Concrete crack-healing materials using biocalcification by ureolytic bacteria isolated in marine environment: An overview

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ABSTRACT

Deterioration of concrete durability is accelerated via penetration of moisture and chemicals into microcracks and pores [1]. Microbial biocalcification is a feasible method for repairing microcracks in concrete, thereby yielding fillers for cracks and pores in concrete. The self-healing technology using microbial-induced calcium carbonate ($CaCO_3$) precipitation (MICCP) is an economic and innovative method to satisfy both durability and environmental demands. Numerous studies on self-healing concrete using microbes performing MICCP isolated from soil have been actively carried out; however, few studies have investigated self-healing concrete in a marine environment. This study reviews previous studies on self-healing concrete, based on bacterial biocalcification. A portion of a feasibility study [8] on identifying bacteria capable of MICCP in a marine environment to be used in self-healing concrete material will be also presented.

Keywords: concrete durability, calcium carbonate precipitation, self-healing concrete, marine envronment

1 INTRODUCTION

Micro-cracks frequently form on the surface of concrete structures owing to external forces and shrinkage [1, 2]. Deterioration of concrete durability is accelerated by the penetration of moisture and chemicals into pores of concrete matrix [2]. Hence, self-healing concrete using polymers, inorganic material, and bacteria has received increasing attention in recent years. In particular, self-healing concrete is an environmental-friendly and sustainable crack-healing technology, in that it undergoes $CaCO_3$ precipitation and seals cracks. Alkaliphilic bacteria that can alkalize their surrounding environment after metabolism in vivo are primarily used for healing concrete cracks [3]. The culture medium is alkalized and bicarbonate is produced owing to the action of urease expressed by ureolytic bacteria [4]. CO_3^{2-} combines with Ca^{2+} proximal to the bacterial cell wall, resulting in the precipitation of $CaCO_3$ [4, 5]. In addition, the charged bacterial cell surface serves as a nucleation site for $CaCO_3$ precipitation.

However, unlike in a soil environment, the degree of concrete deterioration in seawater is significantly influenced by the water depth and location because salinity, temperature, and dissolved oxygen vary with depth [6]. Although a marine environment accelerates the infiltration rate of Cl^-, it is more difficult to repair concrete microcracks in a marine rather than a soil environment [6]. Conventional crack-healing materials, such as chemical synthetic repair agents, have a relatively short repair time; however, they are expensive, environmentally unfriendly, and have a rather short-lived healing effect [7]. Furthermore, determining the exact location of the crack is challenging in water [6]. Therefore, microbial biocalcification is a feasible method for repairing microcracks in concrete, by providing fillers for cracks and pores of concrete in a marine environment. In the present study, previous studies on self-healing concrete based on soil bacteria are reviewed. Moreover, a portion of a feasibility study on marine bacteria for self-healing concrete will be presented [8].

2 LITERATURE REVIEW

2.1 Bacteria used in self-heaing concrete

The metabolic ability of bacteria is an important factor in the healing ability of self-healing concrete. Various studies on factors such as the type of bacteria, metabolism per the external environment, and urea resolution are underway. Soil-dwelling *Bacillus* species have been used as self-healing concrete material and were applied at microcracks in mortar to confirm that the cracks had healed [9, 10]. Self-healing concrete with bacteria should combine Ca^{2+} and CO_3^{2-} formed by urea decomposition; hence, ureolytic bactera are essential for urea decomposition.

The names of bacteria used in previous studies are enlisted in Fig. 1. Although numerous studies regarding self-healing concrete using soil bacteria have been performed, studies on marine bacteria that can survive at high Cl^- and at low temperature are scarce. The high salt and low-temperature marine environment affect the survival and activity of bacteria. Thus, it is necessary to investigate the effect of the marine environment on soil bacteria and to study isolating ureolytic marine bacteria that $CaCO_3$ can be precipitated for self-healing concrete.

