CRACK SELF HEALING CONCRETE BY NATIVE MICROBIAL CALCIUM CARBONATE

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DEDICATION

This thesis is dedicated to my father, who taught me that the best kind of knowledge to have is that which is learned for its own sake. It is also dedicated to my mother, who taught me that even the largest task can be accomplished if it is done one step at a time.

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ABSTRACT

Inevitable concrete microcracks remain as a challenge to civil engineers as they are considered as a threat to structures durability. One of the most common approach is to incorporate ureolytic bacteria in concrete matrix to hydrolyse urea resulting in the self-healing of concrete cracks through the formation of calcium carbonate. Despite that, the issue revolving around the efficacy of crack self-healing remains important. The existing works are still suffering from a better understanding of the factors affecting the fundamental reactions involved as well as bacterial growth in concrete environment. In this study, a comprehensive investigation was conducted to explore the bacterial growth and the influential factors on the evolution of urea hydrolysis aimed to accurately promote calcium carbonate precipitation inside concrete using native bacteria. Subsequently, native ureolytic bacterium species was isolated, identified by 16S rRNA gene sequencing and deposited in the gene bank database under the accession number of MK357893. The bacterial growth was examined in a condition similar to that of concrete in which modified Luria Bertani (LB) broth was utilised to cultivate the bacteria with static incubation. The ureolytic activity was also investigated at pH values of 7 - 13 as well as different concentrations of urea, calcium and nutrient. The Nessler method and an inductively coupled plasma atomic emission spectroscopy (Agilent 700 ICP-OES) technique were used to measure the evolution of urea hydrolysis and calcium carbonate changes in such conditions. In addition, the extent of microbial activity impact on the compressive strength of concrete incorporated with spores, vegetative cells and urea-vegetative cells solution was also evaluated separately. Similarly, the self-healing of an artificial cracked bio-concrete of 0.4 mm was also monitored and evaluated every two weeks by scanning electron microscopy (SEM) with energy dispersive X-ray analysis (EDX) and X-ray diffraction (XRD). In the same context, a system of equations, rationally based on physic-bio-chemical issues, was developed in order to quickly predict a complete understanding of the bio-based healing process. Later, both finite element and finite difference methods were implemented to solve these equations. The results indicated that the bacterium was able to survive as dormant without any reproduction at pH of 12 - 13. While, the optimum bacterial cells concentration was found to be 2×10^7 cells/mL at pH of 9 - 11. In addition, the favoured urea hydrolysis culture conditions were obtained as follows: pH of 9, concentration of calcium ions not exceeding 150 mM, urea concentration of 333 mM and optimum cells concentration of 2×10^8 cells/mL. Subsequent findings also revealed that compressive strength of the concrete incorporated with spores, vegetative cells and urea-vegetative cells was improved by 9%, 10% and 15% compared to that of the control specimens respectively. Moreover, the predicted healing ratio of 0.4 mm crack width was completely achieved after 60 d at the crack mouth, whereas the healing ratio was less than 15% at the deeper part of the concrete surface. This finding was also proved through the experimental work in which the actual crack mouth was fully healed after 70 d. In addition, further studies could be focused on providing a suitable technique to host bacteria for a long term as well as to encourage the bacteria to effectively implement its ureolytic activity inside the concrete matrix.

