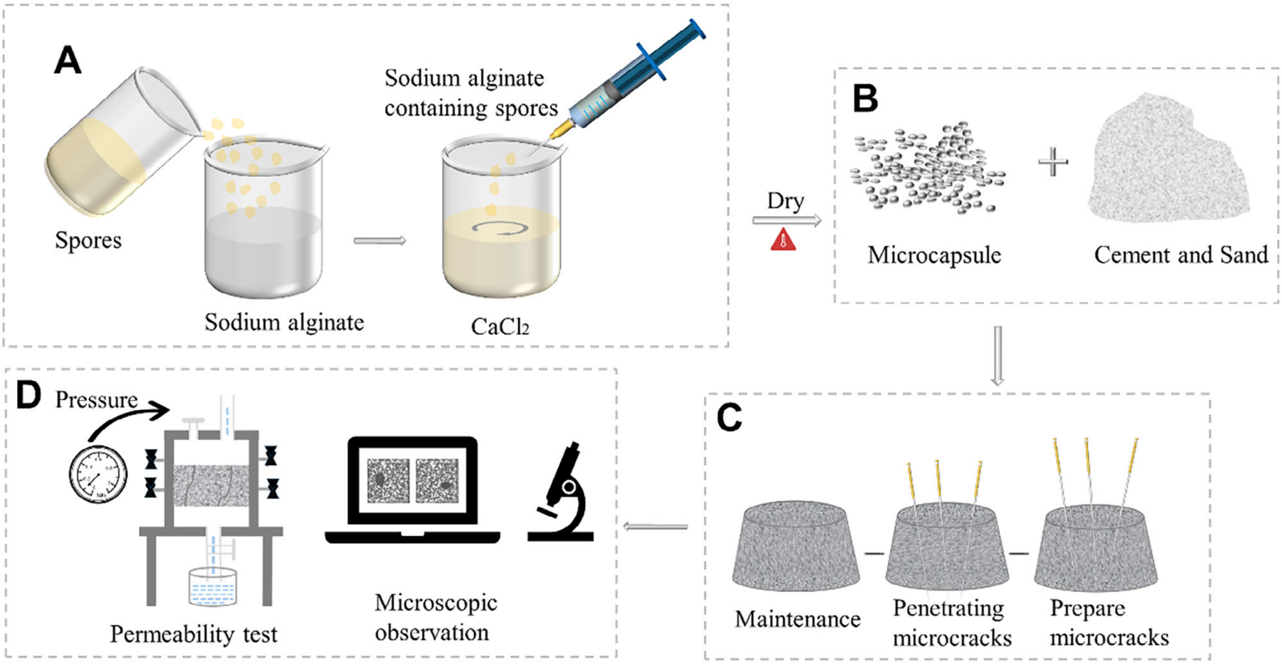

FIGURE 1 | Preparation and testing process of self-healing cement. (A) Preparation of sodium alginate microcapsules containing spores. (B) Preparation of microcapsule specimens. (C) Prepare microcracks, penetrating microcracks and maintenance. (D) Permeability test and microscopic observation.

was purchased from Tangshan Jidong Cement Co., Ltd. Calcium chloride ($CaCl_2$) was purchased from Tianjin Jinke Fine Chemical Research Institute. A cement bending compression testing machine was purchased from Wuxi Dongyi Manufacturing Co., Ltd. China ISO standard sand was purchased from Xiamen ISO standard sand Co, Ltd.

Control specimens: Cement, sand, and water were mixed in a standard ratio of 1:1.5:0.5. After stirring, the mixture was poured into molds, tapped, and leveled to form the base concrete samples. (The upper surface diameter: is 7 cm, the lower surface diameter: is 8 cm, and the height: is 3 cm.)

Crystal penetration specimens: The preparation of crystal penetration specimens involved adding a crystal penetration additive, XYPEX ADMIX C-1000 NF, to the base concrete sample preparation process.

Microcapsule specimens: Bacteria used for self-healing of concrete must withstand high alkalinity and survive for a long time. When cracks appear, the $CaCO_3$ induces precipitation through bacterial metabolic activity, and the cracks are bridged. Microcapsules were prepared using the method described in Gao et al., 2020 for microbial self-healing capsules, and *B. pseudofirmus* was selected, which can survive under extreme conditions and form spores. The bacteria were cultured at 30°C, 200 rpm for 48 h in a specially designed medium, we successfully induced the formation of bacterial spores. These spores are highly adaptable to the environment and can remain active in harsh conditions. The spores were collected using a centrifuge. The bacterial spores ($1.85–2.24 \times 10^7$ spores/g, wet weight) were dissolved in 2.0% (w/v) sodium alginate solution to a 3.51% (w/v) concentration of bacteria. Then dropwise into 3.0% (w/v) calcium chloride solution, the calcium alginate beads containing spores were dried in a fume hood for 24 h. These microcapsules immobilized the spores and formed a stable structure upon drying. The microcapsule specimens were prepared by adding these microcapsules to the process used for the control specimens. Depending on the experimental design, the weight of microbeads was 1% or 2% (w/w) of the total sample, that is, 2.04×10^5 or 4.08×10^5 spores were added to 1 cm^3 of concrete.