Table 1 : List of applicable ureolytic bacteria for self-healing concrete

Bacteria	References
Sporosarcina Pasteurii (or Bacillus Pasteurii)	3, 11, 12,13
Halomonas euryhaline	14, 15, 16
Deleya halophila	4
Bacillus lentus	17
Pseudomonas aeruginosa	18
Bacillus amyloliquefaciens	19
Bacillus sphaericus	20
Bacillus alkalinitrilicus	1

2.2 Factors affecting self-healing efficiency

The effect of crack-healing differs in accordance with changes in external conditions and the rate of self-healing concrete, such as microbial species. Ice crystal formation during freezing causes cellular damage, such as in *Bacillus subtilis*, which is used in self-healing concrete and low temperature under 4◦C, decrease urease activity for $CaCO_3$ precipitation [21]. In addition, the parameters such as pressure and pH influence bacterial spore formation and germination for concrete incorporation. A higher inactivation rate was observed among spores pre-treated with high pressure [22].

Not only the external environment but also the environment in concrete such as pH, pressure in concrete hardening, etc., influences the efficiency of self-healing [4]. In particular, the size of microcracks predominantly affects crack-healing performance. Luo et al. reported that healing efficiency is reduced as the crack width increases, and it is difficult to heal cracks larger than 0.8 mm in the experimental conditions [23]. In addition, as the cracking age of specimens increased, self-healing performance decreased sharply and the healing ratio decreased when the crack was more than 60-days-old [23]. The maximum healing crack with for each study with microcapsule containing bacteria showed different results. Hence, the ability of bacteria to decompose urea and form $CaCO_3$ may have been influenced by environmental conditions of the crack specimens, e.g., temperature, humidity, etc.

2.3 Improvement of self-healing concrete performance

In particular, various studies have attempted to improve bacterial viability at high pH of concrete and pressure generated at curing. In addition, bacterial spore germination is reduced when the spores are surrounded by $CaCO_3$ or the nutrients required for growth disapper. Jonker et al. reported that using AE agent for producing micropores in a concrete matrix can improve bacterial viability [4]. Wang et al. applied microcapsules to encapsulate bacterial spores in mortal specimens [24]. Specimens with microencapsulated bacteria displayed a higher crack-healing ratio than those without microencapsulated bacteria. In addition, the fastest healing effect was observed when repeated dry and wet conditions were provided [24]. However, different contents of microcapsules in concrete specimens resulted in increased porosity, which decreased their compressive strength [24]. Since the porosity in concrete specimens was increased by using AE or microcapsules, the density of the gel in the matrix might be decreased and the compressive strength decreased.

Studies on $CaCO_3$ precipitation in concrete containing polyurethane and silica gel-immobilized bacteria as bacterial capsules have reported that polyurethane-immobilized bacteria have higher healing ability [25]. Jonker investigated the crack-healing efficiency with the self-healing agent embedded in porous expanded clay particles and displayed healing of a 0.46 mm-wide crack, while the water-exposed control only displayed crack healing of 0.18 mm [26]. Wang et al. reported that bio-hydrogels improve healing efficiency compared to pure hydrogels [27]. When bio-hydrogel was used, it displayed a 100% crack-healing effect at cracks less than 0.3 mm, indicating that the healing ratio improved by more than 50% compared to pure hydrogel alone [27].

Wang et al. reported that diatomaceous earth (DE) could protect bacteria at a high pH in concrete [28]. Since DE particles have high specific surface area, they can absorb bacteria and protects bacteria from harsh environmental conditions such as high pH, thereby maintaining the urea decomposition performance of bacteria [28]. Certain studies have aimed to improve the self-healing performance of bacteria by controlling the environment in concrete.

3 PRELIMINARY STUDY [8]

A preliminary study [8] was conducted to identify the effect of calcium $CaCO_3$ precipitation on self-healing by identifying ureolytic marine bacteria and a portion of the study is here recapitulated [8].

Seawater was collected in the western sea of Korea. Ureolytic bacteria were identified on the basis of color changes after incubation in urea-containing medium. The identified bacteria were cultured in a Urea-NB medium to obtain a sufficient bacterial population to be incorporated into cement mortal.

