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**DEVELOPMENT OF BACTERIA-BASED
MICROCAPSULES FOR SELF-HEALING CONCRETE**

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DEVELOPMENT OF BACTERIA-BASED MICROCAPSULES FOR SELF-HEALING CONCRETE

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School of Civil and Environmental Engineering

A thesis submitted to the Nanyang Technological University in
partial fulfilment of the requirement for the degree of
Doctor of Philosophy

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Statement of Originality

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Authorship Attribution Statement

This thesis contains material from 1 paper published in the following peer-reviewed journal(s) / from papers accepted at conferences in which I am listed as an author.

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ABSTRACT

Over the past 15 years, bacteria-based self-healing concrete is developed to eliminate the costly and labor-intensive manual repair methods for the concrete cracks. Conventional bacteria immobilization/encapsulation techniques used in self-healing concrete usually lead to significant strength loss to the matrix because of the low strength and/or incompatibility of the protective materials with the matrix. And the encapsulation of concentrated bacteria spores in a single capsule can lead to uneven dispersion of bacteria in the matrix. Accordingly, the main objectives of the present study are to: (1) develop bacteria-based capsules for self-healing concrete which are compatible with matrix and could maintain the strength of the matrix while engaging self-healing, and (2) develop single bacteria spore encapsulation technology for self-healing concrete to realize the better dispersion of bacteria in the matrix thus leads to more stable and robust healing performance. The first objective is realized by using reactive magnesia cement (RMC) to encapsulate bacteria, where bacteria are encapsulated in RMC-based capsules alone or with nutrients, resulting in two types of capsules, namely RMC-B and RMC-BN capsules. Results show that the RMC-based bacterial capsules are functionally graded, highly compatible with the matrix, and able to engage self-healing while maintaining the strength of the matrix. Afterwards, further studies are carried out on single spore encapsulation technology in accordance with the second objective. Results show that layer-by-layer assembly can successfully encapsulate single bacteria spore, and the resulted capsules can engage stable and robust healing due to the better dispersion of bacteria. Overall, this study presents novel self-healing techniques that are possible to be applied to real world owing to its superior self-healing performance and its compatibility with PC-based mixes.

LIST OF PUBLICATIONS

Published journal papers

- [1] **Xiao, X.**, Goh, L. X., Unluer, C., & Yang, E. H. (2021). Bacteria-induced internal carbonation of reactive magnesia cement. *Construction and Building Materials*, 267, 121748.
- [2] **Xiao, X.**, Unluer, C., & Yang, E. H. (2022). Study on the viability of unprotected bacterial spores directly embedded in a reactive magnesia cement matrix for potential crack healing. *Construction and Building Materials*, 346, 128424.

Patent

- [1] Yang, E. H., **Xiao, X.**, Unluer, C. and Nguyen, T. D., Nanyang Technological University, 2022. Carbonation of reactive magnesia cement (rmc)-based systems. U.S. Patent Application 17/455,203.

Submitted manuscript

- [1] **Xiao, X.**, Tan, C. Y. Ashley, Unluer, C. & Yang, E. H. Development of a functionally graded reactive magnesia cement-based bacteria spores capsule for self-healing concrete.

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- [2] **Xiao, X.**, Tan, Umairah, S., Unluer, C. & Yang, E. H. Development of a novel functionally graded one-part capsule containing bacterial spores and nutrients for autonomous healing in cement-based composites.

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- [1] **Xiao, X.**, Unluer, C., Chu, S. H. & Yang, E. H. Single bacteria spore encapsulation through layer-by-layer self-assembly of poly(dimethyldiallyl ammonium chloride) and silica nanoparticles for self-healing concrete.

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LIST OF ABBREVIATIONS AND SYMBOLS

CFU	Colony-forming unit
CaL	Calcium lactate
EDS	Energy dispersive spectroscopy
HMC	Hydrated magnesium carbonate
MICP	Microbially induced calcite precipitation
MgL	Magnesium lactate
PC	Portland cement
PVC	Polyvinyl chloride
RMC	Reactive magnesia cement
RIR	Reference intensity ratio
RH	Relative humidity
SEM	Scanning electron microscopy
UPV	Ultrasonic pulse velocity
XRD	X-ray powder diffraction
YE	Yeast extract
V	volume of the water passing through the specimen
L	specimen thickness in the direction of flow
A	cross-sectional area subject to flow
h	constant water head
t	duration of flow
W_{Ms}	weight fraction of magnesite
$Mw(Ms)$	molecular weight of magnesite
$Mw(CO_2)$	molecular weight of CO_2 .
A_{or}	crack area before conditioning
A_i	crack area after i days of condoning.
V_s	ultrasonic pulse velocity of the specimen
L_s	length of the cubic specimen
T_w	time that the wave traveled through the specimen
i_1	volume fraction of matrix
i_2	volume fraction of microcracks

i_3	volume fraction of macrocracks
V_1	UPV of the matrix
V_2	UPV of the microcracks
V_3	UPV of the macrocracks

CHAPTER 1 INTRODUCTION

1.1 Research background

Concrete is the most used construction material in the world, with an estimated consumption at over 10 billion cubic meters per year (Coffetti, Crotti et al. 2022). Due to its brittle nature, cracks are inevitable in most concrete structures, which allow the ingress of moisture, carbon dioxide, and aggressive substances (i.e., Cl^- and SO_4^{2-}) to reduce the service life of the infrastructure (Fattuhi and Hughes 1988, Bellmann, Erfurt et al. 2012, Berrocal, Lundgren et al. 2016). Currently, the manual detection and repair of the cracks are labor-intensive, inconvenient and costly. It is reported that the estimated annual cost of concrete repair in the U.S. is 18-21 billion dollars (Li and Li 2011). With the goal of eliminating these drawbacks of manual repair, a novel and environmentally friendly bacteria-based technique has been proposed to realize the self-healing of cracks in concrete structures (Jonkers and Schlangen 2007, Jonkers and Schlangen 2007). The basic mechanism of this method is to apply dormant bacteria spores with necessary compounds (e.g., nutrients and calcium source) into concrete during mixing. When cracks develop, bacteria spores are exposed and activated upon the ingress of oxygen and water. Afterwards, the activated bacteria precipitates through its metabolic activities, leading to crack closure.

After this method was proposed, studies have been conducted to investigate its feasibility, whereas the disability of bacteria precipitation after being embedded into Portland cement (PC)-based mixes for 28 days was reported (Jonkers, Thijssen et al. 2010). The inactivation of bacteria in concrete is generally believed to be associated with the high alkalinity and the densification of the microstructure during hydration (Jonkers, Thijssen et al. 2010, Wiktor and Jonkers 2011, Wang, Mignon et al. 2018). Due to this harsh environment of concrete, encapsulation/immobilization is

necessary for the protection of bacteria. Up to now, the most commonly used encapsulation/immobilization materials in literatures are polymers (e.g., hydrogel (Wang, Mignon et al. 2018, Gao, Guo et al. 2020), melamine (Wang, Soens et al. 2014) and epoxy (Liu, Ke et al. 2015)) and porous carriers (e.g., expanded clay (Wiktor and Jonkers 2011), cellulose fibers (Singh and Gupta 2020) and biochar (Kua, Gupta et al. 2019)). In the polymer-based encapsulation methods, bacteria were usually incorporated into the polymer during the polymerization reaction of monomers, and the size of the capsules was controlled by the synthesis setup or the subsequent grinding. As for the porous carriers, the bacteria were usually loaded to the carriers by soaking or vacuum impregnation and followed by drying. It has been reported that both protection measures were able to prolong the longevity of bacteria in the concrete and engage self-healing of cracks.

However, significant strength loss of the matrix occurred due to the incorporation of these capsules or carriers. For example, it was reported that the use of 5% melamine-based capsules by cement mass has led to 34% decrease in 28-day compressive strength of matrix (Wang, Soens et al. 2014). In another study, the addition of 0.5% (volume ratio) of cellulose fibers with bacteria absorption has decrease the 28-day compressive strength of the matrix by 33% (Singh and Gupta 2020). The significant strength decrease in these systems was generally related to the low strength of the encapsulation/immobilization materials or/and the incompatibility of these materials with the PC-based matrix. Specifically, the polymers that were adopted in the encapsulation of bacteria were usually soft materials, which mostly were hydrogels. Besides, these polymers are incompatible with PC-based matrix due to its different nature from cementitious materials. As for the carriers, due to their high porosity, the strength of this type of material was also much lower than the matrix. After encapsulated/immobilized by afore-mentioned materials, the protected bacteria were presented to be the weak point in the matrix. Meanwhile, the size of the capsules/carriers were usually not well controlled, which

exacerbated the weakening effect. As mentioned above, the size control was usually done by grinding, extruding, or synthesis reaction control. Limited by the capability of the fabrication setup, the size of the capsules/carriers were not able to be controlled to an ideal level. Currently, the smallest bacteria capsule size that reported in the literature was 5 μm (Wang, Soens et al. 2014), which was still five times of the size of a bacteria spore cell (around 1 μm). Due to the relatively large size of the capsules/carriers, the weakening effect of the materials in the matrix was amplified.

Meanwhile, polymers (mainly hydrogels) and porous carriers did not provide full protection for the bacteria. For porous carriers, after the immobilizing bacteria through soaking or vacuum impregnation, there was no surface treatment conducted to seal the opening of the carriers. Therefore, the alkaline components in the matrix can still penetrate through the high porosity of the carriers to contact and inactivate bacteria. In hydrogel-based capsules, similar issue existed as hydrogel can absorb water during the mixing so the alkaline components dissolved in water can be absorbed into hydrogels and then contact with bacteria cells.

To overcome these drawbacks and to address concerns regarding mechanical performance, the use of low alkalinity cementitious materials for encapsulation has emerged as a new technology recently. These materials not only have lower alkalinity which potentially allows the survival of bacteria inside but also have high compatibility with the PC-based matrix. The encapsulation of bacteria using this type of material was usually by mixing bacteria with the material and water to form a homogeneous paste. After the paste was hardened, the size of the capsule was controlled by grinding or extruding. Su et al. (Su, Zheng et al. 2021) has reported that using low alkaline sulphoaluminate cement to encapsulate bacteria can enable the self-healing of the crack. At the same time, the 28-day compressive strength of the matrix was maintained when the dosage of the capsules was 12% by cement

mass. Wu et al. (Wu, Hu et al. 2020) has investigated using carbide slag, fly ash, and desulfurized gypsum to prepare a cementitious material to encapsulate bacteria for self-healing. The results showed that the biocapsule can engage self-healing of cracks around 500 μm , and the strength of the matrix can be maintained when the dosage of the capsules was below 5% of cement mass. Besides, the study has observed the capsule breakage after cracking, suggesting the high compatibility of the cementitious materials with the PC-based matrix. Nevertheless, the continuous hydration of those cementitious material could lead to microstructure densification, resulting in the reduction of the porosity of the bacteria capsules. Therefore, the long-term viability of the bacteria inside the capsules might be reduced.

Apart from the major strength concern, a key factor that affects the performance of capsules, i.e., bacteria loading amount control in each individual capsule/carrier, has yet been addressed and investigated in literatures. Restricted by the methods that encapsulation/immobilization were conducted, i.e., generally were mechanical ways similar to gridding, the number of spores that were loaded in one capsule/carrier usually tended to be random and remain uncertain. That is, multiple bacteria spores were in one capsule/carrier, and the number of bacteria could be different in individual capsule/carrier. It was even possible that there were empty capsule/carrier existed in the system. As a result, the dispersion of bacteria spores in the matrix were uneven, where some of the locations were highly concentrated with bacteria which potentially led to better healing, while some of the locations were with less bacteria or no bacteria at all which could lead to limited healing or no healing. So far, no study has paid attention to the bacteria loading control within the capsule/carrier. The uneven distribution of bacteria and the resulted inconsistent healing performance present to be a potential problem faced by all current methods.

1.2 Research motivation

In the studies of bacteria-based self-healing, researchers have developed several systems to engage the crack healing using bacteria encapsulation/immobilization methods. However, the major problem that most studies encounter is how to maintain the mechanical strength of the matrix while possessing self-healing function. The strength decrease of the matrix is usually related to: (1) the low strength of the encapsulation/immobilization materials, (2) incompatibility of the protection materials with the matrix, or/and (3) the inadequate size control of the resulting capsules/carriers. Few recent studies have shown low alkalinity cementitious materials could be a solution to this problem, however the continuous hydration of the material could lead to the densification of the pore structure of the capsules and the reduction of the pore size could threaten the long-term viability of bacteria. Besides, a key factor that influence self-healing performance, i.e., bacteria loading control in each capsule/carrier, has yet been addressed and studied by existing literatures. The uncontrolled bacteria loading is likely to cause uneven dispersion of bacteria and inconsistent healing performance in the matrix, which is a potential problem that all the current bacteria-based self-healing techniques are facing with.

Overall, though bacteria-based technique has been proved to be an effective method to engage self-healing, the investigation of related technologies is still at an early age, and more research is needed for the development of the bacteria-based self-healing concrete. To sum up, following research gaps are identified in this thesis:

- The incorporation of the bacteria-based capsule/carrier significantly reduces the strength of the matrix due to: (1) incompatibility of the capsule/carrier with the matrix, (2) low strength of the capsule/carrier, and/or (3) relatively large size of the capsule/carrier.

- Bacteria loading is uncontrolled and the number of bacteria in individual capsule/carrier is unknown, leading to uneven dispersion of bacteria in the matrix and inconsistent healing performance.

1.3 Objectives

In accordance with the research gaps identified in Section 1.2, the main purpose of this thesis is to develop bacteria-based microcapsules for self-healing concrete where the strength of the matrix can be maintained. Specifically, two objectives are established:

- Selecting proper encapsulation material to improve the compatibility of the capsule with the matrix.
- Improving current bacteria encapsulation method to control the bacteria capsule size and bacteria loading amount in individual capsule.

1.4 Methodology

To accomplish the objectives of the study, following methodology has been adopted, as shown in Fig. 1.1. As the first step of the study, proper protective material was selected for the encapsulation. The material should be compatible with the PC-based matrix, and not bringing any loss to the strength of the matrix. In this thesis, reactive magnesia cement and silica nanoparticles were selected as the encapsulation materials. Subsequently, specific technologies were adopted to encapsulate bacteria to form microcapsules, e.g., carbonation or layer-by-layer technique. After the microcapsules were fabricated, characterizations were conducted to study the properties of the capsules including the morphology, composition, particle size, etc. Then, studies were carried out to understand the mechanism of the microcapsule, for example why the bacteria can survive in the

capsule and how the bacteria was exposed during cracking. After fabricating and understanding the microcapsules, the microcapsules were incorporated in the PC-based matrix to check its performance. The influence of the capsules on the matrix strength was investigated and the self-healing performance of the capsules were evaluated.

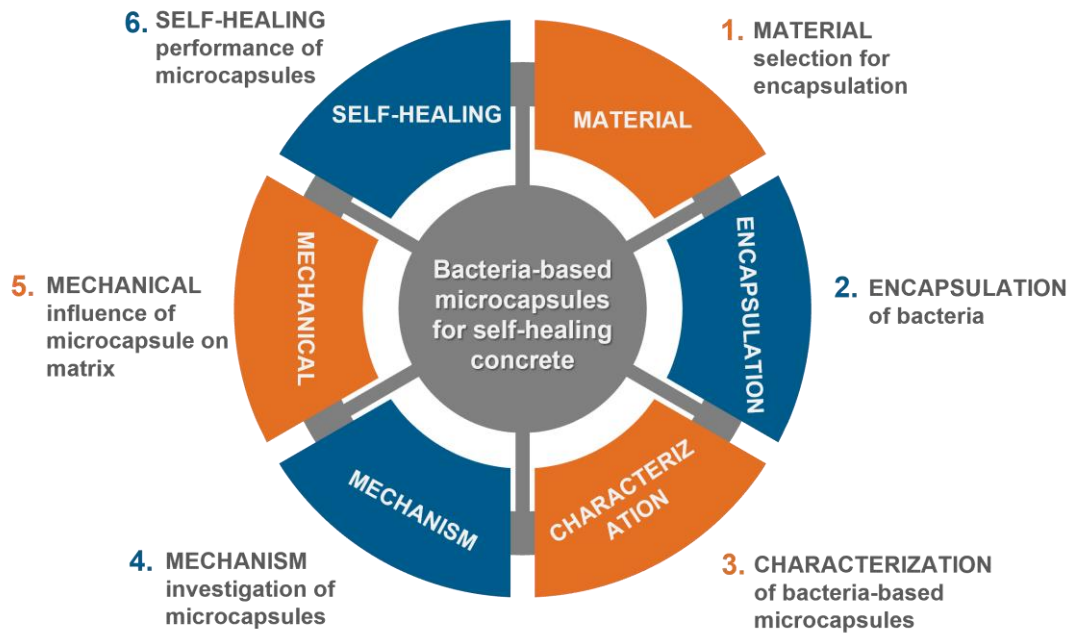


Figure 1.1 Methodology of the study

1.5 Research tasks

The study started with the accomplishment of the first objective in Section 1.3 by developing a functionally graded bacteria-based microcapsule through the use of low alkalinity reactive magnesia cement (RMC) as encapsulation material. RMC is a low alkaline binder (i.e., pH ~10.5 (Hay and Celik 2020)). Unlike most cementitious materials where the strength gain is through hydration, RMC develops its strength mainly via carbonation which makes the resulting RMC-based capsules to possess a unique stable gradient structure with a dense shell and a porous core (Unluer 2018). The porous core accommodates bacteria spores while the dense shell protects bacteria spores from direct contact with the surrounding cement matrix and

provides mechanical strength. The stable microstructure of the RMC-based capsule shall promote long-term viability of bacteria spores. Specifically, 3 tasks were completed as follows:

Task 1: To Investigate the feasibility of encapsulating bacteria in RMC to result a RMC-B capsule for self-healing

In Task 1, bacteria spores were encapsulated in RMC to fabricate a RMC-B capsule, and the RMC-B capsules were incorporated into PC paste while nutrients (yeast extract and calcium lactate) directly dispersed in the matrix. The morphology and the compositions of the RMC-B capsules were characterized, the bacteria survival in the capsule was quantified, the influence of the capsule on the fresh and harden properties of the matrix were examined, and the self-healing performance engaged by the RMC-B capsule was evaluated.

Task 2: To fundamentally study the bacteria survival and precipitation ability in RMC-based environment

To understand the mechanism of the results revealed in Task 1, a fundamental study was conducted in Task 2. In this task, the response of the bacteria viability to various alkalinity levels were evaluated. The survival and precipitation capability of bacteria in RMC/PC paste were investigated. The influence of the internal environments of the RMC paste on the survival of bacteria was tested and discussed.

Task 3: To develop an one-part microcapsule system by encapsulating bacteria together with nutrients in RMC to result a RMC-BN capsule

On the basis of RMC-B capsule, a further development was carried out by encapsulating bacteria together with nutrients (yeast extract and calcium lactate) together in RMC paste to form a RMC-BN capsule. The major advantage of the

RMC-BN capsule was to alleviate the negative influence of the nutrients on the properties of the matrix by avoiding its direct contact with the matrix. The morphology and composition of the capsules were characterized, and the bacteria survival in the capsule was quantified. After incorporating the RMC-BN capsule to the matrix, the influence of the capsule on the fresh and hardened properties of the matrix were examined, and the self-healing performance engaged by the RMC-BN capsule was evaluated.

Upon the completion of Task 1 to Task 3, further research was conducted to accomplish the second objective of this thesis. Similarly, encapsulation material was firstly selected to ensure its compatibility with the matrix. Specifically, silica nanoparticles were chosen as the protection material for the bacteria due to its high compatibility with PC-based mixes. Up to now, extensive studies (Kumar, Singh et al. 2017, Palla, Karade et al. 2017) have investigated the incorporation of silica nanoparticles into concrete to enhance the performance of the concrete, which is a solid indication of its high compatibility with the PC-based matrix. Afterwards, Layer-by-Layer (LBL) self-assembly technology was adopted to encapsulate single bacteria cell into silica nanoparticles. Through LBL, each bacteria cell presented to be an individual capsule and the shell thickness of the capsule can be controlled at nanoscale. Specifically, the following tasks were completed:

Task 4: Fabrication and characterization of the single bacteria capsules

To control the capsule size and bacteria loading to the capsule, single bacteria spore cell was encapsulated with silica nanoparticles while using poly(dimethyldiallyl ammonium chloride) as an intermediate in Task 4. The fabrication process was detailly monitored and presented with zeta potential measurement and morphology observation. Afterwards, the resulted single capsules were incorporated into PC paste to investigate the bacteria exposure after cracking and the interaction of the

capsule shell with the matrix.

Task 5: To investigate the influence of the single capsule on the strength of the matrix and its self-healing performance

Based on Task 4, the fabricated single bacteria spore capsules were incorporated into PC paste with nutrients (yeast extract and calcium lactate). The influence of the capsule on the strength of the matrix was measured, and the self-healing performance of the capsule was evaluated. To highlight the robustness of crack healing engaged by the better bacteria dispersion in the matrix, the consistency of the healing was examined by measuring the crack length that was fully healed and observing the internal healing products along the crack depth direction.

1.6 Thesis layout

This thesis incorporates 8 chapters in total, the details of which are provided below:

Chapter 1 introduces the background, motivation, objectives, methodology and research tasks of the study.

Chapter 2 gives a detailed literature review of the study. First of all, the existing self-healing techniques reported in literature are introduced and compared, where the advantages of bacteria-based self-healing technique is addressed. Afterwards, the development of bacteria-based self-healing is summarized in chronological order from the discover of bacteria precipitation ability, to the external use of the bacteria to repair cracks, and then to the internal use of bacteria in the concrete for crack self-healing. Lastly, the existing bacteria encapsulation/immobilization techniques are introduced and discussed.

Chapter 3 presents the feasibility study of using RMC to encapsulate bacteria spores to form a functionally graded RMC-B capsule for self-healing concrete. The

fabrication and characterization of the RMC-B capsule, the influence of the capsules on the fresh properties and mechanical strength of the matrix, and the self-healing performance of the RMC-B capsules in the matrix are investigated and discussed.

Chapter 4 presents the fundamental investigation of the results revealed in the RMC-B capsule study. The bacteria survival under different alkalinities, the bacteria viability and precipitation capability in RMC/PC pastes, and the internal environments, mainly pH of the pore solution, of RMC/PC are studied and discussed.

Chapter 5 presents the study on developing a self-contained microcapsule system by encapsulating bacteria together with nutrients in RMC to form a RMC-BN capsule for self-healing. The fabrication and characterization of the RMC-BN capsule, the influence of the capsules on the fresh properties and mechanical strength of the matrix, and the self-healing performance of the RMC-BN capsules in the matrix are investigated and discussed.

Chapter 6 presents the development of a single bacteria spore capsule. The capsule is fabricated through LBL self-assembly of encapsulation poly(dimethyldiallyl ammonium chloride) and silica nanoparticles to the bacteria cell surface. The fabrication and the characterization of the capsule are investigated and discussed.

Chapter 7 presents the investigation on the influence of the single capsule on the strength of the matrix, and the self-healing performance of the single capsule. Meanwhile, the consistent healing of the capsule is discussed by observing the crack healing length and internal healing along the crack depth direction.

Chapter 8 summarizes the conclusions reached in this thesis and presents a guideline of future work.

CHAPTER 2 LITERATURE REVIEW

2.1 Self-healing techniques in concrete

Existing self-healing approaches for designing self-healing concrete can be divided into 4 categories: (1) autogenous self-healing, (2) mineral admixture-based self-healing, (3) adhesive agent-based self-healing, and (4) bacteria-based self-healing (Huang, Ye et al. 2016, Hossain, Sultana et al. 2022). This section introduces the concept of the four existing self-healing approaches and discusses their advantages and limitations, respectively.

2.1.1 Autogenous self-healing

As early as nineteenth century, the autogenous crack healing phenomenon in PC concrete has been noticed in water retaining structures, culverts and pipes by Hyde (Hyde and Smith 1889). Later, more and more researchers have observed such phenomenon and started the investigations on the autogenous healing property of concrete, as shown in Fig. 2.1 (Homma, Mihashi et al. 2009, Qian, Zhou et al. 2009). According to the healing products characterization results that reported in literature, different healing products were detected by researchers worldwide. Some researchers detected C-S-H formed within cracks after concrete cracks were cured in water for a certain period, so they concluded that the autogenous crack healing was induced by the rehydration of the unreacted cement particles (Jacobsen, Marchand et al. 1995, Schlangen, Ter Heide et al. 2006). In some other studies, large amount of portlandite was detected together with C-S-H in the healed crack. Huang et al. has reported that portlandite accounted for almost 80% by mass of the healing products in their studies (Huang, Ye et al. 2013). The results revealed that not only further hydration of cement particles is responsible for autogenous self-healing, but also the recrystallization of portlandite leached from the matrix. Meanwhile, Edvardsen (Edvardsen 1999) has found CaCO_3 from autogenous crack

healing. The detection of CaCO_3 in the crack was also reported by Yang et al (Yang, Lepech et al. 2009), which indicated the carbonation of the matrix also contributed to the autogenous healing.

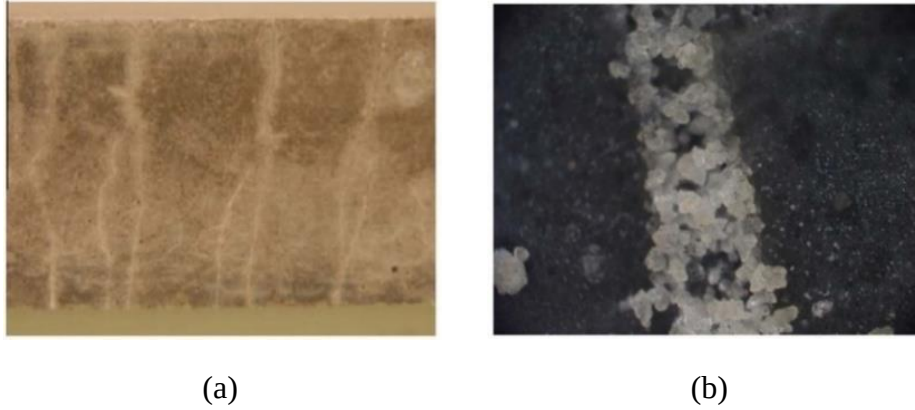


Figure 2.1 Natural crack healing was observed (a) on the surface of specimen with crack width of 40 μm (Qian, Zhou et al. 2009), and (b) in the cracks with width of about 100 μm (Homma, Mihashi et al. 2009)

In summary, the autogenous self-healing process follows 3 mechanisms: (1) further hydration of unhydrated cement, (2) recrystallization of portlandite from the matrix and (3) formation of calcite due to the carbonation of the matrix. As can be seen that autogenous healing relies on the reaction of the available phases in the matrix, no additional chemicals or capsules need to be introduced into matrix externally. The optimization of the healing performance was mainly based on tailoring mix composition, e.g., alter water-to-binder ratio or incorporating supplementary cementitious materials. Therefore, autogenous healing is safe and easy to complement, which has greater potential to be applied in full-scale.

However, autogenous self-healing performance is restricted by several reasons. First of all, there were limited unhydrated cement particles in the system as the hydration progressed. Generally, with higher water-to-cement ratio and longer

curing age, there will be less unhydrated cement particles available to the re-hydration reaction. Secondly, due to the limited CO₂ content in the air and the low dissolution of CO₂ in the water, the carbonation reaction of the hydration phase in the matrix is very limited. Once those phases that exposed on the cracking surface are reacted and consumed, the autogenous healing will be saturated and terminated. As a result, autogenous healing was limited to fine cracks. As reported by Yang et al., when crack width was larger than 150 µm (Yang, Lepech et al. 2009), the crack showed no recovery. Besides, the higher demand of cement used in the matrix potentially would result in an increased hydration heat, higher shrinkage, increased crack risk, and a higher CO₂ footprint (Rajczakowska, Habermehl-Cwirzen et al. 2019).

Since smaller crack favors autogenous healing, to control the crack width of the matrix and engage complete crack healing, fibers were incorporated into PC-based mixes to develop various fiber reinforced cementitious composites, e.g., Engineered Cementitious Composite (ECC). Generally, typical ECC can maintain a tight crack width of about 60 µm up to failure (Li, Wang et al. 2001), recently ECC with even better crack control ability which can control crack as low as 20 µm has been developed (Yang, Yang et al. 2007). With such fine cracks, the crack specimen can engage fully healing when water was applied (Yang, Lepech et al. 2009, Kan and Shi 2012). However, the incorporation of fiber brings some practical issues. Firstly, the fiber could lead to the loss of workability of the fresh mix. Secondly, the even dispersion of fiber in the matrix are difficult to achieve, thus special mixing procedure or dispersion agents need to be applied, which largely complicates the fabrication procedure of the composites. Thirdly, due to the high price of the commercial fibers, the incorporation of fibers significantly increases the cost of concrete (Arce, Noorvand et al. 2021).

2.1.2 Mineral admixture-based self-healing

Self-healing of crack can be engaged by incorporating certain types of mineral admixtures to the matrix. Generally, these admixtures were added to the mixture during mixing. After the hardened concrete is cracked, water can penetrate into crack and react with the unreacted mineral admixtures in the matrix. Then the reaction products will seal the crack, as shown in Fig. 2.2 (Ahn and Kishi 2010).

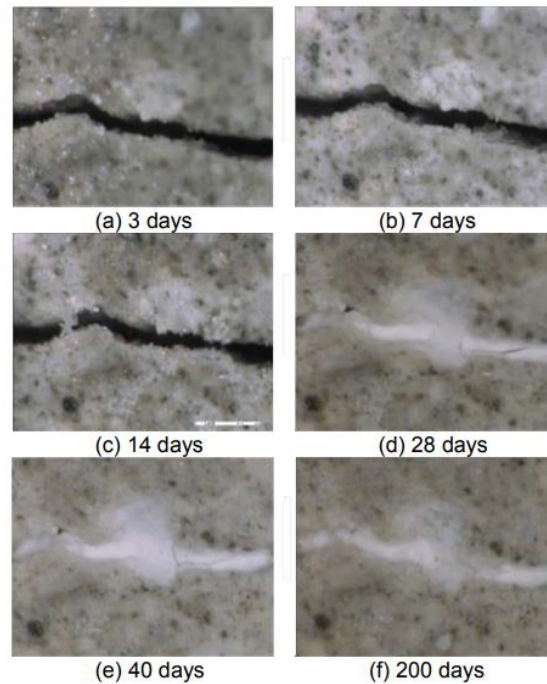


Figure 2.2 Self-healing process of a 0.2mm crack based on mineral admixture

(Kishi 2007, Ahn and Kishi 2010)

Overall, the mineral admixtures for self-healing can be divided into 2 groups: expansive additive and crystalline additive (Sisomphon, Copuroglu et al. 2012). In the case of expansive additive, the crack healing is induced by the volume expansion causing by chemical reaction of the additive. For example, MgO is a common expansion agent used in dam construction because of the volume expansion occurred when forming magnesium hydroxide and magnesium carbonates (Mo, Deng et al. 2010). Regarding the crystalline additive, usually the components of the additives can react with Ca(OH)_2 in the matrix to form

crystalline products then seal the cracks. For example, a synthetic cementitious material which contains reactive silica and some crystalline catalysts was applied for self-healing (Sisomphon, Copuroglu et al. 2012, Roig-Flores, Moscato et al. 2015). The mineral admixtures generally used for self-healing are summarized in Table 2.1.

Table 2.1 Mineral admixtures used in self-healing studies

Category	Materials
Expansive	Calcium sulfoaluminate ($4\text{CaO} \cdot 3\text{Al}_2\text{O}_3 \cdot \text{SO}_3$) (Qian, Zhou et al. 2009)
	Free lime (Qian, Zhou et al. 2009)
	Anhydrite (Qian, Zhou et al. 2009)
	United expanding agent (Jiang, Li et al. 2015)
	Bentonite (Jiang, Li et al. 2015)
	Composites of silicon dioxide, sodium aluminum silicate hydroxide and montmorillonite clay (Nagataki and Gomi 1998, Kishi 2007, Ahn and Kishi 2010)
Crystalline	Types of carbonates: NaHCO_3 , Na_2CO_3 (Kishi 2007)
	Talcum powder (Roig-Flores, Moscato et al. 2015)
	Sodium silicate (Yildirim, Sahmaran et al. 2015)

The use of mineral admixtures as self-healing agent has several advantages. Firstly, since the minerals are directly dispersed in the matrix, it is able to react intensively with water, so that the self-healing of cracks can proceed fast. Besides, due to the expansion of the expansive agent, the crack healing efficiency is improved. However, the mineral admixture-based self-healing technology is also facing some technical problems. As the mineral admixtures are directly added into matrix, once they contact with water in mixing, the admixture will react with water thus be consumed (Nagataki and Gomi 1998). As the result, the available amount of mineral admixture left for crack healing is limited. Besides, after each time of crack healing, the additives will be consumed, therefore the repeated healing would be a potential issue. Furthermore, in the case of expansive agent being used, the agent will cause expansion inside the matrix during hydration and hardening process,

which could lead to damage to the matrix (Sahamitmongkol and Kishi 2011). Accordingly, encapsulation is necessary.

2.1.3 Adhesive agent-based self-healing

The adhesive agent-based self-healing usually relies on the hardening of adhesive agents within the cracks. The agents can be either one-component (Joseph, Jefferson et al. 2010) or two-component agent (Yang, Hollar et al. 2011), or even a multi-component agent (Dry and McMillan 1996). These agents generally are encapsulated with capsules or vessels and then imbedded into concrete. When cracks propagate, the capsules/vessels will be ruptured. Afterwards, the adhesive agent will be released and heal the cracks, as shown in Fig. 2.3 (Joseph, Jefferson et al. 2010). Generally, the hardening process varies with the properties of the agent. Up to now, different kinds of adhesive agents have been applied for self-healing concrete. For example, epoxy is one of the commonly used adhesive agent in concrete, and either two-component or one component can be used. In some studies, two-component epoxy was adopted. The two components were encapsulated separately in capsules and embedded in concrete for self-healing (Feng, Zhuo et al. 2008, Thao, Johnson et al. 2009). When the capsules are ruptured by cracking, both components will be release and contact with each other. Afterwards, the hardening of the epoxy will seal the crack. Meanwhile, one-component epoxy also has been widely studied. Thao et al. (Thao, Johnson et al. 2009) sealed one-component epoxy in glass tubes. After the tubes were broken during cracking, the epoxy was released and exposed to air. Afterwards, the epoxy started to harden and sealed the crack. Similarly, Fig. 2.4 has shown a one-component adhesive agent (cyanoacrylate) which was sealed in hollow capillary tubes (Joseph, Jefferson et al. 2010). After tubes were embedded into concrete, the tube would be ruptured once crack developed, then cyanoacrylate would be released and harden within a short time under the exposure to air. Beside of the mentioned materials, other adhesive agents

like Methylmethacrylate (MMA) (Dry and McMillan 1996, Yang, Hollar et al. 2011) and tung oil (Cailleux and Pollet 2009).

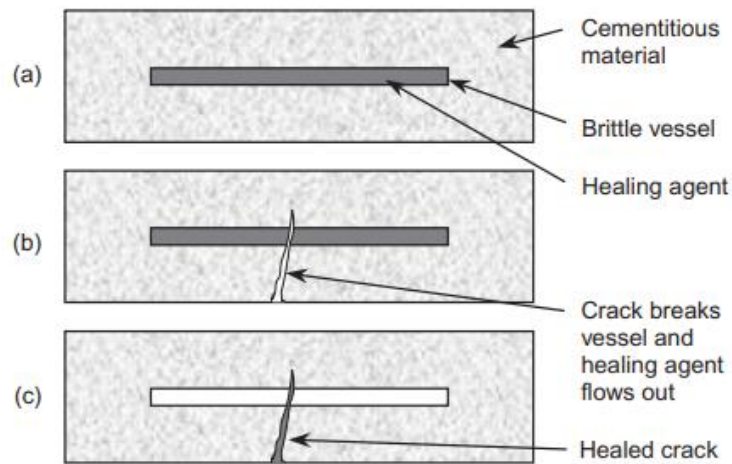


Figure 2.3 Sketch of self-healing realized by adhesive agent encapsulated in vessel (Joseph, Jefferson et al. 2010)



Figure 2.4 Pictures of 1.5 mm and 3.0 mm diameter tubes filled with cyanoacrylate and plugged with wax used for self-healing concrete (Joseph, Jefferson et al. 2010)

As can be seen from above discussion, the adhesive agents used for self-healing mostly are polymers. Therefore, the adhesive agent-based self-healing is fast as the reaction of the components and the hardening of the polymer-based materials only take few hours. However, the limitation of this technology is significant. Firstly, the healing agent needs to be carried in capsules or vessels, which complicates the

fabrication process a lot. Especially for the vessels, by now it is still difficult to install a vascular system inside the concrete matrix (Cailleux and Pollet 2009). Secondly, as the adhesive agents are mostly polymer-based, the healing products are not compatible with cementitious materials, which potentially can cause delamination issues. Thirdly, in multiple component adhesive agent system, the healing products forming reaction cannot be well controlled. As the amount of each component that released from the capsules tends to be random and not under control, the ratio of each component is not optimal. Therefore, the reaction to form healing products might be negatively influenced and the healing efficiency is affected.

2.1.4 Bacteria-based self-healing

Bacteria-based self-healing is utilizing the metabolism of bacteria to induce the formation of precipitation within the crack. PC is an extreme alkaline environment which does not allow the survival of bacteria inside the matrix (Jonkers, Thijssen et al. 2010). Therefore, encapsulation or immobilization of bacteria spore are needed as a protection for the bacteria. Generally, encapsulated/immobilized spores and necessary substances including nutrients for bacteria and the precursor for the precipitation are incorporated into PC-based blends together as self-healing agent. When cracks propagate, under the presence of water, nutrient will facilitate the conversion of spore to vegetative bacteria. Then the metabolic activities of the bacteria will induce the conversion of the precursor to precipitation within the cracks. The bacteria-based self-healing agents that are commonly reported in the literature follow three different metabolic routes: the enzymatic hydrolysis of urea (Wang, Soens et al. 2014), the oxidation of organic carbon (Jonkers, Thijssen et al. 2010), and the anoxic oxidation of organic carbon (Erşan, Hernandez-Sanabria et al. 2016). Specifically, in the urea hydrolysis route, urea is decomposed by the bacteria and forms CO_3^{2-} . Afterwards, CO_3^{2-} will react with the Ca^{2+} in the matrix to form calcium carbonate precipitation, as shown in Eqn. (2.1) and Eqn. (2.2). In the oxidation of organic carbon route, the provided organic carbon (e.g., calcium lactate) is directly oxidized by the bacteria to form calcium carbonate. Meanwhile,

there will be CO_2 formed simultaneously in the reaction, so a secondary reaction can happen where CO_2 dissolved in water to form CO_3^{2-} and react with the Ca^{2+} in the matrix to form calcium carbonate precipitation, as shown from Eqn. (2.3) to Eqn. (2.5). In the anoxic oxidation of organic carbon (e.g., calcium formate) rout, the reaction is a denitrification process where the biological reduction of NO_3^- (e.g., calcium nitrate) generates CO_3^{2-} and HCO_3^- ions, and then the CO_3^{2-} formed in the denitrification and the further ionization of HCO_3^- will react with Ca^{2+} in the matrix to form calcium carbonate precipitation, as shown from Eqn. (2.6) to Eqn. (2.8). For example, Fig. 2.5 has shown the self-healing process of a specimen with the use of melamine-based bacterial microcapsules (Wang, Soens et al. 2014). Table 2.2 has listed some bacteria species under each metabolic rout and their corresponding capsulation materials used in literatures as examples.

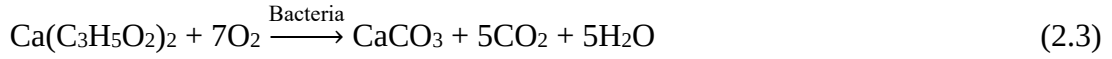




Figure 2.5 Self-healing process induced by melamine-based polymer encapsulated bacteria spores (Wang, Soens et al. 2014)

Table 2.2 Metabolic routes and corresponding bacteria species and encapsulation materials commonly used in literatures

Metabolic routes	Bacteria species	Encapsulation materials
Hydrolysis of urea	<i>Bacillus Sphaericus</i> (Wang, Snoeck et al. 2014, Wang, Soens et al. 2014)	Melamine-based polymer (Wang, Soens et al. 2014), diatomaceous earth (Wang, De Belie et al. 2012), hydrogel (Wang, Snoeck et al. 2014)
	<i>Bacillus cereus</i> (Wu, Hu et al. 2020)	
Oxidation of organic carbon	<i>Bacillus cohnii</i> (Wiktor and Jonkers 2011, Xu and Yao 2014)	Expanded clay (Wiktor and Jonkers 2011)
Anoxic oxidation of organic carbon	<i>Pseudomonas aeruginosa</i> (Erşan, Hernandez-Sanabria et al. 2016)	Diatomaceous earth, expanded clay (Erşan, Hernandez-Sanabria et al. 2016)

The main advantages of bacteria-based method are fourfold. Firstly, the main healing products calcium carbonate is highly compatible with PC-based matrix, as calcium carbonate is one of the phases that originally exists in the concrete. Secondly, the dormant spore can stay alive for hundreds even thousands of years, such shelf life was more matchable with the service life of concrete structures (Cano and Borucki 1995, Gould 2006). Thirdly, it is reported that bacteria-based

method is able to heal larger cracks than other existing self-healing techniques. While the capability of other methods to engage complete crack healing are around 200 μm or below (Muhammad, Shafaghat et al. 2016), the capability of bacteria-based methods can reach up to 970 μm (Wang, Soens et al. 2014). Lastly, after the dormant spore is activated and transformed to vegetative bacteria, the vegetative bacteria is able to transform back to spore, which potentially could facilitate the repeated healing behavior. Due to the harsh environment in concrete (e.g., high alkalinity and dense pore structure), bacteria cannot survive in concrete more than 28 days when no protection is applied (Jonkers, Thijssen et al. 2010). Therefore, encapsulation/immobilization are needed to protect bacteria from the chemical attack and microstructure densification of the matrix.