2.2 | Microcrack Preparation

When studying the resistance to water penetration performance of cement, preparing microcracks is an important step to simulate material damage and assess its repair ability. In this study, we used a technique of embedding fine needles as shown in Figure 1 to create mortar blocks through microcracks. Specifically, we selected needles with diameters of 0.25, 0.3, and 0.35 mm, which were embedded in the mortar blocks beforehand. After the mortar was cured, the needles were removed, creating microcracks in the blocks. The timing for removing the needles was chosen one day after, based on the best time point determined by experiments, to ensure that the formation of cracks is both stable and controllable. In the actual construction industry, concrete cracks less than 0.2 mm are considered harmless and can be left untreated. Concrete cracks in the 0.2–0.5 mm can be closed with embedded joints to deal with, greater than 0.5 mm, you can use the grouting repair method to deal with [29]. Therefore, we chose to prepare microcracks with different pore sizes by pre-embedding fine needles to investigate the range of repair ability of the bio-based microcapsules in this study for different pore sizes.

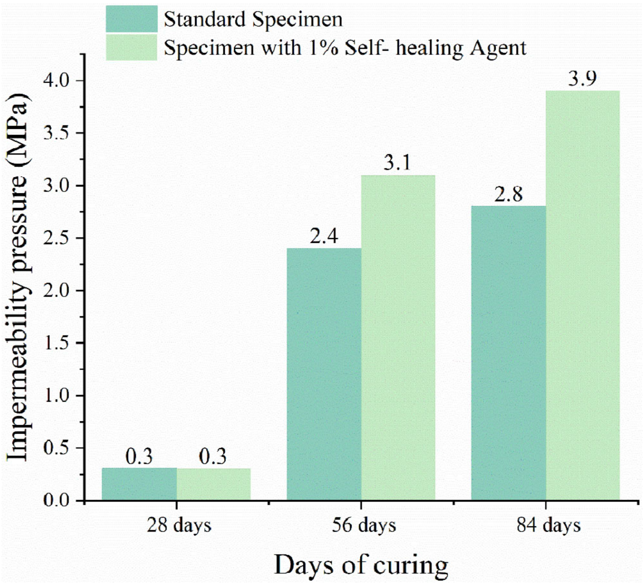

FIGURE 2 | The resistance to water penetration pressure of standard specimens and formed specimens with added self-healing microcapsules. The standard specimen had an impermeable pressure of 0.3 MPa after the first 28 days, 2.4 MPa after 56 days of conditioning and 2.8 MPa after 84 days of conditioning. The Specimen with 1% self-healing Agent had the same impermeable pressure as the standard specimens at 28 days, 0.3 MPa, and the impermeable pressure gradually recovered after 56 days conditioning to 3.1 MPa after 56 days. After 84 days it had recovered to 3.9 MPa.

2.3 | Resistance to Water Penetration Performance Test

According to the GB/T 50082–2009 "Standard for test methods of long-term performance and durability of ordinary concrete" [30, 31]. The SJS-1.5S digital display mortar resistance to water penetration meter from WuXi JianYi Instrument Machinery Corporation Limited was used to test the resistance to water penetration performance of the aged concrete samples. The purpose was to study the effect of adding microcapsules on the cement's resistance to water penetration properties.

3 | Results and Discussion

3.1 | The Resistance to Water Penetration Effect of Self-Healing Capsules on Mortar

To evaluate the performance of microcapsule self-healing agents in mortar, both the control specimens and the specimens with added self-healing microcapsules were subjected to resistance to water penetration pressure test according to the JGJ/T 70–2009 "Standard for test method of basic properties of construction mortar". As shown in Figure 2, the initial results at 28 days showed that both the control specimens and those with capsules had resistance to water penetration pressure of 0.3 MPa. After 56 days of curing with needles punctured, the control specimens' resistance to water penetration pressure increased to 2.4 MPa, while the specimens with Capsules reached 3.1 MPa, showing a 29.2% higher resistance to water penetration ability than the control specimens. Further cured for 84 days, the control specimens' resistance to water penetration pressure was 2.8 MPa, and the specimens with Capsules increased to 3.9 MPa, which is 39.3% higher than the control specimens. According to the GB 55030-2022 "standard for general specifications for waterproofing in buildings and municipal engineering", the resistance to water penetration pressure of mortar specimens for polymer cement waterproof mortar and polymer cement waterproof slurry should be greater than 1.0 MPa. The experimental results show that both the control specimens and those with Capsules far exceeded the standard requirement of 1.0 MPa during the 56 and 84 days of curing. The data indicate that the mortar specimens with 1% added Capsules significantly improved in resistance to water penetration performance compared to the control specimens and met the resistance to water penetration pressure requirements shown in GB 55030-2022.