ABSTRAK

Retak-retak konkrit mikro yang tidak dapat dielakkan kekal sebagai cabaran kepada jurutera awam kerana ia dianggap sebagai ancaman kepada ketahanan struktur. Salah satu pendekatan yang paling biasa adalah untuk memasukkan bakteria ureolitik dalam matriks konkrit untuk menghidrolisis urea yang mengakibatkan pemulihan konkrit sendiri melalui pembentukan kalsium karbonat. Dengan itu juga, isu berkaitan keberkesanan pemulihan-sendiri di sekeliling retak adalah sangat penting. Kerja-kerja yang ada masih kurang pemahaman yang lebih jelas mengenai faktor-faktor yang mempengaruhi tindakbalas asas yang terlibat serta pertumbuhan bakteria dalam keadaan yang keras seperti persekitaran konkrit. Dalam kajian ini, siasatan menyeluruh telah dijalankan untuk meneroka pertumbuhan bakteria dan faktor-faktor yang berpengaruh terhadap evolusi hidrolisis urea yang bertujuan untuk menggalakkan pemendakan kalsium karbonat dengan tepat di dalam konkrit menggunakan bakteria tempatan. Selepas itu, spesies bakteria ureolitik asli telah diasingkan, yang dikenalpasti melalui penjelmaan gen 16S rRNA dan didepositkan dalam pangkalan data bank gen di bawah nombor penyertaan MK357893. Pertumbuhan bakteria diperiksa dalam keadaan yang sama dengan konkrit di mana campuran Luria Bertani (LB) diubahsuai digunakan untuk memupuk bakteria dengan inkubasi statik. Aktiviti ureolitik juga dikaji pada nilai pH 7 - 13 serta kepekatan urea, kalsium, dan nutrien yang berbeza. Kaedah Nessler dan teknik spektroskopi pelepasan atom plasma (Agilent 700 ICP-OES) secara penyatuan induktif digunakan untuk mengukur evolusi urea hidrolisis dan perubahan kalsium karbonat dalam keadaan sedemikian. Di samping itu, sejauh mana pengaruh aktiviti mikrob pada kekuatan konkrit yang digabungkan dengan spora, sel-sel vegetatif dan larutan sel urea-vegetatif juga dinilai secara berasingan. Begitu juga pemulihan-sendiri retak konkrit-bio buatan 0.4 mm juga dipantau dan dinilai setiap dua minggu dengan mengimbas mikroskop elektron (SEM) dengan analisis sinar-X penyebaran tenaga (EDX) dan difraksi sinar-X (XRD). Dalam konteks yang sama, sistem persamaan, secara rasional berdasarkan ciri-ciri fizik-bio-kimia, telah dibangunkan untuk meramalkan dengan cepat pemahaman lengkap tentang proses pemulihan berasaskan bio. Kemudian, kedua-dua kaedah unsur terhingga dan kaedah pembezaan terhingga telah dilaksanakan untuk menyelesaikan persamaan ini. Keputusan menunjukkan bahawa bakteria dapat bertahan hidup sebagai tidak aktif tanpa sebarang pembiakan pada pH 12 - 13. Juga, kepekatan sel bakteria optimum didapati 2×10^7 sel/ml pada pH 9 - 11. Selain itu, keadaan semaian hidrolisis urea yang didapati adalah seperti berikut: pH 9, kepekatan ion kalsium tidak melebihi 150 mM, kepekatan urea 333 mM dan kepekatan sel optimum 2×10^8 sel/ml. Penemuan selanjutnya menunjukkan kekuatan mampatan konkrit yang dicampurkan dengan spora, sel-sel vegetatif dan sel-sel urea-vegetatif telah masing-masing meningkat sebanyak 9%, 10%, dan 15% berbanding spesimen kawalan. Selain itu, ramalan nisbah penyembuhan lebar retak pada bukaan retak yang dicapai selepas 60 hari ialah 0.4 mm, manakala nisbah penyembuhan adalah kurang daripada 15% pada bahagian permukaan konkrit yang lebih dalam. Penemuan ini juga dibuktikan melalui kerja eksperimen di mana bukaan retak sebenar pulih sepenuhnya selepas 70 d. Di samping itu, kajian lanjutan boleh difokuskan untuk menyediakan teknik yang sesuai untuk menyimpan bakteria untuk jangkamasa panjang serta menggalakkan bakteria untuk melaksanakan secara berkesan aktiviti ureolitiknya di dalam matriks konkrit.

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X	-	Spectrophotometer Reading at OD_{600}
Y	-	Bacterial Cell Concentration
OD	-	Optical Density
R	-	Concentration of Urea Hydrolysis
c	-	Urea Concentration
a	-	Maximum Capacity of Urea Hydrolysis
T	-	Time
b	-	Time at Maximum Urea Hydrolysis
k_1		Rate Constant of Urea Hydrolysis
k_2		Rate Constant of Calcium Carbonate Precipitation
P		Failure Load
A_c		Cross Section Area of Concrete Specimen
f_c		Compressive Strength of Concrete
F		Net Flux in unit of M/d
F_m		Flux in unit of $M/m^2.d$
D		Diffusion Coefficient
α		Bacterial Cells Concentration Ratio
α_m		Optimum Cells Concentration in Crack Mouth
α_i		Cells Concentration in Each Node inside Crack Zone
h		hour
min		minute
d		Day
V_h		Crack Healed Volume
V		Volume of Crack
ρ		Density
S		Healing Ratio
N		Shape Function
M		Mole
P		Concentration of Calcium Carbonate

material. Such notable results could inhibit the water or aggressive chemical flow to attack the concrete reinforcement.