2.1.5 Summary of different self-healing techniques

Based on the results reported in literatures, Table 2.3 has summarized the some of the properties of each self-healing technique and listed some of the test results reported in literatures. For the mineral mixture-based self-healing, mineral additives are usually directly incorporated into matrix, which could lead to pre-consumption of self-healing agents. As for adhesive agent-based and bacteria-based self-healing, adhesive agent and bacteria cells are immobilized in carriers or encapsulated in capsules.

Generally, crack closure capacity, transport property recovery, mechanical properties recovery and the time needed for self-healing to take effect are measured to evaluate the self-healing performance. For autogenous self-healing, the crack closure is only limited to fine cracks below 60 μm (Yang, Lepech et al. 2009, Parks, Edwards et al. 2010). The autogenous healing is mainly realized by the hydration of unhydrated cement particles in the matrix, and the amount of unhydrated cement particles is limited along the cross section of the crack as the matrix develops

strength continuously through hydration. Once those unhydrated particles are fully consumed, the self-healing will be terminated. Therefore, the autogenous healing is very limited, and only fine cracks can be healed. For mineral admixture and adhesive agent-based self-healing, cracks up to 200~300 μm are reported to be fully healed. The main disadvantage of those two types of healing agents is that the amount of healing agent was limited at certain location, once the healing agent is consumed up, no further healing can be achieved. Therefore, the crack closure capacity is limited below 300 μm . Different from these three self-healing approaches, bacteria-based self-healing technique has shown its superiority on repairing large cracks, which might relate to the continuous metabolism of bacteria. Unlike above-mentioned methods where healing is terminated once the agent is consumed or released, bacteria can continuously precipitate healing products. Up to now, the maximum crack width that can be healed by encapsulated bacteria was 0.97 mm (Wang, Soens et al. 2014), which is around 3~4 times of mineral admixture and adhesive agent-based self-healing methods. In line with the surface crack closure performance, the transport properties recovery is also differentiated among the four approaches. While the other three methods have limited transport properties recovery, the water permeability recovery engaged by bacteria-based approach is reported to reach 98% (Tziviloglou, Wiktor et al. 2016).

Besides, when mineral or adhesive-based materials are incorporated for self-healing, once the capsules break and the healing agents are released, the capsule lose its availability and cannot function again, which brings concern to repeated healing. Different from them, bacteria can be transformed back to dormant form (spore) after the vegetative bacteria cell finish sealing the crack, and then the spore can be activated again when a new crack develops, which is preferable for repeated crack healing. Besides, the healing products formed from the bacteria-based self-healing is calcium carbonate, which is a phase that originally exists in the matrix thus highly compatible with matrix.

Among the four self-healing approaches, the production cost and the potential to scale-up are different since the materials and technology involved in each method vary significantly. Since autogenous self-healing is an inherent property of concrete, it does not require any additional materials or equipment, and therefore, it is the most cost-effective approach to self-healing. However, the effectiveness of autogenous self-healing is limited to fine microcracks, and it may not be effective for larger cracks that require repair, which greatly limits its scalability. The cost of mineral admixture-based self-healing depends on the type and quantity of the mineral admixture used. Mineral admixtures such as fly ash, slag, and silica fume are commonly used and are relatively inexpensive, but their availability and cost can vary depending on the location and market demand. Besides, due to the pre-reaction of the minerals in the matrix, the matrix compositions and properties might be significantly altered, which limits the application of this method in industry. The cost of adhesive agent-based self-healing depends on the type and quantity of the adhesive agent used. Adhesive agents such as epoxy and polyurethane are commonly used and are relatively expensive, but their effectiveness in repairing larger cracks can reduce the overall cost of concrete repair and maintenance. For bacteria-based self-healing concrete, due to the high cost of organics for bacteria growth and relatively complicated bacteria preparation and encapsulation procedure, the cost of bacteria-based self-healing concrete is roughly twice of the price of conventional concrete. With the further optimization of the bacteria preparation process, the cost of bacteria-based self-healing concrete potentially can be brought down by using sugar-based food nutrient.

Table 2.3 Summary of different self-healing techniques

	Autogenous healing	Mineral admixture-based self-healing	Adhesive agent-based self-healing	Bacteria-based self-healing
Methods to apply healing agent	No special procedure is needed.	Mineral admixture can be directly applied to the matrix, but it will cause pre-consumption of the healing agent.	Adhesive agent needs to be encapsulated in capsules or immobilized in vascular systems.	Bacteria needs to be encapsulated in capsules or immobilized in porous carriers.
Crack closure capability	Complete crack closure of crack width below 60 μm is reported. (Yang, Lepech et al. 2009, Parks, Edwards et al. 2010)	Complete crack closure of crack width around 200 μm is reported. (Ahn and Kishi 2010, Wang, Soens et al. 2014)	Complete crack closure of crack width 300 μm is reported. (Xue, Li et al. 2019)	Complete crack closure of crack width 970 μm is reported. (Wang, Soens et al. 2014)
Transport property recovery	The water permeability recovery reached 57% condition is reported. (Nishiwaki, Koda et al. 2012)	The gas permeability recovery reached 69% is reported. (Kanellopoulos, Qureshi et al. 2015)	The gas permeability recovery reached 66.8% is reported. (Yang, Hollar et al. 2011)	The water permeability recovery reached 98% is reported. (Tziviloglou, Wiktor et al. 2016)
Mechanical property recovery	Compressive strength regaining capacity of 60% is reported. (Zhang, Zhang et al. 2019)	Compressive strength regaining capacity of 75~95% is reported. (Reddy and Ravitheja 2019)	Compressive strength regaining capacity of 78% is reported. (Zaerkabeh, Sadeghi et al. 2022)	Compressive strength regaining capacity of 60% is reported. (Achal, Mukerjee et al. 2013)
Repeatability	Once the available phases in the matrix are reacted, the self-healing will be terminated.	Once the mineral admixture in the cracking location is used up, the self-healing process will be terminated, and the crack healing cannot be repeated.	The capsule/tube are single-use, once it is broken and the heal agent is released, it cannot be used again for the next healing.	Bacteria is able to transform back to spore and stay alive in the matrix after self-healing, therefore repeated healing is feasible.
Compatibility	The healing reaction is originated from the matrix, thus the healing products are highly compatible with matrix.	Most of minerals are inorganic and compatible with matrix.	Most of adhesive agents are polymer-based, which are not compatible with matrix.	Healing products are calcium carbonate, which is highly compatible with matrix.

2.2 Development of bacteria-based self-healing concrete

2.2.1 External use of bacteria for repairing cracks

Although the internal application of bacteria in concrete for self-healing started until after 2000, the calcium carbonate precipitation ability of bacteria was actually noticed by researchers as early as 1900s (Drew 1911, Drew 1913, Kellerman and Smith 1914, Black 1933). Starting with Russian investigator Nadson in 1903 (Ehrlich, Newman et al. 2002), the important role of microbials in the formation of calcium carbonate in the environments began to be noted. Continued by George H. Drew (Drew 1911) in 1913, he found bacterial precipitation of calcium carbonate in the tropical sea. He isolated the bacteria from the sea and cultivated in the laboratory, and then successfully precipitated calcium carbonate from synthetic culture media. Afterwards, more and more researchers have drawn their attention to this phenomenon. Accordingly, various bacteria strains were isolated from the natural environments and laboratory investigations were conducted on the bacterial carbonate precipitation (McCallum and Guhathakurta 1970, Krumbein 1979, Morita 1980).

From 1980s to early 1990s, the adoption of bacterial precipitation to form a plugging system by using injection methods has been studied by researchers (Jack 1988, Kantzas, Stehmeier et al. 1992, Jack, Ferris et al. 1993, Zhong and Islam 1995). At that time, the plugging system was mainly used for oil recovery and sand consolidation. Later in 1998, on the basis of studies of the microbial plugging systems, Ramakrishnan. V. et al. has adopted this mechanism to repair cracks of concrete structures by externally use bacteria. This novel concept has received attentions in academia, and more studies on the use of bacteria to remediate concrete cracks have emerged (V. Ramakrishnan 1998, Bang, Galinat et al. 2001, Ramachandran, Ramakrishnan et al. 2001, Heirman, Herremans et al. 2003).

Generally, in these studies, the bacteria precipitation route for crack repairing was the hydrolysis of urea. The mechanism of the crack repairing was that the urease-producing bacteria (mainly *Bacillus pasteurii*) catalyzed the hydrolysis of urea ($\text{CH}_4\text{N}_2\text{O}$) into NH_3 and CO_2 . Then, the formed CO_2 reacts with the externally provided calcium minerals (e.g., CaCl_2 , $\text{Ca}(\text{NH}_4)_2$) to form CaCO_3 and fill the crack gap. To engage such repairing effect, cracked concrete specimens were submerged in urea- CaCl_2 solution for the precipitation to take place. According to literatures, this submersion method can induce the calcium carbonate precipitation formation within the cracks (Ramachandran, Ramakrishnan et al. 2001) and sample surface (Heirman, Herremans et al. 2003), as shown in Fig 2.6.

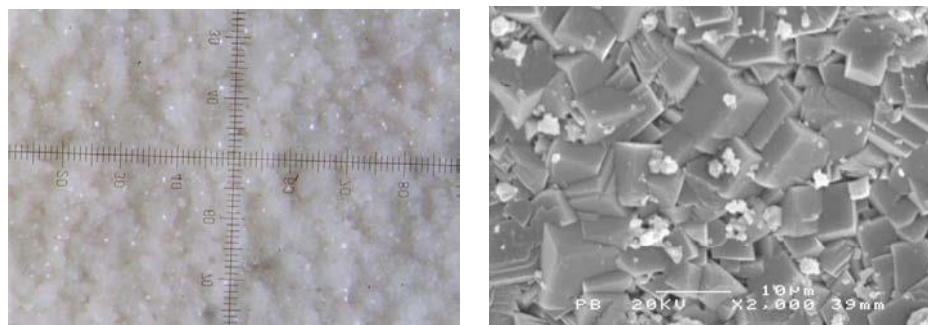


Figure 2.6 (a) Surface layer as seen through the light-microscope (magnification x120), and (b) Surface layers as seen through the SEM (magnification x2000) (Heirman, Herremans et al. 2003)

Nevertheless, since the repair was realized by external application of bacteria and nutrients medium, human interfering was still required. Therefore, the healing of the crack is not a genuine ‘self-healing’ process. In addition, the metabolic route that was adopted in these studies led to the formation of ammonia in the precipitation process. Every one mole urea decomposition caused two moles formation of NH_3 , which may result in excessive environmental ammonia burden. Besides, vegetative bacteria were used in the studies, which had very limited lifetime.

2.2.2 Internal use of bacteria for repairing cracks

In 2007, Jonkers firstly proposed embedding spore-forming bacteria of the *Bacillus* genus and precipitation precursors together into the matrix to realize true self-healing of cracks (Jonkers and Schlangen 2007). Different from active vegetative bacteria cells, the bacteria spores that are formed within mother vegetative cells and released into environments are dormant and highly resistant to harsh environments. Spores have very different morphology and structures from vegetative cells, it has several layers not found in vegetative cells as shown in Figure 2.7. These layers, especially the outmost exosporium, have played a major role in protecting spores so that spores can keep dormant and resistant to harsh environments (Setlow 2014). When nutrients are provided, spores can rapidly lose their dormancy and germinate to active vegetative cells, which are able to precipitate through its metabolic activities. Based on this nature of spores, Jonkers proposed to adopt spores for the self-healing instead of vegetative cells used in the afore-mentioned studies considering concrete is a harsh environment for microbials. Meanwhile, necessary substances (e.g., nutrients and calcium source) were applied together with spores to the matrix, aiming to facilitate the conversion of spore to vegetative cell and induce precipitation when cracks propagate. In addition, different from previous ureolytic metabolic route, the oxidation of organic carbon was adopted as the calcium carbonate precipitation route, which is a more environment-friendly precipitation mechanism since it does not bring any ammonia burden to the matrix and environment.

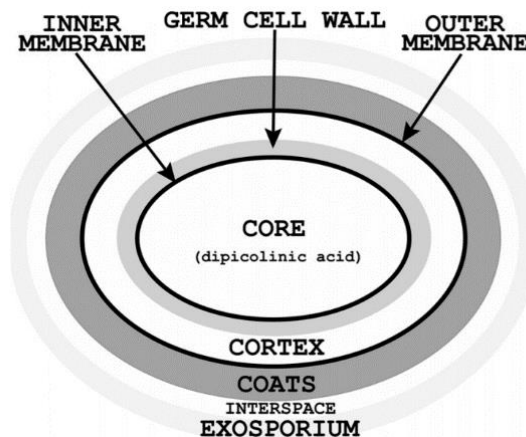


Figure 2.7 Outline of the structure of a Bacillus spore (Setlow 2014)

Upon this idea was proposed, Jonkers has conducted series of experiments to investigate the feasibility. The study was started with incorporating bacteria spore in concrete matrix only (Jonkers and Schlangen 2007). Specifically, spores were added into concrete and the samples were cured for 10 days. Afterwards, the sample was broken into chips and submerged in rich nutrient medium for the observation of precipitation. The results showed that there were calcium carbonate crystals grew on the chip surface as shown in Fig. 2.8, indicating the survival of bacteria in the concrete. On the basis of this results, further studies have been carried out where bacteria spores and nutrients were incorporated into concrete together (Jonkers, Thijssen et al. 2010). Afterwards, the samples were cured to 3, 7 and 28 days. The samples with different curing age were broken into small chips and submerged in water to observe the precipitation, respectively. It has been found that copious amounts of larger 20–80 μm -sized mineral-like particles were formed at the crack surface of early age samples (7 days) but not on those of 28 days cured specimens, as shown in Figure 2.9. The results suggested that the viability of spores in concrete only limited to young age specimens. For the inactivation of spores in concrete, researchers including Jonkers generally believed that the potential reasons lie in three aspects: (1) the high alkalinity of PC, (2) the mechanical force from the

mixing procedure and (3) the pore size decrease during hydration. To sum up, the protection of bacteria is necessary to prolong its longevity in concrete for self-healing.

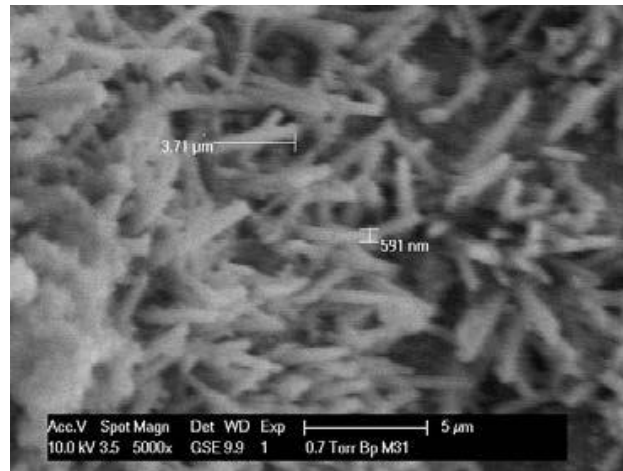


Figure 2.8 Concrete containing spores showed massive calcite-like crystals formed on the concrete chip surface (Jonkers and Schlangen 2007)

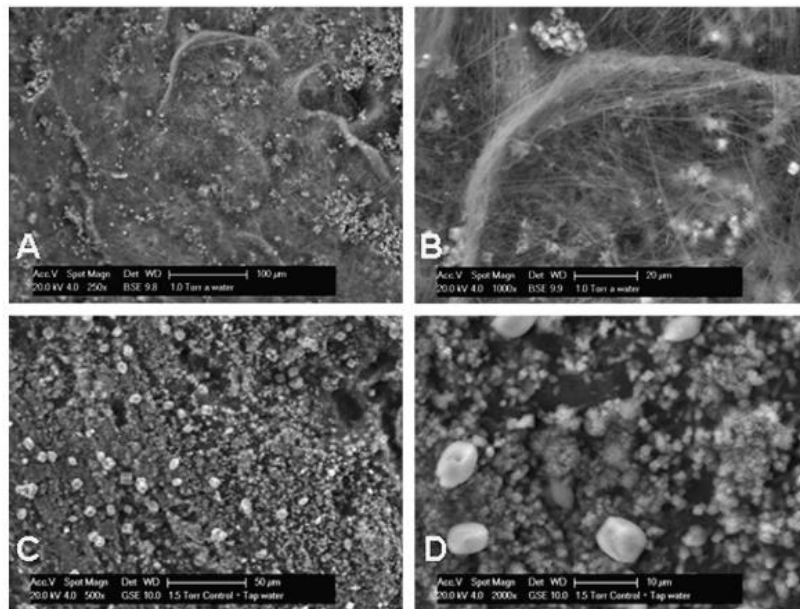


Figure 2.9 Cement stone specimens without incorporated healing agent (controls), cracked after 7 (panels A: 250× and B: 1000× magnification) or 28 days curing (panels C: 500× and D: 2000× magnification) (Jonkers, Thijssen et al. 2010)

2.3 Immobilization/encapsulation materials used for bacteria-based self-healing concrete

The studies on the immobilization/encapsulation of bacteria spores for self-healing concrete started in 2014 (Jonkers 2011). Up to now, the commonly used immobilization/encapsulation materials used in literature can be classified into three categories: (1) porous carriers, (2) polymers and (3) low alkalinity cementitious materials. In this section, the properties of the materials, immobilization/encapsulation methods and the advantages and limitations of each material are introduced, respectively.

2.3.1 Porous carriers

Immobilization of bacteria in porous carriers is a way to protect bacterial spores from being crushed during the mixing and hardening of concrete, since a solid carrier can act as a shield for spores and help to maintain the viability of spores in concrete. Generally, the immobilization of bacteria in the carriers is realized by soaking or vacuum impregnation, where bacteria penetrate and stay in the interior of the carrier through the permeable channels. According to literatures, commonly used porous carriers can be further divided into three types: (1) inorganic carriers, (2) organic carriers and (3) nanoparticles.

Among all the carriers reported in literatures, the most commonly used type is inorganic carriers, including expanded clay (Jonkers 2011, Wiktor and Jonkers 2011, Tziviloglou, Wiktor et al. 2016, Han, Choi et al. 2019), ceramsite (Chen, Qian et al. 2016, Xu, Wang et al. 2018), granular activated carbon (Erşan, Hernandez-Sanabria et al. 2016), expanded perlite (Alazhari, Sharma et al. 2018), biochar (Kua, Gupta et al. 2019), etc. For example, Wiktor et al. (Wiktor and Jonkers 2011) used expanded clay to immobilize 6% (by weight) calcium lactate and 1.7×10^5 bacterial spores in expanded clay. After submersing the cracked sample in water for 100 days, the

bacteria loaded expanded clay engaged a fully healing of crack up to 0.46 mm wide, while control group only had healing of 0.18mm, as shown in Fig. 2.10. Erşan et al. (Erşan, Hernandez-Sanabria et al. 2016) used 22.5 g granular activated carbon particles to immobilize 2.25 g bacteria spores through vacuum impregnation. After the cracked specimens with addition of the particles was immersed in water for 56 days, crack up to 480 μm can be fully healed. The crack healing process engaged by the bacteria loaded granular activated carbon is shown in Fig. 2.11. From 2011 to now, similar studies of using inorganic carriers to immobilize bacteria are extensively reported. Other than the above listed examples, ceramsite (Chen, Qian et al. 2016, Xu, Wang et al. 2018), zeolite (Bhaskar, Hossain et al. 2017), expanded perlite (Alazhari, Sharma et al. 2018), limestone powder (Shaheen and Khushnood 2018), biochar (Kua, Gupta et al. 2019), and recycled coarse aggregate (Khushnood, Qureshi et al. 2020) have been investigated in published papers.

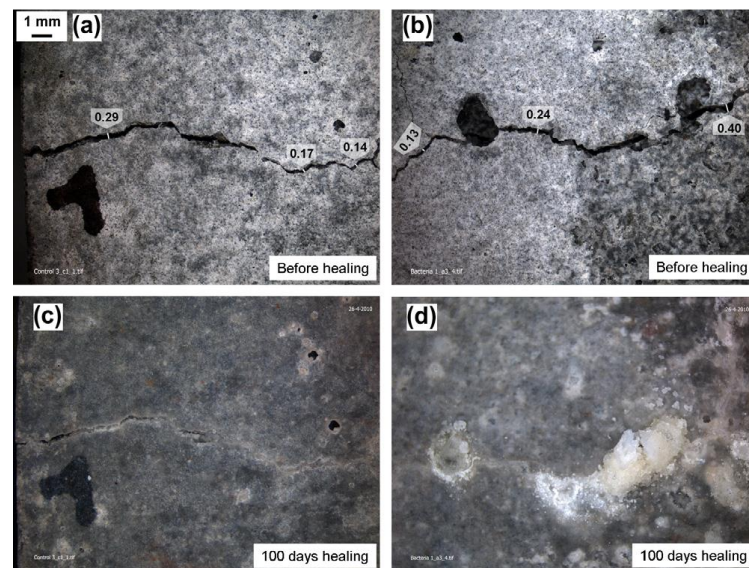


Figure 2.10 Stereomicroscopic images of crack-healing process in control mortar specimen before (a), and after 100 days healing (c); in bio expanded clay-based specimen before (b), and after 100 days healing (d) (Wiktor and Jonkers 2011)

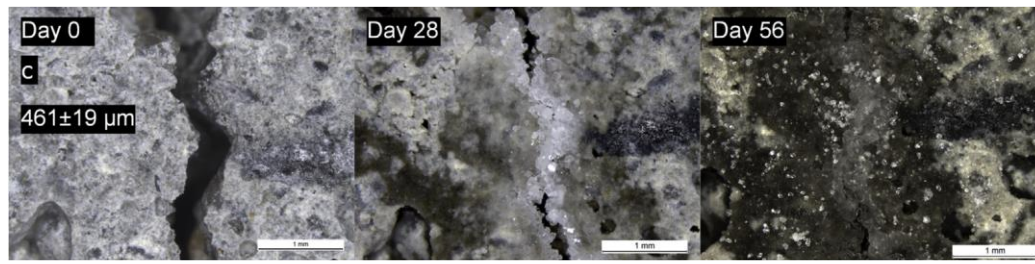


Figure 2.11 Crack healing on the mortar containing bacteria loaded granular activated carbon particles (Erşan, Hernandez-Sanabria et al. 2016)

Other than afore-mentioned inorganic materials, some organic materials such as rubber (Xu, Lian et al. 2019), cellulose fibers (Singh and Gupta 2020) and some natural fibers (Rauf, Khaliq et al. 2020) are used as protective carriers for bacteria. The general way of the immobilization is by soaking. For example, Fig. 2.12 has shown the immobilization process of bacteria in cellulose fiber. As can be seen from the figure, bacteria were mainly absorbed to the fiber by soaking and followed by drying. Same method also applies to other carriers. After immobilization, automatic healing of cracks can be observed. For instance, Xu et al. (Xu, Lian et al. 2019) immobilized 3.75×10^{10} spores in 33 g of rubber particles, and then added into concrete mix with nutrients, calcium source and urea. After soak in water for 28 days, the crack on the sample was healed due to the incorporation of the bacteria loaded rubber, as shown in Fig. 2.13. Besides, some nanoparticles like graphite nanoplatelets (Khaliq and Ehsan 2016) and iron oxide nanoparticle (Shaheen, Khushnood et al. 2019) were also used for the protection of bacteria through soaking and immersion. As an example, Fig. 2.14 has shown the immobilization process of bacteria into iron oxide particles. However, as nanoparticles usually are expensive, so the cost of nanoparticle immobilized bacterial self-healing agents have relatively higher costs compared with regular porous materials.



Figure 2.12 Bacteria absorbed in cellulose fiber (Singh and Gupta 2020)

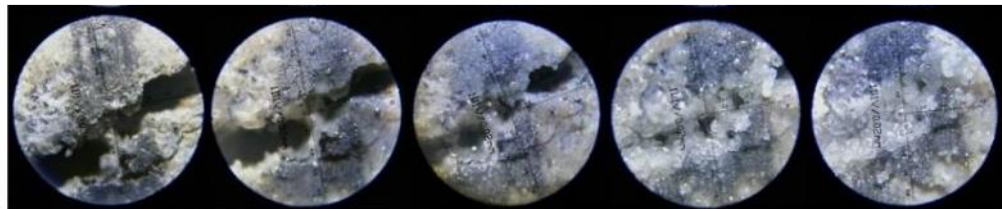


Figure 2.13 Crack (0.48 mm wide) healing process of specimen with incorporation of bacteria loaded rubber particles (Xu, Lian et al. 2019)

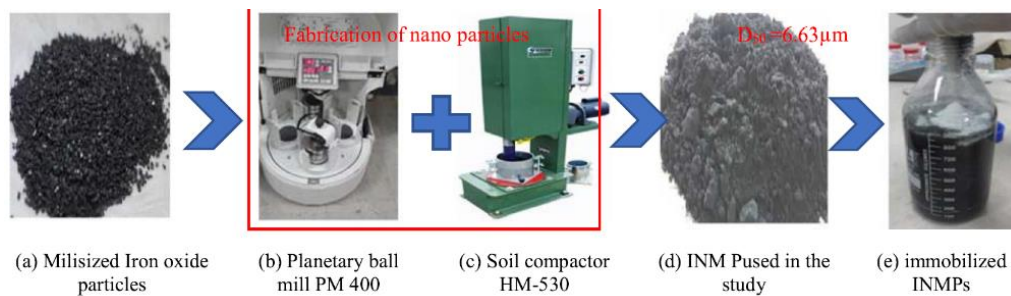


Figure 2.14 Bacteria immobilization process using iron oxide particles (Shaheen, Khushnood et al. 2019)

Though porous carriers show its protection for bacteria to some extents, some major disadvantage cannot be neglected. One concern of porous material as immobilization carriers is that it does not provide full protection for bacteria. As the permeable channels of the carriers allow bacteria and nutrients to penetrate during

immobilization, these channels also provide a way for alkaline components in the concrete to penetrate inside during the mixing and hydration. As a result, the bacteria inside would contact the alkaline components and then be inactivated by these alkaline phases. Another major disadvantage of porous carriers is that the incorporation of these materials in the matrix significantly decrease the strength of the matrix. Due to the high porosity of the materials, the carriers usually have much lower strength than the matrix. After incorporation to the matrix, the carriers presented to be the weak points in the matrix. As a result, the strength of the matrix is significant compromised. For example, Jonkers used bacteria loaded expanded clay to replace 50% of the original aggregates, and 50% decrease in compressive strength was observed after 28 days curing when compared to specimens without replacement of aggregates with expanded clay particles (Jonkers 2011). Xu et al. used rubber to immobilized bacteria spores. When the dosage of the rubber was 9.4% by cement mass, the 28-day strength of the matrix was reduced by 20.1% (Xu, Lian et al. 2019). Singh et al. used cellulose fiber to immobilize bacteria spores. After adding 0.5% (by volume) cellulose fiber to the mortar, the 28-day compressive strength of the matrix was reduced by 33.1% (Singh and Gupta 2020).

2.3.2 Polymers

From 2014 to present, the use of polymers to encapsulate bacteria spores is also a popular direction in the studies on self-healing concrete as polymers have more functionalities compare to normal porous carriers and the functions of the polymer can be customized through specific polymerization reactions. Overall, the polymer-based materials reported in literatures include melamine (Wang, Soens et al. 2014) and hydrogel (Wang, Snoeck et al. 2014, Wang, Mignon et al. 2015, Palin, Wiktor et al. 2016, Wang, Mignon et al. 2018, Hassan, Milla et al. 2019, Fahimizadeh, Diane Abeyratne et al. 2020, Gao, Guo et al. 2020), among which hydrogel is mainstream. In 2014, Wang et al. (Wang, Soens et al. 2014) encapsulated spores in

melamine-based materials and obtained a microcapsule with size around 5 μm . In 1 g of the dry capsules, there were around 10^9 cells loaded. After cracked samples were immersed in water for 8 weeks, a maximum crack up to 970 μm can be healed. The healing process of crack engaged by the microcapsule is shown in Fig. 2.15. However, significant strength lose due to the addition of the microcapsules was observed. When the dosage of the microcapsules increased from 1% to 5%, the 28-day compressive strength decreased by 15% to 34%, as shown in Fig. 2.16.

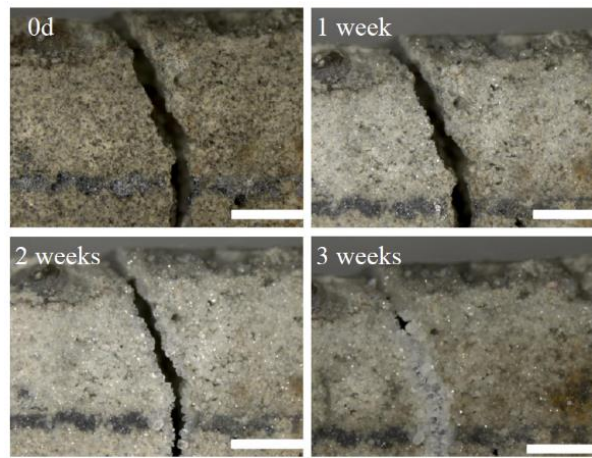


Figure 2.15 Crack healing engaged by the melamine-based bacterial microcapsules
(Wang, Soens et al. 2014)

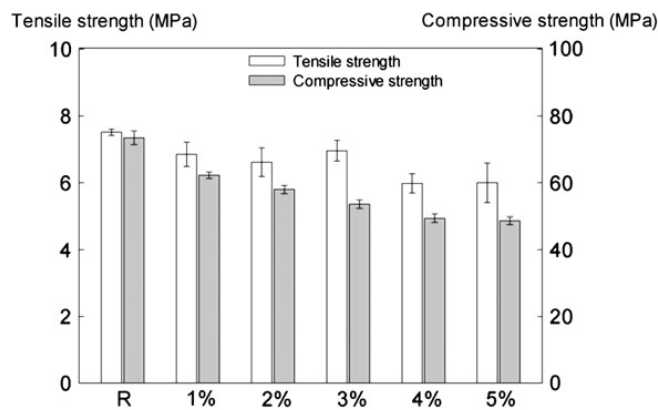


Figure 2.16 Strength properties of the specimens (with different additions of microcapsules, 0% (R), 1%, 2%, 3%, 4% and 5% of cement by mass, respectively) at the age of 28 days (Wang, Soens et al. 2014)

In the other works conducted by Wang et al., hydrogel was used to encapsulate bacteria spore was developed (Wang, Snoeck et al. 2014). Bacteria cells were encapsulated through the incorporation into polymers during polymerization reaction. Afterwards, the resulted hydrogel sheets were grinded and dried. According to the study, the advantage of using hydrogel was that the hydrogel can absorb water so it can serve as a water reservoir, which may facilitate the bacteria activity. Although the bacteria loaded hydrogel can engage self-healing as shown in Fig. 2.17, however, it was also because of hydrogel can absorb water and swell, the addition of hydrogel brought significant strength loss to the matrix. During the mixing, the hydrogel absorbed water and the volume increased. As the hydration proceeded, the water in the hydrogel was released gradually, and the volume of the hydrogel was reduced accordingly. As a result, pores would be created around the hydrogel and the strength of the matrix was compromised. Wang et al. reported in the study that with only 1% incorporation of hydrogel in the mortar, the 28-day compressive strength of the matrix reduced 23.4% (Wang, Mignon et al. 2015).

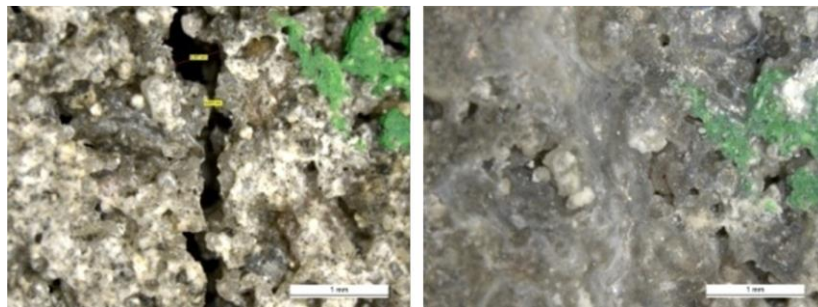


Figure 2.17 The crack (width: 507 μm) healing process of the specimen with addition of the bacteria encapsulated hydrogel (Wang, Snoeck et al. 2014)

On the basis of that, different types of hydrogels with different compositions were further studied by Wang herself and other researchers over the world, including chitosan-based pH-responsive hydrogel (Wang, Mignon et al. 2018), calcium alginate beads (Palin, Wiktor et al. 2016, Hassan, Milla et al. 2019) and sodium

alginate beads (Shahid, Aslam et al. 2020). The encapsulation process of the bacteria in the hydrogels were similar, mainly realized by the addition of bacteria into the mixture and encapsulated through reactions. For example, Fig. 2.18 has shown the encapsulation of bacteria in alginate hydrogels (Fahimizadeh, Diane Abeyratne et al. 2020). Firstly, the bacteria and needed nutrients were mixed with sodium alginate. Afterwards, the mixture was injected into calcium lactate, and bacteria loaded calcium alginate beads were formed through ionic gelation of alginate upon contact with Ca^{2+} ions. In these studies, similar phenomenon was observed, where the addition of the hydrogels significantly decreased the strength of the matrix. For example, Wang et al. further developed a chitosan-based hydrogel, and they found that the 28-day strength of the matrix decrease was around 5–20% with the addition dosage of 0.5m%–2 m%. Shahid et al. used sodium alginate beads to encapsulate bacteria and reported that the addition of 2% to 15% of the beads can lead to strength decrease by 4.9% to 40.5%. Except for the afore-mentioned water absorption property of hydrogel, another key reason that leads to the strength decrease is that hydrogel is a soft material with much lower strength than concrete. Therefore, the bacteria encapsulated hydrogels are not compatible with the matrix and present to be the weak points in the matrix, decreasing the strength. Besides of the negative effects on the mechanical properties, other drawbacks of polymer-based encapsulation include the complicated processing procedure and the relatively high cost of the materials.

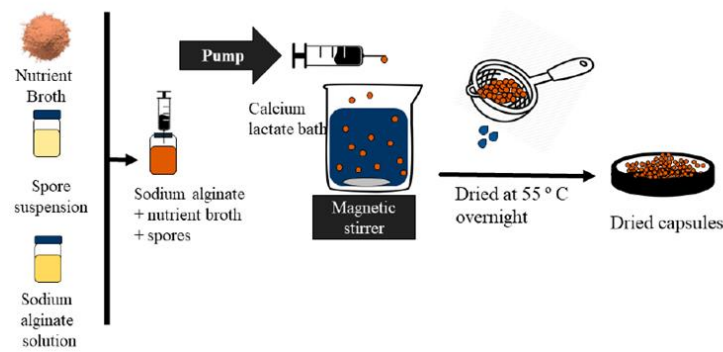


Figure 2.18 Encapsulation procedure of the bacterial calcium alginate capsules
(Fahimizadeh, Diane Abeyratne et al. 2020)

2.3.3 Low alkalinity cementitious materials

To overcome the concerns regarding mechanical performance as mentioned in Section 2.3.1 and Section 2.3.2, the use of low alkalinity cementitious materials for encapsulation has emerged as a new technology recently. These materials not only have lower alkalinity which potentially allows the survival of bacteria inside but also have high compatibility with the PC-based matrix. Generally, the bacteria were added into the cement-based encapsulation material during the cement mixing with water procedure. After the paste was hardened, the hardened mixture was further grinded or extruded into favorable size. Su et al. (Su, Zheng et al. 2021) has reported that using low alkaline sulphoaluminate cement to encapsulate bacteria, as shown in Fig. 2.19. After the cracked sample was immersed in water for 28 days, the healing of the crack was observed, as shown in Fig. 2.20. At the same time, the 28-day compressive strength of the matrix was maintained when the dosage of the capsules was 12% by cement mass. Wu et al. (Wu, Hu et al. 2020) has investigated using carbide slag, fly ash, and desulfurized gypsum to prepare a cementitious material to encapsulate bacteria for self-healing, and the resulted bacterial capsule was shown in Fig. 21. The results showed that the biocapsule can engage self-healing of cracks around 500 μm , and the strength of the matrix can be maintained when the dosage of the capsules was below 5% of cement mass. Besides, the study

has observed the capsule breakage after cracking, suggesting the high compatibility of the cementitious materials with the PC-based matrix. However, continuous hydration of those cementitious materials could still result in microstructure densification and reduction of porosity of the bacteria capsules. Therefore, the long-term viability of the bacteria inside the capsules might be reduced.

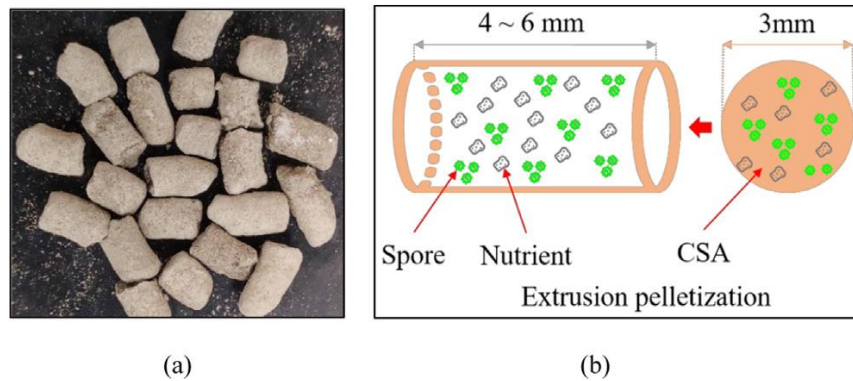


Figure 2.19 The sizes of sulphoaluminate cement-based self-healing agent, (a) real microbial pellets; (b) schematic diagram of microbial pellets (Su, Zheng et al. 2021)

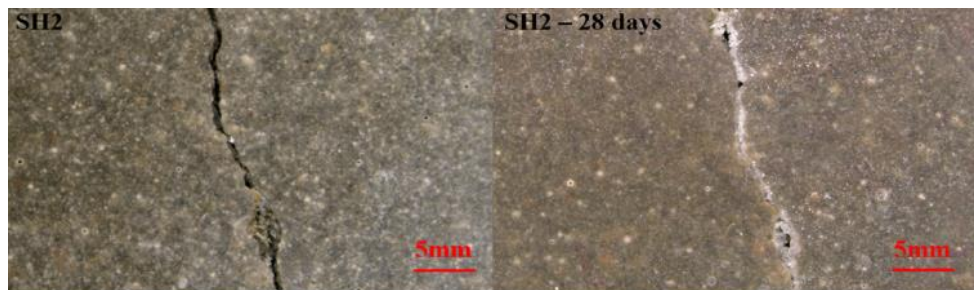


Figure 2.20 Surface crack healing of the sample with addition of sulphoaluminate cement encapsulated bacteria healing agent (Su, Zheng et al. 2021)

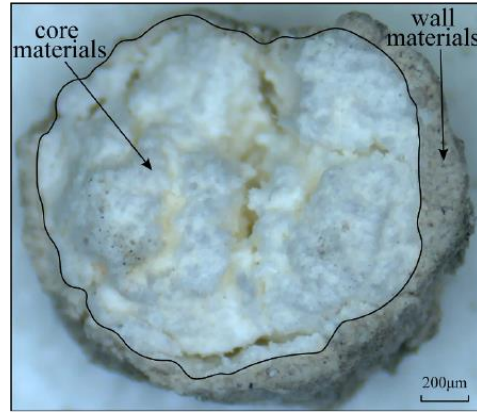


Figure 2.21 Cross-section of the carbide slag, fly ash, and desulfurized gypsum-based bacteria capsule (Wu, Hu et al. 2020)

2.4 Test methods for self-healing concrete

One of the critical aspects in the self-healing concrete research is the proper evaluation of the self-healing efficiency. According to Van Tittelboom and De Belie, the evaluation of the self-healing performance can be classified into three broad categories, (i) visualization and determination, (ii) assessment of the regained resistance, and (iii) assessment of regained mechanical properties (Van Tittelboom and De Belie 2013). In the following section, the three types of evaluation and their corresponding tests are introduced.