TABLE 1 | Compressive strength of uncracked and cracked standard specimens and the specimens with 1% self-healing agent after 6 months of water curing.

Project: Compressive strength	Standard specimen	Specimen with 1% self-healing agent
Without cracks	51.9 MPa	50.2 MPa
Cracked, 6 months water curing	Not measured (not formed)	44.4 MPa

3.2 | The Reinforcing Effect of Self-Healing Microcapsules on Mortar

To evaluate the reinforcing effect of self-healing microcapsules in mortar, standard mortar was used to prepare rectangular (40 mm × 40 mm × 160 mm) standard specimens and specimens with a 1% agent. The specimens with 1% agent were cracked, then joined and fixed with plastic rope, and cured in water for 90 days. As shown in Figure S1, the self-healing microcapsules showed obvious fracture repair.

According to the GB/T 17671-2021 standard "Test Method of Cement Mortar Strength (ISO Method)", we conducted compressive strength tests on standard specimens and specimens with 1% agent added. The test results are shown in Table 1. After 6 months of water curing, the compressive strength of the specimens with the agent reached 44.4 MPa, with a recovery rate of 88.4%, respectively. These results are similar to those of Xu Jing et al., 2017, who loaded microbial spores and organic nutrients on expanded shale and mixed them into concrete. The compressive strength recovery rate of the cracked specimens after 28 days of self-healing was nearly 63% [32]. When low-alkali cementitious materials loaded with microbial repair agents were applied to concrete specimens, the compressive strength recovered to 84% of the pre-cracking level after 28 days of self-heal [33]. The results of the compressive strength tests show that the addition of agents significantly enhanced the self-healing ability of the specimens after cracking and enabled them to withstand the corresponding pressure.

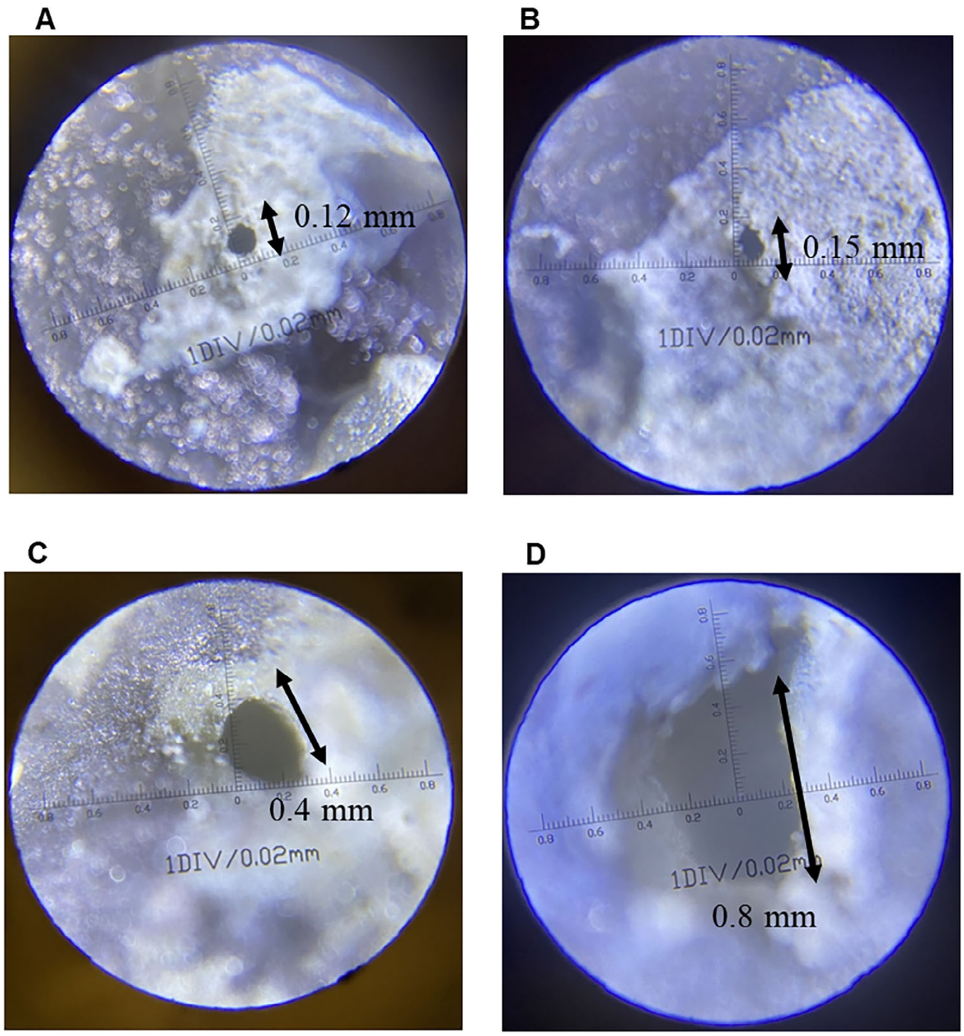

FIGURE 3 | Typical hole diameters on the upper and lower surfaces of artificially prepared microcracks. (A) Typical hole diameter on the lower surface: 0.12 mm. (B) Typical small hole diameter on the upper surface: 0.15 mm. (C) Typical medium hole diameter on the upper surface: 0.4 mm. (D) Typical large hole diameter on the upper surface: 0.8 mm.