The potential ability of bacteria to seal cracks through the formation of calcium carbonate was intensively investigated through different mechanisms such as sulphate-reduction bacteria (Jonkers *et al.*, 2010; O'Connell *et al.*, 2010), oxidation of organic acids (Khaliq and Ehsan, 2016; Lors *et al.*, 2017; Luo *et al.*, 2015a), nitrate reduction bacteria (Erşan *et al.*, 2016a; Erşan *et al.*, 2016b) and ureolytic bacteria (Achal *et al.*, 2013; Balam *et al.*, 2017). The formation of microbial calcium carbonate is fully dependent on the bacterial activity. For example, ureolytic bacteria releases urease enzyme to decompose the urea into carbonate ion, which eventually precipitates $CaCO_3$ through the reaction between carbonate and calcium ions. This phenomenon is commonly known as metabolic activity or ureolytic activity. In addition, ureolytic bacteria is able to form spore in concrete, which enables it to survive the harsh concrete environment for many years until cracks occur. Subsequently, with water, the nutrient spores activate, multiply and produce limestone in response to the hydrolysis of urea in the presence of sufficient amount of calcium ion, as shown in Figure 1.1. This microbial self-healing technique presents several benefits including the possibility to last for a longer period of time and both fast and active crack repair properties. In addition, it is also environmentally friendly.

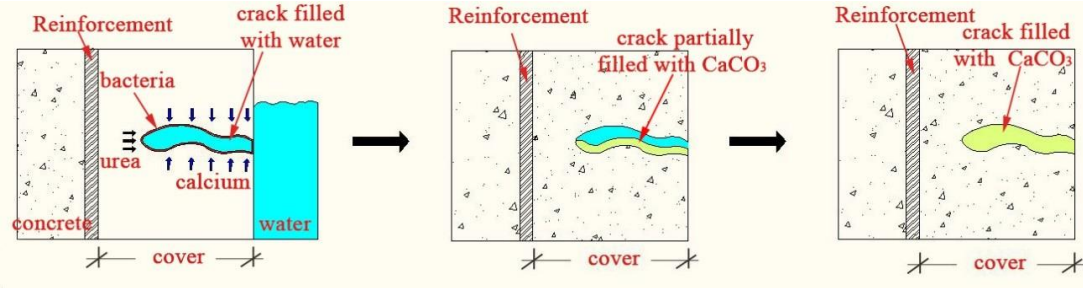

Figure 1.1 Crack healing evolution via ureolytic bacteria

The effectiveness of microbial calcium carbonate was evaluated through either roughly quantifying the width of sealed cracks using microscopic technique or assessing concrete strength enhancement and durability aspects in the literature. From the viewpoint of cracks remediation visualisation using photographic imaging, it was reported that a 0.46 mm of concrete crack-width was completely healed after 100 d via

Bacillus alkalinitrilicus (Wiktor and Jonkers, 2011). At the age of 28 d, crack widths of up to 0.79 mm were also completely healed via another bacterial species namely *Bacillus cohnii* (Zhang et al., 2017). On a similar note, the ability of ureolytic bacterial to heal widths of up to 0.97 mm in 8 weeks of water submission has been proven (Wang *et al.*, 2014c). The same ureolytic bacterial species has also demonstrated its ability to almost completely heal crack widths of 0.5 mm in just 7 d (Wang *et al.*, 2014a). In addition, Krishnapriya and Babu (2015) also demonstrated that after 61 d, the bio-calcite product started to precipitate via ureolytic bacteria. In addition, they emphasised that at the age of 81 d, crack widths of 0.3 mm were completely healed. In the same context, nitrate reducing bacteria also showed its capability to heal crack widths of 0.46 mm in 56 d (Erşan*, et al.*, 2016a).