2.4.1 Visualization and determination

Under this category, the main techniques are qualitative methods to visualize the healing occurrence. Few quantitative tests can be done by using the measurement function of the equipment. Based on the specific procedure, the visualization and determination can be further divided into three subcategories: microscopy, imaging and spectroscopy.

Microscopic methods are the most common evaluation techniques for self-healing concrete as the visual observation of crack closure is necessary in any self-healing

studies. The major tool used is optical microscope, which can be used to observe the healing products formation within the crack and measure the crack width change before/after the healing. Usually, scanning electron microscopy (SEM) and environmental scanning electron microscopy (ESEM) are adopted together to determine the morphology, shape and size of the healing products.

While microscopic methods are mainly used for surface observation, imaging methods are able to conduct interior structural inspection without destruct specimens. The major techniques to be used in this category includes X-ray radiography/tomography, neutron radiography/tomography and digital image correlation. The source of the electromagnetic radiation can be X-ray or neutron radiation. In radiography, 2D images can be produced, as shown in Fig. 2.22. With tomography, 3D images can be generated by using the digital geometry from 2D images, as shown in Fig. 2.23. Lastly, digital image correlation (DIC) is an optical method to measure the two-dimensional and three-dimensional changes in images via tracking and image registration techniques. DIC can measure displacement and strain, therefore the use of DIC can monitor the crack development and closure (Feiteira, Tsangouri et al. 2017).

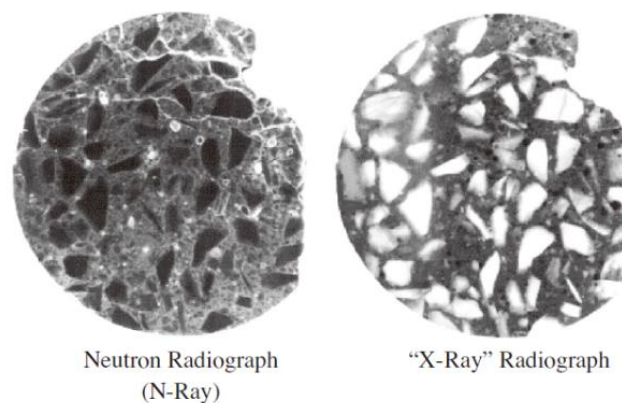


Figure 2.22 2D images by radiography (Najjar, Aderhold et al. 1986)

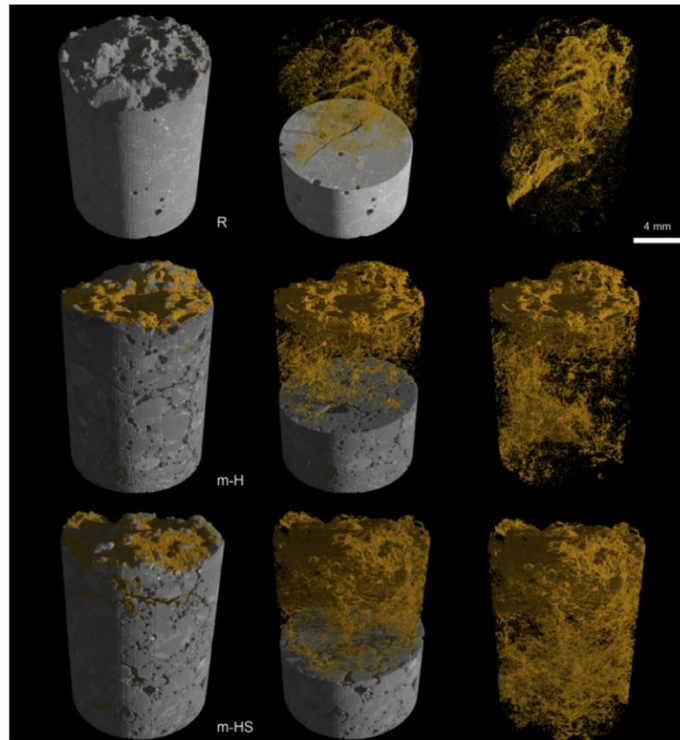


Figure 2.23 3D view of the spatial distribution of healing products (in yellow) in the different specimens (left: outlook of samples plus the precipitation; middle: distribution of precipitates inside; right: the whole precipitates in the sample).

Spectroscopic methods are mostly used to determine the chemical composition of the self-healing products. In some cases, some spectroscopic tests can deliver detailed data about the healing process (Tang, Kardani et al. 2015). Generally, the mechanism of spectroscopic techniques is to transmit a beam of light through specimen and then record the spectrum of the transmitted light. By analyzing the position, shape and intensity of the peaks in the received spectrum, the information of the specimen of interest, e.g., molecular structure, can be provided. Commonly used techniques for spectroscopic examination includes x-ray spectroscopy, infrared spectroscopy, Raman spectroscopy and x-ray diffraction analysis. Fig. 2.24 has summarized the working principles of each method. Among these techniques, x-ray spectroscopy (Fig. 2.24(a)) and infrared spectroscopy (Fig. 2.24(b)) help to detect and determine the healing products where x-ray spectroscopy transmit x-ray

spectrum and infrared spectroscopy transmit light beams in the infrared region of the electromagnetic spectrum (Wiktor and Jonkers 2011). Besides, both Raman spectroscopy (Fig. 2.24(c)) and x-ray diffraction analysis can be used to characterize the chemical composition of the healing products while x-ray diffraction analysis can further investigate the 3D structure of a crystalline specimen by measuring the properties of x-ray beams diffracted due to the atomic structure of the specimen (Homma, Mihashi et al. 2009, Ahn and Kishi 2010).

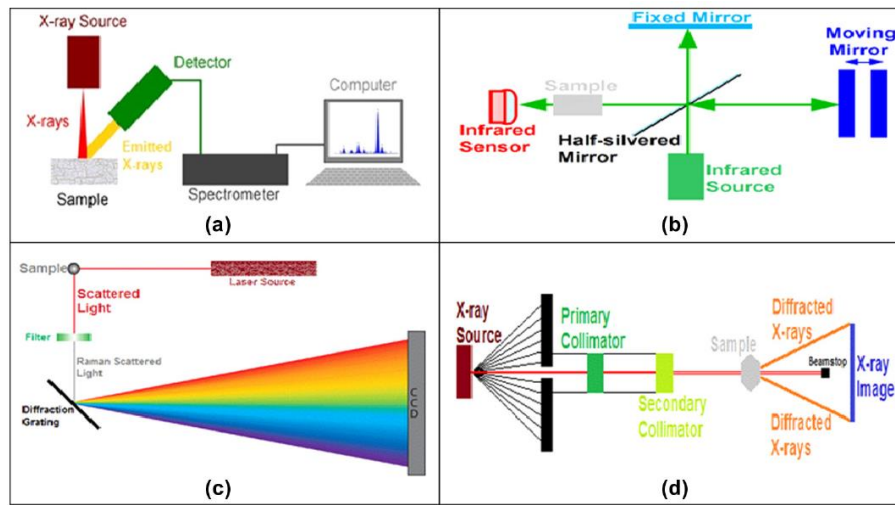


Figure 2.24 Four types of spectroscopy techniques: (a) x-ray spectroscopy, (b) infrared spectroscopy, (c) Raman spectroscopy, and (d) x-ray diffraction analysis

2.4.2 Assessment of the regained resistance

The propagate of cracks in concrete leads to the discontinuity of the matrix, as a results, the matrix becomes vulnerable to the penetration of moisture and harmful substances. Therefore, one of the important criteria to evaluate the healing performance is the recovery of the tightness of the matrix. Accordingly, the assessment of the regained resistance needs to be measured. Specifically, the regained resistance can be further evaluated by transport properties, resistivity and continuity recovery of the matrix after healing.

Transport property of concrete describes the ability of ions and fluids to move through concrete material (Claisse 2014). To quantify the transport properties, water and air permeability, sorptivity, chloride diffusivity and osmotic pressure usually are tested and measured. Among these tests, water and air permeability are the most commonly used methods in literature for the testing (Guo, Wang et al. 2019, Hou, Li et al. 2022). By comparing the water/air permeability before and after the healing, the regained tightness against water and air penetration can be quantified. The normally used set-up for the measurement of water permeability is shown in Fig. 2.25.

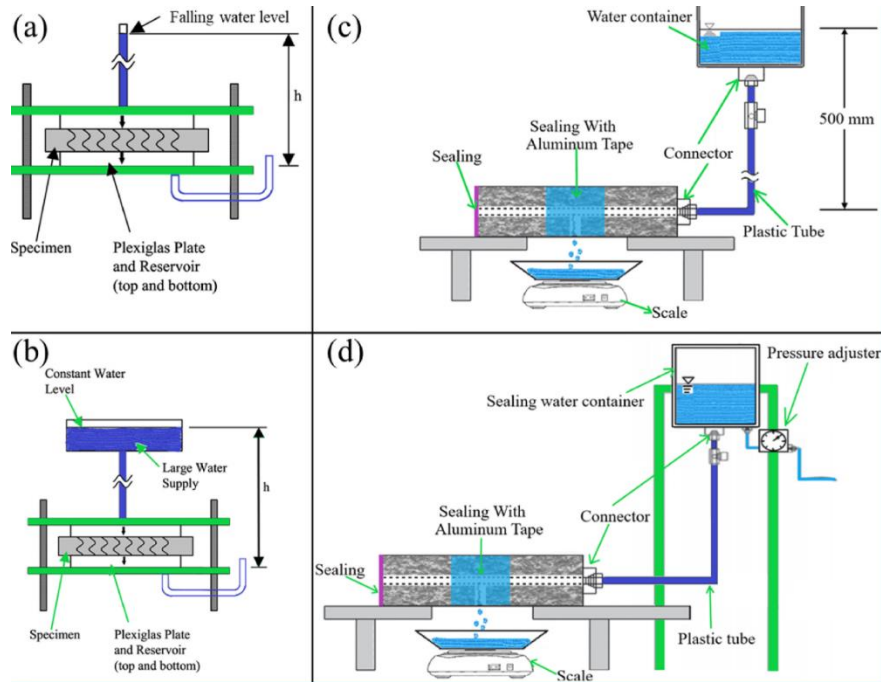


Figure 2.25 Different water permeability measurement setup reported in literatures:

(a) Falling head test (Yang, Lepech et al. 2009), (b) Constant head test (Yang, Lepech et al. 2009), (c) Low constant pressure test (Gruyaert, Debbaut et al. 2016) and (d) High constant pressure test (Gruyaert, Debbaut et al. 2016).

Sorptivity describes the capillary water suction of the concrete, which is a standard procedure to measure the rate of capillary water uptake of the specimen (Standard

2011), as shown in Fig. 2.26. Another important transport property is the resistance of concrete to the chloride attack as the presence of chloride ions can cause the corrosion of reinforcement bars in concrete. The testing of rapid chloride permeability follows ASTM C1202, and the setup is shown in Fig. 2.27. Besides, osmotic pressure test also has been adopted in self-healing studies to measure the selective permeability of the concrete against water, solutes and ions (Sangadji and Schlangen 2012).

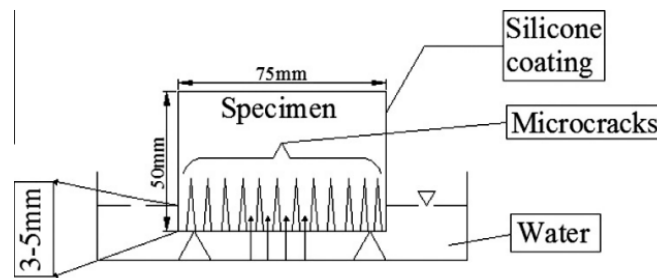


Figure 2.26 Sketch of setup for sorptivity test (Zhang, Qian et al. 2014)



Figure 2.27 Rapid chloride permeability apparatus (Chahal, Siddique et al. 2012)

Another series of properties which relates to the durability of concrete include its continuity and the resistance to all kinds of hazards which may cause degradation of concrete (e.g., corrosion and freeze-thaw). Generally, the tests to be done include ultrasonic waves to testing the internal flaws of concrete (Singh and Gupta 2020), corrosion test to test the ability of concrete to resist corrosion (Pelletier, Brown et al.

2011) and frost/salt test to investigate the resistance to freeze-thaw cycles (Sisomphon, Çopuroğlu et al. 2011). Overall, the evaluation of transport property recovery is much more extensive and common than the tests for continuity and resistivity in existing self-healing studies.

2.4.3 Assessment of regained mechanical properties

To evaluate the mechanical properties after self-healing, series of destructive mechanical tests and non-destructive tests have been done to measure the recovery of cracked specimens. The major destructive tests include bending tests, compression test, tensile strength test, horizontal deformation test and impact loading test.

Bending test is a common test in self-healing study to generate cracks on concrete specimens. By 3-point bending or 4-point bending test, the flexural strength of the concrete can be measured, as shown in Fig. 2.28 and Fig. 2.29. Through the comparison the flexural strength before and after the healing, the regain of the mechanical strength can be evaluated. Some studies also observe the location of the crack occurrence before and after healing to evaluate the healing performance by investigating whether healed crack would crack again under loading (Chen, Peters et al. 2013). Besides for the bending test, compression test (Yang, Hollar et al. 2011) and tensile test (Qian, Zhou et al. 2009) are also commonly used tests to evaluate the regained compressive strength, tensile strength, stiffness and ductility of cracked concrete after healing. The setup for tensile strength test is shown in Fig. 2.30.

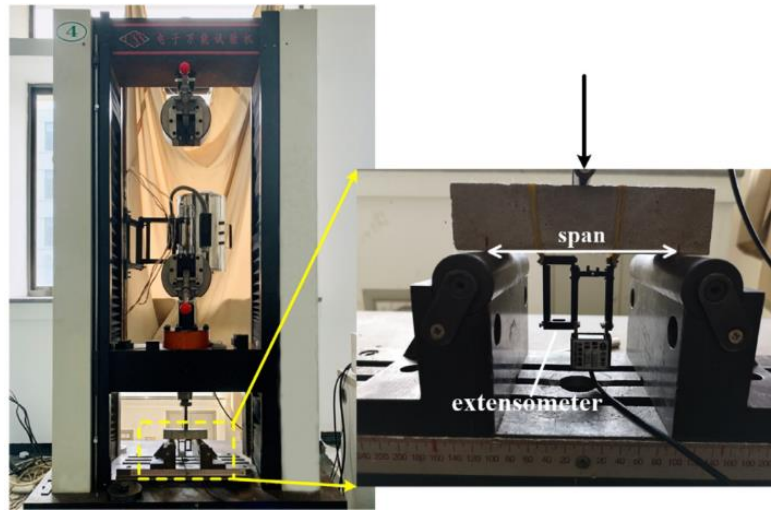


Figure 2.28 3-point bending test setup(Feng, Chen et al. 2021)

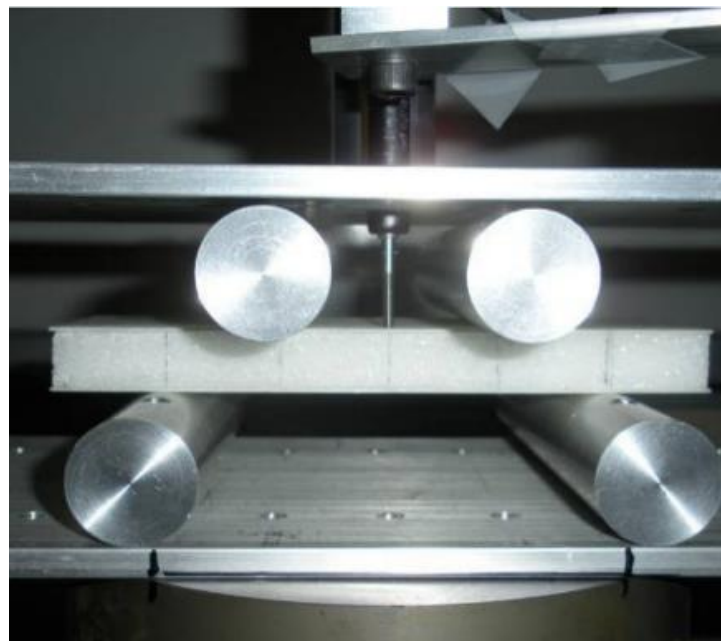


Figure 2.29 4-point bending test setup (Chen, Peters et al. 2013)

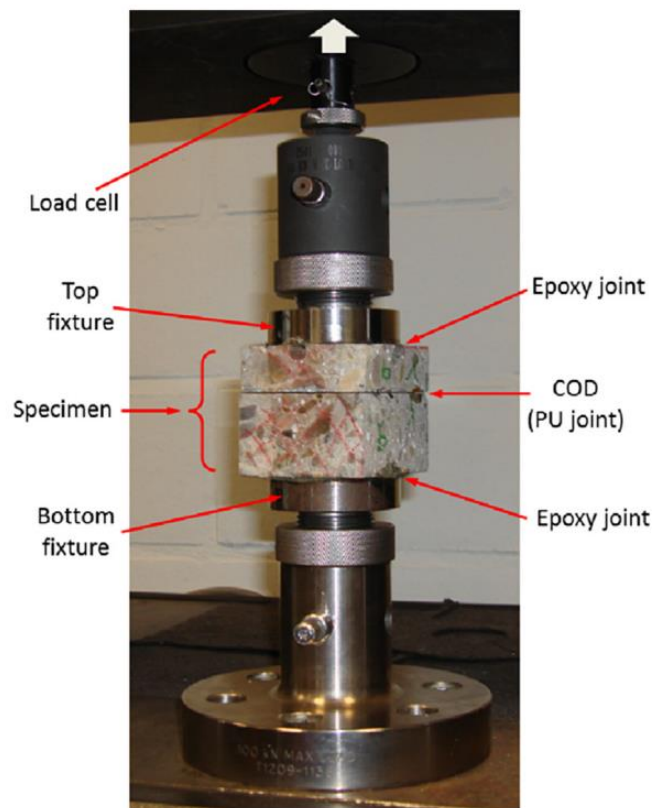


Figure 2.30 Tensile test setup (Gilabert, Van Tittelboom et al. 2017)

Another two tests which are less common than the aforementioned mechanical tests in literatures are horizontal deformation test and impact loading test. In the horizontal deformation test (THAO 2011), horizontal load with controlled displacement is applied to the top of column specimen to generate cracks. After healing, same loading is applied again to the column, and the obtained load-displacement relationship can be used to quantify regained tangent stiffness of the healed specimen, as shown in Fig. 2.31. As for the impact loading, the impact energy is created by drop a mass from a certain height, and the test is used to evaluate the regained static stiffness of healed specimen (Dry 1997).

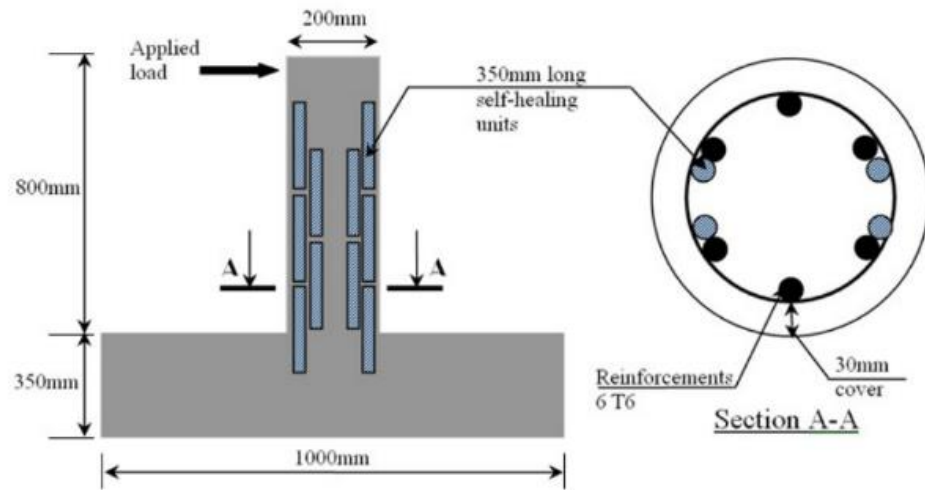


Figure 2.31 Horizontal deformation test setup (THAO 2011)

For the non-destructive tests, acoustic emission (AE) and resonance frequency (RF) analysis are usually used. AE analysis is realized by externally stimulating the matrix and then receiving the signals from the specimen (Van Tittelboom, De Belie et al. 2012). As for RF, it is an indirect method used to evaluate the regained stiffness and quantify the degree of damage and recovery (Jacobsen and Sellevold 1996). Overall, the general methods used in the evaluation of self-healing performance is summarized in Table 2.4.

Table 2.4 Summary of current test methods for the evaluation of self-healing
concrete

	Microcopy	Optical microscopy, ESEM
Visualization and determination	Imaging	Radiography, Tomography, Digital image correlation
	Spectroscopy	X-ray diffraction analysis, X-ray spectroscopy, Raman spectroscopy, Infrared analysis
Regained resistance	Transport properties	Water permeability, Air permeability, Sorptivity, Chloride diffusion, Osmotic pressure
	Resistivity and continuity	Corrosion, Frost/salt scaling, Ultrasonic measurement
Regained mechanical properties	Destructive mechanical tests	Compression test, Bending test, Tensile test, Horizontal deformation, Impact loading
	Non-destructive tests	Acoustic emission, Resonance frequency analysis

2.5 Introduction of reactive magnesia cement (RMC)

The main component of magnesia cement is MgO. In the past, the applications of MgO cement in construction industry mainly include magnesium oxychloride (MOC) cements used in fiber-reinforced fire-resistant boards (Sorre and Armstrong 1976, Urwongse and Sorrell 1980), magnesium oxysulfate (MOS) cements used in lightweight insulation panels (Beaudoin and Feldman 1977, Beaudoin and Ramachandran 1978), magnesium phosphate (MAP) cements used in fire protection and rapid repairs (Ribeiro and Morelli 2009, Mestres and Ginebra 2011), and MgO-based expansive additives (MEAs) used to compensate concrete shrinkage in dam construction (Zheng, Xuehua et al. 1991, Gao, Lu et al. 2008). MgO cement can be obtained through two methods: 1. calcination of magnesia-based minerals (dry-

route), 2. extraction from brine or seawater (wet-route). According to the different calcination temperature of MgCO_3 , the obtained MgO can be divided into four categories: light-burned ($700\sim 1000\text{ }^\circ\text{C}$), hard-burned ($1000\sim 1400\text{ }^\circ\text{C}$), dead-burned ($1400\sim 2000\text{ }^\circ\text{C}$), and fused ($>2800\text{ }^\circ\text{C}$). The lower calcination temperature is, the higher activity of MgO is. Therefore, light-burned MgO has highest activity and is used as cement binder.

Recently, using RMC as an alternative binder of Portland cement and gain strength from accelerated carbonation has been extensively studied (Unluer and Al-Tabbaa 2014, Dung and Unluer 2018, Hay and Celik 2020). Comparing to PC, the main advantages of RMC include its (1) lower calcination temperature, (2) ability to sequester CO_2 and gain strength by the formation of HMCs, (3) high-durability performance in extreme and aggressive environments, (4) lower sensitivity to impurities, enabling the use of industrial by-products, and (5) potential to be fully recycled when RMC is used alone as a binder.

The strength gain process of RMC blends involves two procedures: hydration and carbonation, as shown in Fig. 2.32. Firstly, MgO hydrates and forms $\text{Mg}(\text{OH})_2$ (brucite). The pore volume reduces due to the volume expansion associated with the brucite formation. Unlike PC blends, the hydrate phase brucite has a porous structure and does not make a significant contribution to strength development (Liska and Al-Tabbaa 2009, Unluer and Al-Tabbaa 2012, Li 2013). Afterwards, the carbonation of $\text{Mg}(\text{OH})_2$ leads to the formation of a range of hydrated magnesium carbonates (HMCs) such as nesquehonite ($\text{MgCO}_3\cdot 3\text{H}_2\text{O}$), artinite ($\text{Mg}_2(\text{CO}_3)(\text{OH})_2\cdot 3\text{H}_2\text{O}$), hydromagnesite ($\text{Mg}_5(\text{CO}_3)_4(\text{OH})_2\cdot 4\text{H}_2\text{O}$), and dypingite ($\text{Mg}_5(\text{CO}_3)_4(\text{OH})_2\cdot 5\text{H}_2\text{O}$). HMCs contain different number of MgO , H_2O , and CO_2 . They can be defined with the general formula $x\text{MgCO}_3\cdot y\text{Mg}(\text{OH})_2\cdot z\text{H}_2\text{O}$, as listed in Fig. 2.33 (Liska and Al-Tabbaa 2009). The SEM images of some common HMCs are shown in Figure 2.34 (Giammar, Bruant Jr et al. 2005, Ian, Siobhan et al. 2007,

Ferrini, De Vito et al. 2009, Teir, Eloneva et al. 2009). Accordingly, the formation of HMCs on the surface is dense and strong, which is the main strength source of RMC-based mixes. Besides, it further reduces the pore volume since it is a volume expansion process. Altogether, the morphology and the binding strength of the HMCs contribute the most to the interlocked network of microstructure (Unluer 2018).

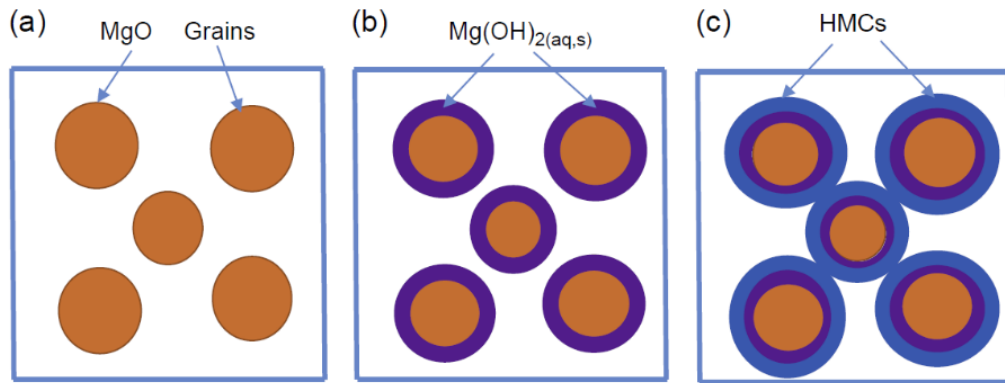


Figure 2.32 Schematic representation of the hydration and carbonation processes of RMC

Group	No. of MgO moles	No. of H ₂ O moles	No. of CO ₂ moles	Compound	Chemical formula
I	1	1	—	Brucite	Mg(OH) ₂
		—	1	Magnesite	MgCO ₃
		2	1	Barringtonite	MgCO ₃ ·2H ₂ O
		3	1	Nesquehonite	MgCO ₃ ·3H ₂ O
		5	1	Lansfordite	MgCO ₃ ·5H ₂ O
II	2	1.5	1	Pokrovskite	Mg ₂ (CO ₃)(OH) ₂ ·0.5H ₂ O
III	5	4	1	Artinite	Mg ₂ (CO ₃)(OH) ₂ ·3H ₂ O
		5	4	Hydromagnesite	Mg ₅ (CO ₃) ₄ (OH) ₂ ·4H ₂ O
		6	4	Dypingite	Mg ₅ (CO ₃) ₄ (OH) ₂ ·5H ₂ O
IV	7	6–7	4	Giorgiosite	Mg ₅ (CO ₃) ₄ (OH) ₂ ·5–6H ₂ O
		25	5	Shelkovite	Mg ₇ (CO ₃) ₅ (OH) ₄ ·24H ₂ O

Figure 2.33 Phases forming of carbonated RMC blends

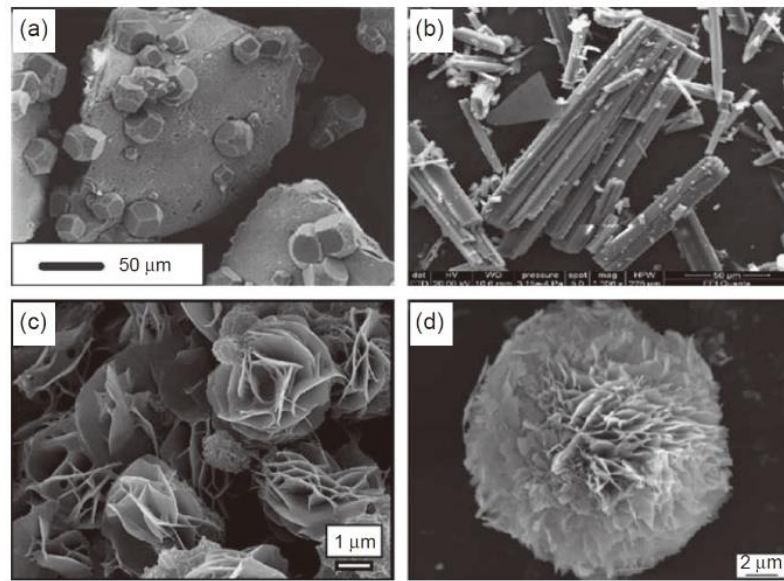


Figure 2.34 SEM images of some of the most common magnesium carbonates: (a) magnesite, (b) nesquehonite, (c) dypingite, and (d) hydromagnesite

Similar to the structure of MgO grains, RMC-based components present same structure where the surface of the component is dense HMCs and the core region is consisted of porous MgO and brucite. Therefore, RMC-based components generally present a gradient structure with a gradual decreased density and strength from surface to the core. Besides, due to the nature of brucite and strength gain mechanism of RMC blends, RMC systems are low alkalinity systems. $\text{Mg}(\text{OH})_2$ has very low solubility (0.00064 g/100 Ml at 25 °C), therefore, the pH of uncarbonated of RMC blends is mild. After carbonation curing, the pH of the system potentially can further reduce since the consumption of OH^- . Latest research on the pH of RMC blends has reveal that the uncarbonated blends had pH ranging from 10.2~10.5. And the pH of carbonated RMC blends is between 9.9~10.3 (Hay and Celik 2020).

2.6 Introduction of layer-by-layer (LBL) technique in single cell encapsulation

Layer-by-layer (LBL) is a technique to assemble functional thin films to the surface of substrates. Generally, LBL assembly is realized by cyclically absorbing

oppositely charged materials to the surface of the substrate with washing steps in between of each layer of deposition. Normally, thickness of a single layer of coating is on the order of nanometers, and the deposition process can be repeated until a desired function or thickness of the coating is reached. Currently, the assembly technology can be classified into five categories: (i) immersive, (ii) spin, (iii) spray, (iv) electromagnetic, and (v) fluidic assembly, as shown in Fig. 2.35.

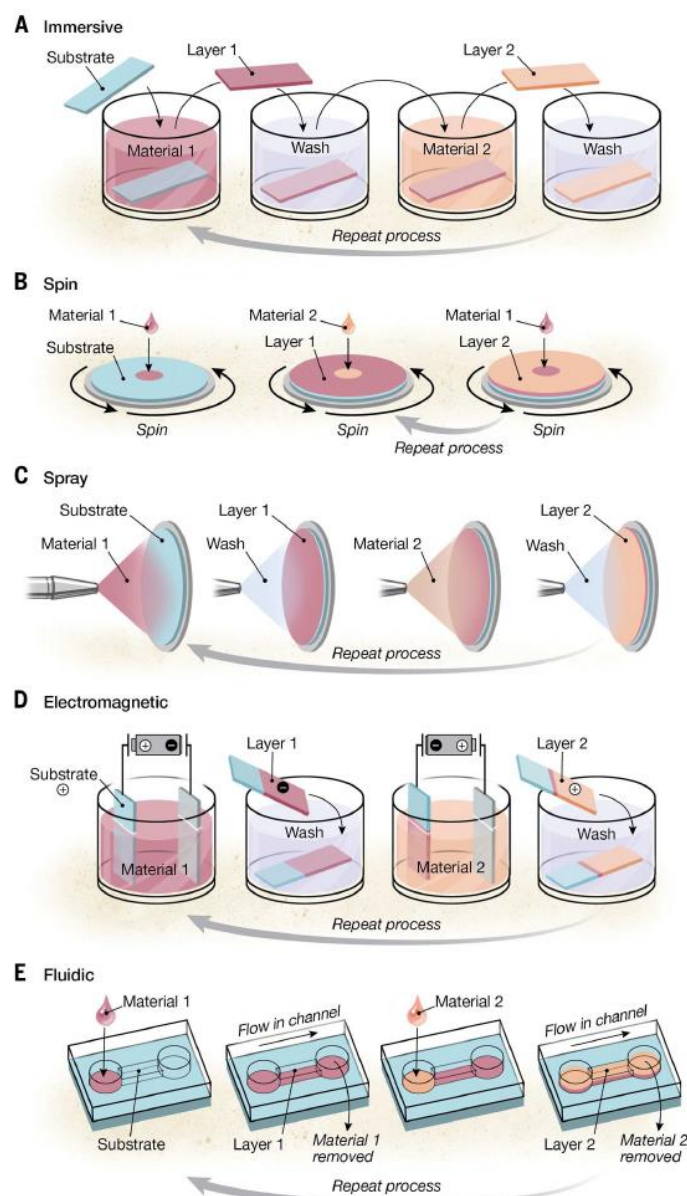


Figure 2.35 Different layer-by-layer assembly technologies (Richardson, Björnmalm et al. 2015)

Theoretically, any charged materials can be used as substrate and coating materials. For example, melamine formaldehyde (Gao, Donath et al. 2002, Katagiri and Caruso 2004, Angelatos, Radt et al. 2005), polystyrene (Yap, Quinn et al. 2005), silica particles (Wang, Yu et al. 2005), organic (Caruso, Trau et al. 2000), and inorganic microcrystals (Holt, Lam et al. 2007) have been coated with a large variety of polyelectrolytes and other charged materials, including metal nanoparticles (Kahraman, Zamaleeva et al. 2009). The formed functional composites have wide range of applications, e.g., tissue engineering and drug delivery. Overall, the LBL assembly process and its application are shown in sketch Fig. 2.36.

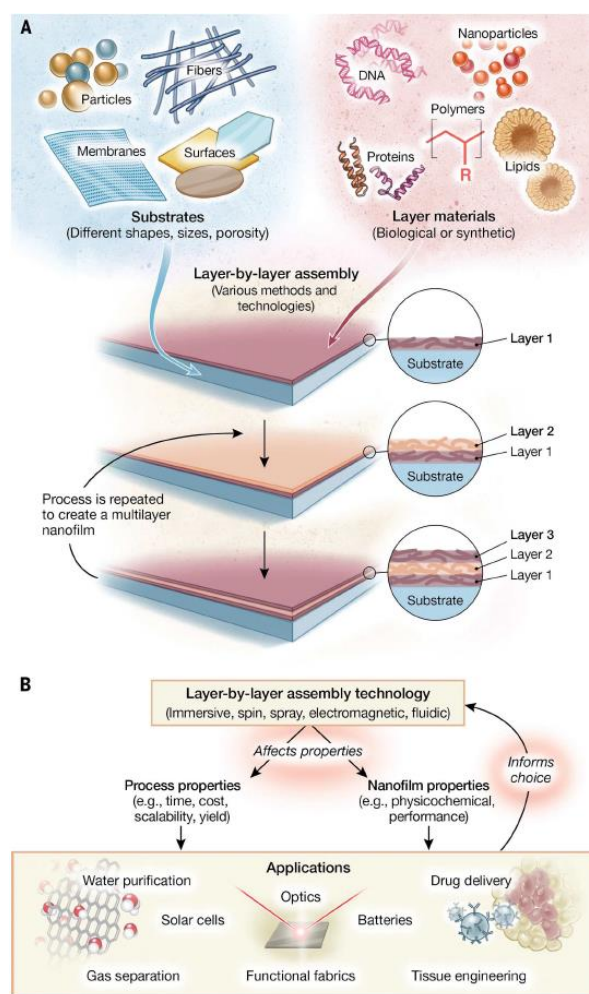


Figure 2.36 (A) Schematic overview of LbL assembly and (B) application areas of LBL techniques (Richardson, Björnmalm et al. 2015)

Due to the small size (typically around 1 μm) and the fluid wall of the biological cell (Balkundi, Veerabadrán et al. 2009), retaining the function of the biological cell while modifying its surface architecture has been a challenge. Over the past years, LbL self-assembly technique has been investigated in cell encapsulation and demonstrated to be an effective solution (Balkundi, Veerabadrán et al. 2009, Fakhrullin, Zamaleeva et al. 2009, Franz, Balkundi et al. 2010), as shown in Fig. 2.37. The very early study on cell surface coating in 2002 has reported to use cationic poly(dimethyldiallylammonium chloride) (PDADMAC) and anionic sodium poly(styrenesulfonate) (PSS) to coat bovine platelets (Ai, Fang et al. 2002). In the study, the researchers managed to prevent the cell aggregation and secretion by controlling the thickness and composition of the coating, indicating the possibility to modify the function of single cell by LBL technique. Later on, different types of cells were encapsulated by LBL including yeasts (Yang, Lee et al. 2009), stem cells (Wilson, Cui et al. 2011) red blood cells (Fakhrullin and Lvov 2012), fibroblasts (Xin, Zeng et al. 2017), endothelial cells (Gentile, Carmagnola et al. 2015), and dormant bacterial spores (Balkundi, Veerabadrán et al. 2009), and the viability and metabolic activity changes of the encapsulated cells were investigated.

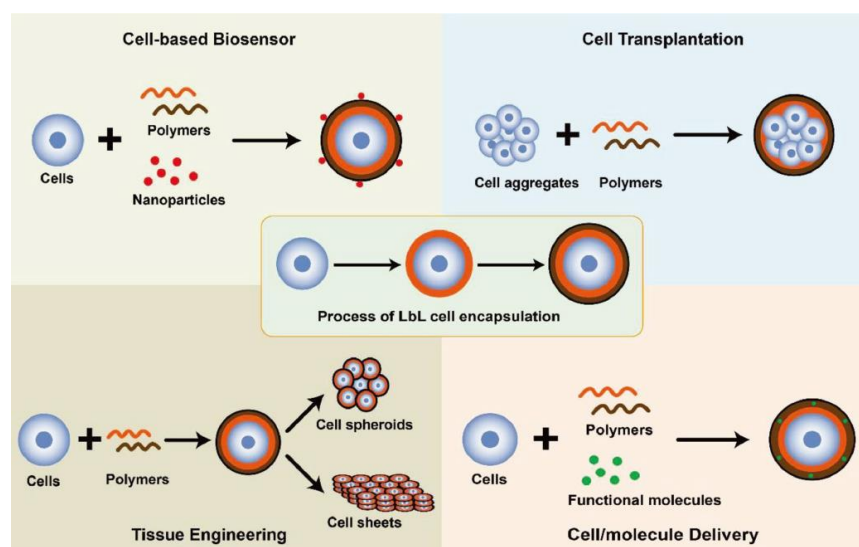


Figure 2.37 Overview of the cell encapsulation process by LBL and the applications of cell encapsulation

Among different cells, the investigation on single dormant spore encapsulation by LBL was firstly reported by Balkundi et al (Balkundi, Veerabadran et al. 2009). In the study, spores of *Bacillus subtilis* was encapsulated with different pair of cationic and anionic polyelectrolytes, namely poly(dimethyldiallyl ammonium chloride) (PDDA)/ sodium poly(styrene sulfonate) (PSS) and polyelectrolytes polyglutamic Acid(PGA)/ polylysine(PLL). Through alternative deposition of coating materials and a final layer of silica nanoparticles, spores were successfully encapsulated, as shown in Fig. 2.38. The study also reported that, after encapsulation, the germination of the dormant spores and its metabolism are not inhibited. Similarly, bacteria cells of *Escherichia coli*, *Staphylococcus cohnii* and other types of bacteria are also encapsulated in literatures (Kahraman, Zamaleeva et al. 2009).

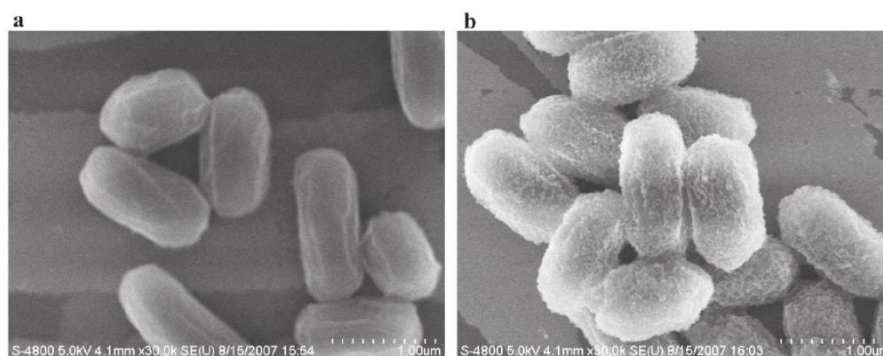


Figure 2.38 SEM images of (a) *Bacillus subtilis* spores without encapsulation and (b) *Bacillus subtilis* encapsulated with (PLL/PGA)₄ + PLL + 22 nm silica nanoparticles (Balkundi, Veerabadran et al. 2009)

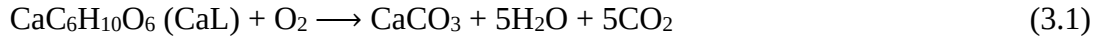
CHAPTER 3 FUNCTIONALLY GRADED REACTIVE MAGNESIA CEMENT (RMC)-BASED BACTERIA SPORE CAPSULES (RMC-B)

3.1 Introduction

This chapter reported a novel functionally graded reactive magnesia cement (RMC)-based bacteria spores capsules for self-healing concrete. RMC, a low alkaline binder (i.e., pH ~10.5 (Hay and Celik 2020)), was used to encapsulate bacteria spores. Unlike most cementitious materials where the strength gain is through hydration, RMC develops its strength mainly via carbonation which makes the resulting RMC-based bacteria (RMC-B) capsules to possess a unique stable gradient structure with a dense shell of HMCs and a porous core that mainly consists of brucite and MgO (Unluer 2018). The composition and density change from surface to the core region enables the capsule to be a functionally graded capsule. While the porous core accommodates bacteria spores, the dense shell protects bacteria spores from direct contact with the surrounding cement matrix and provides mechanical strength. The stable microstructure of the RMC-B capsule shall promote long-term viability of bacteria spores.