3.3 | Results of Microcrack Preparation

This study successfully prepared mortar blocks through microcracks by embedding fine needles. After the needles were removed, the resulting cracks had openings with diameters ranging from 0.1–0.3 mm on the lower surface, which were independent of the needle's diameter. Additionally, the diameters on the upper surface were categorized into three sizes based on the crack size: small holes (0.1–0.3 mm), medium holes (0.3–0.6 mm), and large holes (0.6–1.0 mm). This categorized hole diameter design provides us with an experimental model of microcracks of different sizes, facilitating the study of the impact of different crack sizes on material properties.

Using a 100× reading microscope model MG10085-1A and optical microscopy techniques, the microstructure of artificially prepared microcracks was further observed. As shown in Figure 3, the typical hole diameter on the lower surface was 0.12 mm, while the typical hole diameters on the upper surface were 0.15 mm for small holes, 0.4 mm for medium holes, and 0.8 mm for large holes.

3.4 | Self-Healing Resistance to Water Penetration Restoration Effect of Micro-Cracked Bio-Based Mortar

In this study, we compared the resistance to water penetration performance of bio-based self-healing mortar and cement crystallization mortar.

The resistance to water penetration performance of the bio-based self-healing mortar blocks is shown in Figure 4. In the ordinary mortar without agents, the resistance to water penetration pressure reached 1.6 MPa after 28 days. When a 1% agent was added to the mortar, samples with medium and large hole diameters showed lower resistance to water penetration pressure, while samples with small hole diameters (0.1–0.3 mm) showed resistance to water penetration pressure as high as 2.0 MPa, indicating the advantage of the agent in repairing small hole diameter cracks in the cement. Bio-based cements show better crack healing than high-volume fly ash self-compacting concrete (HVFA-SCC) [34]. According to the design requirements of GB/T 50164, the water penetration resistance of the bio-based self-repairing

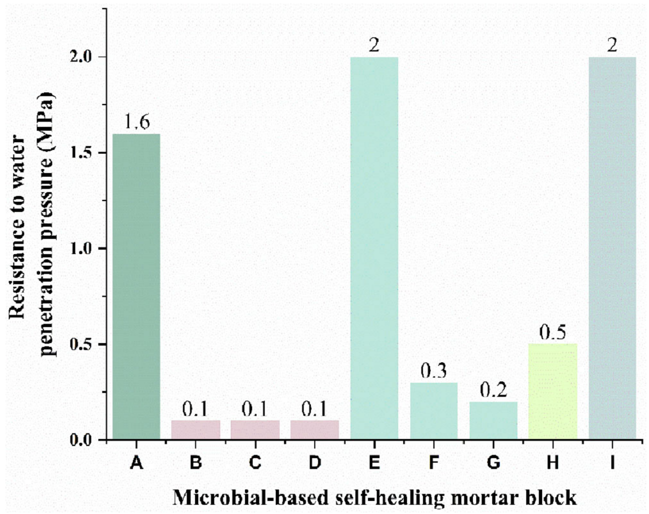

FIGURE 4 | Resistance to water penetration pressure of Bio-based Self-healing Mortar Blocks. The specimens are labelled as "Mortar + the amount of self-healing agent added + lower surface hole size (the diameters on the upper surface were categorized into three sizes based on the crack size: small holes [0.1–0.3 mm], medium holes [0.3–0.6 mm), and large holes [0.6–1.0 mm]) + Resistance to water penetration pressure". (A) Mortar (28d)*3 + 1.6 MPa. (B) Mortar + small holes (28d)*3 + 0.1 MPa. (C) Mortar + medium holes (28d)*3 + 0.1 MPa. (D) Mortar + large holes (28d)*3 + 0.1 MPa. (E) Mortar + 1% self-healing agent + small holes (28d)*3 + 2 MPa. (F) Mortar + 1% self-healing agent + medium holes (28d)*3 + 0.3 MPa. (G) Mortar + 1% self-healing agent + large holes (28d)*3 + 0.2 MPa. (H) Mortar + 1% self-healing agent + small holes (56d)*3 + 0.5 MPa. (I) Mortar + 2% self-healing agent + small holes (28d)*3 + 2 MPa (*3 means three parallel specimens in each group).

mortar with cracks in this experiment reached the standard of water penetration-resistant concrete (>P6: can withstand 0.6Mpa hydrostatic pressure without water penetration) [35]. As time extended to 56 days, the resistance to water penetration pressure of samples with 1% agent decreased to 0.5 MPa, indicating that the resistance to water penetration effect of the agent may decay over time. Furthermore, when the dosage of the agent was increased to 2%, the observed resistance to water penetration pressure was similar to that with a 1% dosage, suggesting that the dosage of the self-healing agent has a limited effect on enhancing the resistance to water penetration repair effect within a certain range.