From the point of bacterial efficiency in terms of concrete strength, there are uneven improvements in the compressive strength of bio-concrete. The improvements in the compressive strength are approximately ranging from 6% to 53%. For example, *Lysinibacillus sp.* I13 has proven its capability to increase the compressive strength of concrete by 34.6%, compared to that of the control specimens (Vashisht *et al.*, 2018). The enhancement of concrete strength incorporating with *B. megaterium* MTCC 1684 was also 16% higher than control specimens (Krishnapriya and Babu, 2015). On contrast, live cells and dead cells of *B. subtilis* ATCC 168 did not show any improvement in compressive strength of cement mortar (Pei *et al.*, 2013). In addition, concrete specimens incorporating encapsulated *B. subtilis* showed noticeable improvement in their compressive strength, from 9.8% to 12%, in comparison to the control specimens.

On the other hand, limited researchers have also focused on the factors affecting $CaCO_3$ precipitation such as curing condition, cracking age and reactants concentration (Ling and Qian, 2017; Xu *et al.*, 2018; Zhang, *et al.*, 2017). These researches were sought to explore the effect of these conditions on the microbial calcium carbonate productivity. However, further studies are still urgently required to fully understand the evolution and behaviour of calcium carbonate precipitation in minimal conditions such as high pH.

1.2 Problem Statement

Inevitable microcracks remain as a challenge to civil engineers. They are considered as a threat to the durability of structures. This fact has fuelled researchers to explore a solution to produce smart sustainable and green concrete materials. In recent years, bacteria-based crack healing was extensively investigated in the literature. However, the evolution of microbial calcium carbonate inside the concrete crack is still questionable and unravelled. More knowledge regarding the factors affecting the rate of the microbial calcium carbonate precipitation as well as the bacterial growth are still in urgent demand. This is because the existing literatures have only evaluated the effectiveness of crack-self-healing through either approximately quantifying the width of sealed cracks using photographic imaging or assessing concrete strength enhancement and durability aspects.

Indeed, these methods are not able to shed light on microbial activity, which is the main sole of inducing calcium carbonate in a harsh condition, such as concrete. The fundamental reactions involved as well as bacterial growth are still not understood. The extent of ureolytic activity impact and their environmental factors on crack remediation are still not unclear. Relatedly, there is a need to further examine the extent of the possibility of the concrete environment to host bacteria and their influential factors on the metabolic activity. This is very important to accurately assess the productivity of bio-based healing, prior to the real application of bacterial concrete. Such findings can promote the precipitation of $CaCO_3$ as a promising sustainable strategy to prolong concrete life span.

Therefore, a comprehensive investigation was developed to assess the precipitation of microbial calcium carbonate inside the concrete using native bacteria. In addition, a mathematical model was also developed to quickly predict the complete understanding of crack-healing behaviours without wasting cost and time.

1.3 Objectives of Research

The aim of this research was to assess the evolution of microbial calcium carbonate precipitation in concrete cracks. Four objectives were taken into account to achieve the aim of this research, including:

(a) To examine the growth, characteristics and ureolytic activity of the bacteria in a harsh condition, such as the concrete environment.

(b) To investigate the influential factors on microbial calcium carbonate precipitation in a condition similar to that of concrete.

(c) To assess the extent of the microbial calcium carbonate precipitation in concrete by evaluation of the concrete strength and the crack-self healing.

(d) To predict the bio-based crack healing results and the related factor affecting the microbial calcium carbonate precipitation through a mathematical model.

1.4 Scope of the Research

The scope of this study involved several phases to evaluate the influential factors on the productivity of calcium carbonate inside the cementitious material by the native bacteria. These investigations were developed to further promote the incorporation of the bacteria in the concrete for the purpose of healing structural cracks, and thus, prolonging concrete life span.

In the first phase of the present work, the aerobic bacteria, which was isolated from the soil, was examined through gram stain, endospore, urease enzyme and $CaCO_3$ productivity. LB broth, urea, as well as calcium nitrate were used for bacterial growth, ureolytic activity and calcium carbonate production. The bacteria that fulfilled the requirement was identified and further used in the patch experiment.