Accordingly, bacteria spores capsules were fabricated by encapsulating *Bacillus cohnii* spore in RMC pastes. *Bacillus cohnii* is a non-ureolytic bacteria, which can oxidize calcium lactate to form calcium carbonate, as shown in Eqn. (3.1) (Xu and Yao 2014, Tziviloglou, Wiktor et al. 2017). Unlike ureolytic bacteria which lead to ammonia formation during calcium carbonate precipitation, the metabolic precipitating route of *Bacillus cohnii* does not cause any ammonia burden to the environment. The compositions and morphology of the RMC-B capsules were characterized. Viability of bacteria spores in the RMC capsules was evaluated. Effects of RMC-B capsule addition on hydration and fresh and hardened properties of Portland cement (PC) paste were investigated. The capsules were then

incorporated together with the nutrients into a PC paste to evaluate the self-healing performance. The nutrients included calcium lactate (CaL) and yeast extract (YE), where YE helped the activation of the spores and CaL was the precursor for calcium carbonate precipitation. The self-healing performance was evaluated via observation of crack closure and tests on water passing rate.



3.2 Experimental program

3.2.1 Preparation of bacteria spores

Bacillus cohnii ATCC 51227 from American Type Culture Collection was used in this study. As a mesophilic bacterium, *Bacillus cohnii* is capable of growing at moderate temperatures between 20-45°C with an optimal growth temperature of around 37°C, and the optimal humidity range for the growth and survival of *Bacillus cohnii* colony is around 30-60% relative humidity. The vegetative cells were grown aerobically in a sterile growth medium consisting of 5 g/L peptone, 3 g/L meat extract, 0.42 g/L NaHCO₃ and 0.53 g/L Na₂CO₃. After 24 hours of incubation on an orbital shaker (200 rpm) at 25°C, the vegetative cultures were inoculated into a sterile sporulation medium (1% inoculum by volume) for 3-7 days under the same incubation environment. The sporulation medium consisted of 0.2 g/L NH₄Cl, 0.02 g/L KH₂PO₄, 0.225 g/L CaCl₂, 0.2 g/L KCl, 0.2 g/L MgCl₂·6H₂O, 1 ml trace elements solution SL12B (for 1L sporulation medium), 0.1 g/L yeast extract, 4.2 g/L NaHCO₃, 5.3 g/L Na₂CO₃, and 5.16 g/L sodium citrate (Tziviloglou, Wiktor et al. 2017). The spores were harvested by centrifugation at 8000-9000 rpm for 10 minutes and the pellets were re-suspended in sterile water. The spore suspension was then subjected to pasteurization (20 minutes in an 80°C water bath, followed by 5 minutes in meshed ice) in order to eliminate the remaining vegetative

cells (Wang, Soens et al. 2014).

Serial dilutions and spread plate technique were used to determine the colony-forming unit (CFU) concentration of the spore suspension (Sanders 2012). Furthermore, 2 μL spore suspension was dropped on a clean glass plate and air dried. The sample was then sputter coated with platinum by means of a sputter coater (JEOL JFC-1600) under 20 mA current for 40 seconds and examined with a scanning electron microscope (SEM, JEOL JSM-7600 F) under the secondary electron image (SEI) mode at a voltage of 5.0 kV to reveal the morphology of the spores (Fig. 3.1). The suspension was stored in a fridge at 4°C for further use.

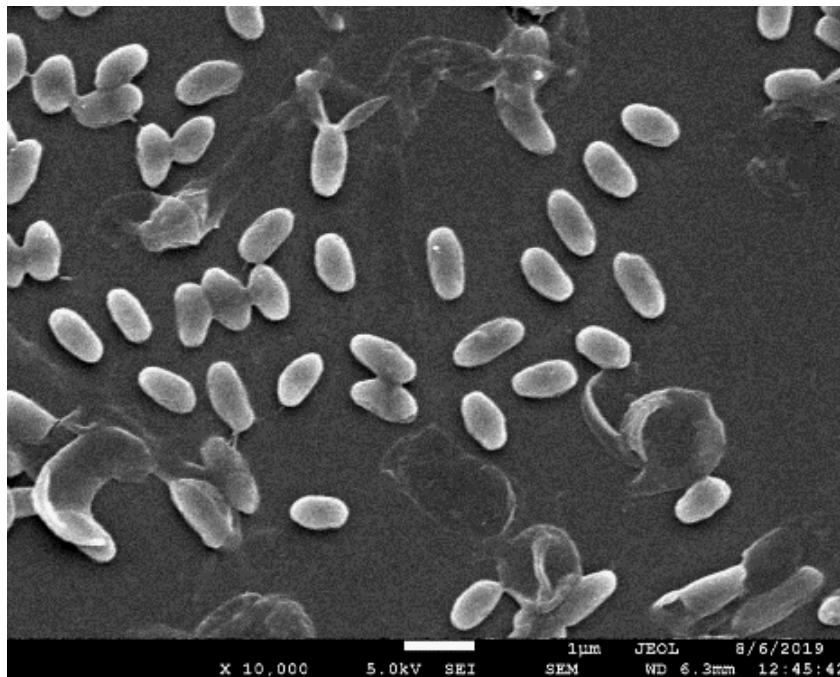


Figure 3.1 Morphology of the resulting *Bacillus cohnii* spores

3.2.2 Fabrication of functionally graded RMC-based bacteria spores capsule (RMC-B capsule)

To fabricate the functionally graded RMC-based bacteria spores capsule (RMC-B capsule), the spore pellets (1.02×10^{11} CFU) were first mixed with 20 g water,

forming a well-dispersed suspension solution. The spore suspension was then added into 40 g RMC powder and mixed with a hand mixer for around 5 minutes until a homogeneous state was reached. The RMC used in this study, whose chemical composition is shown in Table 3.1, was purchased from Richard Baker Harrison Ltd. (UK). Its reactivity, measured by the time required for the neutralization of 0.25 M of acetic acid by 5 g of RMC, was recorded as 520 s (Dung and Unluer 2018).

Table 3.1 Chemical composition of RMC

Composition	Weight percentage (%)
MgO	> 91.5
SiO ₂	2.0
CaO	1.6
R ₂ O ₃	1.0
LOI	4.0

The fresh pastes were then cast into 2.5 cm cube molds and hardened for 3 days under ambient conditions. After demolding, the cubes were crushed into powders using a mortar, and sieved through a 150 μ m mesh. The powders were spread onto a pan and subjected to accelerated carbonation curing under 30°C with 10% CO₂ and 80% relative humidity (RH) for 7 days. After carbonation was completed, the particle size distribution of the capsules was investigated by a laser diffraction particle size analyzer (Beckman Coulter LS 13 320) and the results are shown in Fig. 3.2.

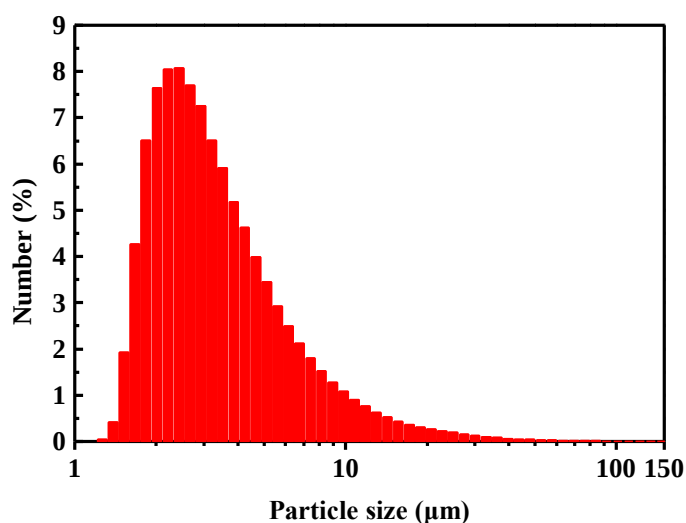


Figure 3.2 Particle size distribution of the RMC-B capsules

Around 0.1 g capsules were freeze-dried for 1 day, and then coated with platinum using an auto fine coater (JEOL JFC1600) under 20 mA current for 40 seconds. The coated capsules were examined by scanning electron microscopy (SEM, JEOL JSM-7600 F) under the secondary electrons imaging (SEI) mode at a voltage of 5.0 kV.

To quantify the crystallographic structure of the capsules, x-ray diffraction (XRD) and reference intensity ratio (RIR) method were adopted (Snyder 1992, Johnson and Zhou 2000). The carbonated capsules were further crushed and sieved with a 75 μm mesh. 20 wt.% fluorite (CaF_2) was introduced to the crushed and sieved capsules as the internal standard. The mixture was freeze dried for 1 day, and then scanned by Panalytical Xpert Pro using Cu K α radiation (40 kV, 30 mA), with a scanning rate of 0.017° 2θ /step from 5° to 80° 2θ . In the RIR calculations, calibration curves for magnesite and brucite, which were the components used for quantitative analysis, were straight lines through the origin i.e. $y = kx$, where x is the RIR of the phase analyzed and y is the weight fraction of the component investigated. The value of k was 0.2886 for magnesite and 0.3651 for brucite (Liska and Al-Tabbaa 2009, Unluer

and Al-Tabbaa 2013). The RIR was acquired through dividing the integrated intensity of the strongest line of the phase with that of the standard. For the calculation of HMC phases in the capsules, the amount allocated to magnesite originally present in the raw material (RMC) was deducted from the rest. To obtain the magnesite content in RMC, thermogravimetric analysis (TGA) was conducted on a Perkin Elmer TGA 4000 equipment from 50 to 900°C, with a heating rate of 10 °C/min under nitrogen flow.

The viability of the bacteria spores in the RMC-B capsules was quantified according to the methods suggested in the literatures (Jonkers, Thijssen et al. 2010, Bundur, Amiri et al. 2017). Firstly, the RMC-B capsules were crushed with a mortar and sieved with a 53 µm mesh. 1 g of the sieved powder was then suspended in 10 ml distilled water and vortexed for around 1 minute. Afterwards, the suspension was treated in an ultrasonic water bath (70 W, 42 kHz) for 20 minutes. The supernate was then taken out for serial dilution and spread plate counting. The followed procedure is shown Fig. 3.3. Three replicates were prepared for each test. The viability of bacteria spores in the PC capsules was also evaluated as the control. 1.15×10^9 CFU bacteria spores were added in a PC paste (50g cement with 16g of water) followed the same mixing procedure as the RMC-B capsules and crushed into powder. The same quantification procedure was carried out to investigate the viability of bacteria spores in the PC-B capsules at the age of 1, 8, 14 and 28 days.



Figure 3.3 The procedure to quantify the bacteria viability in the RMC-B and the PC-B capsules

3.2.3 Effects of RMC-B capsule addition on hydration and fresh and hardened properties of PC paste

RMC-B capsules were incorporated into a PC paste to study their effects on heat of hydration, flowability, initial setting time, and compressive strength of the PC paste. Table 3.2 shows the mix design of the PC paste with the inclusion of RMC-B capsules. A control mix with the same mix design but without the inclusion of RMC-B capsule was also prepared.

Table 3.2 Mix design to study effects of RMC-B capsule addition on hydration and fresh and hardened properties of PC paste

Mix ID	PC (g)	Water (g)	RMC-B capsule (g)
P-C	1500	480	35
P	1500	480	-

To prepare the P-C mix, the RMC-B capsules were first mixed in water. The suspension was then added into PC and mixed in a mixer for ~5 minutes until homogeneous. The heat of hydration was measured by means of the isothermal calorimetry test at 30°C in an I-Cal 8000 HPC calorimeter (Calmetrix) in accordance with ASTM C1679. The flowability of the paste was determined in accordance with ASTM C1437 using a flow table (63-L0040/A, Controls). The initial setting time was measured in accordance with ASTM 191 using an automatic Vicat apparatus (EN 196-3, Testing Bluhm & Feuerherdt GmbH).

To determine the compressive strength, fresh pastes were cast into 50 mm cubic molds, sealed in an air-tight plastic to prevent water evaporation, and placed in ambient conditions (25°C, 80% RH). The cubic specimens were demolded after 2 days and cured under the same conditions for another 26 days. Afterwards, the compressive strength of the specimens was tested at the age of 7, 14 and 28 days on a compression machine (ToniTechnik Baustoffprüfsysteme) operated at a loading rate of 55 kN/min.

3.2.4 Self-healing performance

The fabricated RMC-B capsules were used to prepare specimens (Mix PNC) to evaluate the self-healing performance as shown in Table 3.3. Two control mixes were also prepared: (i) Mix P, a PC paste without the inclusion of RMC-B capsules and the nutrient, was to reveal crack healing (if any) due to further hydration or carbonation of the PC matrix; and (ii) Mix PNS was designed to study the self-healing performance of PC paste with the inclusion of unprotected bacteria spores at the same concentration (CFU) level.

Table 3.3 Mix designs of specimens prepared for self-healing tests

Mix ID	PC (g)	Water (g)	CaL (g)	YE (g)	RMC-B capsule (g)	Bacteria spores (CFU)
PNC	1500	480	30	15	35	-
PNS	1500	480	30	15	-	5.95×10^{10}
P	1500	480	-	-	-	-

PC used in this study was purchased from EnGro Ltd (Singapore), complying with SS EN 197-1: 2014. The chemical composition of PC is shown in Table 3.4. The calcium lactate and yeast extract were purchased from Sigma-Aldrich.

Table 3.4 Chemical compositions of the PC

Composition	Weight percentage (%)
SiO ₂	20-25
Al ₂ O ₃	5-6
Fe ₂ O ₃	2-3
CaO	60-65
MgO	1-2
Na ₂ O	0.2-0.3
K ₂ O	0.4-0.5
C ₃ A	5-10
Free lime	0.5-1.0
LOI	2-3

To prepare the mix, the nutrients (CaL and YE) were first mixed with water until they were fully dissolved. RMC-based capsules (or bacteria spores) were then

added into the solution and mixed until they were well dispersed. The suspension was added into the dry PC powder and mixed for around 5 minutes until homogeneous. The fresh paste was cast into cylinder molds (height of 2.5 cm, diameter of 7.5 cm), initially filling half of the mold. A circular steel mesh (diameter of 7 cm) was then placed inside, followed by covering the steel mesh with the fresh paste and filling the mold until full as shown in Fig. 3.4. The use of the steel mesh in the middle of specimen was to prevent the complete splitting of specimen in the following crack generation process. The specimens were sealed in an air-tight plastic to prevent water evaporation. The specimens were demolded after 2 days and cured in the same condition for another 26 days.

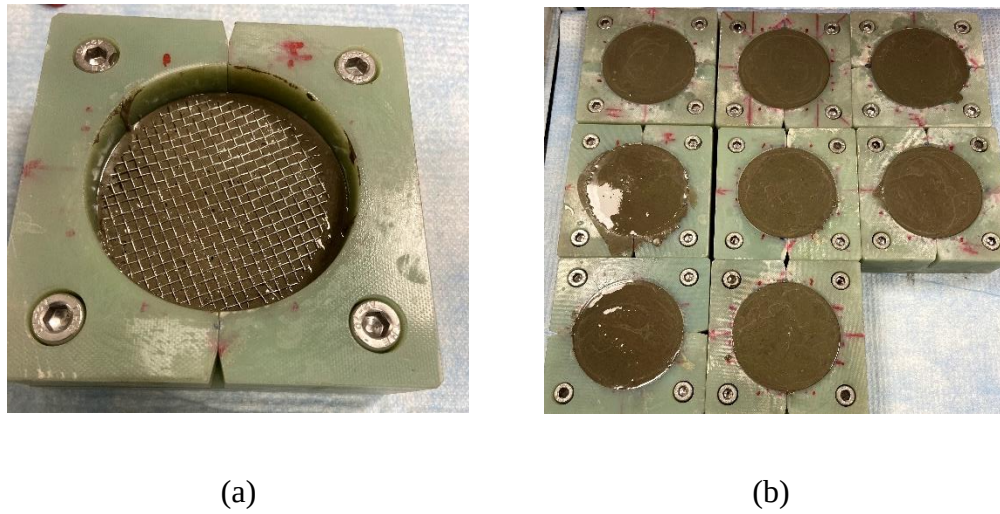


Figure 3.4 Self-healing specimen preparation: (a) Steel mesh placed in the half-full mold, and (b) molds fully filled

Pre-cracking of the cylinder specimens at 28 days was carried out by means of splitting tension via a servo-hydraulic universal testing machine (MTS Landmark 250 kN), operated at a constant rate of 0.01 mm/s, as shown in Fig. 3.5(a). After pre-cracking and unloading, a split crack was form in the center of the specimen.

The crack width was measured at five marked locations along the crack (Fig. 3.5(b)) by a stereoscopic microscope (Nikon SMZ 745T).

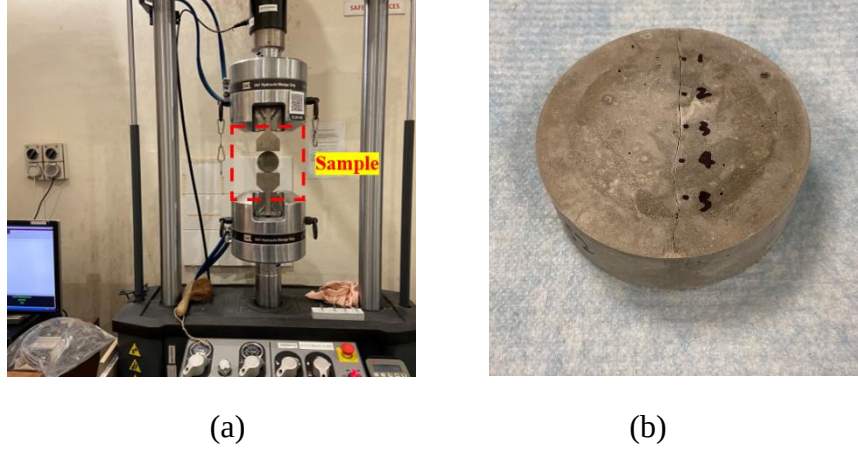


Figure 3.5 (a) Set up for pre-cracking of cylinder specimens and (b) pre-cracked specimens with marks

Furthermore, the water passing rate of the pre-cracked specimens was determined by the constant water head method, in accordance with previous studies (Sisomphon, Copuroglu et al. 2012, Zheng and Qian 2020). As shown in Fig. 3.6, the constant water head was created by a PVC pipe with a height of 10 cm and diameter of 6 cm. The pipe was attached to the top surface of the specimen and sealed with waterproof plasticine. A constant water head of 10 cm was maintained for a duration of 5 minutes to allow water to pass through the pre-cracked specimens. The amount of water collected in the bowl below was measured and the water passing rate was calculated as shown in Eqn. (3.2), where V is the volume of the water passing through the specimen; L is the specimen thickness in the direction of flow; A is the cross-sectional area subject to flow; h is the constant water head; and t is the duration of flow. For each specimen, the test was carried out 3 times, and the mean and standard deviation values were reported.

$$\text{Water passing rate} = \frac{VL}{Aht} \quad (3.2)$$

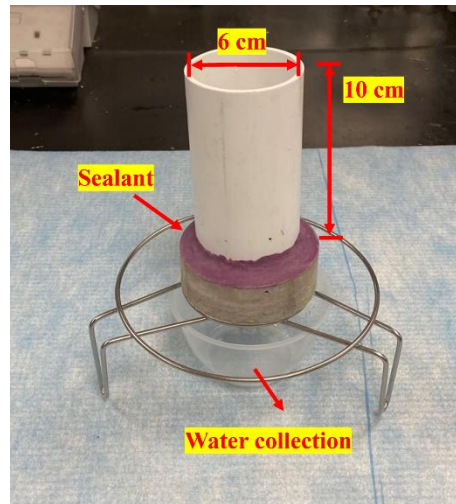


Figure 3.6 Water passing rate measurement setup

Three specimens were prepared for each group, and the specimens were then subjected to wet/dry conditioning regimes, during which they were fully submerged in water (25°C) for 24 hours, followed by drying in the ambient air (25°C, 80% RH) for another 24 hours. Specimens of different mix designs were not placed in the same containers for wet/dry conditioning to avoid cross-contamination between different mixes. Conditioning water was replaced and replenished after each cycle. Crack widths at the five marked locations and the water passing rate after five wet/dry conditioning cycles were determined. The crack closure (%) and water passing rate reduction (%) were calculated in accordance with Eqn. (3.3) and (3.4), respectively.

$$\text{Crack closure (\%)} = \frac{\text{Crack width (pre-cracking)} - \text{crack width after wet/dry cycles}}{\text{Crack width (pre-cracking)}} \quad (3.3)$$

Water passing rate reduction (%) =

$$\frac{\text{Water passing rate (pre-cracking)} - \text{water passing rate after wet/dry cycles}}{\text{Water passing rate (pre-cracking)}} \quad (3.4)$$

Afterwards, small samples were cut from the specimens of Mix PNC by means of a

precision saw (Buehler IsoMet 1000), as shown in Fig. 3.7. Samples were extracted from the cracked location to observe healing products (if any) on the specimen surface (Fig. 3.7(a)). To check the internal healing products, specimens were fractured to expose the interior of the healed crack (Fig. 3.7(b)). Samples were extracted from the uncracked location to observe any precipitation on the specimen surface (Fig. 3.7(c)). The samples were freeze-dried for 3 days to completely remove internal moisture, followed by coating with platinum using an auto fine coater (JEOL JFC1600) under 20 mA current for 40 seconds. All samples were examined by SEM (JEOL JSM-7600 F) under the SEI mode at a voltage of 5.0 kV.

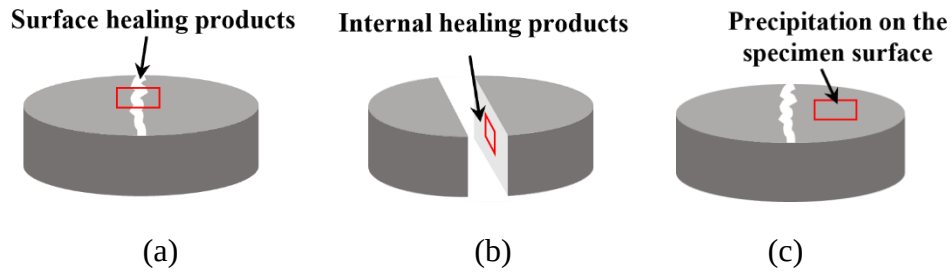


Figure 3.7 Sketch of sample extraction for the examination of healing products under SEM

3.3 Results and Discussion

3.3.1 RMC-B capsule characterizations

The SEM images of the fabricated RMC-B capsules are shown in Fig. 3.8. The surface morphology of the capsules composed of disk-like crystals, which resembled the morphology of hydromagnesite (Unluer 2018).

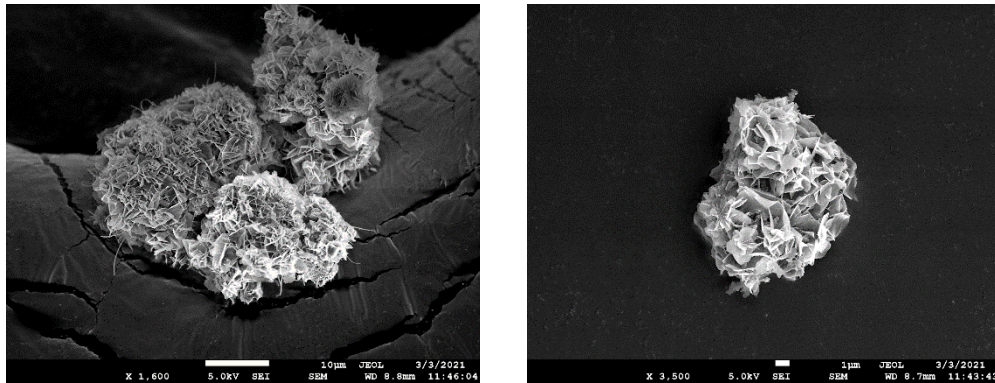


Figure 3.8 SEM images of the RMC-B capsules

The XRD pattern of the capsules is shown in Fig. 3.9. Intense fluorite peaks were observed at 28.3° and 47.0° 2θ due to the addition of 20% standard phase. Major peaks of MgO (at 42.9° and 62.3° 2θ) and brucite (at 18.6° and 38.0° 2θ) were also recorded, indicating the presence of uncarbonated brucite even after accelerated carbonation curing. Minor peaks of hydromagnesite and magnesite were found at 15.2° and 32.8° 2θ , respectively. The existence of magnesite was attributed to the incomplete calcination of the parent material during the production of RMC, while hydromagnesite was the main carbonation product that formed during accelerated carbonation curing.

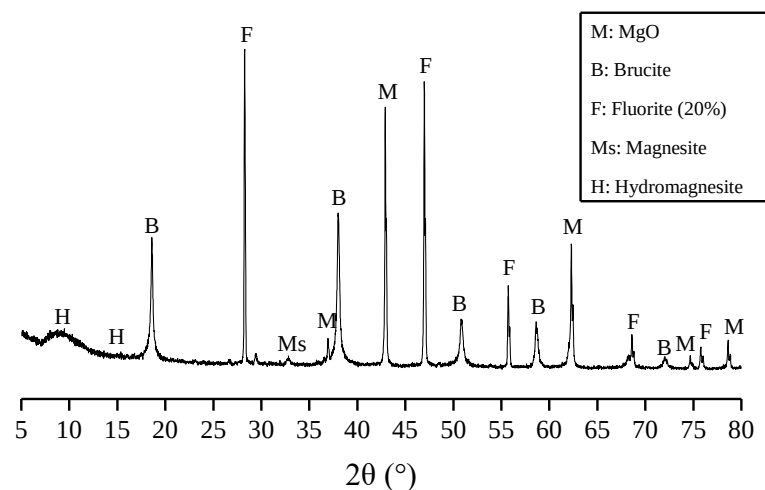


Figure 3.9 XRD pattern of the RMC-B capsules containing 20 wt.% fluorite

To calculate the content of carbonate phase (hydromagnesite) in RMC-B capsules, the amount of magnesite (MgCO_3) needed to be deducted. Accordingly, the percentage of magnesite in the raw material (RMC) was calculated based on the weight loss of RMC between 500-900°C, as shown in Fig. 3.10. The decomposed phases at different temperatures and the corresponding weight loss are marked accordingly in the figure. From 50°C to 300°C, the main weight loss was due to the loss of free water in RMC, followed by the weight loss caused by the decomposition of brucite from 300°C to 500°C. From 500°C to 900°C, the loss was related to the decarbonation of magnesite in the RMC, as shown in Eqn. (3.5) (Dung and Unluer 2018). The weight loss associated with decarbonation was 2.61%, corresponding to 5% magnesite content in RMC, which was calculated by using Eqn. (3.6), where W_{Ms} is the weight fraction of magnesite, $M_w(Ms)$ is the molecular weight of magnesite and $M_w(\text{CO}_2)$ is the molecular weight of CO_2 . The composition of the capsule calculated by XRD analysis is shown in Table 3.5. The RMC-B capsules consisted of around 31% unhydrated MgO , 51% brucite and 13% HMC phases.

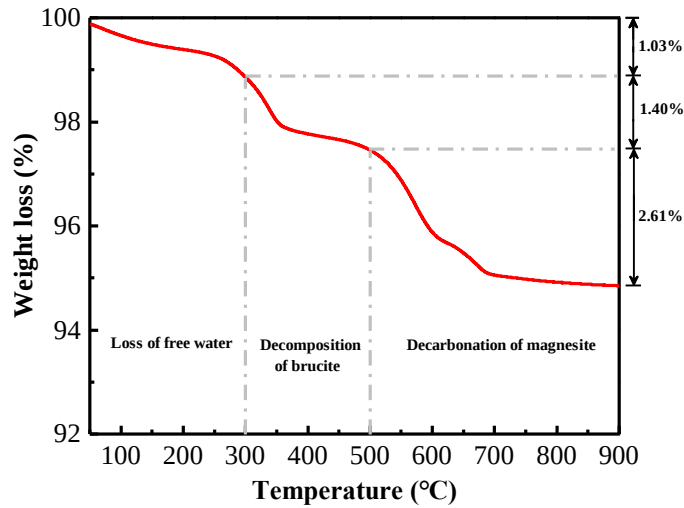


Figure 3.10 Weight loss of raw RMC material



$$W_{Ms} = \frac{M_w(Ms)}{M_w(CO_2)} \times 2.61\% \quad (3.6)$$

Table 3.5 Composition of the RMC-B capsules

Composition	Weight fraction (%)
MgO	30.9
Mg(OH) ₂	51.5
Magnesite	5.0
HMCs	12.6

From the surface morphology observations and phase composition results, the structure of the RMC-B capsule is shown schematically in Fig. 3.11. As can be seen, the resulting RMC-B capsule had a gradient structure with a dense HMCs shell (12.6%) and a porous core (52% brucite and 31% unreacted MgO). This was because MgO hydrated and formed brucite first. After carbonation, brucite near the capsule surface transformed to a range of HMCs with dense microstructure (Unluer and Al-Tabbaa 2014, Dung and Unluer 2017) and the core consisted of mainly brucite and unhydrated RMC with high porosity (Unluer 2018). The dense HMCs formed on the capsule surface (i.e., shell) hindered further penetration of CO₂ towards the capsule core. As a result, the RMC-B capsules shall have a stable microstructure (i.e., a hard shell and soft core) unlike other cement-based capsules where hydration continuous to refine the internal microstructure. The porous core accommodated bacteria spores while the dense shell protected bacteria spores from direct contact with the surrounding cement matrix and provided mechanical strength. The stable microstructure of the resulting RMC-B capsule shall promote long-term viability of bacteria spores.

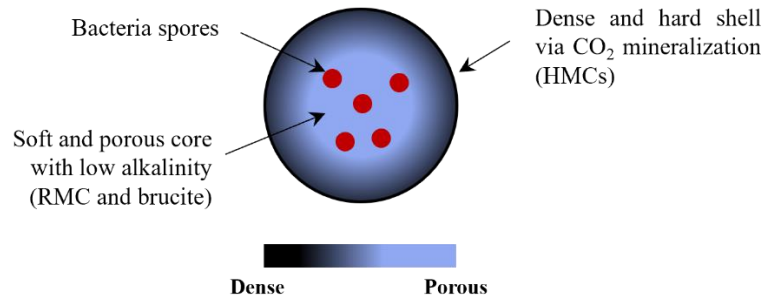


Figure 3.11 Schematic diagram of the gradient structure of the RMC-B capsule with dense shell and porous core

3.3.2 Viability of bacteria spores in RMC-B capsule

Fig. 3.12 has plotted the number of viable bacteria spores in the PC-B capsule. As can be seen, the number of viable bacteria spores reduced dramatically by four orders after 1 day in the PC matrix. The number of viable bacteria spores continued to reduce with the age of PC until lower than the detection limit of the adopted method at the age of 28 days. This highlighted the vulnerability of bacteria spores in PC-based matrix due to the harsh environment of PC (Jonkers, Thijssen et al. 2010).

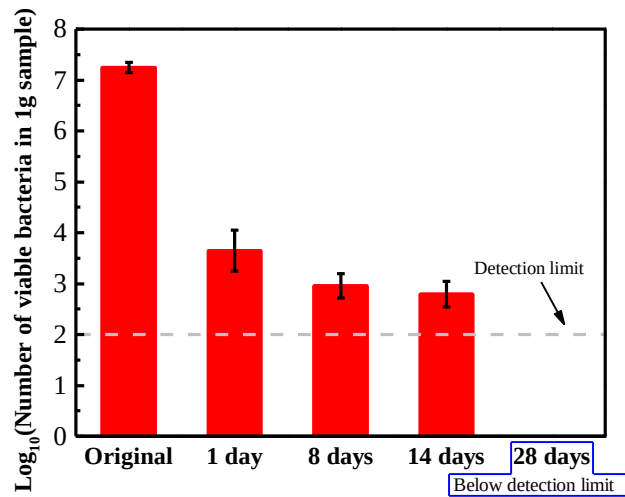


Figure 3.12 Viability of bacteria spores in the PC-B capsules

Fig. 3.13 shows the number of viable bacteria in the RMC-B capsule. As can be

seen, the viability of the bacteria spores in the RMC matrix was much higher than that in the PC matrix. This may be attributed to the low alkalinity of the RMC system which was reported to have an internal pH of around 9.9-10.5 (Dung and Unluer 2019, Hay and Celik 2020), providing a more friendly environment for the survival of bacteria spores. Furthermore, the porous core of the RMC-B capsule may preferably provide space to accommodate bacteria spores.

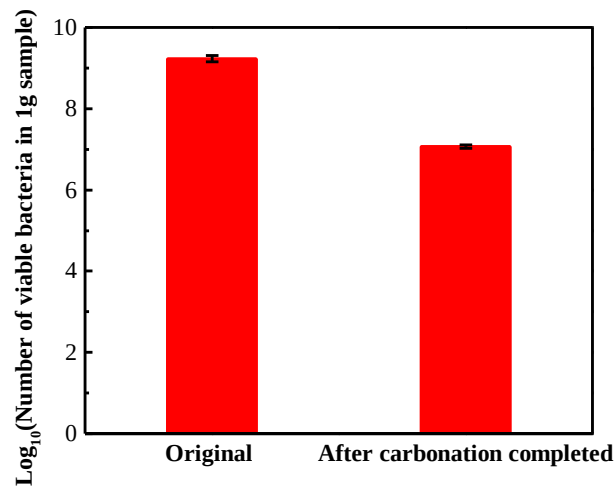


Figure 3.13 Viability of bacteria spores in the RMC-B capsules

3.3.3 Effects of RMC-B capsule addition on hydration and fresh and hardened properties of PC paste

Fig. 3.14 shows the evolution of hydration heat and the corresponding cumulative heat during the first 72 hours for the PC paste with (Mix P-C) and without (Mix P) the inclusion of RMC-B capsules. As can be seen, peak of hydration of both mixes took place at around the 9th hour, indicating that the addition of RMC-B capsules did not alter the hydration rate of PC paste. Furthermore, both mixes showed comparable accumulated heat after 72 hours of hydration, suggesting the minimal effect of the capsules on the hydration process at early ages.

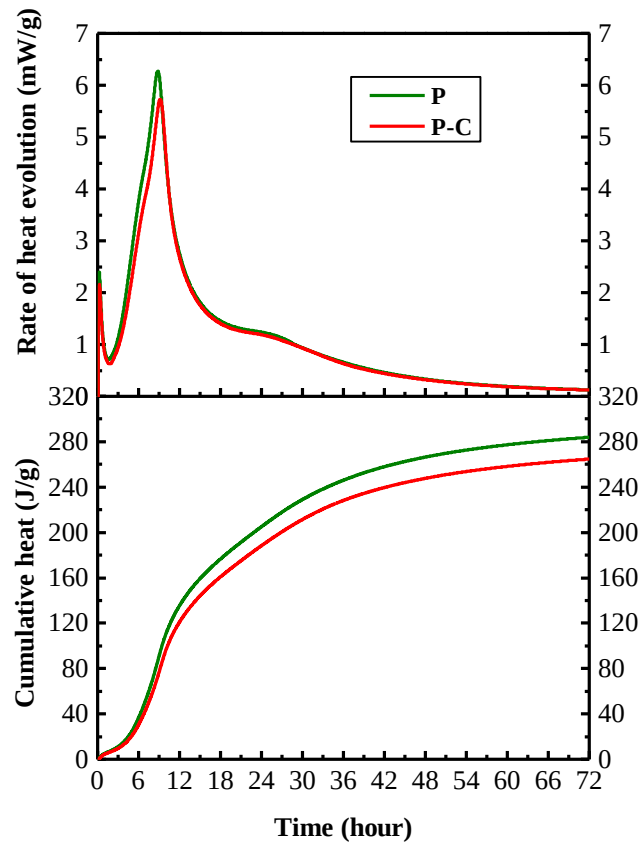


Figure 3.14 Isothermal calorimetry results of the Mix P and Mix P-C

Table 3.6 summarizes the flowability and initial setting time of the P-C and P mixes. As can be seen, the control group (Mix P) had a flowability of 69% and an initial setting time of 187 min. The inclusion of RMC-B capsules (Mix P-C) did not introduce significant impacts on flowability and initial setting time of the PC paste. This may be attributed to the formation of HMCs (mainly hydromagnesite) shell, which is chemically stable in the alkaline environments such as PC paste (Braithwaite and Zedef 1996, Lin, Zheng et al. 2017), of the functionally graded RMC-B capsule via carbonation. As a result, the inclusion of RMC-B capsules did not introduce significant impacts on the hydration and fresh properties of the PC paste.

Table 3.6 Flowability and initial setting time of the Mix P and Mix P-C

Mix ID	Flowability (%)	Initial setting (min)
P-C	67	209
P	69	187

Fig. 3.15 compares the compressive strength of the two mixes at the age of 7, 14 and 28 days. As can be seen, PC paste with the inclusion of RMC-B capsules (Mix P-C) showed higher compressive strength than the control mix (Mix P). This was particularly pronounced at early ages. The 7-day strength of Mix P-C (63.4 MPa) was 26% higher than that of Mix P (50.3 MPa). At the age of 14 days, Mix P-C (69.5 MPa) was 21% stronger than Mix P (57.6 MPa). When it reached 28 days, the strength of Mix P-C (74.1 MPa) was still higher than Mix P (62.8 MPa) by 18%. This indicated the RMC-B capsules were not the strength limiting phase in the hardened PC paste unlike the typical soft self-healing capsules. Furthermore, this suggested the resulting functionally graded RMC-B capsules were strong and highly compatible to the PC matrix perhaps due to the formation of hard HMCs shell via carbonation. Further study is necessary to better understand the interface transition zone between the RMC-B capsule and the surrounding PC matrix to reveal the strength enhancement mechanisms.

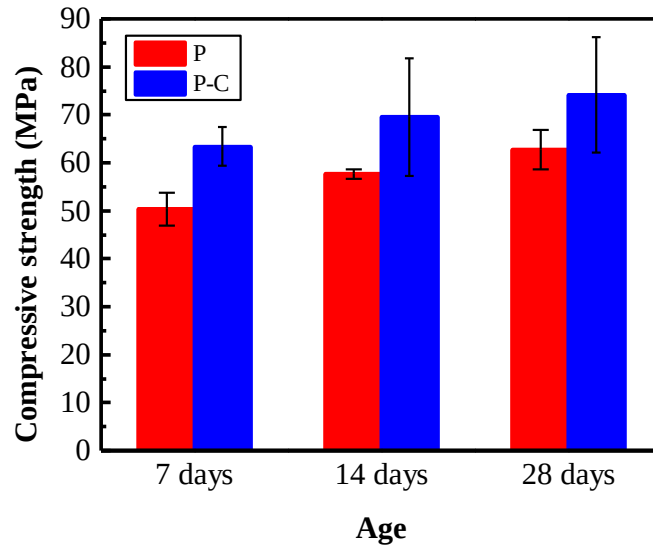


Figure 3.15 Compressive strength of PC paste with and without the addition of RMC-B capsules

Overall, these results suggested that the addition of the RMC-B capsules did not alter the heat of hydration, flowability, and initial setting time of PC paste. Furthermore, the incorporation of the capsules increased the compressive strength of the matrix. This suggested the newly developed functionally graded RMC-B capsules with hard HMCs shell were chemically stable and compatible with the surrounding PC matrix.

3.3.4 Self-healing performance

1.1.1.1 Surface crack closure

Crack width after 5 wet/dry conditioning cycles was plotted against the original crack width, as shown in Fig. 3.16. For each mix, 3 specimens were measured, with 5 observation points on each specimen. Thus, overall, there are 15 data points in each plot. The 45° black solid line represents no crack closure, whereas the grey dashed line indicates 50% crack closure. As can be seen, all cracks in the Mix PNC

reduced their width significantly after 5 wet/dry conditioning cycles while minimum crack closure rate were found in Mix PNS and Mix P. Table 3.7 calculates the average crack closure rate of each mix. As can be seen, the three mixes had comparable crack width of around 220-250 μm before the wet/dry conditioning. After 5 cycles of wet/dry conditioning, both Mix PNS and Mix P revealed marginal crack closure rates of about 20% while Mix PNC achieved a significant crack closure rate of around 92%. This highlighted the effectiveness of the RMC-B capsules to engage self-healing in PC paste. The marginal crack closure in Mix PNS and Mix P specimens was likely due to further hydration and carbonation as the mix had a low water-to-cement ratio of 0.32 and thus plenty unhydrated cement can be expected at the age of 28 days.