The resistance to water penetration performance of the cement crystallization mortar blocks is shown in Table 2. For the cement crystallization mortar blocks, even with the application of cement crystallization materials on both the inner and outer surfaces or by incorporating cement crystallization materials internally, the resistance to water penetration pressure of all samples did not exceed 0.1 MPa. This result indicates that although crystallization materials provide some degree of Resistance to water penetration, their effect is not significant.

Comparing the performance of the two types of mortar, it is clear that the resistance to water penetration pressure of the bio-based self-healing mortar significantly increased after the addition of the self-healing agent, especially in the case of microcracks with small hole diameters. In contrast, the resistance to water penetration pressure of the cement crystallization mortar was generally low and showed limited improvement. These results indicate that the bio-based self-healing mortar with microcracks is markedly superior to the cement crystallization mortar in terms of resistance to water penetration recovery capability.

3.5 | Analysis of the Resistance to Water Penetration Mechanism of Microcapsule Self-Healing Agents

To deeply understand the resistance to water penetration effect of microcapsule self-healing agents, we characterized the microstructure of the sample hole diameters after the resistance to water penetration test in detail. As shown in Figure 5, in the samples where the resistance to water penetration ability was significantly restored after the addition of the self-healing agent, the hole diameters on the lower surface were generally less than or equal to 0.3 mm. The mechanism of the self-healing agent's repair action could be that the microcapsule self-healing agent introduced precipitations of crystals at the microcracks, effectively blocking the cracks and preventing water penetration. This phenomenon is similar to the microbial-induced calcium carbonate precipitation (MICP) mechanism [36, 37]. Due to the infiltration of external water solutions, the hydrogel swells, stimulating the revival of bacterial spores. Bacteria grow and reproduce by metabolizing alginate in an alkaline environment, producing carbon dioxide. In the presence of Ca^{2+}, OH^-, and CO_2, calcium carbonate is produced, which precipitates in the cracks, filling and reinforcing them [38].

However, for cracks with medium to large hole diameters, the self-healing agent's repair ability seemed weaker. It is hypothesized that this phenomenon is due to the limitation of calcium carbonate production by carbon dioxide produced by the growth of spores in the self-healing agent incorporated in the cement and by substances in the alkaline environment. Once the pore diameter on the lower surface is greater than 0.3 mm, the extent of repair by the microencapsulated self-healer is limited. This indicated that the repair effect of the microcapsule self-healing agent was better in small hole diameter cracks than in medium to large hole diameter cracks, and its resistance to water penetration repair ability might be limited by the size of the hole diameter. In practice, the formation of cracks in cement is a gradual process, occurring in stages from small to large. The prompt repair of minor cracks that form is of significant importance in actual production, as it prevents the emergence of larger cracks.

4 | Conclusions

This study thoroughly investigated the application of microbial self-healing cement in repairing cracks in cement-based materials and enhancing its resistance to water penetration performance. The experimental results showed that adding 1% self-healing agent to microbial self-healing cement significantly increased the resistance to water penetration pressure of the mortar blocks. The incorporation of *Sporosarcina pasteurii* immobilized on polyurethane increased the resistance to water penetration pressure of concrete by 12% [39]. Bacterial cells genetically modified with a spore-forming strain of *Bacillus subtilis* by incorporation

TABLE 2 | Resistance to water penetration performance of cement crystallization mortar blocks.

Type	Upper surface holes	Upper surface hole diameter	Testing time	Resistance to water penetration pressure
Mortar*3	No holes	None	28d	1.6 Mpa
Mortar*3	Small holes	0.1–0.3 mm	28d	0.1 MPa
Mortar*3	Medium holes	0.3–0.6 mm	28d	0.1 MPa
Mortar*3	Large holes	0.6–1 mm	28d	0.1 MPa
Mortar + External Cement Crystallization Coating*3	Small holes	0.1–0.3 mm	28d	0.1 MPa
Mortar + External Cement Crystallization Coating*3	Medium holes	0.3–0.6 mm	28d	0.1 MPa
Mortar + External Cement Crystallization Coating *3	Large holes	0.6–1 mm	28d	0.1 MPa
Mortar + External Cement Mortar Coating*3	Small holes	0.1–0.3 mm	56d	0.1 MPa
Mortar + External Cement Mortar Coating*3	Medium holes	0.3–0.6 mm	28d	0.1 MPa
Mortar + External Cement Mortar Coating*3	Large holes	0.6–1 mm	28d	0.1 MPa
Mortar + Internal Cement Crystallization*3	No holes	None	28d	1.6 MPa
Mortar + Internal Cement Crystallization*3	Small holes	0.1–0.3 mm	28d	0.1 MPa
Mortar + Internal Cement Crystallization*3	Medium holes	0.3–0.6 mm	28d	0.1 MPa
Mortar + Internal Cement Crystallization*3	Large holes	0.6–1 mm	28d	0.1 MPa