The second phase dealt with influential factors affecting the hydrolysis of urea and inducing the microbial calcium carbonate at different conditions. These included calcium concentration of 50 mM - 2 M, urea concentration of 50 mM - 2 M, bacterial cells concentration of $10^6 – 10^8$ cells/mL and pH of 9 - 13. The effect of these values on the rate of urea hydrolysis and $CaCO_3$ precipitation were evaluated in a condition similar to that of concrete matrix. The rate of ureolysis and calcium carbonate precipitation were also calculated based on a logistic equation.

A concrete mixture was designed to achieve a compressive strength of 30 MPa at 28 d with a slump of 150 - 200 mm in the third phase of this work. The proportions of ordinary Portland cement (OPC), local natural sand, crushed granite type 10 mm aggregates and tap water were obtained according to DOE method. In addition, bacterial spore, bacterial vegetative cells and urea-vegetative cells solution were also incorporated separately during concrete mixing with different concentrations. The change in concrete strength using 100 × 100 × 100 mm cubes was evaluated after 7, 14 and 28 d. Other mechanical concrete and durability characteristics were not considered in this study. This, of course, was not to neglect them, but rather, it was believed that the main goal of the microbes was to heal cracks only. Relatedly, a crack width of 0.4 mm was developed in the mortar specimen with the bacteria as well as other relevant chemical compounds. Water immersion curing condition was used when monitoring the crack healing process for three months. The resulting microbial product was identified and quantified through both XRD and SEM-EXD.

In the last phase, microbial calcium carbonate evolution in concrete crack through the hydrolysis of urea was simulated by developing a system of equations. It involved two first order ordinary differential equations to represent the hydrolysis of urea and $CaCO_3$ evolution in the crack. In addition, the reaction-diffusion equation (second order partial differential equation) was also utilised to simulate the diffusion of urea along the crack domain. The system of equation was numerically solved using one dimensional Galerkin finite element as well as finite difference method. Moreover, the numerical model was further developed to obtain the main influential factor on the hydrolysis of urea. The predicted result was compared against the experimental work for the purpose of validation.

1.5 Research Significance

The research findings from this project could benefit the community in several ways, including:

(a) The microbial calcium product could provide a sustainable solution to alleviate crack repairs, which would then save a considerable amount of money.

(b) It would protect concrete structures for over 200 yr.

(c) It is also environmentally friendly as it would reduce the carbon dioxide emission in the cement industry.

(d) The proposed model would provide significant contribution to better understand the evolution of crack healing.

(e) The developed model would also reduce the cost of experimental tests through the prediction of the healing process in the crack mouth, as compared to experimental efforts.

(f) The model is capable to predict the relevant processes involved without being time consuming and it is not tedious to conduct.

(g) The proposed model could reduce human errors associated with experimental test.

1.6 Layout of Thesis

This thesis has five chapters. Firstly, Chapter 1 describes the overall assessment and provides a concise explanation of the research background. The aim, objectives, scope, research hypothesis and research significance are also spelt out in this chapter.

Chapter 2 provides the latest known knowledge to justify the problem statement of this research in terms of evidence, lack or thereof as well as the theoretical and mathematical framework behind this study. This is then followed by highlighting the research gap of this research and also detailing what it could contribute to.

Next, Chapter 3 discusses the research methodology, which was adopted to implement this work. It was divided into four basic tasks. The first task was to briefly highlight the isolation and identification of the bacteria. The second task was to explore the influential factors on the ureolysis and microbial calcium precipitation in a condition similar to that of concrete. In addition, the investigation pertaining the suitability of the bacteria to be used in concrete was taken into account in task 3, in terms of compressive strength and crack healing. Finally, the development of a mathematical model designed to predict crack healing was discussed in task 4, followed by a comparison between the actual crack healing results obtained from experiment and those obtained from the proposed numerical model.

Chapter 4 then discusses the results of the four tasks in detail and deals with the core of the thesis. Specifically, it clearly and cohesively highlights the research's contribution to the existing knowledge through tables, figures and charts.

Finally, Chapter 5 draws conclusion from the entire findings of this study and discusses future works that are needed to continue the investigation before large scale application can be implemented.

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