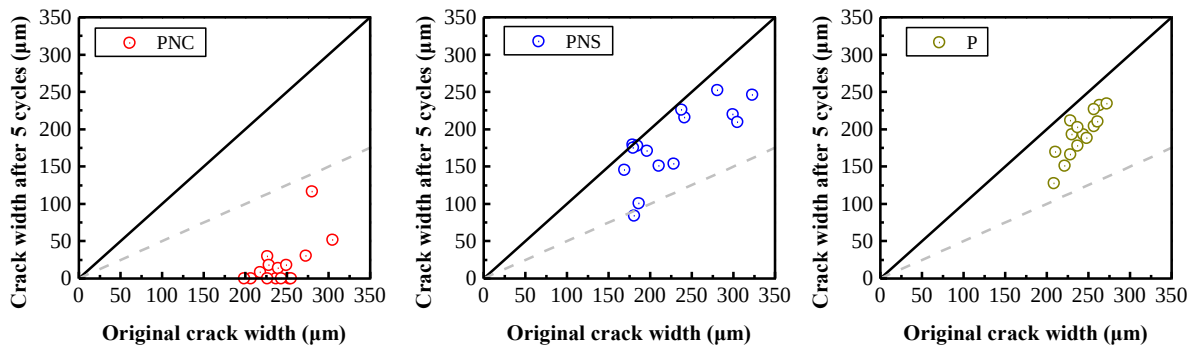


Figure 3.16 Crack widths of different specimens before and after 5 wet/dry cycles

Table 3.7 Average crack closure rate after 5 wet/dry conditioning cycles

Mix ID	Original crack width (μm)	Crack width after 5 wet/dry cycles (μm)	Average crack closure rate (%)
PNC	243 ± 28	19 ± 31	92.8 ± 11.0
PNS	227 ± 52	180 ± 49	20.1 ± 16.2
P	240 ± 19	192 ± 30	20.2 ± 8.3

Representative optical microscope images of a crack in each mix before and after 5 cycles of wet/dry conditioning are presented in Fig. 3.17. Mix PNC (Fig. 3.17(a)) revealed a notable healing when compared to Mix PNS (Fig. 3.17(b)) and Mix P

(Fig. 3.17(c)). Accordingly, Mix PNC achieved a complete crack closure enabled by the abundant formation of healing products within the crack, whereas Mix PNS and Mix P showed sparse formation of white crystals along the edge of the crack. It was worth noting that the formation of healing products was not only limited to within the crack, but also on the entire surface of the PNC specimen after the wet/dry conditioning cycles. This indicated the high bioactivity of the RMC-B capsules in the PC paste.

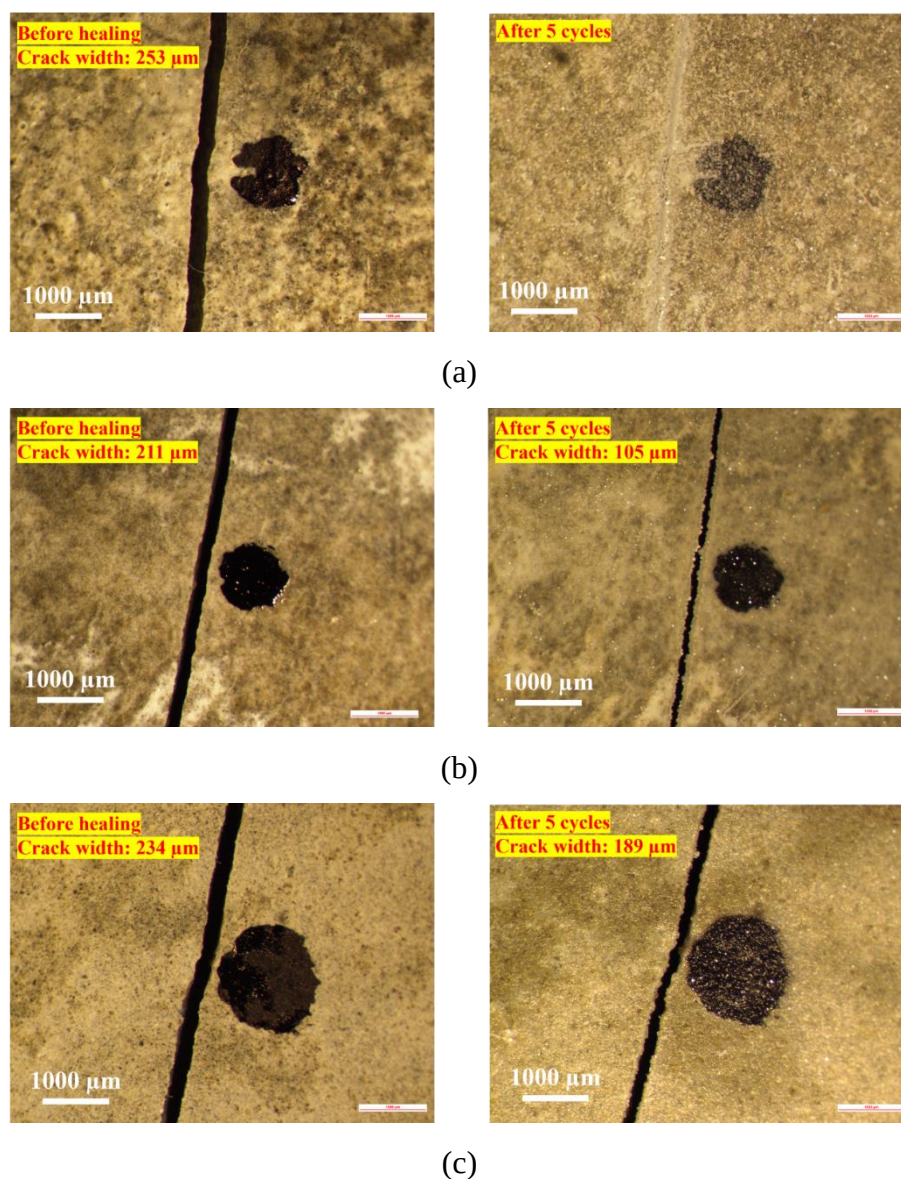


Figure 3.17 Representative optical microscope images of a crack in (a) Mix PNC, (b) Mix PNS and (c) Mix P before and after 5 wet/dry conditioning cycles

3.3.4.2 Water passing rate

Fig. 3.18 compares the average water passing rate reduction of the 3 mixes. Overall, the results were in agreement with the crack closure rate results. The water passing rate reduction of Mix PNS and Mix P were below 25% while that of the Mix PNC was more than 80%. The resistance of the healed crack to the water passing is a key parameter to evaluate the healing performance of the crack as the crack can present a weak point in the matrix, enabling the ingress of water and aggressive substances. The resistance is directly associated with the crack closure performance and the density of the crystal formation within the crack. The high resistance of the healed Mix PNC to the water passing has indicated the dense nature of the healing products and the overall high healing efficiency of the RMC-B capsules in the PC paste.

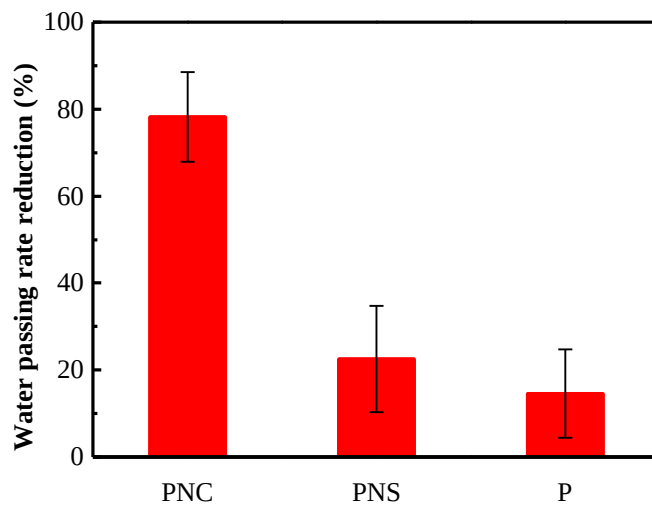
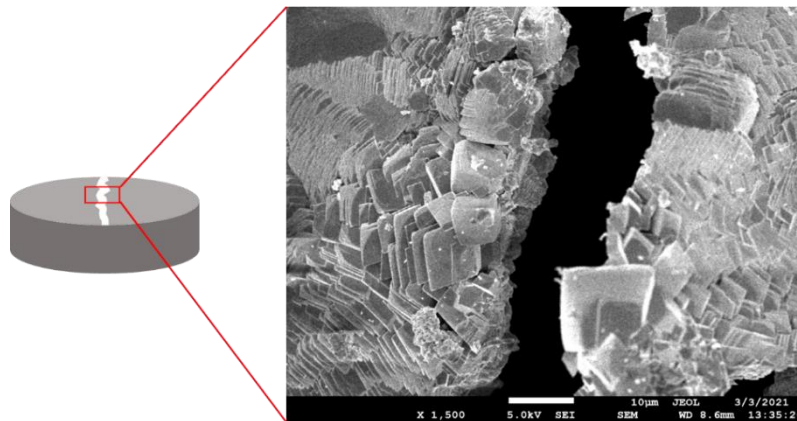


Figure 3.18 Water passing rate reduction of Mixes PNC, PNS and P after 5 wet/dry conditioning cycles

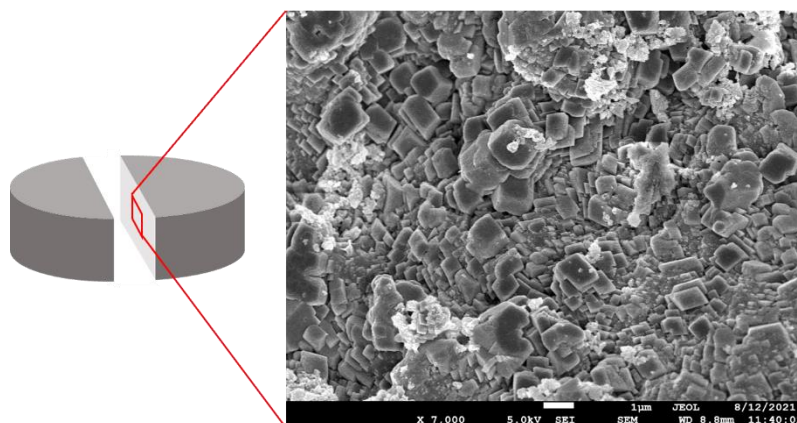
3.3.4.3 Microstructure of healing products

Fig. 3.19 shows the morphology and microstructure of healing products from different location of the Mix PNC specimen. As can be seen, all 3 figures revealed

the formation of layered rhombohedral calcite, which was in line with the findings of previous studies using the same type of bacteria and nutrient (Tziviloglou, Wiktor et al. 2016). The healing products not only formed on the crack surface, but also along the crack depth direction. The dense formation of calcite along the entire crack from the surface to the interior led to the significant reduction of water passing rate in the Mix PNC specimen. The abundant formation of healing products was not just within the crack, but also on the specimen surface as shown in Fig. 3.19(c), where the dense formation of calcite crystals and the presence of gel-like C-S-H from further hydration were observed.



(a)



(b)

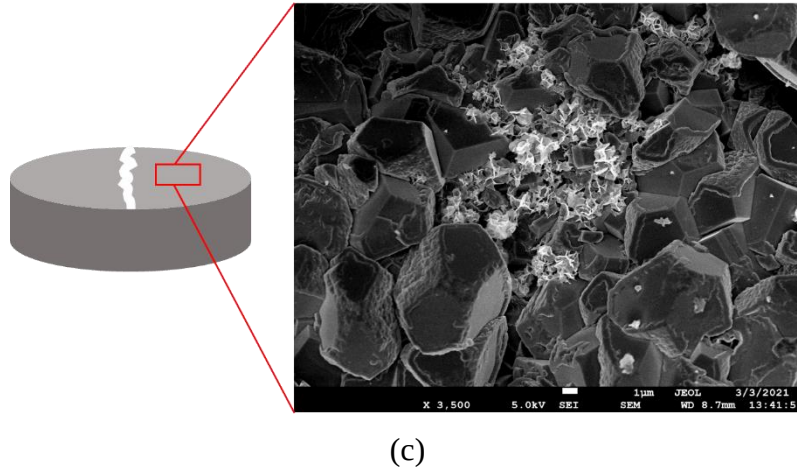


Figure 3.19 SEM of the healing products on the (a) crack surface, (b) crack interior and (c) surface of a PNC specimen

3.4 Summary

This chapter reported the development of a novel functionally graded RMC-B capsule for self-healing concrete. The capsule had a gradient structure with a dense HMCs shell and a porous and low alkaline core to better protect and to enhance the viability of the bacteria spores. Results showed that the inclusion of the RMC-B capsules did not negatively impact the hydration, the initial setting time, and the flowability of the PC paste. Furthermore, the 28-day compressive strength of the hardened PC paste incorporating the RMC-B capsules was 18% stronger than that of the control. This suggested the capsules were strong and highly compatible to the PC matrix. The newly developed functionally graded RMC-B capsules with hard HMCs shell were chemically stable and compatible with the surrounding PC matrix. Specimens with the RMC-B capsules exhibited strong crack healing performance as evidenced by the high crack closure rate of 93% and high water passing rate

reduction rate of 80% for wide crack of few hundred microns after only five wet/dry conditioning cycles. Microstructure observations revealed the dense formation of layered rhombohedral calcite along the entire crack from the surface to the interior. These highlighted the effectiveness of the proposed functionally graded RMC-B capsules for self-healing concrete.

CHAPTER 4 MECHANISM INVESTIGATION OF BACTERIA SURVIVAL AND PRECIPITATION CAPABILITY IN RMC-BASED PASTE

4.1 Introduction

As reported in Chapter 3, the RMC-B capsule can engage self-healing in PC-based matrix. To fundamentally understand the mechanism, studies have been conducted to reveal the influence of alkalinity on bacteria viability, the bacteria survivability/precipitation capability in RMC paste, and how the internal conditions of RMC paste influence the bacteria survival.

Accordingly, unprotected *Bacillus cohnii* spores were directly embedded in RMC matrix. To keep the consistency of the element between carbonate precipitation and the matrix, magnesium lactate (MgL) was adopted as the precipitation precursor. The precipitation reaction between the bacteria and MgL was validated first, followed by investigating the impacts of solution alkalinity on the viability of bacteria spores. Carbonate precipitation due to the MICP of unprotected *Bacillus cohnii* spores in the RMC paste was examined, and the viability of bacteria spores in the hardened matrix was quantified and discussed.

4.2 Experimental program

Fig. 4.1 shows the experimental program of the current study. While the oxidation of CaL to calcium carbonate by *Bacillus cohnii* has been extensively studied, the oxidation of MgL to magnesium-based carbonates by *Bacillus cohnii* remains uncertain. Therefore, MICP of *Bacillus cohnii* in MgL solution was examined first. Thereafter, the impacts of alkalinity on the viability of bacteria spores was evaluated. Finally, carbonate precipitation of RMC paste with direct incorporation of unprotected bacteria spores was evaluated by monitoring the precipitation formation within artificially generated cracks, investigating the precipitation

products, studying the viability of bacteria in the hardened pastes, and examining the pore solution pH of the hardened pastes.

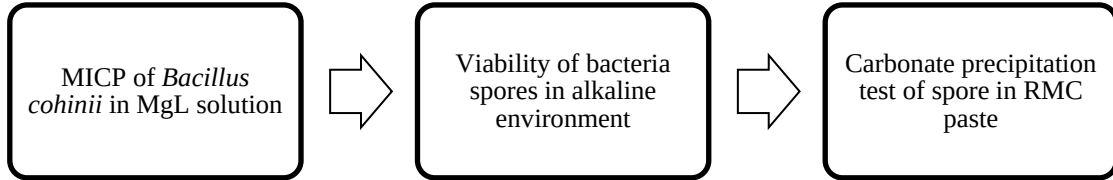


Figure 4.1 Summary of experimental program

4.2.1 Preparation of bacteria spores

Bacillus cohnii ATCC 51227 from American Type Culture Collection was used in this study. The preparation of the bacteria spores followed the same procedure as described in Chapter 3.2.1.

4.2.2 MICP of *Bacillus cohnii* spores in MgL solution

An in vitro precipitation test was conducted to validate the MICP of *Bacillus cohnii* spores in the MgL solution. 20 g MgL and 10 g yeast extract were added into 400 ml spore suspension (1.7×10^8 CFU/ml) and incubated on an orbital shaker (200 rpm) at 25°C. After 10 days of incubation, precipitation was observed at the bottom of the flask. The upper solution was removed, and around 400 ml distilled water was added into the flask to wash the precipitation. The flask was placed on the same orbital shaker for 1 minute, and then stood still on the bench to allow the precipitation to settle down to the bottom of the flask. Afterwards, the upper solution was removed, and the precipitates were taken out and freeze-dried for 3 days in preparation for the SEM analysis. Around 0.1 g of dried precipitates were sputter coated with platinum by means of a sputter coater (JEOL JFC-1600) under 20 mA current for 40 seconds. The coated samples were analyzed by means of a SEM (JEOL JSM-7600 F) under the SEI mode at a voltage of 5.0 kV. Around 5 g of dried precipitates was ground using a mortar and sieved through a 75 μ m mesh

for the x-ray diffraction (XRD, Panalytical Xpert Pro) analysis. The XRD scan was conducted under Cu Ka radiation (40 kV, 30 mA) with a scanning rate of 0.017° 2 θ /step from 5° to 80° (2 θ).

4.2.3 Viability of *Bacillus cohnii* spores in alkaline solutions

To study the effects of alkalinity on the viability of *Bacillus cohnii* spores, 1 ml of spore suspension (1.09×10^9 CFU/ml) was added into 20 ml of NaOH solution with pH ranging from 10 to 14, resulting in an initial spore concentration of 5.19×10^7 CFU/ml (i.e., 1.09×10^9 CFU spores in 21 ml solution). The solutions were then kept under room temperature ($25 \pm 2^\circ\text{C}$). 2 μl of solution was pipetted out after 1 day and 30 days for serial dilutions and 100 μl diluted solution was taken out for spread plate counting, thereby determining the viable spores after alkaline exposure. When the concentration of viable bacteria in the solution was too low and no dilution was needed, 100 μl of solution was directly pipetted out for spread plate counting. Accordingly, 10 CFU/ml (i.e., only one bacteria colony was observed on the Petri dish) was considered to be the measuring limit, meaning that when the concentration of bacteria dropped below 10 CFU/ml there will be no bacteria colony could be observed.

4.2.4 Precipitation test of bacteria in RMC paste

4.2.4.1 Specimen preparation

To examine the precipitation ability of unprotected bacteria spores in RMC matrix, four paste mixes were prepared and Table 4.1 summarizes the mix designs. PC mixes were also prepared as the control. Water-to-cement ratios (w/c) of 0.5 and 0.33 were adopted for the RMC and the PC mixes, respectively, so that the mixes have comparable workability.

Table 4.1 Mix design of the RMC and the PC pastes

Mix	RMC (g)	PC (g)	Water (g)	Spore (CFU/g paste)
M	400	-	200	0
MS	400	-	200	1.74×10^7
P	-	400	132	0
PS	-	400	132	1.74×10^7

RMC used in this study was purchased from Richard Baker Harrison Ltd. (UK), and PC (CEM I) used in this study was purchased from EnGro Ltd (Singapore). The RMC/PC used in this study were the same as the RMC/PC used in Chapter 3, and the chemical compositions of the RMC and PC were complying with Table 3.1 and Table 3.4 in Chapter 3, respectively.

To prepare the mixes, the spores were first mixed with water to form a uniform suspension. The spores-containing water was then added into the cement and mixed with a hand mixer for around 5 minutes until reaching a homogenous status. Afterwards, the fresh paste was cast into 25 mm cube molds. To better observe the precipitation formation, an artificial crack was generated on each mix by inserting a 0.2 mm thick steel sheet into each cube specimen (Fig. 4.2). The depth of the pre-fabricated crack was around 5-10 mm. In order to better investigate the bacteria spore survival and precipitation ability in the RMC-based system, the matrix was preferably to be kept as clean as possible with the inclusion of bacteria spores only. Comparing to generate a realistic crack where reinforcement or fibers are needed, this study has chosen to generate an artificial crack to avoid bringing in anything that potentially would influence the observation. In literatures, it was reported that the bacteria and fiber have coupled effects on crack healing (Feng, Su et al. 2019, Vijay and Murmu 2019) as the fiber provides a physical network in the gap of the crack and the bacteria can precipitate on the fiber bridging. Accordingly, the inclusion of reinforcement or fiber might have influence on the observation of actual bacteria precipitation performance. Differences do exist between an artificial

crack and a realistic crack, for example an artificial crack has smoother and flatter cross-section along the crack depth direction therefore the artificial crack is less tortuous than a real crack, which may cause healing products not adhere well to the cross section, reducing its effectiveness. Considering the main purpose of this study is to observe the bacteria spore survival and precipitation ability in RMC-based system, an artificial crack would be sufficient to achieve the goal. The fresh pastes without the bacteria spores addition (i.e., mixes M and P) were also cast into cylinder molds (diameter: 40 mm, height: 85 mm) for the pore solution extraction test. The specimens were sealed in plastic bags and cured in the laboratory ambient condition for 24 hours before demolding and removing the steel sheets. This was followed by curing the specimens for another 27 days, during which the P and PS specimens were cured in an air-tight plastic container for the entire 27 days; whereas the M and MS specimens were stored in a CO₂ incubator with 10% CO₂ concentration at 30°C and 80% RH for the first 7 days followed by curing in an air-tight plastic container for another 20 days.

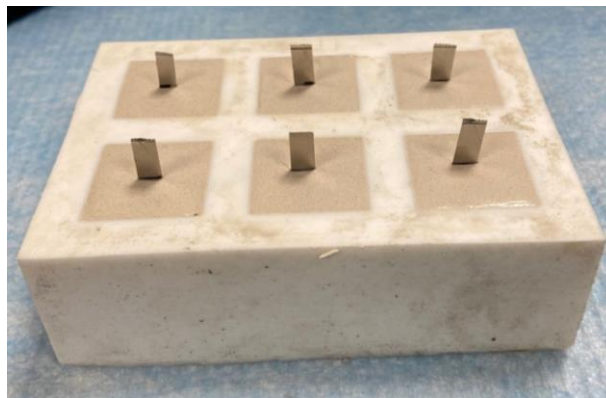


Figure 4.2 Cube specimens with pre-fabricated crack for the carbonate precipitation test

4.2.4.2 Test methods

To engage carbonate precipitation, cube specimens were fully submerged in the rich media, whose compositions are shown in Table 4.2. Accordingly, yeast extract (YE)

enables the activation and germination of the spores, whereas MgL and CaL are the MICP precursors for the RMC and the PC specimens, respectively. To monitor and quantify the carbonate formation by the bacteria, the pre-fabricated crack in each specimen was observed by means of a stereoscopic microscope (Nikon SMZ 745T) before submersion in the medium and after pre-determined durations (i.e., 4, 8, and 12 days) of the medium conditioning. The area of each crack was measured before and after certain conditioning cycles, and the crack area reduction was calculated according to Eqn. (4.1), where A_{or} was the crack area before healing and A_i was the crack area after i days ($i = 4, 8, 12$) of healing conditioning.

$$\text{Crack area reduction} = \frac{A_{or} - A_i}{A_{or}} \times 100\% \quad (4.1)$$

Table 4.2 Compositions of crack healing mediums for the RMC and the PC specimens

Mix	Composition
M, MS	20 g/L YE, 40 g/L MgL
P, PS	20 g/L YE, 40 g/L CaL

The precipitation products on the surface and inside the crack were examined under SEM. The cube specimens were first cut in half perpendicularly to the crack direction (Figure 4.3) with a precision sectioning saw (Buehler, IsoMet 1000) to expose the interior of the crack. Afterwards, the samples were freeze-dried and sputter coated with platinum by means of a sputter coater (JEOL JFC-1600) under 20 mA current for 40 seconds. The coated samples were analyzed by means of a SEM (JEOL JSM-7600 F) under the SEI mode at a voltage of 5.0 kV.

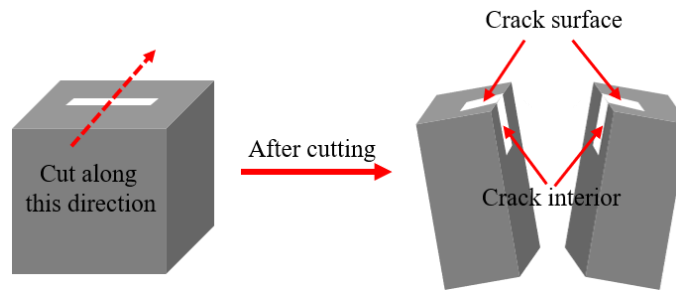


Figure 4.3 Sketch of cutting of the cube specimens for precipitation examination from the surface and the interior of the crack under SEM

The viability of bacteria spores in the hardened MS and the hardened PS specimens after pre-determined curing durations (i.e., 1, 8, 14 and 28 days) were quantified, following the procedure as described in Section 3.2.4.

To examine the alkalinity of the pore solution in the hardened RMC and the hardened PC pastes, an INSTRON 8506 servo hydraulic system was used to extract the pore solution from the hardened M and the hardened P cylinder specimens at pre-determined ages (i.e., 1, 8, 14 and 28 days). The extraction procedure followed the method described by Barneyback and Diamond (Barneyback Jr and Diamond 1981) by using a high-pressure extraction setup (Fig. 4.4). A displacement-controlled extraction was carried out on a 200-tonne dynamic testing frame at a rate of 2 mm/min and terminated at 1500 kN (Fig. 4.4(a)). The setup was equipped with a closed air flow circulation to drive the extracted solution into the collection bottle (Fig. 4.4(b)). Furthermore, the CO₂ in the circulating air was removed by aerating it through 1 mol/L NaOH solution in order to minimize carbonation of pore solution during the extraction process. In detail, the air flowed from the pump through NaOH solution (from label 1 to 2 in Fig. 4.4(b)), followed by the flow of air from the bottle to the back of the extraction setup (from label 3 to 4 in Fig. 4.4(b)). As the air flowed from label 4 to 5, the pore solution was pushed out of the extraction device and collected in the bottle (from label 5 to 6 in Fig. 4.4(b)). Immediately

after the extraction, the pore solution was filtered by using a syringe filter with pore sizes of 0.45 μm to remove particles in the solution. After that, the pH of the solution was measured using a standard pH meter (Mettler Toledo SevenCompact) to determine the pore solution alkalinity.

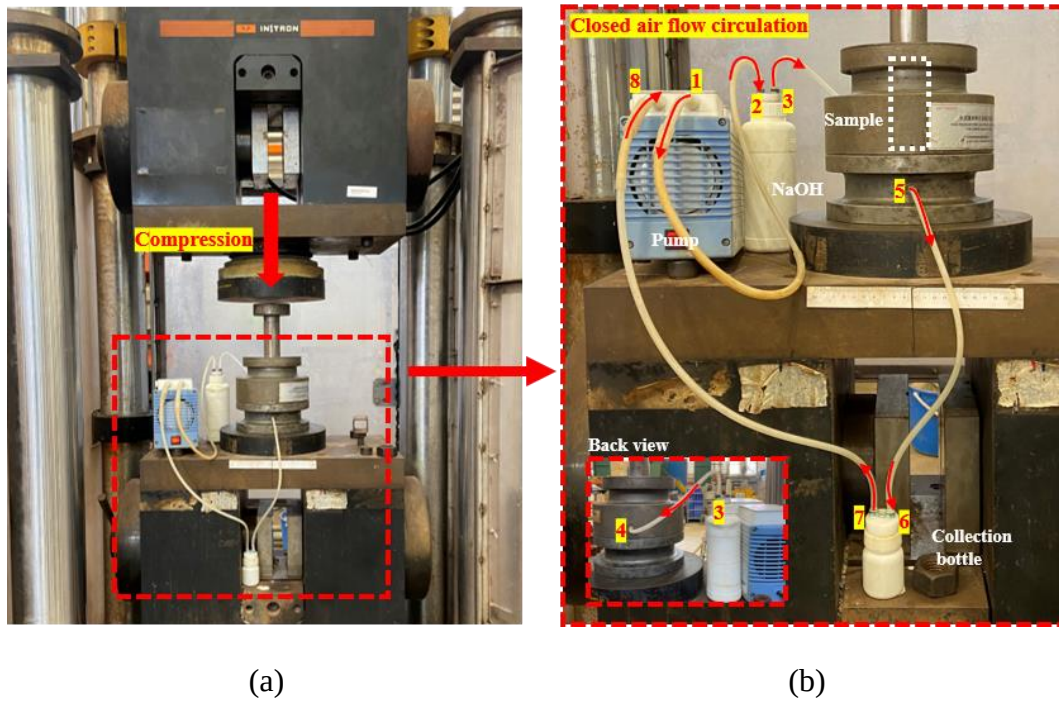


Figure 4.4 (a) Pore solution extraction apparatus and (b) details of the closed air circulation loop.

4.3 Results and discussion

4.3.1 MICP of *Bacillus cohnii* spores in MgL solution

To validate the MICP capability of *Bacillus cohnii* with MgL, in vitro precipitation test was conducted. Following an incubation of 10 days, abundant precipitation was observed at the bottom of flask, suggesting oxidation of MgL ($(\text{C}_3\text{H}_5\text{O}_3)_2\text{Mg}$) under the action of *Bacillus cohnii* spores. The SEM image and XRD pattern of the oxidation products are shown in Fig. 4.5. As can be seen, the SEM image of the precipitation products revealed a rod-like morphology with sharp edges, which

resembled the morphology of nesquehonite ($\text{MgCO}_3 \cdot 3\text{H}_2\text{O}$) (Unluer and Al-Tabbaa 2013). This was verified with the matching of major peaks on the XRD pattern with those of nesquehonite (PDF #70-1433), indicating the main precipitation product as nesquehonite. Except for nesquehonite crystals, there might be small amount of residuals from the medium that presented some minor and weak peaks on the pattern. Since those residuals were not phase of interest, the figure did not include any of those residual phases. Overall, the results suggest that *Bacillus cohnii* spores are able to oxidize MgL and to induce magnesium-based carbonate precipitation.

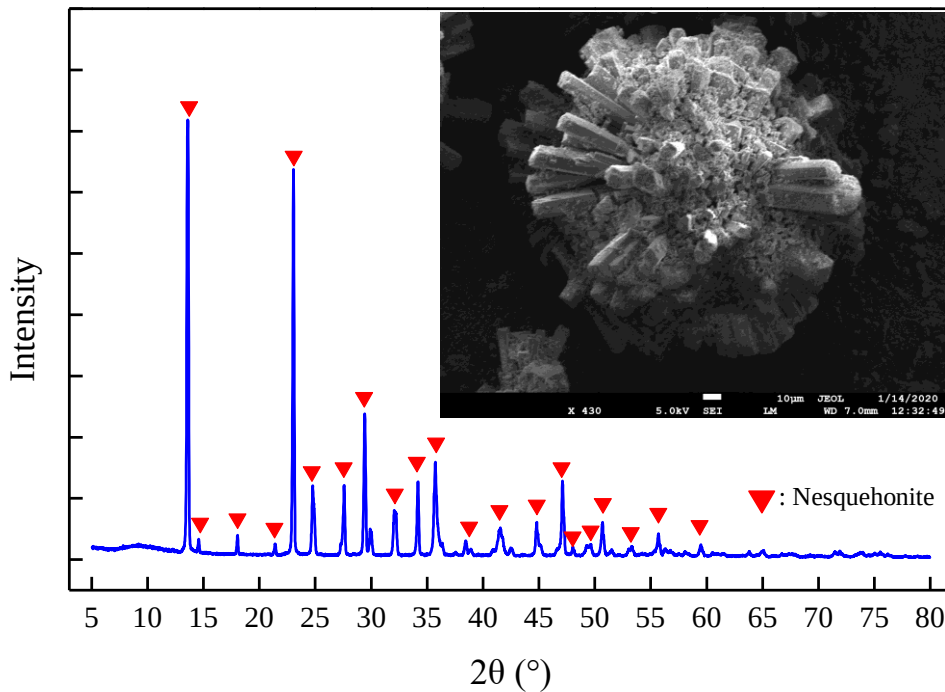


Figure 4.5 Morphology and XRD pattern of the precipitates induced by *Bacillus cohnii* spores in the MgL solution

4.3.2 Viability of *Bacillus cohnii* spores in alkaline solutions

Alkalinity is a crucial factor influencing the bacteria survival rate in cement. Fig. 4.6 shows the viability of *Bacillus cohnii* spores in alkaline solution with pH between 10 and 14. C_s represents the number of viable spores in 1 ml suspension. The initial spore concentration and the measuring limit (i.e., minimum identifiable

concentration) are also marked in Fig. 4.6. As can be seen, the number of viable spores in the suspension have decreased in all alkalinity levels. Significant drop was observed at pH beyond 12 due to the inactivation of the surface proteins of the spores under the high pH level, which led to the destruction of the cell structures of the spores (Setlow, Loshon et al. 2002). The viable spore concentration decreased of 1-2 orders after 1-day exposure at pH of 10-11 and the reduction grew to around 4 orders at pH of 12. Even with prolonged exposure duration up to 30 days, a certain amount of spores could still survive at pH of 10-12, albeit with lower concentrations than those observed after 1-day exposure. It was noticed that the relationship between bacteria viability and alkalinity level was not linear as after 30 days treatment the spore viability was highest at pH of 11, which indicated that *Bacillus cohnii* might prefer environment with pH of 11 the most rather than lower alkalinity level at 10. No meaningful results could be obtained (i.e., below the measuring limit) at pH of 13-14, indicating a dramatic decrease of viable bacteria when alkalinity reached 13. It has been reported that the pH of RMC matrix is around 9.9-10.5 (Dung and Unluer 2019, Hay and Celik 2020). Accordingly, the *Bacillus cohnii* spores should have a much improved viability in the RMC paste than in the PC paste.

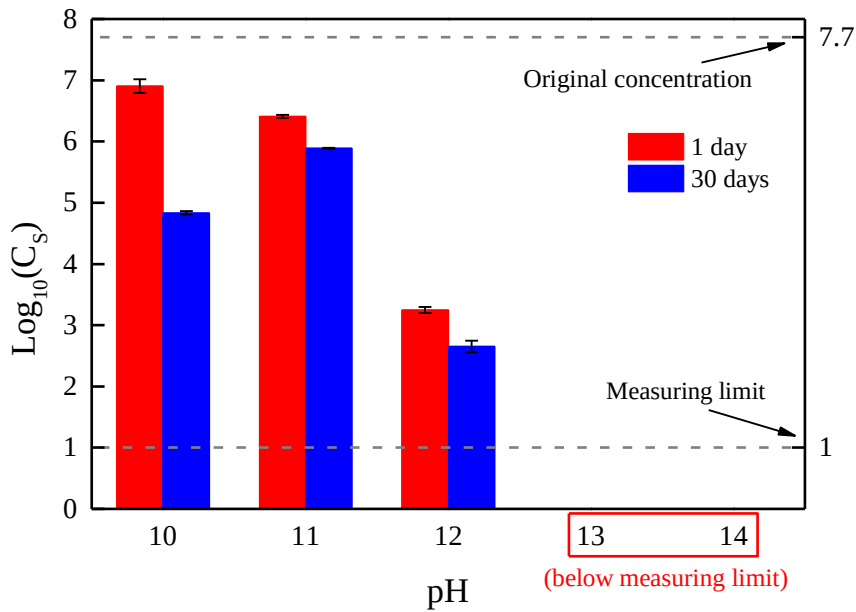


Figure 4.6 Viability of bacteria spores under different alkalinities (pH between 10 and 14)

4.3.3 Precipitation capability of bacteria in RMC paste

4.3.3.1 Precipitation formation within crack

The crack area reduction percentage of the RMC and PC specimens after different exposure durations are used to evaluate the precipitation capability of bacteria in the paste, as shown in Fig. 4.7. Accordingly, the microscopic images of the precipitations within the crack along the conditioning durations were captured and shown in Figs. 4.8 and 4.9, respectively. It can be seen from Fig. 4.7 that, along the whole conditioning period, MS specimen had significantly higher crack sealing performance than all the other groups. After 12 days of conditioning, MS specimen reached a 100% closure of the crack, while all the other groups had crack area reduction only around 10%. Accordingly, Fig. 4.8(b) has visually revealed strong precipitation on the MS specimens (i.e., RMC paste containing bacteria spores), and it can be seen that the 0.2 mm wide crack was completely sealed on the surface after

12 days immersion in the medium. As early as 4 days immersion, the vast formation of crystals inside the crack and on the specimen surface was noticed. While bacteria spores were also included in the PS specimens (i.e., PC paste containing bacteria spores), no or very limited precipitation was observed (Fig. 4.9(b)), potentially indicating the limited viability of unprotected bacteria spores in the PC specimens. This observation was in agreement with previous studies (Wiktor and Jonkers 2011, Rauf, Khaliq et al. 2020). The current study demonstrated the viability of bacteria spores in the RMC paste, and the bacteria can engage fast and abundant precipitation while in RMC paste.

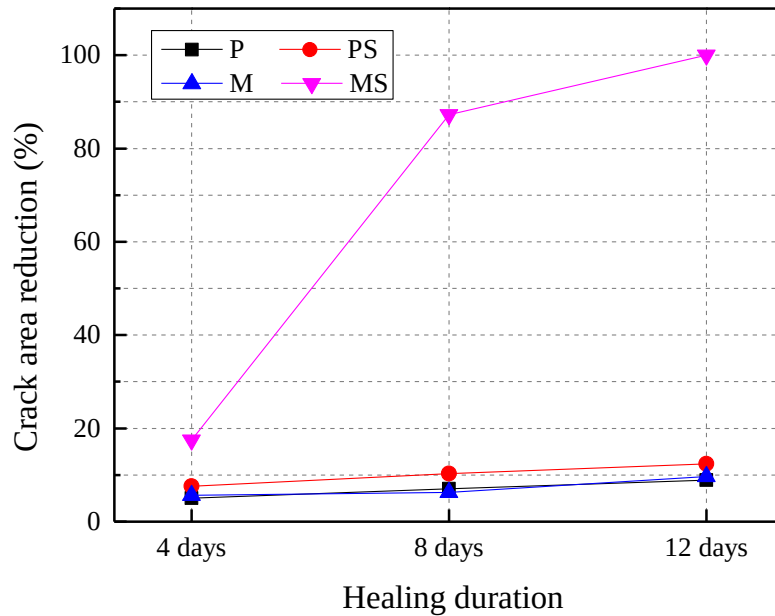


Figure 4.7 Surface crack area reduction after conditioning

In contrary, no or very limited precipitation was observed in the M specimen (i.e., pure RMC paste) and the P specimen (i.e., pure PC paste) as shown in Figs. 4.8(a) and 4.9(a), respectively. After 12 days immersion, only sparse precipitation could be identified at the marked locations. It has been reported that RMC (or PC) is able to engage autogenous healing via chemical reactions by means of further hydration of unreacted RMC (or PC) in the presence of water as well as the carbonation of

brucite (or portlandite) via its reaction with the CO₂ present in air (Qiu, Ruan et al. 2019, Ruan, Qiu et al. 2019). However, under ambient conditions, the crack healing engaged by chemically induced precipitation is limited to only fine microcracks below 30-50 μm (Qiu, Ruan et al. 2019). With a wider crack width of 0.2 mm in the current study, it is difficult to engage chemically induced healing in the RMC and the PC specimens.