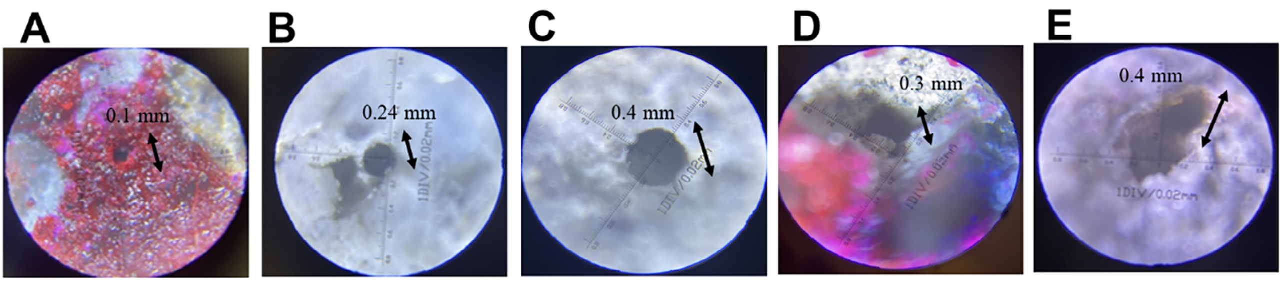

FIGURE 5 | Microstructural characterization of hole diameters in resistance to water penetration test. Evaluation indicators: The amount of self-healing agent added + typical hole diameter on the lower surface + highest permeability strength. (A) 1% + 0.1 mm + 2.0 MPa. (B) 1% + 0.24 mm + 2.0 MPa; (C) 1% + 0.4 mm + 0.3 MPa; (D) 2% + 0.3 mm + 2.0 MPa; (E) 2% + 0.4 mm + 0.1 MPa.

of a bioremediation enzyme-like protein gene survived for a long time in a gelling environment, with a 16.6% increase in the resistance to water penetration pressure [40]. In this study, compared to the standard specimens, the resistance to water penetration pressure increased by 29.2% at 28 days and further increased to 39.3% at 84 days. The use of *B. pseudofirmus* self-healing cement in this study showed high resistance to water penetration pressure. Moreover, in the pre-cracked experiment simulating actual cracks in cement, microbial self-healing cement could effectively restore the resistance to water penetration performance of the mortar blocks, whether 1% or 2% self-healing agents were added. The repair effect was significantly better than the methods of incorporating or coating with cement crystallization materials. For cracks with hole diameters below 0.3 mm, the self-heal effect was particularly evident, which might be related to the crystals formed by the microcapsule self-healing agents in the microcracks. These crystals effectively sealed the cracks and prevented water penetration. Based on fermentation and preparation costs, the cost per cubic meter of bio-based microcapsules (at 1% addition) is $7, which is comparable to the cost of traditional additives. Today, more and more researchers consider bio-based agents as more cost-effective candidates.

This study provides a basis for an efficient and environmentally friendly solution for crack repair in cement-based materials. In the field of building cement crack repair, bio-based self-healing cement has shown excellent performance in terms of durability, intelligent repair, and economic cost. Although the effect on repairing cracks larger than the pore size is limited, this certainly points the way to the development of bio-based self-healing cement. Future work will focus on further optimizing the formulation and process of microbial self-healing technology to improve repair efficiency and durability. At the same time, the performance of microbial self-healing cement under different environmental conditions will be explored to ensure its stability and reliability under variable environments.

Acknowledgments

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Conflicts of Interest

The authors have declared no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

References

1. S. Van der Zwaag, N. Van Dijk, H. Jonkers, S. Mookhoek, and W. Sloof, "Self-Healing Behaviour in Man-Made Engineering Materials: Bioinspired but Taking Into Account Their Intrinsic Character," *Philosophical Transactions of the Royal Society A: Mathematical, Physical and Engineering Sciences* 3671894 (2009): 1689–1704.

2. S. Altin, T. Tankut, Ö. Anil, and Y. Demirel, "Response of Reinforced Concrete Beams With Clamps Applied Externally: An Experimental Study," *Engineering Structures* 259 (2003): 1217–1229.