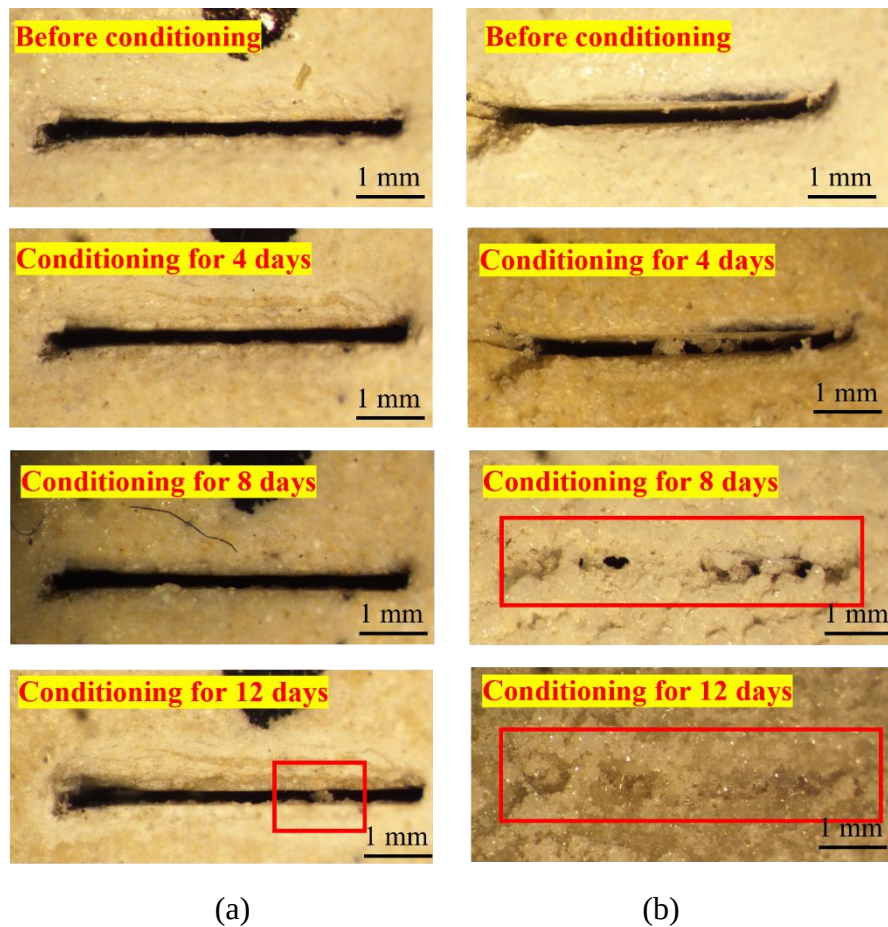


Figure 4.8 Images of crack on the surface of (a) the M specimen (average crack width of 0.20 mm) and (b) the MS specimen (average crack width of 0.21 mm) after pre-determined durations

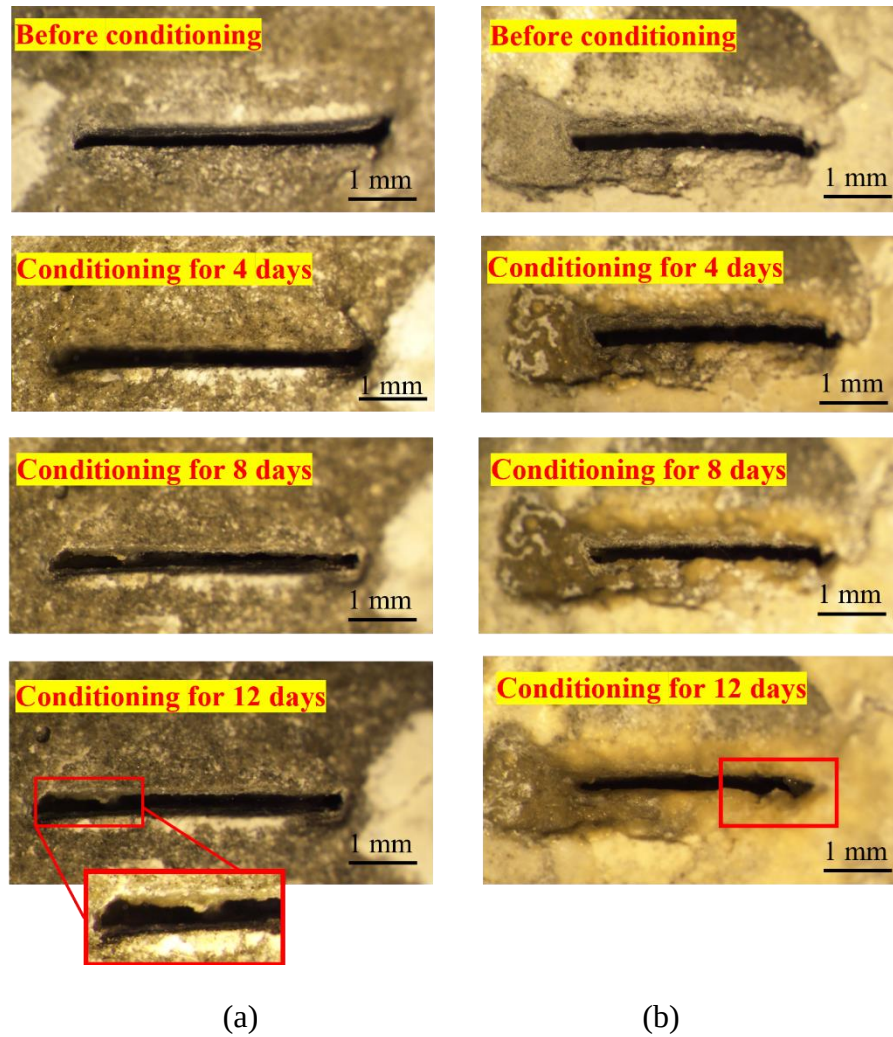


Figure 4.9 Images of crack on the surface of (a) the P specimen (average crack width of 0.21 mm) and (b) the PS specimen (average crack width of 0.21 mm) after pre-determined durations

The morphology of the carbonate precipitation on the specimen surface and crack interior were examined by means of the SEM as shown in Figs. 4.10 and 4.11. As can be seen, the products possessed a rod-like morphology with sharp edges resembling nesquehonite that has been observed in the in vitro precipitation test (Fig. 4.5). This suggested the bacteria spores in the RMC paste remained viable and were able to germinate into vegetative cells on surfaces contacting the conditioning medium for MICP.

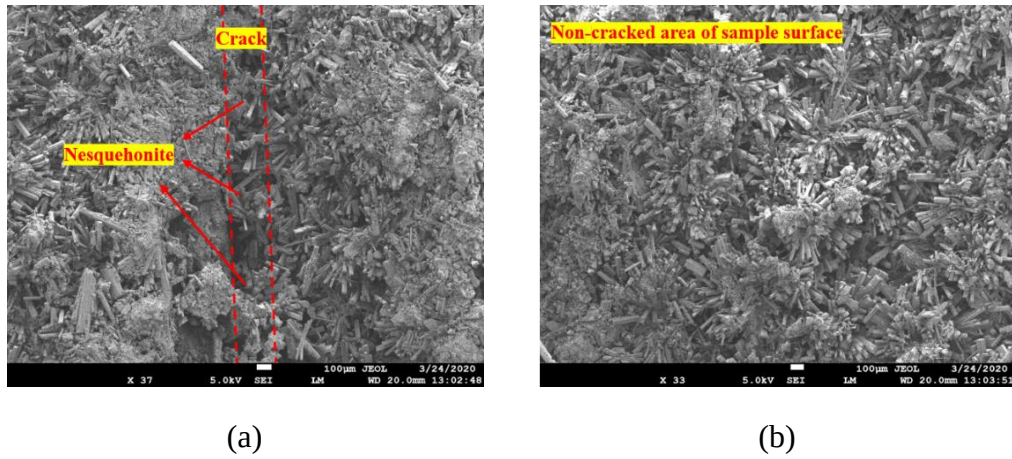


Figure 4.10 Morphology of MICP products observed on the surface of the MS specimen after 8 days conditioning: (a) The pre-fabricated crack area; and (b) other surface region.

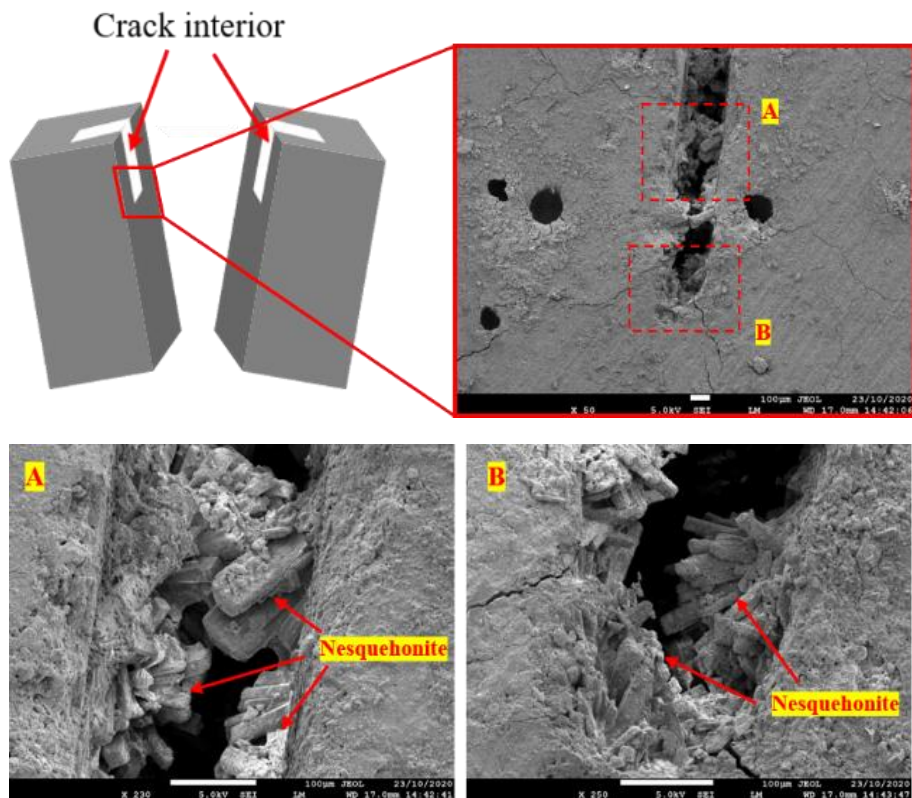


Figure 4.11 Morphology of MICP products observed inside the crack of the MS specimen after 12 days conditioning.

4.3.3.2 Viability of bacteria spores in the hardened RMC and PC pastes

Fig. 4.12 shows the concentration of the viable bacteria spores C_s in the hardened MS and PS specimens at different ages. The initial spores concentration and the measuring limit are also marked. As can be seen, viable spores concentration in the PS specimen continuously reduced with prolonged age. The viable spore concentration decreased by 4 orders after only 1 day, and further decreased by another 1.3 orders from 1 day to 8 days. At the age of 28 days, the viable spore concentration in the PS specimen dropped to be lower than the measuring limit. The continuous reduction of bacteria could be associated with the high pH in the PC system and the continuous pH increase caused by hydration. The MS specimen, on the other hand, possessed much higher viable spores concentrations at all ages when compared to the PS specimen. In fact, the reduction of viable spores mainly happened before 8 days, after the age of 8 days, the spore concentration remained stable. The reduction before 8 days were associated with the hydration and carbonation of the RMC specimen, which potentially could lead to the internal pH and space change within the matrix therefore lead to inactivation of bacteria spores. After 8 days, the carbonation was completed, thus the internal environment of the MS specimen was stable, and the viable spore concentration was not changed. Accordingly, the strong MICP and crack healing of the MS specimen could thus be attributed to the higher viability of bacteria spores in the RMC paste.

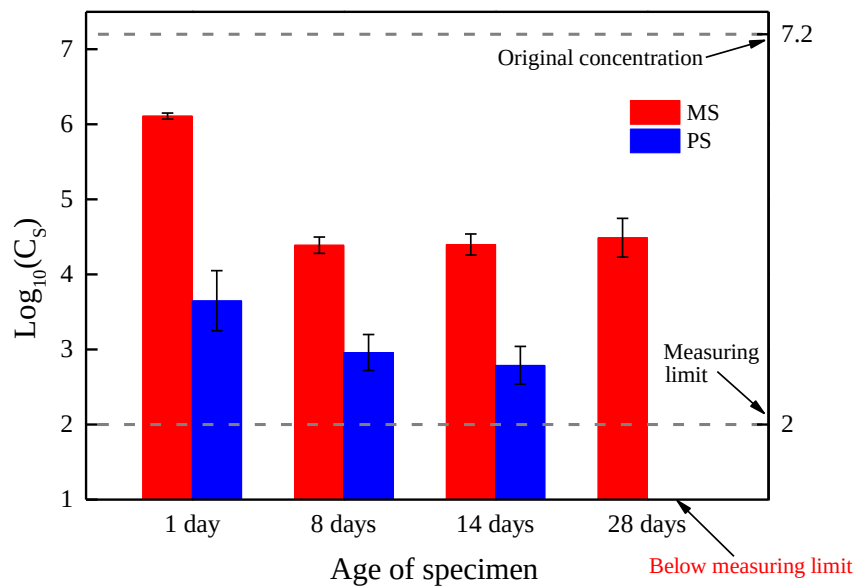


Figure 4.12 Viable bacteria spores in the hardened MS and the hardened PS specimens at different ages

4.3.3.3 Pore solution alkalinity of the hardened RMC and PC pastes

RMC is considered a low-alkalinity system (pH of 9.9-10.5) according to the published literatures (Dung and Unluer 2019, Hay and Celik 2020). However, instead of measuring the pH of the extracted pore solution directly, the alkalinity of RMC was often obtained by grinding hardened RMC samples into powder, soaking the ground powder in water, and measuring the pH of the resulting solution. Table 4.3 summarizes the pH of the pore solutions of the M and the P specimens at different ages. As can be seen, the pH of the pore solution extracted from the PC paste (i.e., around 4.5 ml solution can be extracted from a specimen) remained above 13 regardless of age, which was in line with the findings of earlier studies (Andersson, Allard et al. 1989, Larbi and Bijen 1990, Plusquellec, Geiker et al. 2017). The slightly increased pH with age from 13.1 to 13.4 was associated with the continuous hydration of PC (Larbi and Bijen 1990, Ortolan, Mancio et al. 2016).

As for the pH of the pore solution of the RMC paste, a lower pH of 12 was obtained at the age of 1 day. To our surprise, no solution could be extracted from the M specimens at the age of 8 days and beyond. It was worth noting that the M mix actually had a higher water-to-binder ratio than the P mix. This suggested that there was no free water in the M specimen. This could be attributed to the additional self-desiccation mechanism of the RMC paste. Besides the hydration of RMC, the carbonation of brucite also consumed water which was chemically combined with magnesium carbonates to form HMCs such as dypingite ($4\text{MgCO}_3 \cdot \text{Mg}(\text{OH})_2 \cdot 5\text{H}_2\text{O}$), hydromagnesite ($4\text{MgCO}_3 \cdot \text{Mg}(\text{OH})_2 \cdot 4\text{H}_2\text{O}$), and nesquehonite ($\text{MgCO}_3 \cdot 3\text{H}_2\text{O}$) (Unluer and Al-Tabbaa 2014).

Table 4.3 Pore solution pH values of the M and the P specimens

Mix	1 day	8 days	14 days	28 days
M	12.0	-	-	-
P	13.1	13.1	13.3	13.4

The dry environment creates a more favorable condition for the habitation and survival of bacteria in the RMC paste for the following two reasons: 1) Spores are stable and able to keep dormancy under dry environment (Gest and Mandelstam 1987, Cano and Borucki 1995, Marquis 1998). Thus, spores shall remain metabolically inactive and viable in the RMC matrix. 2) Dry internal environment of RMC leads to less contact between spores and alkaline components that dissolved in pore solution, resulting in reduced OH^- attack to the bacteria spores. As pointed out in the previous section, the viable spores concentration in the RMC paste remained stable after the age of 8 days (Fig. 4.11), which corresponded well with the observation from the pore solution extraction that the RMC matrix was dry and no solution could be extracted from the RMC paste at the age of 8 days and beyond (Table 4.4). This implies a potential long-term viability of bacteria spores in the RMC matrix due to its stable internal environment which is dry with lower alkalinity.

4.4 Summary

This chapter studied the mechanism of the self-healing ability of the RMC-B capsules by investigating the survivability and carbonate precipitation ability of bacteria spores in RMC paste. The precipitation reaction between the bacteria and MgL was validated first, followed by investigating the impact of solution alkalinity on the viability of bacteria spores. Carbonate precipitation of the RMC paste with direct incorporation of bacteria spores was evaluated by observing precipitation within cracks. Furthermore, viability of bacteria spores in the hardened pastes and pore solution pH of the hardened pastes were examined. The main conclusions can be summarized as follows:

- Solution alkalinity had a strong influence on the viability of bacteria spores. Dramatic reduction of viable *Bacillus cohnii* spores can be expected for pH beyond 13. With 30 days treatment at pH of 13, spores were inactivated completely.
- Bacteria showed strong MICP in RMC paste evidenced by the massive formation of nesquehonite within the cracks, which led to complete crack closure after 12 days conditioning, and
- The higher viability of *Bacillus cohnii* spores in hardened RMC paste was associated with the low alkalinity and dry internal environment of the RMC matrix.

CHAPTER 5 DEVELOPMENT OF FUNCTIONALLY GRADED ONE-PART CAPSULES CONTAINING BACTERIAL SPORES AND NUTRIENTS (RMC-BN CAPSULE)

5.1 Introduction

In previous work, RMC-B capsule was developed. To engage self-healing, RMC-B capsule was incorporated into PC paste while the nutrients (YE and CaL) were directly mixed in the paste. During the mixing, it was noticed that the addition of nutrients directly into the paste could cause some changes on the fresh properties of the paste, for example the flowability and setting time were increased and there were some small air bubbles were generated in the paste. Besides, literatures have extensively reported that the direct addition of yeast extract in the matrix could significantly delay the hydration of the mix and compromise the strength of the matrix (Jonkers and Schlangen 2008, Bundur, Kirisits et al. 2015). Therefore, a proper way to apply the nutrients to the matrix needs to be investigated.

In this chapter, a self-contained RMC-BN capsule was proposed to solve this issue by encapsulating nutrients together with bacteria in the capsule. Accordingly, spores of *Bacillus cohnii*, yeast extract (YE) and calcium lactate (CaL) are encapsulated together in RMC-based capsules to form a self-contained RMC-BN capsule, as shown in Fig. 1. The incorporation of YE was for the activation and germinate of spores to active vegetative cells and CaL was for the calcium carbonate precipitation precursor. The morphology and composition of the RMC-BN capsule were studied by scanning electron microscope (SEM) and x-ray diffraction (XRD), and the bacteria spore viability in the capsules was quantified. To study the influence of RMC-BN capsules on the fresh and hardened properties of the matrix, initial setting time, flowability and compressive strength were tested by adding the capsules into PC paste. Lastly, the self-healing performance of the RMC-BN

capsules were evaluated by conditioning cracked specimens to wet/dry cycles in water. Surface crack closure, water passing rate through the crack, and ultrasonic pulse velocity were tested.

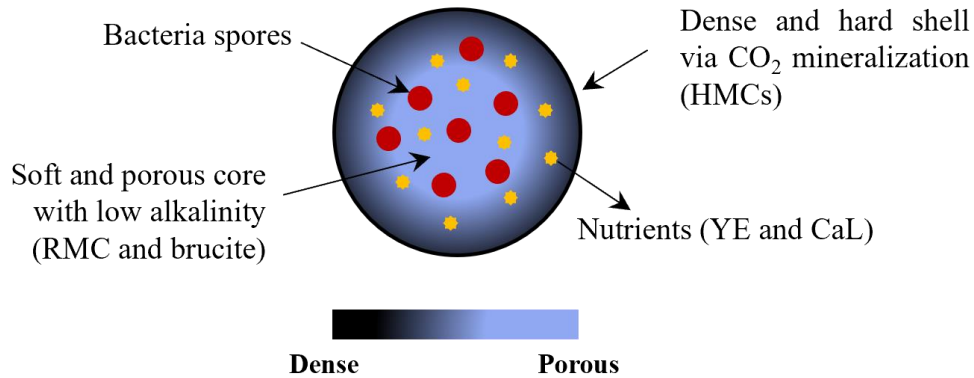


Figure 5.1 Schematic illustration of the proposed functionally graded one-part RMC-BN capsule with a dense shell and a porous core

5.2 Experimental program

5.2.1 Bacteria spore preparation

Bacteria strain *Bacillus cohnii* from American Type Culture Collection was used in this study. The preparation of the bacteria spores followed the same procedure as described in Chapter 3.2.1.

5.2.2 Fabrication and characterization of the RMC-BN capsule

The RMC-BN capsule was prepared according to the mix design in Table 5.1. The RMC used in this study was purchased from Richard Baker Harrison Ltd. (UK). Its chemical composition was the same as the RMC used in Chapter 3, Section 3.2.2. To prepare the capsule, the bacteria cells were first evenly suspended in water, and then the suspension was mixed with RMC with a hand mixer for around 5 mins until it achieved a homogeneous state. The paste was continuously mixed while the

pre-mixed yeast extract and calcium lactate powder were slowly added into the paste until all the ingredients were well mixed and the paste was homogeneous again. The resulting paste was placed in a plastic container under ambient conditions (25°C and 80% relative humidity (RH)) for 2-3 days, before being broken into smaller pieces and cured in a carbonation incubator under 10% CO₂, 30°C and 80% RH for 1 day. Subsequently, the samples were manually ground into powder form with a mortar. The obtained powder was sieved with a 150 µm mesh and then cured in the carbonation incubator under same conditions for another 6 days.

Table 5.1 Mix design of RMC-BN capsule

MgO (g)	Water (g)	Bacteria (CFU)	Yeast extract (g)	Calcium lactate (g)
40	20	1.02×10 ¹¹	25.7	51.4

To study the morphology of the capsule, around 0.1g sample was investigated with SEM. To quantitatively analyze the composition of the RMC-BN capsule, XRD-RIR technique was adopted. To study the survival of bacterial spores in the RMC-BN capsule, the viability of spores in the capsules was quantified. The sample preparation and testing procedure of the three tests followed the same procedure described in Section 3.2.2.

5.2.3 Effects of capsule addition on fresh and hardened properties of PC paste

The influence of capsule addition on the flowability, initial setting time and compressive strength of a PC paste were studied. Mix design of the PC paste can be found in Table 2. The Portland cement (PC) used in this study was purchased from EnGro Ltd (Singapore), complying with SS EN 197-1: 2014. The chemical composition of PC is the same as reported in Chapter 3, Section 3.2.4, Table 3.4. To prepare the mix (P-BNC), water was added into cement powder and mixed in a mixer until homogenous. The RMC-BN capsules were then slowly added into the fresh mixture and mixing for another 5 minutes until the paste was homogeneous.

Similar procedure was used to prepare the control mix (P), except no capsules were included.

Portion of the fresh mixture was taken out for the flowability test and the initial setting time test. The flowability of the paste was determined in accordance with the ASTM C1437 using a flow table (63-L0040/A, Controls). The initial setting time was measured in accordance with the ASTM 191 using an automatic Vicat apparatus (EN 196-3, Testing Bluhm & Feuerherdt GmbH). Meanwhile, the remaining fresh paste was cast into 50 mm cubes and cured under ambient condition (25°C, 80% RH) for 2 d. The cubes were then demolded and sealed in plastic bag for curing. The compressive strength tests were carried out on a compression machine (ToniTechnik Baustoffprüfsysteme) operated at a loading rate of 55 kN/min when the age of cubes reached 7, 14 and 28 days, respectively. Three replicates were prepared for each test.

Table 5.2 Mix design of the samples for flowability, initial setting and compressive strength tests

Mix ID	Portland cement (g)	Water (g)	RMC-BN capsule (g)
P	1500	480	-
P-BNC	1500	480	80

5.2.4 Self-healing performance of PC paste incorporating RMC-BN capsules

The self-healing performance of PC paste incorporating RMC-BN capsules was assessed via crack width reduction that revealed surface crack sealing. Water passing rate test was conducted to evaluate the transport property recovery, and ultrasonic pulse velocity (UPV) method was adopted as a non-destructive approach to evaluate the degree of healing.

The preparation of the fresh paste followed the same procedure as described in Section 5.2.3. To prepare specimens for crack width measurement and water

passing rate tests, the fresh paste was cast into cylinder molds with a diameter of 7.5 cm and a height of 2.5 cm. Firstly, half of the mold was filled with fresh paste. A circular steel mesh with a diameter of 7 cm was then placed in the middle height of the mold. Afterwards, the mold was filled up with paste as shown in Fig. 5.2. The specimens were cured under ambient condition for 2 days, and then demolded and sealed in plastic bag until 28 days. For each mix, three specimens were prepared. To prepare specimens for the UPV test, 50 mm cubes were casted. Firstly, half of the mold was filled with fresh paste, followed by placing a 50 mm square plastic mesh in the middle height of the mold. Afterwards, the mold was fully filled with paste as shown in Fig. 5.3. For each mix, three specimens were prepared. The demolding and curing procedure were the same as the cylinder specimens.

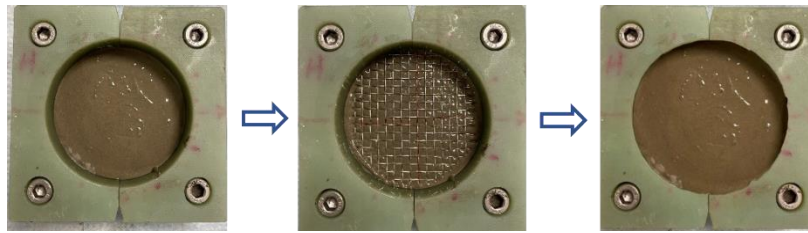


Figure 5.2 Casting procedure of cylinder specimens for crack width measurement and water passing rate test

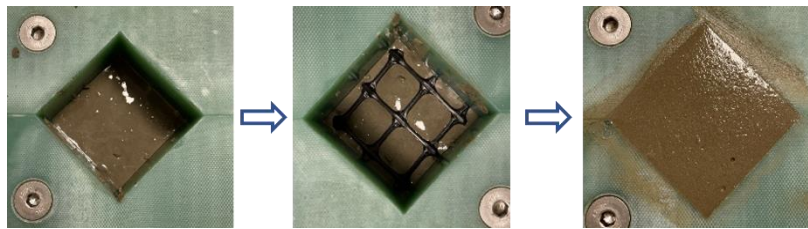


Figure 5.3 Casting procedure of cubic specimens for crack width measurement and water passing rate test

At the age of 28 days, a crack was produced in cylinder specimens following the same procedure as described in Section 3.2.4. After a single crack was produced throughout the center of the specimen, five locations were marked on each cylinder

specimen along the crack length for surface crack width monitoring. It was worth noting that in this section, the generated crack was relatively wider than crack generated in Chapter 3 for RMC-B capsule and more conditioning cycles was conducted accordingly. In the study of RMC-B capsule, a relatively conservative crack generation process was conducted with crack width below 350 μm since it was the very first study. After 5 cycles, the crack healing performance of the RMC-B capsule mix has been already significantly differentiated with control groups, therefore conditioning cycles were terminated. On the basis of crack around 200~300 μm can be effectively healed within 5 cycles, larger cracks up to 500 μm was generated and the condition cycle was prolonged to 50 cycles accordingly in the studies of RMC-BN capsule and single bacteria capsule in the following chapters. To evaluate degree of healing, surface crack width and water passing rate before and after wet-dry conditioning of the cylinder specimens were measured. The surface crack width at each observation location on the disc specimen was measured by a Nikon stereoscopic microscope (SMZ 745T), and the water passing rate of the was determined via the constant water head method in accordance with Section 3.2.4.

At the age of 28 days, a crack was produced in the cubic specimens via a similar pre-cracking setup of the cylinder specimens, except a V-shaped cutting edge, as suggested in previous studies (Singh and Gupta 2020), was used, as shown in Fig. 5.4(a). Same loading rate and loading procedure for the pre-cracking were adopted to generate an edge crack in the cubic specimens, as shown in Fig. 5.4(b).

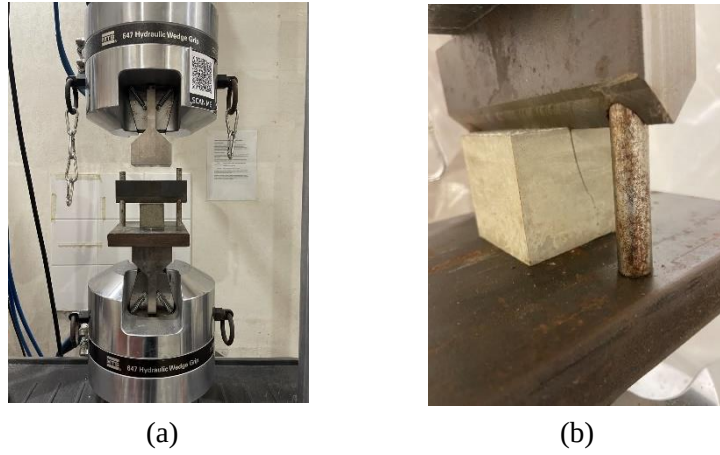


Figure 5.4 (a) Setup for pre-cracking of cubic specimens, and (b) an edge crack in the specimen

UPV test is a widely used non-destructive technique to evaluate damage (e.g. cracks and flaws) in cement-based composites. UPV test was carried out by means of the Pundit Lab (+) ultrasonic pulse velocity machine to gauge the degree of healing of the specimens, in line with the procedure outlined in previous studies (Institute 2013, Xu and Yao 2014, Camara, Wons et al. 2019). The two transducers were positioned on the opposite surfaces of the cube specimen. One of the transducers transmitted the ultrasonic wave and the other one received it, where the transmission of the ultrasonic pulse was perpendicular to the crack as shown in Fig. 5.5. Coupling gel was applied between the transducer and specimen surfaces to ensure good contact. The test was performed at frequency of 150 kHz according to ASTM C597-16. The time that the ultrasonic wave traveled across the specimen from one transducer to other was recorded, and ultrasonic pulse velocity was calculated as Eqn. (5.1), where V_s is the ultrasonic pulse velocity of the specimen, L_s is the length of the cubic specimen (50 mm), and T_w is the time that the wave traveled through the specimen.

$$V_s = L_s / T_w \quad (5.1)$$

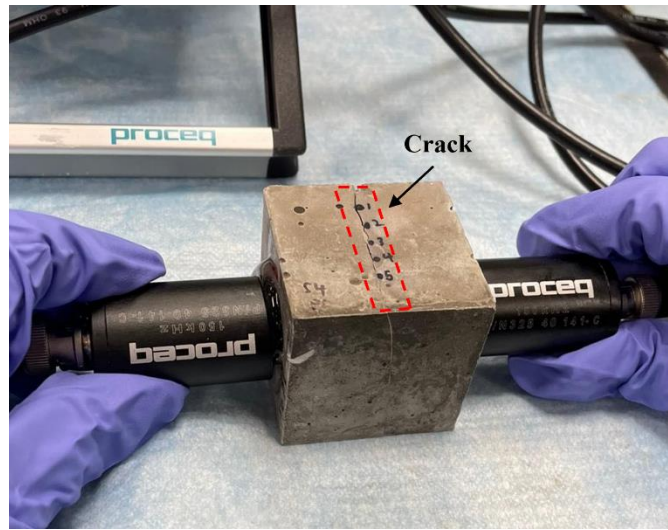


Figure 5.5 UPV test set-up

The pre-cracked specimens were then conditioned in wet and dry cycles. Each cycle consisted of 1 day of immersion in water (25°C), followed by another day of drying in ambient air (25°C, 80% RH). Specimens from different mixes were placed in different containers for wet/dry conditioning to avoid cross-contamination, and the conditioning water was replaced and replenished after each cycle. Surface crack width, water passing rate, and UPV were measured after every 10 wet/dry conditioning cycles. Accordingly, the surface crack width reduction (%) and the water passing rate reduction (%) were calculated according to Eqn. (3.3) and Eqn. (3.4) in Section 3.2.4, respectively.

5.3 Results and discussion

5.3.1 RMC-BN capsule characterization

The representative SEM image of the RMC-BN capsule is shown in Fig. 5.6. As can be seen, the capsule surface was fully covered with a needle-like phase, resembling the morphology of nesquehonite ($\text{MgCO}_3 \cdot 3\text{H}_2\text{O}$). The formation of nesquehonite on the surface was associated with the accelerated carbonation curing

of the capsule.

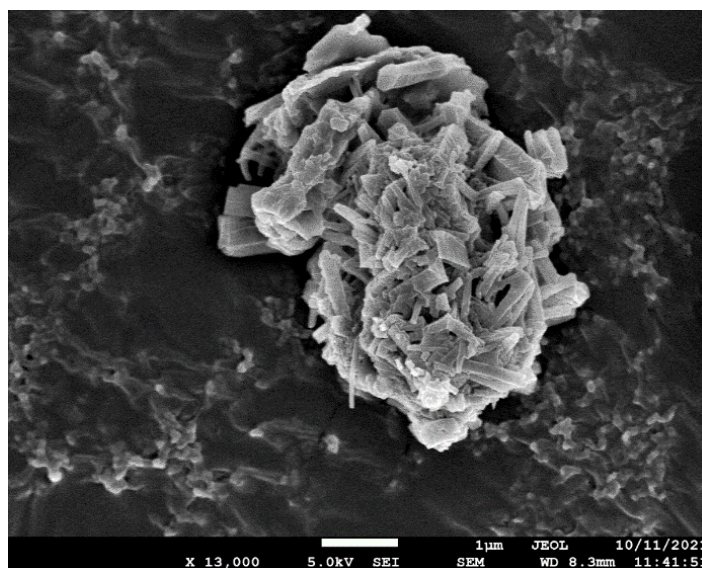


Figure 5.6 Representative SEM image of the RMC-BN capsule

The XRD pattern of the capsule with the inclusion of an internal standard (20 wt.% CaF_2) is shown in Fig. 5.7. Strong peaks of CaF_2 were observed at 28.2° and 47.0° . Peaks of MgO and brucite were found at 18.7° and 62.3° , respectively. The presence of MgO and brucite indicated that incomplete hydration and carbonation of MgO /brucite in the capsules. Nesquehonite peaks were observed at 13.6° , 23.1° and 29.5° , which were in agreement with the surface morphology of the RMC-BN capsule (Fig. 5.6). Magnesite, from the incomplete calcination in the production of MgO cement (around 5 wt.%), was also observed at 43.0° . Based on the mix design of the capsule and RIR method, the weight fraction of each component in the RMC-BN capsule was calculated and summarized in Table 5.3. The major composition of the capsules was nutrients (YE and CaL), followed by HMCs and unreacted RMC and brucite. Overall, the results revealed that the RMC-BN capsule had a shell composed of HMC (20 wt.%), while the remaining core (80 wt.%) consisted of nutrients, unreacted RMC and brucite.

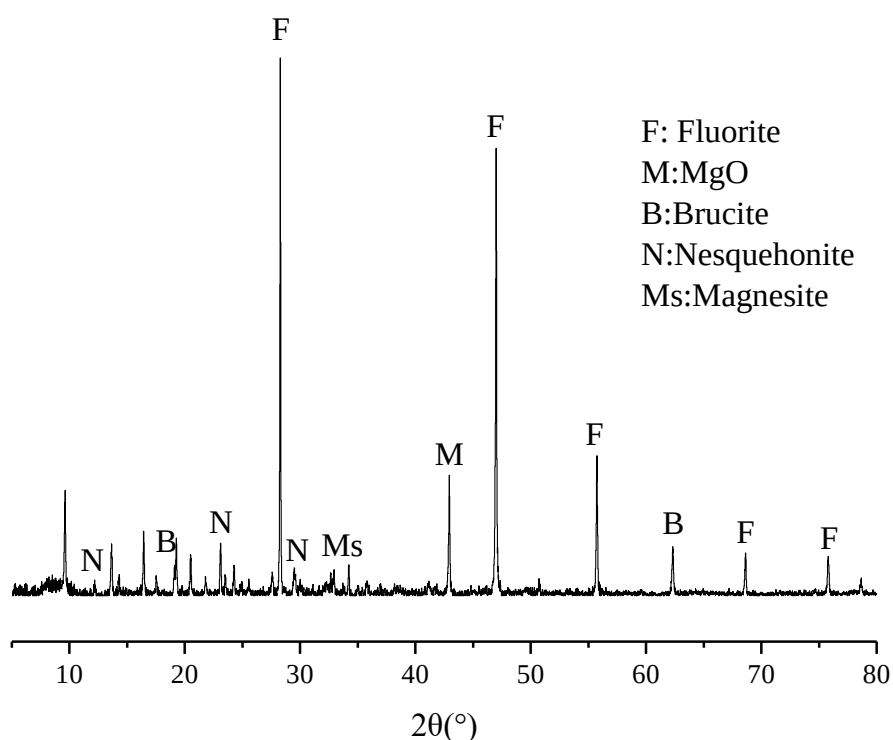


Figure 5.7 XRD pattern of the RMC-BN capsule with the inclusion of 20 wt.% CaF_2

Table 5.3 Composition of RMC-BN capsule

Composition	Weight fraction (%)
MgO	11.9
$\text{Mg}(\text{OH})_2$	7.7
Magnesite	5.0
Nutrients	56.3
Nesquehonite	19.1

The viability of bacteria in the RMC-BN capsule is shown in Fig. 5.8. The (logarithm of the) viable bacterial spores incorporated in 1 g of sample was 8.8 initially, which reduced and stabilized to around 6 in the fresh and the aged RMC-BN capsules. This represents a significant viability enhancement of bacterial spores when compared to the direct addition of unprotected spores into the PC paste, where the viable bacterial spores reduced by more than 6 orders (Xiao, Unluer et al. 2022). It was worth noting that no further reduction of the viable spores was observed in

the aged capsules, indicating the internal environment in the capsule might have reach to a stable condition. This may be attributed to the following reasons. As mentioned in Section 3.1, RMC first hydrated to form weak and porous brucite. Accelerated carbonation converted surface brucite into dense HMCs shell which prevents further carbonation of the internal brucite, leaving a stable core in terms of microstructure and physical and chemical properties, which favored the long-term viability of the bacterial spores in the RMC-BN capsule with a gradient core-shell structure.

The observed moderate reduction of viable bacterial spores in the RMC-BN capsule using the current bacteria counting method may be attributed to the following reasons. The adopted viability test method involved a grinding process to expose the bacterial spores for counting. During this process, some spores might be inactivated due to mechanical grinding induced strong forces. Secondly, during the capsule fabrication process, bacterial spores were mixed with nutrients and RMC binder. Some spores might be activated and germinated to vegetative cells in this process. It is known that vegetative cells were much more vulnerable in the harsh environment, thus being inactivated in the RMC matrix.

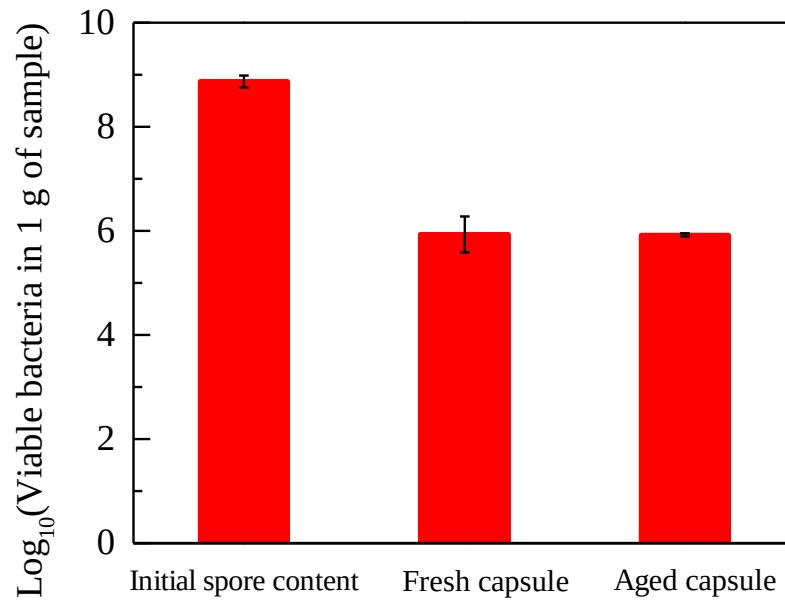


Figure 5.8 Viable bacteria in 1 g of RMC-BN capsule

5.3.2 Effects of capsule addition on fresh and hardened properties of PC paste

The influence of RMC-BN capsule addition on the flowability and the initial setting time of the fresh PC paste are summarized in Table 5.4. As can be seen, capsule addition did not cause major impacts on the flowability of the fresh paste, where P-BNC and the control mix (P) had comparable flowabilities of 61% and 69%, respectively. However, the incorporation of the capsules reduced the initial setting time from 187 min to 115 min. The reduction of the setting time could be attributed to the large surface area of the RMC-BN capsule. In the capsule fabrication process, the size of the capsule was controlled below 150 μm , resulting in large surface area of the capsule. During the mixing procedure, the surface of the capsule could absorb water from the mixture, therefore led to reduced initial setting time.

Table 5.4 Flowability and initial setting time of a PC paste with and without RMC-BN capsules

Mix	Flowability (%)	Initial setting time (min)
P-BNC	61	115
P	69	187

The compressive strength of the PC paste incorporating RMC-BN capsules is shown in Fig. 5.9. As can be seen, the incorporation of RMC-BN capsules results in enhanced or comparable compressive strength of the resulting paste. At 28 days, P-BNC showed a higher compressive strength than the control mix by 10%, where the former had a strength of 68.8 ± 4.4 MPa while the control had a strength of 62.8 ± 4.1 MPa. The results indicated that the RMC-BN capsules have sufficient strength and good compatibility to the surrounding PC matrix. This differentiated the proposed approach in the current work from the previous studies where porous carriers or organic-based material were used for encapsulation and resulted in significant strength loss when the capsules were incorporated into the PC matrix.