3. P. R. K. Soda, E. K. Chakravarthi, A. Mogal, and K. Mini, "Statistical and Experimental Investigation on Self-Healing of Microcracks in Cement Mortar by Encapsulation of Calcite Precipitating Bacteria Into Expanded Perlite," *Construction and Building Materials* 342 (2022): 127985.

4. Q. Zhan, W. Dong, C. Fu, A. Wang, H. Yi, and Z. Pan, "The Self-healing of Marine Concrete Cracks Based on the Synergistic Effect of Microorganisms and Inorganic Minerals," *Journal of Building Engineering* 61 (2022): 105210.

5. D. Li, R. Zhang, Y. Zhang, et al., "Code for Quality Acceptance of Concrete Structure Construction (GB50204-2015)," (Beijing, China: China Architecture and Building Press, 2015).

6. J.-S. Ryu and N. Otsuki, "Crack Closure of Reinforced Concrete by Electrodeposition Technique," *Cement and Concrete Research* 321 (2002): 159–164.

7. M. A. Molod, P. Spyridis, and F.-J. Barthold, "Applications of Shape Memory Alloys in Structural Engineering With a Focus on Concrete Construction–A Comprehensive Review," *Construction and Building Materials* 337 (2022): 127565.

8. S. Raza, B. Shafei, M. S. Saiidi, M. Motavalli, and M. Shahverdi, "Shape Memory Alloy Reinforcement for Strengthening and Self-Centering of Concrete Structures—State of the Art," *Construction and Building Materials* 324 (2022): 126628.

9. Z. Huang, Z. Song, Y. Zhang, S. Donkor, L. Jiang, and H. Chu, "A State-of-the-Art Review of Self-Healing Stimuli-Responsive Microcapsules in Cementitious Materials," *Journal of Industrial and Engineering Chemistry* 119 (2023): 153–180.

10. X. Wang, Q. He, Y. Huang, Q. Song, X. Zhang, and F. Xing, "Mechanical Characteristics of Fiber-Reinforced Microcapsule Self-Healing Cementitious Composites by Orthogonal Tests," *Construction and Building Materials* 383 (2023): 131377.

11. W. De Muynck, N. De Belie, and W. Verstraete, "Microbial Carbonate Precipitation in Construction Materials: A Review," *Ecological Engineering* 362 (2010): 118–136.

12. M. Yokota, "Rehabilitation and Protection of Marine Concrete Structures Using Electrodeposition Method," *Proc of the JCI* 141 (1992): 849–854.

13. C. Czaderski, M. Shahverdi, R. Brönnimann, C. Leinenbach, and M. Motavalli, "Feasibility of Iron-Based Shape Memory Alloy Strips for Prestressed Strengthening of Concrete Structures," *Construction and Building Materials* 56 (2014): 94–105.

14. M. J. Castro-Alonso, L. E. Montañez-Hernandez, M. A. Sanchez-Muñoz, M. R. Macias Franco, R. Narayanasamy, and N. Balagurusamy, "Microbially Induced Calcium Carbonate Precipitation (MICP) and Its Potential in Bioconcrete: Microbiological and Molecular Concepts," *Frontiers in Materials* no. 6 (2019): 126.

15. M. Gao, J. Guo, H. Cao, et al., "Immobilized Bacteria With pH-Response Hydrogel for Self-Healing of Concrete," *Journal of Environmental Management* 261 (2020): 110225.

16. N. R. Buenfeld and J. P. Broomfield, "Influence of Electrochemical Chloride Extraction on the Bond Between Steel and Concrete," *Magazine of Concrete Research* 522 (2000): 79–91.

17. M. S. Alam, M. A. Youssef, and M. Nehdi, "Analytical Prediction of the Seismic Behaviour of Superelastic Shape Memory Alloy Reinforced Concrete Elements," *Engineering Structures* 3012 (2008): 3399–3411.

18. K. Habel and P. Gauvreau, "Response of Ultra-High Performance Fiber Reinforced Concrete (UHPFRC) to Impact and Static Loading," *Cement & Concrete Composites* 3010 (2008): 938–946.

19. S. Pyo, K. Wille, S. El-Tawil, and A. E. Naaman, "Strain Rate Dependent Properties of Ultra High Performance fiber Reinforced Concrete (UHP-FRC) Under Tension," *Cement and Concrete Composites* 56 (2015): 15–24.

20. H. M. Jonkers, A. Thijssen, G. Muyzer, O. Copuroglu, and E. Schlangen, "Application of Bacteria as Self-Healing Agent for the Development of Sustainable Concrete," *Ecological Engineering* 36, no. 2 (2010): 230–235.

21. K. Gao, P. Bick, M. T. Suleiman, et al., "Wind Erosion Mitigation Using Microbial-Induced Carbonate Precipitation," *Journal of Geotechnical and Geoenvironmental Engineering* 2023 (1498): 04023053.

22. H. Lin, M. T. Suleiman, D. G. Brown, and E. Kavazanjian, "Mechanical Behavior of Sands Treated by Microbially Induced Carbonate Precipitation," *Journal of Geotechnical and Geoenvironmental Engineering* 2016 (1422): 04015066.

23. H. M. Jonkers, A. Thijssen, G. Muyzer, O. Copuroglu, and E. Schlangen, "Application of Bacteria as Self-Healing Agent for the Development of Sustainable Concrete," *Ecological Engineering* 362 (2010): 230–235.

24. M. Alazhari, T. Sharma, A. Heath, R. Cooper, and K. Paine, "Application of Expanded Perlite Encapsulated Bacteria and Growth media for Self-Healing Concrete," *Construction and Building Materials* 160 (2018): 610–619.

25. J. Wang, D. Snoeck, S. Van Vlierberghe, W. Verstraete, and N. De Belie, "Application of Hydrogel Encapsulated Carbonate Precipitating Bacteria for Approaching a Realistic Self-Healing in Concrete," *Construction and Building Materials* 68 (2014): 110–119.

26. M. Seifan, A. Khajeh Samani, S. Hewitt, and A. Berenjian, “The Effect of Cell Immobilization by Calcium Alginate on Bacterially Induced Calcium Carbonate Precipitation,” *Fermentation* 34 (2017): 57.

27. L. Cheng and R. Cord-Ruwisch, “Upscaling Effects of Soil Improvement by Microbially Induced Calcite Precipitation by Surface Percolation,” *Geomicrobiology Journal* 315 (2014): 396–406.

28. M. B. Burbank, T. J. Weaver, T. L. Green, B. C. Williams, and R. L. Crawford, “Precipitation of Calcite by Indigenous Microorganisms to Strengthen Liquefiable Soils,” *Geomicrobiology Journal* 284 (2011): 301–312.

29. D. Li, J. Liu, L. Feng, Y. Zhou, P. Liu, and Y. F. Chen, “Terrestrial Laser Scanning Assisted Flatness Quality Assessment for Two Different Types of Concrete Surfaces,” *Measurement* 154 (2020): 107436.

30. P. Chen, Y. Li, J. Zhang, Z. Zhang, and D. Wang, “Influence of Interface Agent and Form on the Bonding Performance and Impermeability of Ordinary Concrete Repaired With Alkali-Activated Slag Cementitious Material,” *Journal of Building Engineering* 94 (2024): 110043.

31. C. Standard, “Test Methods of Long-Term Performance and Durability of Ordinary Concrete (GB/T 50082–2009),” (Beijing, China: China Architecture and Building Press, 2009).

32. J. Xu and B. Wang, “Research on Self-Healing of Concrete Cracks by Ceramsite Immobilized Microorganism,” *Nature Reviews Materials* 31 (2017): 127–131.

33. X. Jing and W. Xianzhi, “Self-Healing of Concrete Cracks by Microorganisms Loaded in Low-Alkali Cementitious Material,” *Journal of Tsinghua University (Science and Technology)* 598, 2019, 601–606.

34. M. Şahmaran, S. B. Keskin, G. Ozerkan, and I. O. Yaman, “Self-Healing of Mechanically-Loaded Self Consolidating Concretes With High Volumes of Fly Ash,” *Cement and Concrete Composites* 3010 (2008): 872–879.

35. L. Zhang, R. Ma, J. Lai, et al., “Performance Buildup of Concrete Cured Under Low-Temperatures: Use of a New Nanocomposite Accelerator and Its Application,” *Construction and Building Materials* 335 (2022): 127529.

36. H. Lin, Y. Dong, J. S. Park, and B. M. Montoya, “Cementation Stress Characteristic Curve for Sands Treated by Microbially Induced Carbonate Precipitation,” *Journal of Geotechnical and Geoenvironmental Engineering* 14912 (2023): 04023115.

37. J. T. DeJong, B. M. Mortensen, B. C. Martinez, and D. C. Nelson, “Bio-Mediated Soil Improvement,” *Ecological Engineering* 362 (2010): 197–210.

38. K. Gao, H. Lin, M. T. Suleiman, et al., “Shear and Tensile Strength Measurements of $CaCO_3$ Cemented Bonds Between Glass Beads Treated by Microbially Induced Carbonate Precipitation,” *Journal of Geotechnical and Geoenvironmental Engineering* 1491 (2023): 04022117.

39. S. S. Bang, J. K. Galinat, and V. Ramakrishnan, “Calcite Precipitation Induced by Polyurethane-Immobilized Bacillus Pasteurii,” *Enzyme and Microbial Technology* 28 (2001): 404–409.

40. M. Sarkar, D. Adak, A. Tamang, B. Chattopadhyay, and S. Mandal, “Genetically-Enriched Microbe-Facilitated Self-Healing Concrete—A Sustainable Material for a New Generation of Construction Technology,” *RSC Advances* 5 (2015): 105363–105371.

Supporting Information

Additional supporting information can be found online in the Supporting Information section.