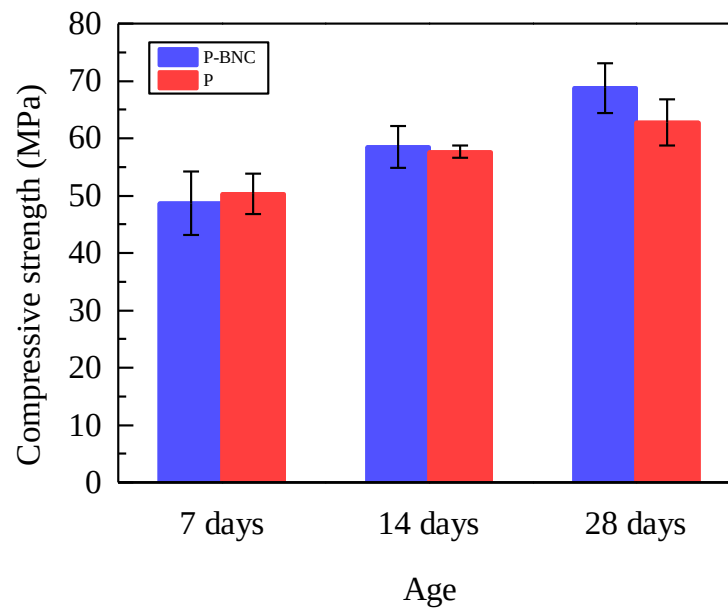


Figure 5.9 Compressive strength of a PC paste with and without RMC-BN capsules

5.3.3 Self-healing performance of PC paste incorporating RMC-BN capsules

5.3.3.1 Surface crack closure

To evaluate the healing performance, the surface crack widths before and after 50 conditioning cycles were plotted for mixes P-BNC and P, as shown in Fig. 5.10.

The 45-degree solid line represents no reduction of surface crack width while the grey dashed line indicates 50% surface crack closure. As can be seen, the crack closure in the P mix exhibits strong dependency on the initial crack width. The capability of crack closure impairs significantly with increasing initial crack width. This is because crack closure in the P mix relies on the hydration of unreacted cement and the carbonation of portlandite in the matrix, where crack width is the critical factor for such intrinsic healing process (Yang, Lepech et al. 2009). The P-BNC mix, on the other hand, shows much significant surface crack closure than the P mix regardless of the initial crack width, indicating the effectiveness of the RMC-BN capsule. This suggests the bacteria-based approach has the capability to heal much larger cracks (Muhammad, Shafaghat et al. 2016).

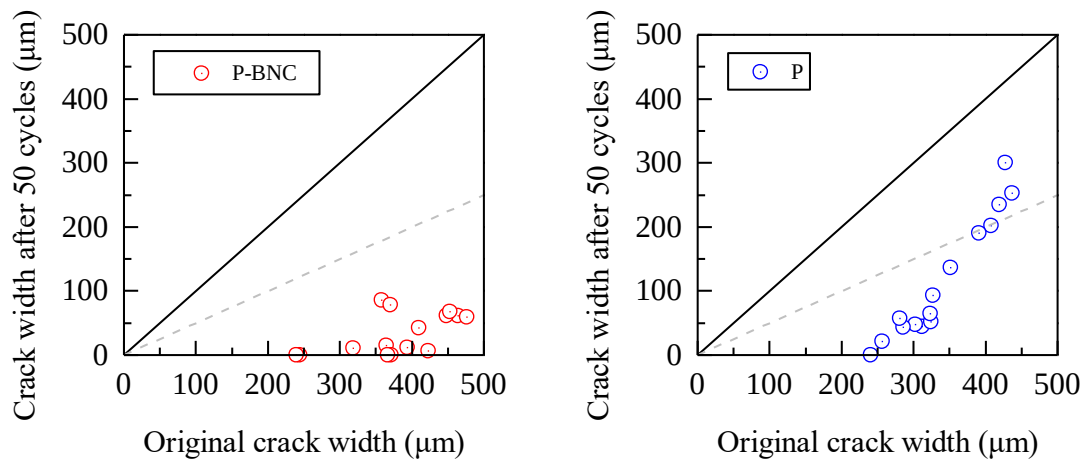


Figure 5.10 Original crack width and crack width after 50 cycles of wet/dry conditioning

Fig. 5.11 compares the average crack width reduction (%) of large cracks ($> 350 \mu\text{m}$) in the two mixes. As can be seen, the average large crack width reduction (%) in the P-BNC mix was about twice that in the control mix. At the end of the 50 conditioning cycles, the average large crack width reduction (%) in the P-BNC mix can reach $90.1 \pm 8.1\%$ while that in the control mix was below 50% ($46.5 \pm 10.5\%$).

This demonstrated the superior capability of large crack repair of the RMC-BN capsules. Fig. 5.12 shows the representative images of a crack with a width of 371 μm in the P-BNC mix that was completely closed after 40 conditioning cycles, and a crack of 390 μm in the P mix that was partially healed after 40 conditioning cycles.

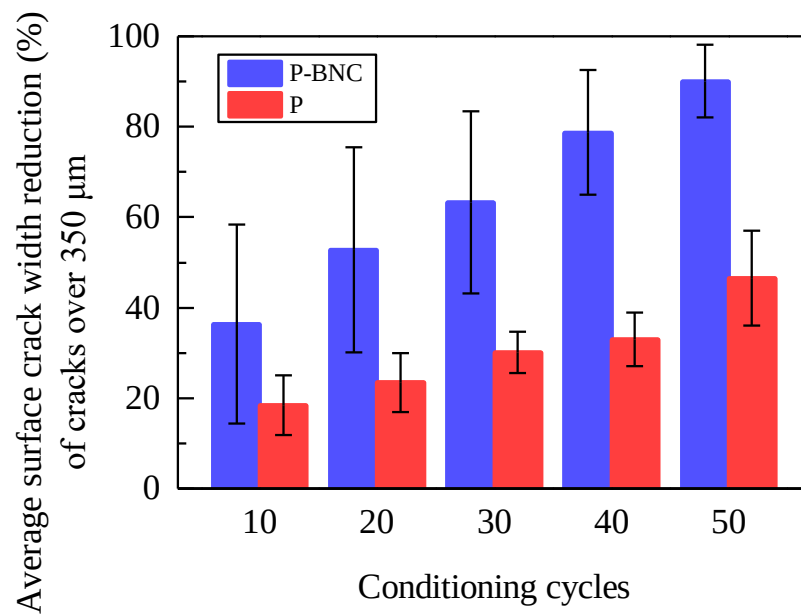
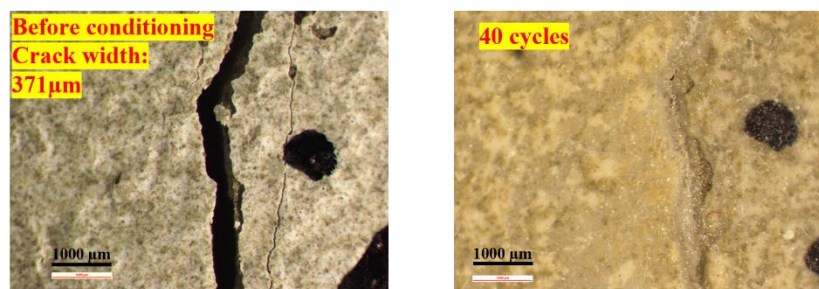


Figure 5.11 Average surface crack width reduction (%) of large cracks ($> 350 \mu\text{m}$)



(a)

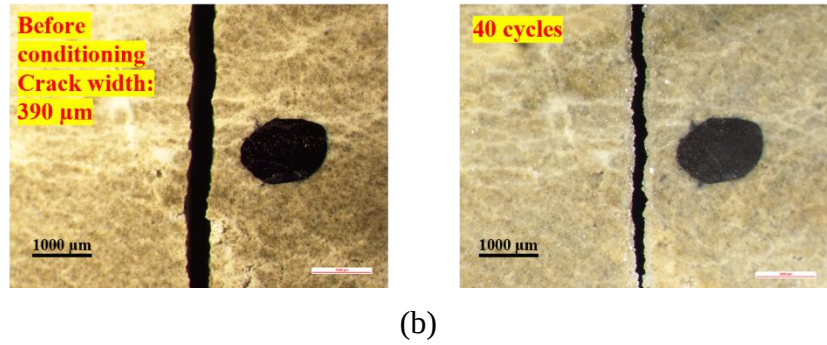


Figure 5.12 Representative images of (a) a crack (371 μm) in mix P-BNC that was completely healed after 40 conditioning cycles, and (b) a crack (390 μm) in mix P that was partially healed after 40 conditioning cycles

5.3.3.2 Water passing rate

To study the transport property recovery, the average water passing rate reduction (%) of the two mixes are plotted against conditioning cycles, as shown in Fig. 5.13. After 10 conditioning cycles, the mix P-BNC demonstrated a significant water passing rate reduction (71.2%) when compared to the control mix P (< 10%). The water passing rate reduction is much more significant than the surface crack width reduction (%) after 10 conditioning cycles as shown in Fig. 5.11. While the water passing rate reduction (%) of mix P-BNC was around 8 times that of mix P, the average surface crack width reduction (%) of mix P-BNC was only about 1.8 times that of mix P. Fundamentally, the transport property recovery was not only related to the surface crack sealing, but also depended on the internal healing along the crack depth. Accordingly, the results suggested that the P-BNC mix might have much better internal healing than the control mix, though the sealing of surface crack was not proportion to the internal healing after 10 wet/dry cycles.

The water passing rate continued to reduce with increasing conditioning cycles. Overall, the P-BNC mix demonstrated a notably higher water passing rate reduction (%) than the control mix P. At the end of 50 conditioning cycles, the average water

passing rate reduction of P-BNC was $89.3 \pm 7.1\%$, which was 2.7 times that of the control mix ($33.3 \pm 2.7\%$). The results demonstrated the effectiveness of RMC-BN on the transport property recovery.

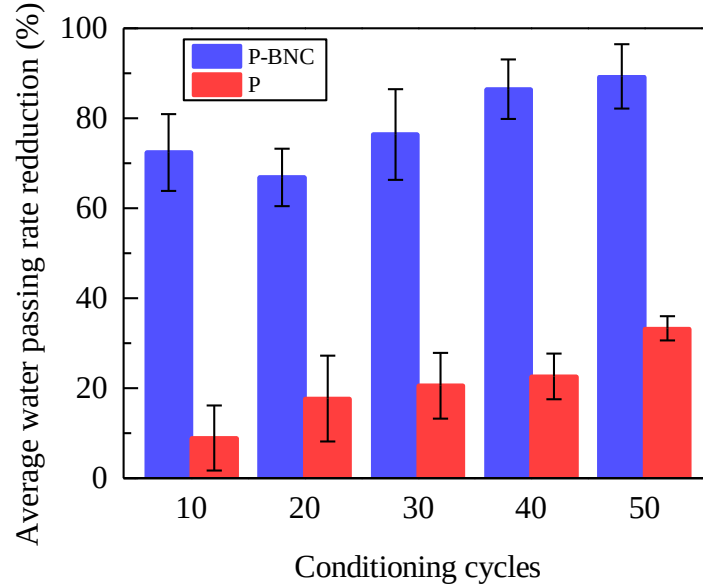


Figure 5.13 The average water passing rate reduction (%) of mix P-BNC and mix P after wet/dry conditioning.

5.3.3.3 Ultrasonic pulsive velocity

Fig. 5.14 shows the UPV test results. As can be seen, UPV of the virgin uncracked cubic specimen was 4885 m/s, regardless of the addition of the RMC-BN capsules. After pre-cracking, the cracks generated on the P specimen ranging from 177-600 μm , and the cracks generated on the P-BNC specimen ranging from 121-566 μm . Pre-cracking led to significant reduction of the measured UPV as shown in the figure. Accordingly, the UPV of the pre-cracked P specimen was 3140 ± 149 m/s (64.3% of the virgin uncracked specimen) and that of the pre-cracked P-BNC specimen was 3317 ± 265 m/s (67.9% of the pristine specimen). This is because the measured UPV represents the combined property of matrix, microcracks and macrocracks, which can be described in Eqn. (5.2),

$$V_s = \frac{1}{\frac{i_1}{V_1} + \frac{i_2}{V_2} + \frac{i_3}{V_3}} \quad (5.2)$$

where V_s is the UPV of the specimen; i_1 , i_2 , i_3 are the volume fraction of matrix, microcracks and macrocracks, respectively; and V_1 , V_2 , V_3 are the UPV of the matrix, microcracks, and macrocracks, respectively (Zhong and Yao 2008). V_1 is a function of the mechanical properties of the matrix, such as elastic modulus, Poisson's ratio, material fracture toughness and inner friction. V_2 is a function of microcrack parameters such as the half-length, shape ratio and density of the microcracks. V_3 is the speed of sound in air, which is 340 m/s. For uncracked specimens, UPV of the specimen is mainly dominated by V_1 as the volume fraction of micro/macro cracks is very small compared to the matrix. The formation of cracks results in the reduction of matrix volume fraction i_1 and increase of the volume fraction of the micro/macro cracks i_2 and i_3 . As a result, UPV of pre-cracked specimens would decrease significantly. The comparable UPV of both the pre-cracked P-BNC and P specimens indicates that the pre-cracking induces similar damage to the specimens.

After 50 conditioning cycles, the P-BNC mix exhibited significant increase of UPV to 4756 ± 599 m/s (97.4 $\pm$ 12.3% of the uncracked specimen) while the control mix showed only very limited recovery (3317 ± 265 m/s, i.e., 67.9 $\pm$ 5.4% of the uncracked specimen). The increase of UPV can be attributed to reduction of micro/macro cracks because of crack healing after the wet/dry conditioning, and thus it can be an indication of damage recovery. Overall, the results highlighted that the RMC-BN capsules not only lead to enhanced healing on the surface, but also engaged in the healing of the interior of the crack.

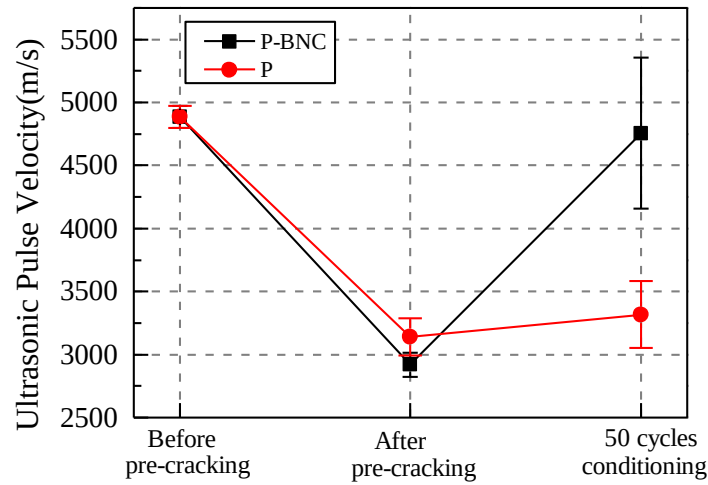


Figure 5.14 UPV results of mixes P-BNC and P

5.4 Summary

On the basis of RMC-B capsule, this chapter developed a novel functionally graded one-part capsule containing bacterial spores and nutrients (RMC-BN). Similarly to RMC-B capsule, the resulting RMC-BN capsule possessed a gradient core-shell structure. The dense HMC (nesquehonite) shell protected bacterial spores from direct contact with the harsh cement matrix, provided mechanical strength of the capsule, and prevented further carbonation of the core brucite to give a stable internal environment for long-term viability of bacterial spores, while the porous brucite/unreacted RMC core accommodated bacterial spores and nutrients.

The incorporation of RMC-BN capsules into a PC paste led to 10% increase of the 28-day compressive strength, indicating the high compatibility of the inorganic-based capsule with the PC matrix. Furthermore, PC pastes with RMC-BN capsules demonstrated superior autonomous healing of large cracks and recovery of transport properties. Specifically, cracks less than 350 μm were completely sealed while cracks $>350 \mu\text{m}$ showed more than 90% width reduction on average. Concurrently, the water passing rate exhibited 89.3% reduction while the UPV recovered by 97% after fifty wet/dry conditioning cycles.

CHAPTER 6 SINGLE BACTERIA SPORE ENCAPSULATION THROUGH LAYER-BY-LAYER SELF-ASSEMBLY OF POLY(DIMETHYLDIALLYL AMMONIUM CHLORIDE) AND SILICA NANOPARTICLES

6.1 Introduction

As summarized in Chapter 1, current immobilization/encapsulation methods were not able to control the loading amount of bacteria in the capsule so that bacteria are likely dispersed unevenly in the matrix and cause inconsistent healing. The probability of capsules hit by a crack in the matrix was extensively studied through numerical methods (Zemskov, Jonkers et al. 2011, Lv and Chen 2013, Mauludin, Zhuang et al. 2018). Lv et al. (Lv and Chen 2013) developed a three-dimensional probability model to establish a mathematical relationship between the capsule size and the chance of the capsules hit by a crack. The results suggested that reducing the capsule size and increasing the number of capsules can enhance the chance of the capsules hit by the crack. Accordingly, when the same number of bacteria were used to engage self-healing in concrete, it is desired to have smaller capsule size with larger number of capsules, which can improve the chance of a crack hitting capsules and thus exposing the bacteria spores as illustrated in Fig. 6.1. Besides, the size of the capsule was relatively large and led to strength loss to the matrix. Accordingly, the encapsulation of single bacteria cell could be a solution to these issues.

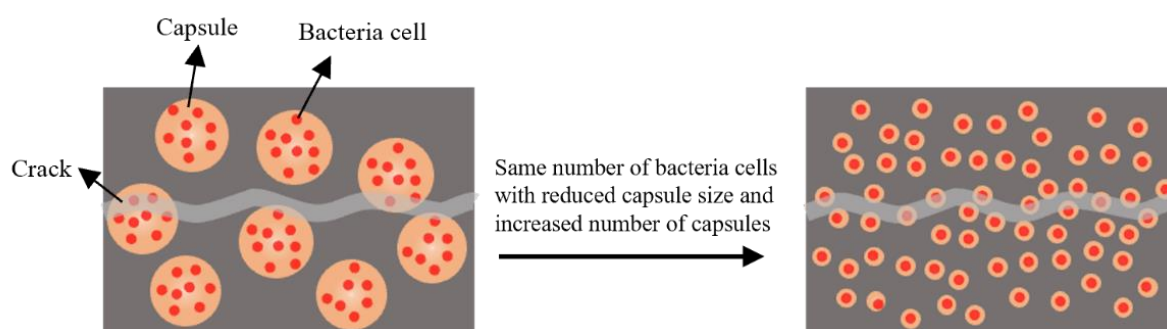


Figure 6.1 Schematic illustration of reduced capsule size and increased number of capsules can improve the chance of a crack hitting capsules and thus exposing the bacteria spores

Retaining the internal working properties of the native system while modifying the surface architecture of a biological cell has been a challenge, ascribing to the small size (typically around $1\mu\text{m}$) and the fluid wall of the biological cell (Balkundi, Veerabadran et al. 2009). To overcome these bottlenecks, LbL self-assembly technique has been proposed for surface modification of biological cells over the past years and demonstrated to be an effective solution (Balkundi, Veerabadran et al. 2009, Fakhrullin, Zamaleeva et al. 2009, Franz, Balkundi et al. 2010). LbL self-assembly process involves the sequential exposure of a charged substrate to polycation and polyanion solutions to achieve adsorption of complementary materials on the substrate surface (Liu, Wang et al. 2019). In this chapter, single *Bacillus cohnii* spores were encapsuled in silica nanoparticles via the LbL self-assembly.

Since the surface of *Bacillus cohnii* spores and silica nanoparticles are both negative-charged, the surface charge of the spore needs to be modified to positive for the assembling of silica nanoparticles. Poly(dimethyl diallyl ammonium chloride) (PDDA) is a commonly used polyelectrolyte in the cell modification, which is positive-charged, harmless to bacteria cells and can retain the metabolism of bacteria (Balkundi, Veerabadran et al. 2009). Accordingly, a layer of PDDA was

first self-assembled onto the bacteria spore surface via electrostatic attraction and the surface charge of the spore was modified from negative to positive. Subsequently, a layer of negatively charged silica nanoparticles was coated on the outer surface to complete the single bacteria spore encapsulation as illustrated in Fig. 6.2.

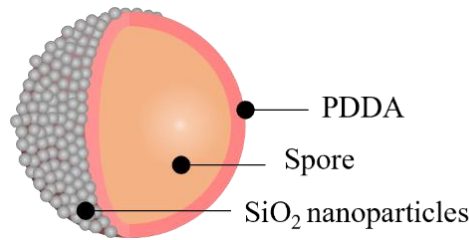


Figure 6.2 Schematic diagram of the section view of the single bacteria capsule

Silica is an inorganic material with high compatibility with PC-based mixes (Kumar, Singh et al. 2017, Palla, Karade et al. 2017). The silica nanoparticles on the capsule shell can potentially react with the surrounding cement via the pozzolanic reaction as illustrated in the conceptional sketch (Fig. 6.3). Specifically, when the single bacteria capsules are incorporated into the mix, the silica nanoparticles shell protects the spore from contacting the surrounding alkaline environment during mixing (Fig. 6.3(a)). As the hydration progresses, the silica nanoparticles on the shell could bond with the surrounding matrix via pozzolanic reaction (Fig. 6.3(b)). When a crack forms in matrix, it could break the shell and pass through the capsule because of the bonding between the silica nanoparticle shell with surrounding matrix. As a result, the spore inside can be exposed to engage healing (Fig. 6.3(c)-(d)).

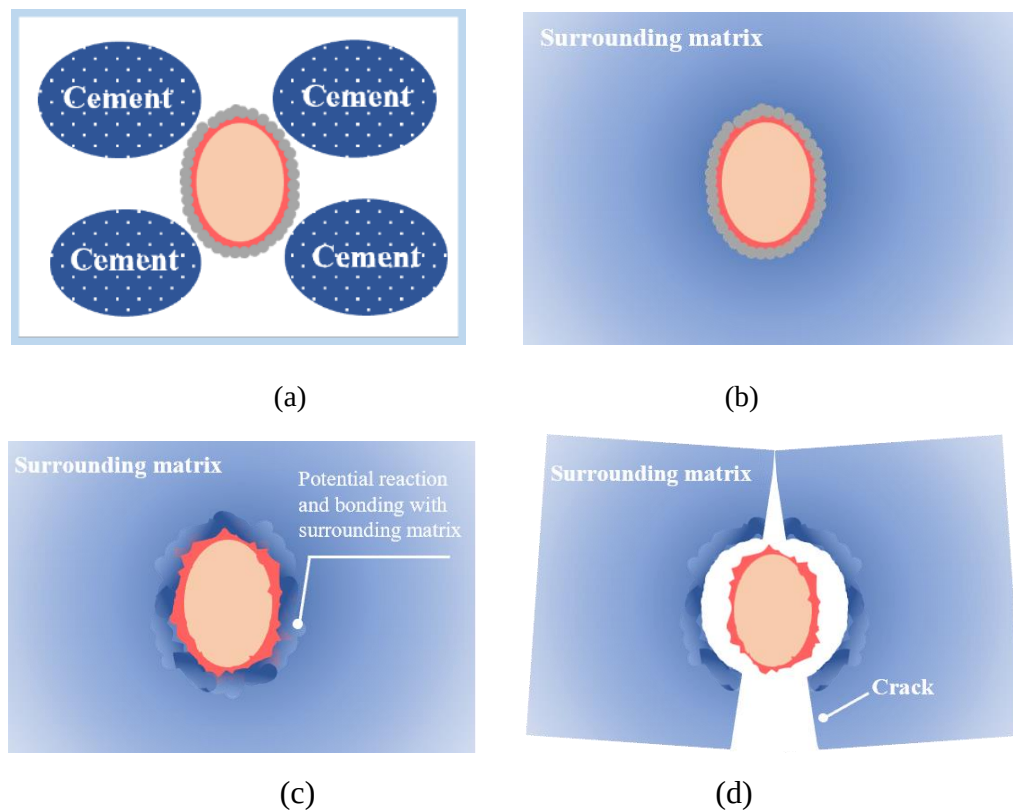


Figure 6.3 The mechanism of spore release and exposure when crack propagates

Overall, the single bacteria spore capsules were fabricated. The fabrication process and the resulted capsule were monitored and characterized via the zeta potential measurement and scanning electron microscope (SEM) observation. Afterwards, the resulted capsules were incorporated into PC paste. The hardened specimen was cracked, and the natural cracking surface was exposed to observe the bacteria releasing and exposure.

6.2 Experimental program

6.2.1 Bacteria spore preparation

Bacteria strain *Bacillus cohnii* from American Type Culture Collection was used in this study. The preparation of the bacteria spores followed the same procedure as described in Chapter 3.2.1.

6.2.2 Encapsulation of single bacteria spore through LbL self-assembly

The process began with cleaning the spores for encapsulation. The prepared spores were centrifuged at 9000 rpm for 10 minutes and the supernate was disposed. The spores were then re-suspended in distilled water. This procedure was repeated 3 times to ensure the spores were clean and free of any medium.

In the second step, the first layer (PDDA) was deposited onto spore surface to modify the surface charge. The prepared clean bacteria spores were first suspended in 20 ml distilled water (with a spore concentration of around 10^7 - 10^8 CFU/ml). The suspension was treated in an ultrasonic bath (70 W, 42kHz) for a few seconds to prevent the aggregation of spores. 20 ml PDDA solution ($M_w < 100$ kDa, Sigma-Aldrich) with a concentration of 1 mg/ml was treated in an ultrasonic bath (70 W, 42kHz) for 10 minutes followed by vortex mixing. The PDDA solution was then added into the spore suspension. After 15 minutes, the PDDA-encapsulated spore (PDDA-S) suspension was centrifuged at 9000 rpm for 10 minutes to remove any excess PDDA in the suspension. The PDDA-S pellet was re-suspended in distilled water and centrifugation was applied again to remove any residual PDDA in the solution. The clean PDDA-S was re-suspended in 10 ml distilled water.

In the third step, the second layer (SiO₂ nanoparticles) were deposited onto the PDDA-S surface. 40 ml SiO₂ nanoparticles suspension (22 nm in diameter, Sigma-Aldrich) with concentration of 2 mg/ml was treated in an ultrasonic bath (70 W, 42kHz) for 10 minutes, followed by vortex mixing to ensure the even dispersion of nano-SiO₂ particles. The SiO₂ nanoparticles suspension was then added into the PDDA-S suspension for the fabrication of SiO₂ nanoparticles and PDDA encapsulated single bacteria spore (nSiO₂-PDDA-S). After 30 minutes, the same centrifugation cleaning procedure was applied to remove the excess nano-SiO₂.

The zeta potential and morphology of the bacteria spores before and after each layer

of encapsulation were analysed by Malvern Nano-ZS Zeta Sizer and SEM (JSM-7600F), respectively. To prepare the samples for zeta potential measurement, 0.7 ml suspension was loaded to a DTS1070 cell for measurement, and each sample was run three times. To prepare the samples for SEM observation, ~50 μL spore suspension was dispensed on a glass slide and air-dried under ambient conditions. Afterwards, the glass slide was coated with platinum using auto fine coater (JEOL JFC1600) under 20 mA current for 40 seconds.

6.2.3 Observation of bacteria exposure on natural cracking surface

To check the bacteria exposure on the natural fracture surface from cracking, a small cylinder specimen (i.e., PNC) with a diameter of 25 mm and height of 20 mm was prepared. The size of the cylinder was designed according to the size of the SEM sample holder so that the sample could be directly loaded to the equipment to be observed via SEM. This approach enabled the preservation of the natural fracture surface and elimination of any disturbance caused by cutting and other sample preparation procedures. The mix design of specimen PNC was shown in Table 6.1. Before casting, nutrients were fully dissolved in the prepared water and followed by suspending single bacteria spore capsules in the solution. After that, the suspension was mixed with cement in a mixer to form a uniform mixture and the paste was casted into the mould. 24 hours after casting, the specimens were demolded and sealed in plastic bag until 28 days.

Table 6.1 Mix design of the samples for self-healing tests

Mix	Cement (g)	Water (g)	YE (g)	CaL (g)	Capsule
PNC	1500	480	15	30	4.65×10^{10}

At 28 days, the specimen was split with a MTS Landmark Servohydraulic Test System, as shown in Fig. 6.4. The loading rate was controlled at 0.01 mm/s, and the loading was terminated when the specimen split. Then the cross section of the

split piece was coated with platinum to check the morphology with SEM, and the elements were checked with energy dispersive X-ray spectroscopy (EDS).

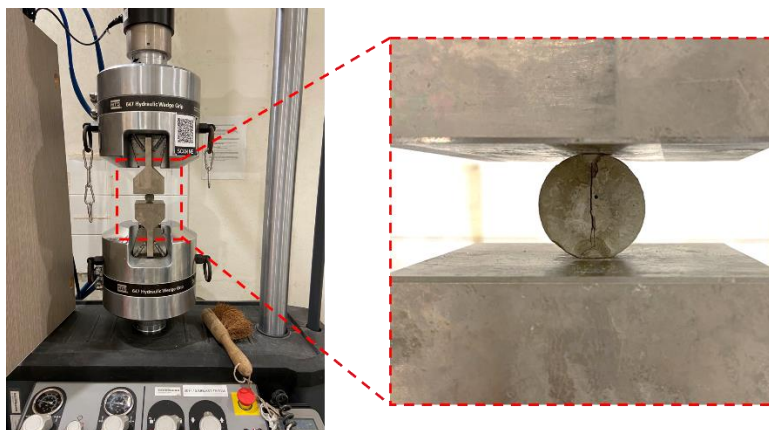


Figure 6.4 Splitting of specimen

6.3 Results and discussion

6.3.1 Encapsulation of single bacteria spore

Fig. 6.5 shows the morphology and zeta potential of the bacteria spores before and after LbL encapsulation. As can be seen, the spores have a size of $\sim 1 \mu\text{m}$ with a smooth surface and a zeta potential of around -27 mV . After the first LbL assembly of the positively charged PDDA, the zeta potential of the resulting spores changed to around $+35 \text{ mV}$. Furthermore, the surface of the resulting spores presented a wrinkled texture, indicating that the spore was wrapped with a polymer layer. The changes in the zeta potential and the morphology of the resulting spores indicated the successful encapsulation of single bacteria spore with the PDDA layer (i.e. PDDA-S) in the first LbL assembly.

After the second LbL assembly of the negatively charged SiO_2 nanoparticles, the zeta potential of the resulting spores changed to around -20 mV . During this process, the morphology of the cell surface was significantly altered. Differing from the

previous wrinkled texture, the surfaces of the resulting spores were rough with densely packed nanoparticles. The changes of the zeta potential and the morphology of the resulting spores indicated the successful encapsulation of PDDA-S with the SiO₂ nanoparticle layer (i.e. nSiO₂-PDDA-S) in the second LbL assembly. Overall, single bacteria spore capsules were successfully fabricated via the LbL self-assembly method. The size of the resulting single bacteria spore capsule remained to be around 1 μ m, indicating the proposed method did not alter the capsule size significantly.

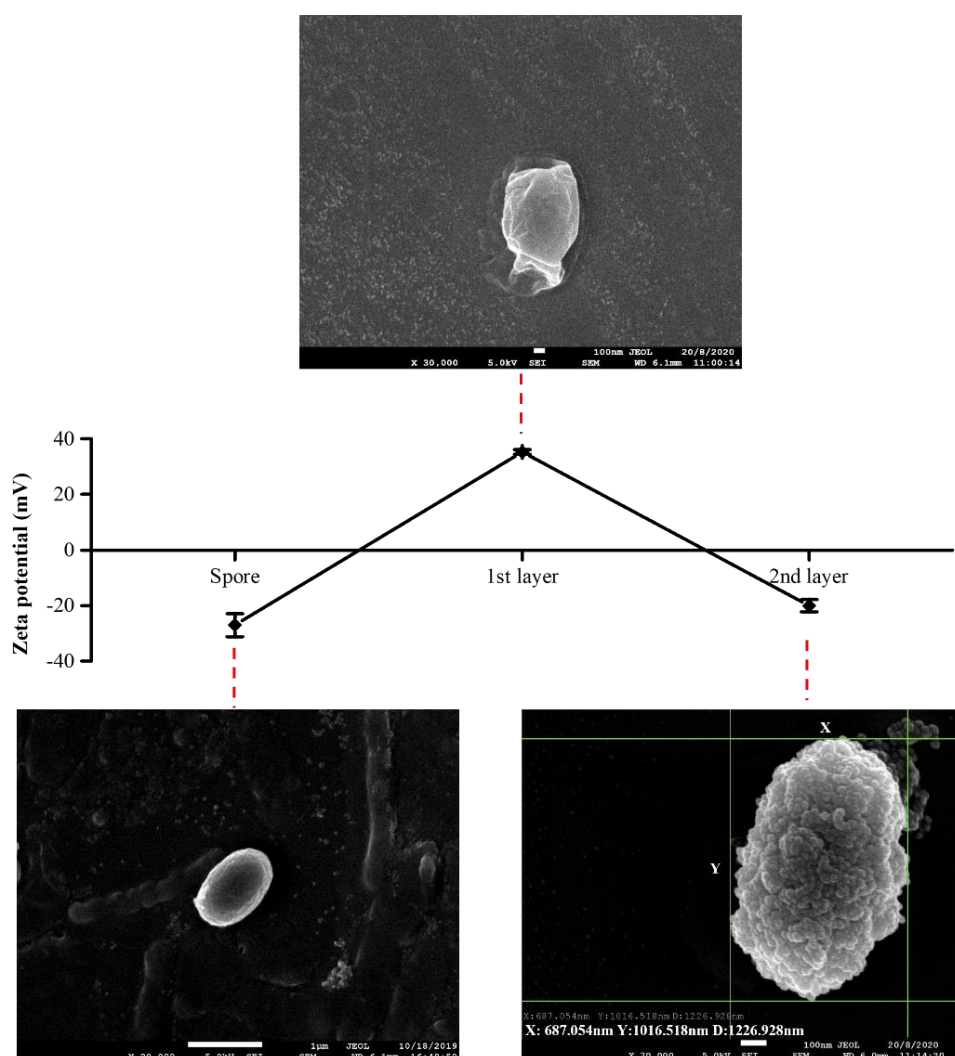


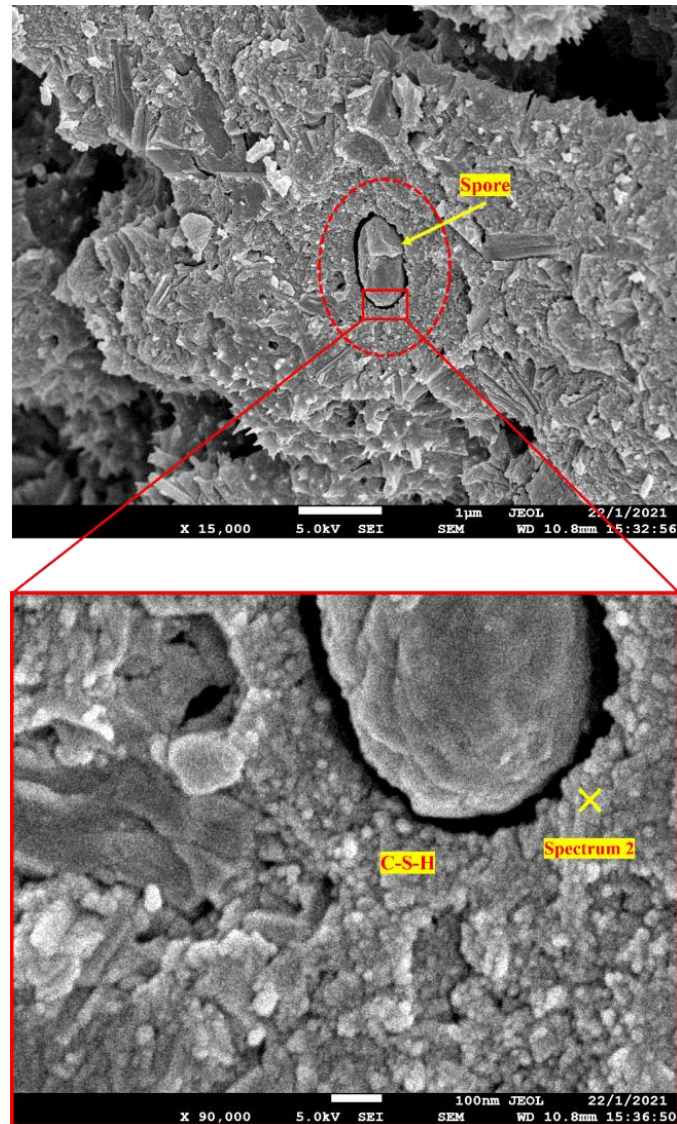
Figure 6.5 Zeta potential and morphology evolution of the bacteria spores at different encapsulation stage

6.3.2 Bacteria exposure on the natural cracking surface

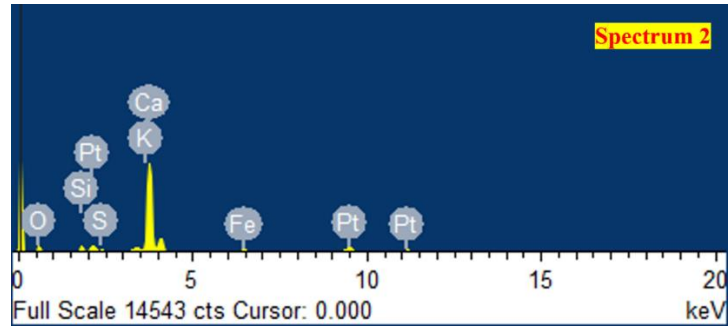
One of the key preconditions of bacteria-induced self-healing is the exposure of bacteria (spores) on the fracture surface. However, the mechanism of the bacteria exposure was rarely mentioned in previous studies because of the difficulties in: (i) determining the strength of the bacteria capsule, (ii) characterizing the bond between the capsule and the surrounding matrix, and (iii) investigating the cracking patterns of the matrix containing capsules. As self-healing is directly related to whether bacteria can be exposed after cracking, in this study, the prepared single bacteria capsules were incorporated into PC pastes and the exposure of bacteria on the fracture surface of the specimens was observed with SEM-EDS (Fig. 6.6).

Fig. 6.6(a) shows the morphology of the spore on the natural fracture surface. It is clear that the surface of the spore presented a wrinkled and rough texture, which was in line with the morphology of the spore surface with a layer of PDDA coating (Fig. 6.5). As marked by the red dashed line, the spore surface was surrounded by a similar phase in the range of 0.15-0.6 μm measured according to the scale bar shown on the figure. The image of the surrounding region of the spores taken at a higher magnification indicated the presence of a gel-like phase. This was supported by the information presented in Fig. 6.6(b), where the presence of Si, Ca, and O was observed within the phase, indicating the formation of C-S-H gel. The determination of this phase was further supported by comparing the image with a typical SEM image of C-S-H provided in the literature (Kim, Kang et al. 2017). Overall, the SEM-EDS results revealed that the shell of the capsule has potentially reacted and bonded with the surround cement matrix via the pozzolanic reaction to form C-S-H. Before the single capsule was added to the paste, the silica shell of the capsule was bonded with bacteria through electrostatic attraction. As the shell gradually participated in hydration, the shell was consumed and bonded with matrix through chemical reactions, which enabled the bonding between capsule

shell and bacteria gradually disappeared. When a crack propagated and hit the capsule, the bacteria will be released since the cell was free of the silica shell, as shown in Fig. 6.6(a).

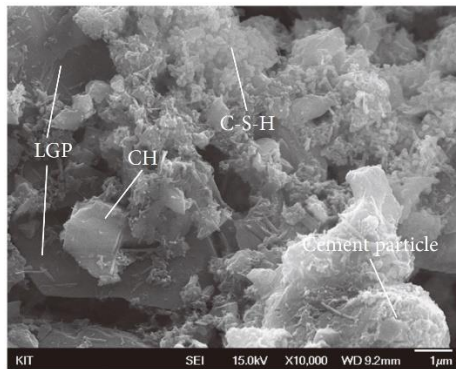


(a)

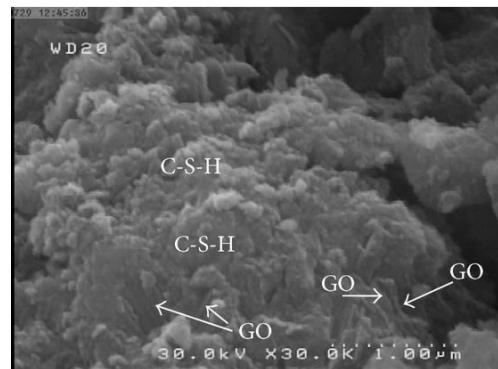


(b)

Figure 6.6 (a) SEM image of the bacteria spore exposure on the fracture surface and
(b) the elements within the phase surrounding bacteria



(a)



(b)

Figure 6.7 SEM image of C-S-H gel in literatures (Babak, Abolfazl et al. 2014, Kim, Kang et al. 2017)

6.4 Summary

This chapter developed a single bacterial capsule by LbL self-assembly of PDDA and silica nanoparticles. The zeta potential measurement and SEM imaging revealed that single bacteria was successfully encapsulated layer-by-layer by PDDA and silica nanoparticles by virtue of the alternatively depositing to the surface of bacteria spore. The natural cracking surface observation revealed that the bacteria spore was released from the capsule and was exposed after cracking. Overall, the

single bacterial capsule was successfully fabricated, and the bacteria was able to exposure upon cracking. In the next step, the self-healing performance and the influence of the capsules on the strength of the matrix shall be examined.

CHAPTER 7 SELF-HEALING PERFORMANCE OF SINGLE BACTERIA CAPSULE AND ITS INFLUENCE ON MATRIX STRENGTH

7.1 Introduction

In this chapter, the single bacterial capsules were incorporated into Portland cement matrix together with yeast extract (YE) and calcium lactate (CaL) to evaluate the self-healing performance by means of crack closure monitoring and water passing rate test. The influence of capsule inclusion on the compressive strength of the matrix was investigated.

7.2 Experimental program

7.2.1 Specimen preparation

To evaluate the self-healing performance, a cement paste (PNC) with the inclusion of single bacteria spore capsules, yeast extract (YE), and calcium lactate (CaL) was prepared as shown in Table 7.1. The Portland cement (PC) used in this study was purchased from EnGro Ltd (Singapore), complying with SS EN 197-1: 2014. The chemical composition of PC is the same as reported in Chapter 3, Section 3.2.4, Table 3.4. A cement paste (PNS) with the inclusion of unprotected bacteria spores, YE, and CaL, and a pure cement paste (P) were also prepared as the control mixes. All the three mixes have the same water-to-cement (w/c) ratio of 0.32. In the current study, the mix was named based on the following convention: P stands for paste, N stands for nutrients, S stands for unprotected bacteria spores, and C stands for single bacteria spore capsules.

Table 7.1 Mix design of the samples for self-healing tests

Mix	Cement (g)	Water (g)	YE (g)	CaL (g)	Spore (CFU)	Encapsulation
P	1500	480	0	0	0	-
PNS	1500	480	15	30	4.65×10^{10}	No
PNC	1500	480	15	30	4.65×10^{10}	Yes

Before casting, nutrients were fully dissolved in the prepared water and followed by suspending single bacteria spore capsules (or unprotected bacteria spores) in the solution. After that, the nutrients and bacterial spores suspension were mixed with cement in a mixer to form a uniform mixture. Cylindrical disc specimens with a height of 25 mm and a diameter of 75 mm were prepared. Firstly, half of the mould was filled with the fresh paste, and a steel mesh (70 mm in diameter) was placed on the half-filled mould, followed by filling up the mould. For each mix, 4 specimens were prepared for the self-healing test. 24 hours after casting, the specimens were demolded and sealed in plastic bag until 28 days.

7.2.2 Pre-cracking and healing conditioning regime

At 28 days, the disc specimen was cracked on a MTS Landmark Servohydraulic Test System following the same procedure as described in Section 3.2.4, as shown in Fig. 7.1. On each sample, 5 observation locations were marked along the length of crack on the surface of the disc specimen as shown in Fig. 7.2.



Figure 7.1 Pre-cracking of disc specimen



Figure 7.2 A split crack at the center of the specimen after pre-cracking and 5 observation locations along the length of crack on the surface of the disc specimen

The pre-cracked disc specimens were then conditioning in wet-dry cycles. One cycle consists of one day full submersion in water (27°C), followed by dry in the ambient air (27°C, 80% RH) for a day. The wet-dry conditioning resembles typical outdoor exposure of concrete structure where alternating wetting (e.g., raining day) and drying (e.g., windy or sunny day) is expected. Each mix was conditioning in its respective container to prevent cross contamination. The conditioning water was

replaced and replenished with clean tap water after each conditioning cycle.

7.2.3 Self-healing performance evaluation

To evaluate degree of healing, surface crack width and water passing rate before and after wet-dry conditioning were measured. The surface crack width at each observation location on the disc specimen was measured by a Nikon stereoscopic microscope (SMZ 745T). The surface crack width reduction of the pre-cracked disc specimen after conditioning was calculated based on the Eqn. (3.3) in Section 3.2.4. The water passing rate of the disc specimen was determined via the constant water head method where the amount of water passing through the specimen is counted (Sisomphon, Copuroglu et al. 2012, Zheng and Qian 2020). The testing procedure and the calculation of water passing rate was in accordance with Section 3.2.4. Water passing rate of each specimen was measured three times and the average value was reported. The water passing rate reduction of the pre-cracked disc specimen after conditioning was calculated as per Eqn. (3.4) in Section 3.2.4.

To check the healing performance within the crack, after 50 cycles of conditioning, specimen was loaded to the same crack generation set-up, and the specimen was completely split to expose the cross section of the crack. To examine the morphology of the self-healing products, the PNC specimens were cut into small pieces with a precision cutter (BUEHLER, IsoMet 4000) after 50 healing cycles were completed. The piece with self-healing products was freeze-dried for 2 days and then coated with platinum to check the morphology with SEM, and the element within the products were checked with energy dispersive X-ray spectroscopy (EDS).

7.2.4 Compressive strength test

The same mixes (P and PNC) for self-healing was used to study the impact of spore capsules addition on the compressive strength of the matrix (Table 7.2). Two

additional mixes, PN (cement paste with YE and CaL) and PC (cement paste with single bacteria spore capsules), were also prepared and tested. The mixing procedure was the same as that described in Section 7.2.1, and the fresh pastes were casted into 50 mm cube moulds. After 24 hours, the specimens were demoulded and sealed in a plastic bag. Compressive strength of the specimen was determined at the age of 7, 14 and 28 days in accordance with ASTM C109/C109M-13 at a loading rate of 55 kN/min. For each mix, three specimens were tested and the average value was reported.

Table 7.2 Mix design for compressive strength test

Mix	Cement (g)	Water (g)	Spore capsules (CFU)	YE (g)	CaL (g)
P	1500	480	-	-	-
PNC	1500	480	4.65×10^{10}	15	30
PN	1500	480	-	15	30
PC	1500	480	4.65×10^{10}	-	-

7.3 Results and discussion

7.3.1 Surface crack width reduction

Overall, the cracks generated on the specimens varied between 200-450 μm . To investigate the crack healing performance, the average crack reduction in the range of 200-350 μm and 350-450 μm were plotted against conditioning cycles. As can be seen from Fig. 7.3, crack width generally reduced with increasing conditioning cycles and smaller cracks exhibited higher crack width reduction under the same conditioning cycles. Among the three mixes, PNC has shown better crack healing performance than the other two groups regardless of healing cycles and crack width, indicating the effectiveness of the capsules. When crack width was below 350 μm , the crack width reduction of PNC reached 100% without any variation at 40 and 50 cycles, meaning that all the cracks in PNC mix reached fully closure after 40 cycles of conditioning. The results suggested that the healing engaged by the capsules

were robust and consistent. When the crack width was even larger, the difference of PNC mix with control mixes regarding the crack healing efficiency and robustness were more significant and telling.

Among the larger cracks of 350-450 μm , very limited surface crack width reduction was observed for mixes P and PNS during the whole conditioning period, with both mixes were below 50% after 50 conditioning cycles. Alternatively, mix PNC achieved a crack reduction of more than twice of the other 2 mixes from 30 cycles onwards, and revealed more than 90% closure for cracks $> 350 \mu\text{m}$ after 50 cycles of conditioning.

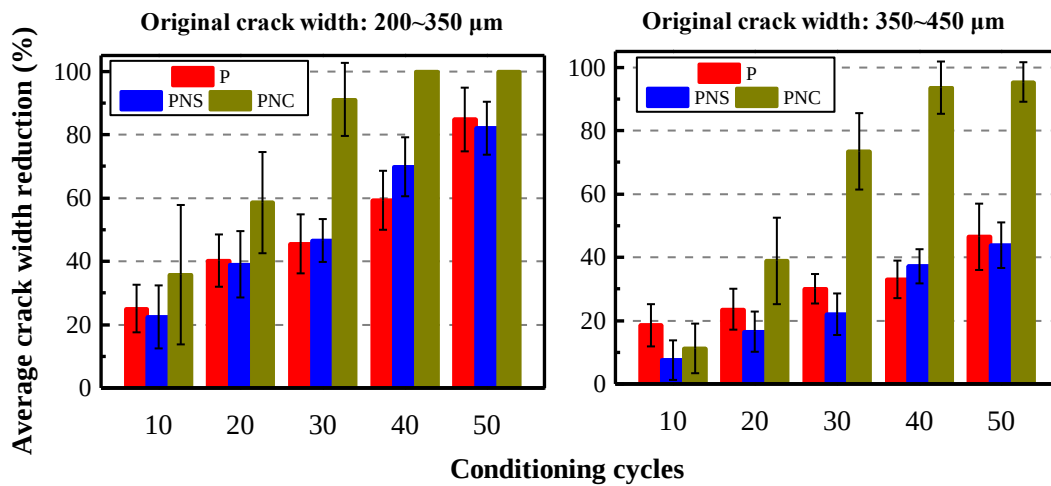


Figure 7.3 Average crack reduction after different condition cycles

Specifically, the surface crack width after 50 wet-dry conditioning cycles was plotted against the original crack width at the same observation location, as shown in Fig. 7.4. The y-axis represents the surface crack width after pre-determined conditioning cycles, and the x-axis represents the surface crack width before conditioning. For each figure, 20 data points were collected from 4 specimens and 5 observation locations on each specimen. The 45 degree solid line represents no reduction of the crack width, while the grey dashed line indicates 50% crack width

reduction. The average surface crack width reduction with standard deviation (%) after pre-determined conditioning cycles was summarized in the table below each figure.

After 50 conditioning cycles, mix PNC showed a significant difference from other two mixes regarding the location of the data points on the figure. In PNC mix, almost all the data points fell on or close to the x-axis, indicating that the healing ratio was close to 100%, while the data points in the other two mixes were mostly in the middle of the figure which represented partial crack healing. According to the average crack width reduction value summarized in the table, the healing of cracks blow 350 μm in PNC mix reached 100% with no variation while other two mixes could not reach fully healing, which was in line with Fig. 7.3. For cracks larger than 350 μm , the crack width of PNC reduced by 95%, while that in mixes P and PNS reduced by 47% and 44%, respectively. Mix PNC not only had more than twice of the crack reduction, but also had less variation of the data which can be told both from the minor dispersion degree of the data points on the figure of PNC and the standard deviation calculated in the table (6.2%). Accordingly, the high crack reduction value has indicated the efficiency of the capsule on crack healing, and the small variation of the data has indicated the consistency of the healing performance. The microscopic image of a crack before and after 50 cycles of conditioning in each mix is shown in Fig. 7.5. Similar crack width around 400 μm was selected to compare the healing performance. The figure clearly shows that the crack from PNC mix was fully healed with white crystal grew within the crack and sealed the gap completely. However in the other 2 mixes, only a few white crystals can be observed along the edge of the crack, revealing very limited crack healing in the Mix PNS and Mix P. Overall, the microscopic images were straightforward and clearly demonstrated the effectiveness of the capsules on crack healing.

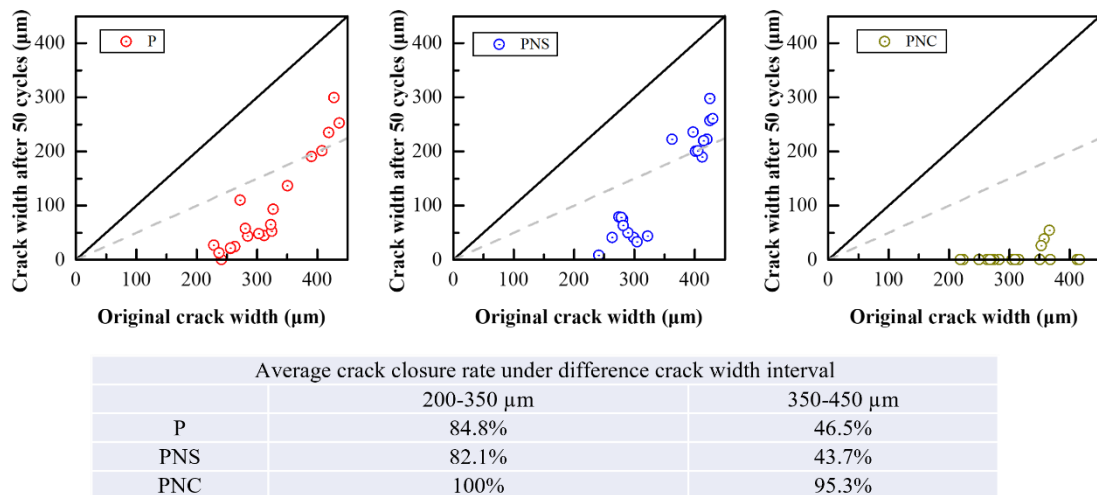


Figure 7.4 Crack width and crack width reduction (%) after 50 conditioning cycles

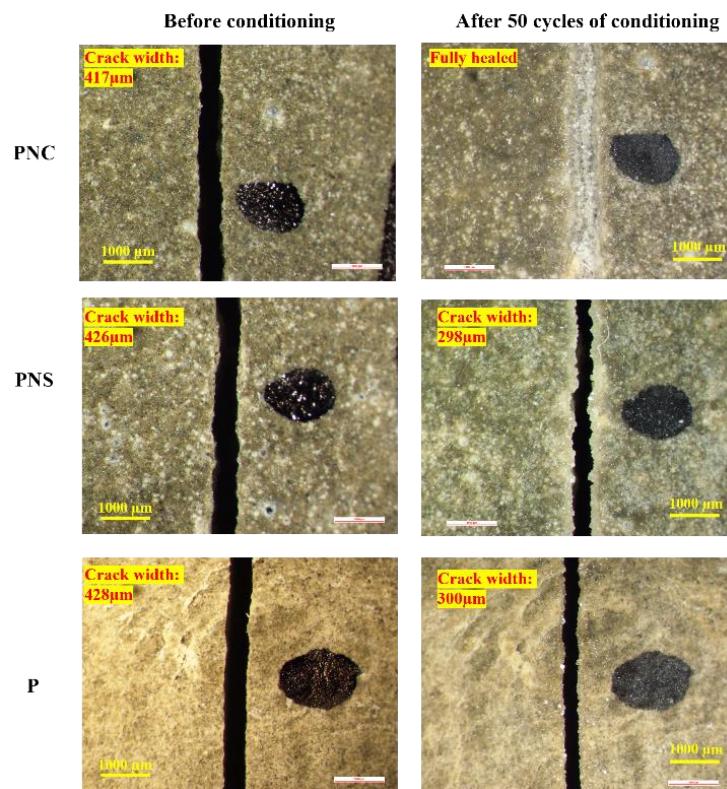
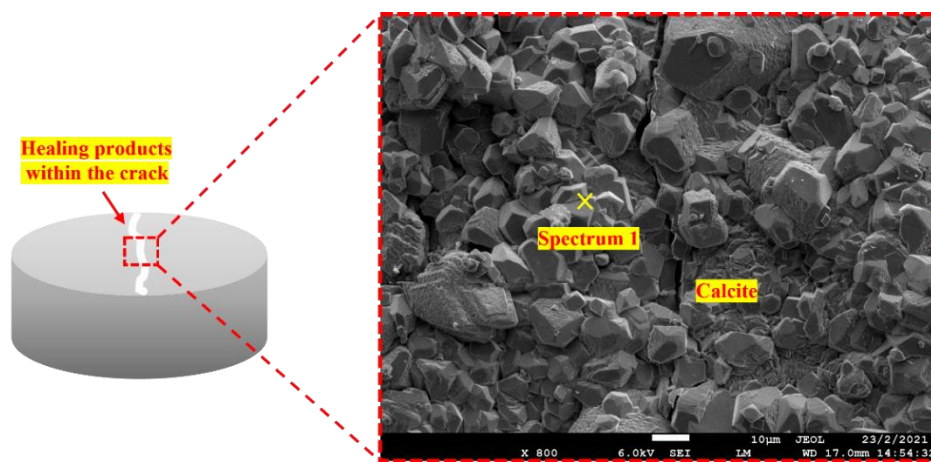


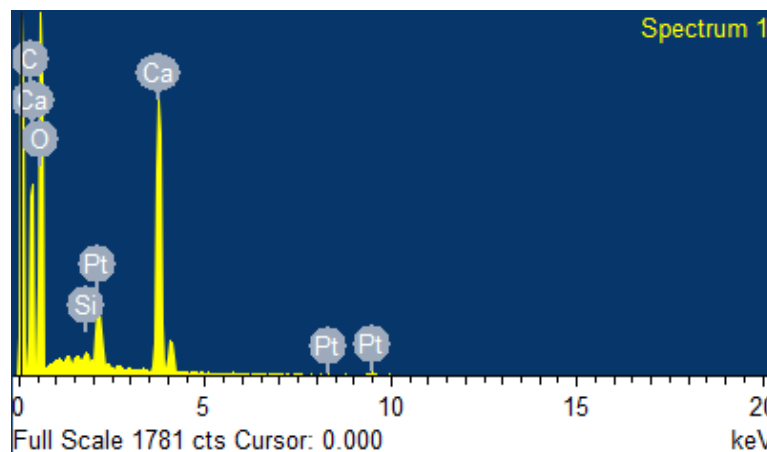
Figure 7.5 Microscopic image of crack before and after 50 cycles of condition in Mix PNC, Mix PNS and Mix P

The morphology of healing products resulting from a single bacteria spore capsule

is shown in Fig. 7.6(a). As can be seen, the healing products presented a layered rhombohedral morphology with a crystal size varying from 4 to 10 μm . With the presence of elements Ca, C, and O, as shown in Fig. 7.6(b), it can be concluded that the healing products from the single bacteria spore capsules were mostly calcite. The morphology of the products was in line with the findings of previous studies that reported the use of the same bacteria and precursor (Tziviloglou, Wiktor et al. 2016).



(a)



(b)

Figure 7.6 (a) SEM image of the healing products resulted from the single bacteria capsules and (b) the elements in the healing products

Fig. 7.7 shows a representative PNC disc specimen after 50 cycles of wet-dry

conditioning. As can be seen from the figure, the entire crack with length of 5.9 cm along the longitudinal direction was fully sealed with white colored healing products. This highlights the consistency of healing along the entire length of the crack in the current study, which may be attributed to the well dispersion of the single bacteria spore capsule in the cement matrix.



Figure 7.7 A representative fully healed crack in PNC mix

Overall, the above results indicate that the newly developed single bacteria spore encapsulation can effectively protect the bacteria spores and engage efficient and robust healing of large crack ($> 400 \mu\text{m}$) in cement-based material. Without proper protection, bacteria spores are not able to survive in cement-based mixes (PNS) (Jonkers, Thijssen et al. 2010), resulting in limited crack width reduction comparable to that in the pure cement paste mixes (P) without the addition of bacteria spores. The crack width reduction observed in mixes PNS and P was attributed to the intrinsic healing of the cement paste, where the re-hydration of unreacted cement particles and the carbonation of portlandite led to precipitation of hydrate/carbonate phases within the crack. This is particularly true for the current study as the cement matrix has a low w/c of 0.32.

7.3.2 Water passing rate reduction

The average water passing rate reduction (%) of each mix was plotted against healing cycles in Fig. 7.8. Each column represented an average of 12 test results (i.e. 4 specimens in each mix, and water passing rate test was performed three times for each specimen). The water passing rate reduction (%) increased with increasing conditioning cycles. Mix PNC showed the highest water passing rate reduction, followed by mixes PNS and P at all conditioning cycles. After 30 cycles of wet-dry conditioning, the water passing rate reduction of mix PNC reached almost 90%. As the conditioning continued to 50 cycles, the water passing rate reduction of PNC mix reached $100\pm0\%$, while that of P mix and PNS mix were $35.1\pm4.3\%$ and $51.3\pm17.9\%$, respectively. The average value of water passing rate reduction of PNC mix was around twice of the other two mixes, demonstrating the efficiency of the capsule on transport property recovery of the cracked specimen. Moreover, after 50 cycles, all four PNC specimens reached 100% water passing reduction without variation. The results reveal that the transport property recovery engaged by the single bacteria spore capsules was consistent and robust.

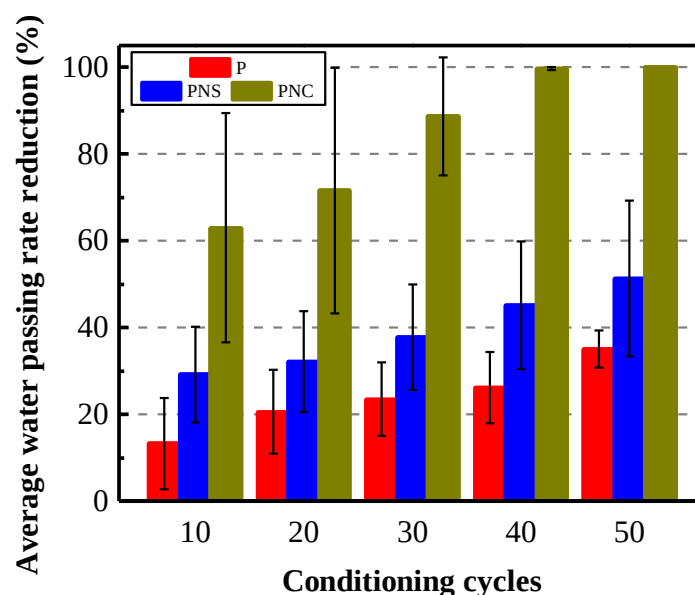
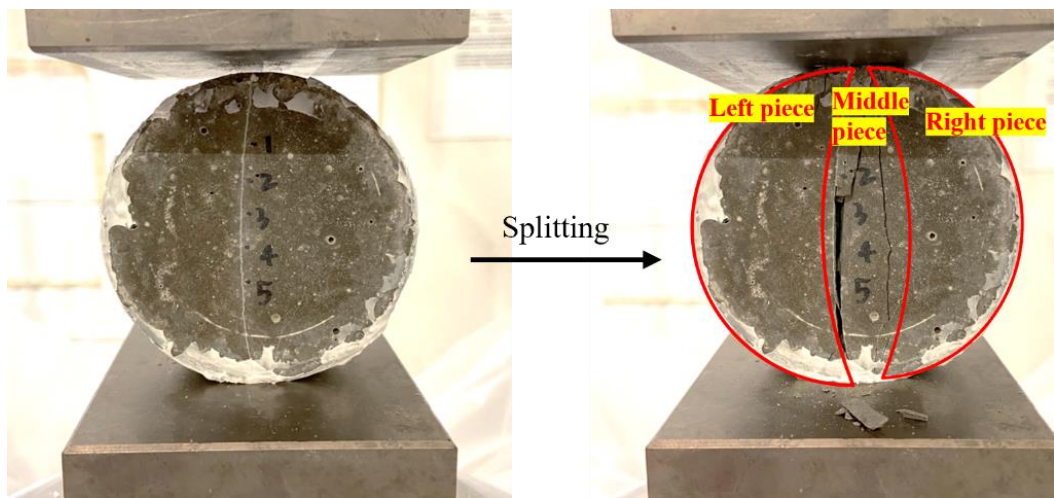


Figure 7.8 Water passing rate reduction rate under different healing cycles of group PC, group PNS and group PNC

After water passing rate test, a healed PNC specimen subjected to 50 conditioning cycles was split open along the healed crack as shown in Fig. 7.9(a). A close examination on the healed crack revealed that the entire crack from surface to the interior was covered with white healing products (Fig. 7.9(b)), resulting complete recovery of the transport properties of the PNC mix. This highlights the efficiency and robustness of healing in the current study.



(a)

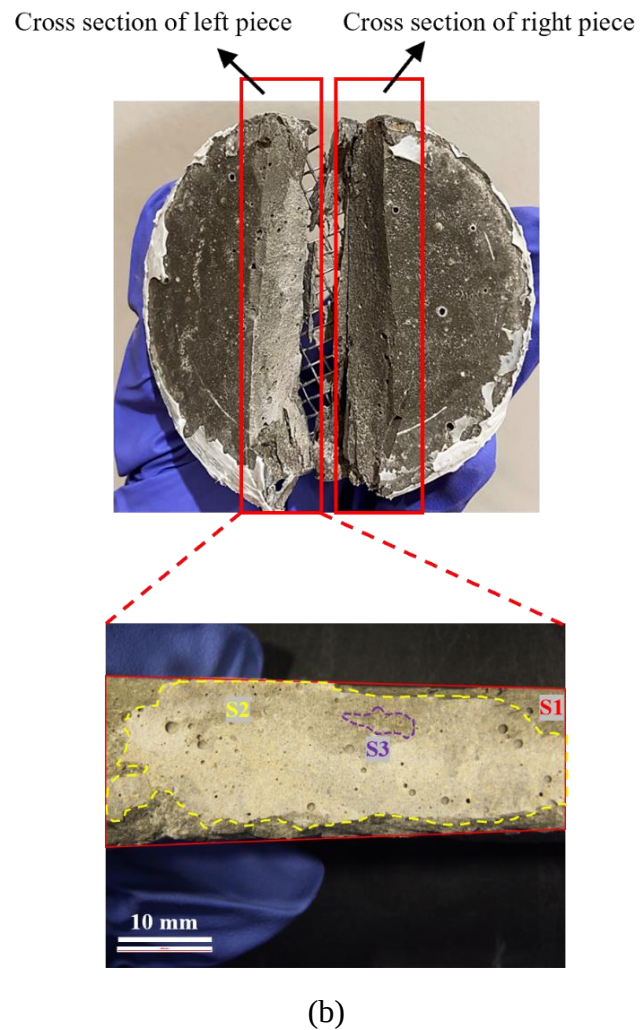


Figure 7.9 (a) A healed PNC specimen subjected to 50 conditioning cycles was split open along the healed crack and (b) white healing products were observed on the entire crack from surface to the interior

7.3.3 Compressive strength

Fig. 7.10 compares the compressive strength of mixes P, PC, PN, and PNC to demonstrate the effect of the inclusion of the capsules and/or nutrients on the mechanical properties of the matrix. Mixes PC and P revealed comparable strengths, showing that the addition of single bacteria spore capsules did not have a negative impact on the compressive strength of the mix. However, the addition of nutrients (PN) led to a strength reduction of ~18% at the age of 28 days. This effect was

reported in earlier studies, where the addition of yeast extract was found to decrease the strength of the matrix while calcium lactate had a minor influence (Jonkers, Thijssen et al. 2010, Schreiberova, Bílý et al. 2019). Accordingly, Mix PNC showed comparable compressive strength to PN which was lower than Mix P due to the presence of nutrients.

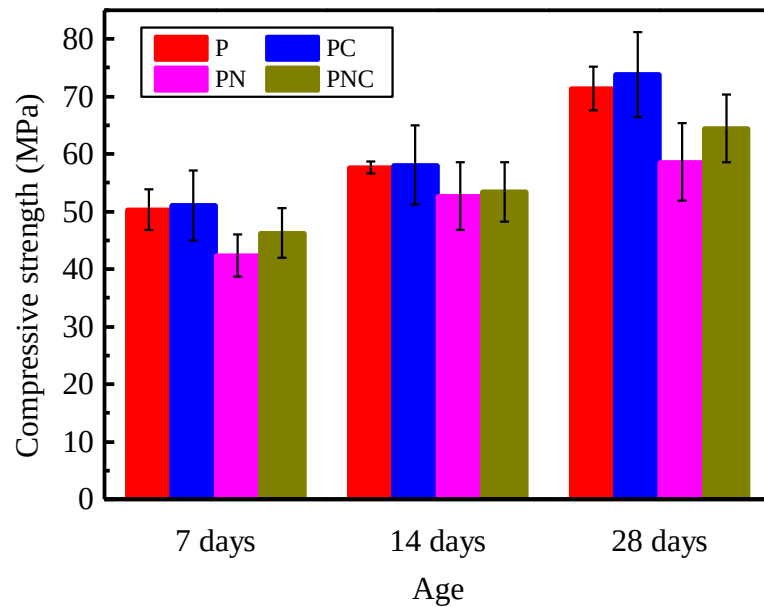


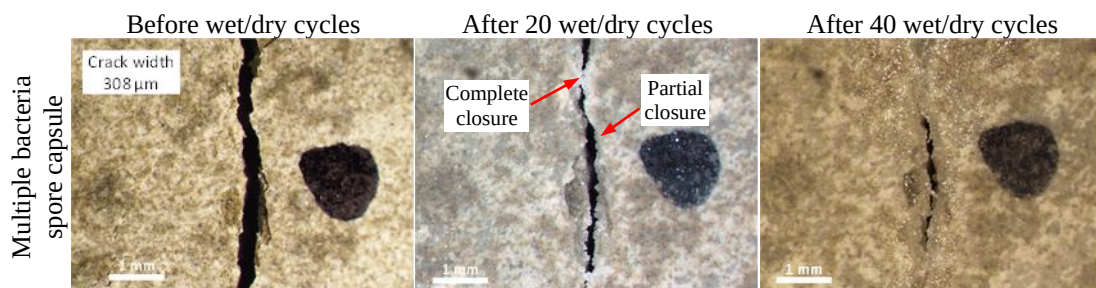
Figure 7.10 Compressive strength tests results

7.3.4 Discussion

It is worth noting that it takes 40-50 wet/dry cycles to reach complete crack closure in the current study which seems to be longer than the reported results in the published literature. One of the reasons is that the study focuses on the healing of large crack with width above 200 μm . For specimens with crack width below 200 μm , complete crack closure can be achieved in 10 wet/dry cycles. Another reason can be related to the variation of bacteria cell concentration along the crack path as illustrated in Fig. 6.1. For previous studies, multiple bacteria spores were encapsulated in one capsule, leading to high variation of bacteria cell concentration along the crack path. At locations where capsules are present, faster healing

performance can be expected because of high local concentration of bacteria cells. At the same bacteria loading, the incorporation of single bacteria spore capsules leads to a more uniform distribution of bacteria cell in the matrix. As a result, the crack can hit more capsules along its path, i.e., bacteria spores would be observed more evenly along the crack path. However, each capsule location only has one bacteria cell. This resulted in a more uniform healing along the entire crack (i.e., improved consistency) but slower healing rate.

Fig. 7.11 compares the surface crack closure engaged by the typical capsule where multiple bacteria were encapsulated in one capsule (top) and the single bacteria spore capsules in the current study (bottom). The two cases used the same bacteria type and bacteria loading. As can be seen, the closure rate and degree along the crack length is not consistent in the multiple bacteria spore capsule specimen. After 20 wet/dry cycles, complete closure in some spots while partial closure in other locations can be observed in the specimen (Fig. 7.11 top). This highlights the inconsistent healing when such capsules are used to engage self-healing. While this may be overcome by increasing the capsule dosage, it can lead to higher cost, reduced workability, and less efficiency use of such technology. On the contrary, the PNC specimen with single bacteria spore capsule shows much more uniform closure rate and degree along the crack (Fig. 7.11 bottom). As revealed above in Fig. 7.7, the entire crack (5.9 cm) was fully sealed with white colored healing products.



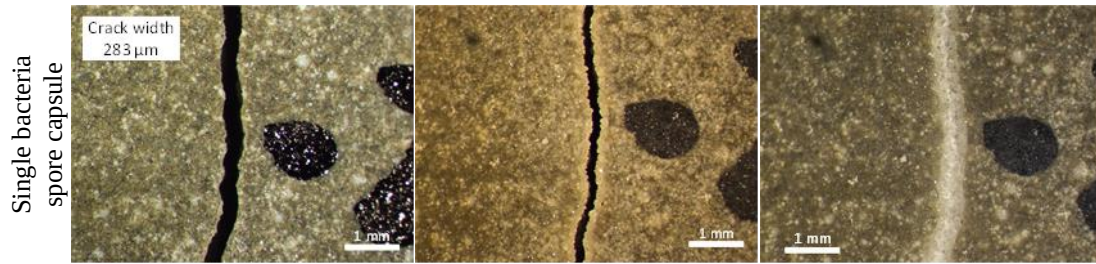


Figure 7.11 Surface crack closure engaged by the typical capsule where multiple bacteria were encapsulated in one capsule (top) and the single bacteria spore capsules in the current study (bottom)

7.4 Summary

In this chapter, the self-healing performance of the single bacterial capsule was evaluated by incorporating the capsules together with YE and CaL into PC paste. The cement paste incorporating the capsules showed efficient and robust healing of large cracks. Cracks with width less than 350 μm exhibited complete closure while cracks with width of 350-450 μm showed 95% closure together with 100% water passing rate reduction after 50 wet-dry conditioning cycles on cracked disc specimens. Healing products in the form of microbial induced calcite precipitation was observed along the entire crack from the surface to the interior. This suggests the newly developed single bacteria spore capsules improves distribution uniformity of the capsule in the matrix and thus the chance of a crack hits the capsules increases because of the small capsule size and increased capsule number. As a result, the efficiency and more important the consistency to engage healing is greatly enhanced in the current study. Besides, the single bacteria spore capsules have good compatibility with cement matrix. The inclusion of the capsules did not compromise the compressive strength of the matrix owing to its small size and good compatibility with the surrounding matrix.

CHAPTER 8 CONCLUSION AND FUTURE WORK

Overall, this study has developed bacteria-based microcapsules for self-healing concrete. Specifically, the study started on the selection of reactive magnesia cement to encapsulate bacteria spore, followed by fundamental studies on investigating the bacteria survivability and precipitation capability in the RMC-based environment. Based on that, the microcapsule was improved to a one-part system by encapsulating nutrients with bacteria in the same capsule. Afterwards, further development has been done by adopting single bacteria encapsulation technology to form a single bacteria microcapsule where the size of capsule can be controlled around 1 μm and the capsules can have better dispersion in the matrix. The major findings that revealed in the studies are summarized in following section.

8.1 Findings of the research

8.1.1 Functionally graded reactive magnesia cement (RMC)-based capsules:

RMC-B capsule and RMC-BN capsule

Reactive magnesia cement (RMC) presented to be a proper material for the encapsulation of bacteria for self-healing concrete. When bacteria was encapsulated in RMC to form a RMC-B capsule, viable bacteria can remain at a certain high level in the capsule. After the fabrication of the capsule was completed for 28 days, the number of the viable bacteria in the capsule kept at same level, indicating the long-term viability of bacteria in the RMC-B capsule. The structure of the RMC-B capsule presented to be gradient strength from surface to core with dense hydromagnesite formed on the surface and porous brucite and RMC in the core. After adding 2.3% RMC-B capsule (by cement mass) together with yeast extract and calcium lactate into PC, it can engage crack closure of 93% with average crack width of 243 μm and water passing rate reduction rate of 80% after subjected to 5

wet/dry cycles in water. Microstructure observations revealed the dense formation of layered rhombohedral calcite along the entire crack from the surface to the interior. Moreover, the addition of the RMC-B capsule increased the matrix 28-day compressive strength by 18%.

Fundamental studies have shown that the high survivability of bacteria in RMC was related to the low alkalinity and dry internal environment of RMC paste after the carbonation was completed. Bacteria spore itself can only tolerate alkalinity up to 12. Once the pH reached 13 or higher, the spores would lose activity completely. The pore solution of fresh RMC paste had pH of 11 while PC paste had pH higher than 13, and the internal environment of the paste presented to be dry after carbonation was completed where no pore solution can be extracted. Therefore, bacteria spores were able to keep its viability in the RMC-based mixes rather than in PC-based mixes. Crack healing engaged by the MICP showed that, the viable bacteria spores in RMC paste were highly active when it was activated by nutrients, and able to precipitate carbonates and seal crack in very short conditioning period. Altogether, RMC was a friendly encapsulation material in terms of the bacteria viability and bioactivity.

On the basis of RMC-B capsule, further development was conducted to form a RMC-BN capsule where nutrients were encapsulated together with bacteria. This self-contained capsule mainly had two advantages. First of all, bacteria spores were easier to reach nutrients during healing process as the nutrients were in the capsule rather than dispersed in the matrix. Secondly, nutrients were found to negatively affect both fresh and hardened properties of the matrix, therefore the encapsulation of nutrients could avoid the direct contact of nutrients with matrix. The RMC-BN capsule was also functionally graded, while the surface of the RMC-BN capsule was dense nesquehonite and the porous core was made up of RMC, brucite and nutrients (YE and CaL). After adding 5.3% RMC-BN capsule to PC paste by

cement mass, the 28-day compressive strength of the matrix was increased by 10%. And the specimens with incorporation of RMC-BN exhibited superior performance on the closure of large cracks, the recovery of transport property, and internal healing of the crack. After conditioned in wet/dry cycles in water for 50 cycles, Mix P-BNC has reached an average 90.1% crack width reduction of all the cracks over 350 μm , which was around twice of the control mix. And the average water passing rate reduction of P-BNC reached 89.3%, which was 2.7 times of that of control mix. And the UPV of Mix P-BNC was recovered 97% while that of Mix P was below 70%.

8.1.2 Single bacteria spore encapsulation through layer-by-layer self-assembly of poly(dimethyldiallyl ammonium chloride) and silica nanoparticles

Single bacteria encapsulation technology has been adopted to control the capsule size and the bacteria loading amount into the capsule to ensure each cell presented to be an individual microcapsule and the size of the capsule remained around 1 μm which was the typical size of the bacteria. Besides, the single spore encapsulation can result in better dispersion of bacteria in the matrix. Accordingly, layer-by-layer technique was used to assemble poly(dimethyldiallyl ammonium chloride) and silica nanoparticles to spore surface. Zeta potential and SEM image have monitored the encapsulation process and showed that each bacteria cell was encapsulated individually, and the size of the microcapsule was controlled around 1 μm . After adding the microcapsule into matrix, it can be observed from the natural cracking surface that spore was released from the capsule due to the surface silica nanoparticles were reacted with the matrix as the hydration progressed.

The strength test showed that the addition of the single capsule to the matrix did not compromise the strength of the matrix. And the incorporation of single bacteria capsules in the PC paste led to complete healing of cracks (<350 μm) and 95%

healing of cracks (350~450 μm) after 50 wet-dry conditioning cycles, outperforming the control mixes without capsules by more than twice. Besides, the incorporation of single bacteria capsules in the PC paste decreased the water passing rate through the crack by 100%, indicating all the cracks were impermeable to water after 50 cycles of conditioning, while the water passing rate reduction of control mixes was both below 50%. The results have shown that the single capsule was compatible with the matrix, and it was able to engage stable and robust healing of large cracks.

8.2 Future work

Some future work regarding the RMC-based capsules is listed as follows:

1. Parametric studies will be conducted on the optimization of the RMC-BN capsules. The dosage of the bacteria concentration, yeast extract and calcium lactate will be varied to establish their relationship with self-healing performance so that the optimal mix design of the capsule can be determined. Afterwards, the dosage of the RMC-BN will be varied to investigate how the capsule dosage influence the matrix properties (i.e., fresh and harden properties) and the self-healing performance.
2. More characterizations on the capsule properties will be carried out. The production process of the RMC-based capsules resulted in an irregular granular shape of the capsule, leading to inhomogeneity of the formed hard shell. Hence, the relationship between the size of RMC-B capsule, the thickness of the carbonized dense shell, strength of the capsule and other properties will be furthered studied.
3. Further investigation and quantification of self-healing along crack depth with the newly developed approach will be conducted, and the repeatability of the self-healing will be studied.

4. The performance of the RMC-based bacteria capsules in real construction project will be carried out. Up to now, the study on the capsule is on a laboratory basis, and the performance of the capsule in a real structure remains unknown. In reality, different grades of concrete are used. Therefore, to examine the universality of the developed capsules so that the capsules are generally compatible with all grades of concrete and can be applied in various scenarios, the testing of the capsules in different grades of concrete will be carried out. Furthermore, the capsules will be applied to real structure to test its healing performance.

5. Feasibility analysis of the commercialization of the RMC-based capsules. The fabrication of the RMC-B and RMC-BN capsules are straightforward and simple, which has the potential to be scaled up and commercialized. Analysis will be carried out to assess the feasibility including the consideration of mass production, cost, market, etc.

The future work regarding the single bacteria spore capsule can be summarized as follows:

1. Fundamental studies will be conducted to investigate the mechanism of bacteria releasing from the capsule. The interaction of the capsule shell with the matrix will be monitored and studied.

2. More characterizations on the capsule will be conducted to understand the properties of the capsule. For example, the amount of encapsulation material that assembled on the capsule surface, the protection efficiency of the capsule, and accurate size of the capsule size, etc.

3. The encapsulation of nutrients will be studied. In the single spore capsule system, nutrients are incorporated into matrix directly, leading to some negative effects on the matrix properties. Therefore, the encapsulation of nutrients is needed. Referring

to the results on the RMC-based capsule, to encapsulate nutrients in RMC individually might be a solution, therefore the combination of the single bacteria capsule with the RMC-based nutrient capsule could be studied. Alternatively, it is also possible to encapsulate nutrients through LBL technique, referring to the studies in drug delivery. Accordingly, some other methods/materials to encapsulate nutrients will also be explored.

4. Optimization on the encapsulation procedure will be studied. Currently, the encapsulation of the single bacteria cell is done in a small scale. Once the fabrication is scaled up, aggregation is observed. Therefore, further studies will be conducted to understand the factors that influencing the encapsulation, and optimization of the encapsulation to increase the capsule fabrication efficiency.

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