The 16S rRNA sequence of the selected ureolytic bacteria were amplified using polymerase chain reaction (PCR) with primers 27F (5'-AGAGTTTGATCMTGGCT CAG-3') and 1492R (5'-CGGTTACCTTGTTACGACTT-3') [29]. The 16S rRNA sequence was assessed for homology in GenBank database and phylogenetic affiliation of selected bacteria is determined. To analyze the mechanism underlying $CaCO_3$ production in the identified bacteria, the concentration of ions such as NH_4^+ and Ca^{2+} in culture media after metabolism was measured via ion chromatography (IC). The morphology and composition of $CaCO_3$ produced by the identified bacteria was characterized using scanning electron microscopy (SEM), X-ray diffractometry (XRD) analysis. The effect of crack healing by bacterial $CaCO_3$ precipitation was determined via microscopic observation after curing the cracked mortar with isolated bacterial culture media. Details of the results and the method of the test can be found in [8].

4 SUMMARY

Self-healing concrete with bacteria can constitute an innovative technology to improve the long-term durability of concrete by hindering the effect of harmful ions such as Cl^- and chemicals penetrating cracks [2, 6]. In recent years, numerous studies have focused on improving crack-healing efficiency, e.g., via bacterial microencapsulation. Although various studies have investigated self-healing concrete using soil bacteria, limited attention has been paid to the marine bacteria for self-healing concrete. This paper have reviewed the previous studies regarding self-healing concrete with bacteria. In addition, self-healing concrete with marine bacteria will be discussed on the basis of the results of the preliminary test [8].

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REFERENCES

[1] V. Wiktor and H.M. Jonkers, "Quantification of crack-healing in novel bacteria-based self-healing concrete," Cem. Concr. Compos., 33(7), 763-770, 2011

[2] S.A. Altoubat and D.A. Lange, "Creep, shrinkage and cracking of restrained concrete at early age" ACI Mater. J. 51, 2001

[3] H.M. Jonkers, "Self healing concrete: a biological approach. In Self Healing Materials" Springer Netherlands, 195-204, 2007

[4] H.M. Jonkers, A. Thijssen, G. Muyzer, O. Copuroglu and E. Schlangen, "Application of bacteria as self-healing agent for the development of sustainable concrete," Ecol Eng., 36, 230-235, 2010

[5] Effect of ureolytic bacteria on concrete properties. Constr. Build. Mater., 25(10), 3791-3801.

[6] M.H.P. Kadam, D.B. Desai and A. K. Gupta, "A review paper on Deterioration of Concrete in Marine Structures," Imperial J. Interd. Res., 3(2). 2017

[7] L. Basheer, D. Cleland, "Freeze–thaw resistance of concretes treated with pore liners," Constr. Build. Mater., 20, 990-998, 2006

[8] H.Y. Kim, H.M. Son, H.K. Lee, "Isolation of ureolytic bacteria in marine for concrete crack healing material," 2018 (In preparation)

[9] V. Achal and X. Pan, "Characterization of urease and carbonic anhydrase producing bacteria and their role in calcite precipitation," Curr. Microbiol., 62, 894-902, 2011

[10] Bansal, R., N.K. Dhami, A. Mukherjee and M.S. Reddy, "Biocalcification by halophilic bacteria for remediation of concrete structures in marine environment," J Ind. Microbiol. Biotechnol., 43, 1497-1505, 2016

[11] S.S. Bang, J.K. Galinat and V. Ramakrishnan, "Calcite precipitation induced by polyurethane-immobilized Bacillus pasteurii," Enzyme Microb. Technol., 28(4-5), 404-409, 2011

[12]V. Ramakrishnan, R.K. Panchalan and S.S. Bangs, "Improvement of concrete durability by Bacterial Mineral Precipitation," Proceedings ICF 11. Torino, Italy, 2005

[13] S.M. Al-Thawadi, "Ureolytic bacteria and calcium carbonate formation as a mechanism of strength enhancement of sand," J. Adv. Sci. Eng. Res., 1(1), 98-114, 2011

[14] R. Siddique and N.K. Chahal, "Effect of ureolytic bacteria on concrete properties," Constr. Build. Mater., 25(10), 3791-3801, 2011

[15] M.A. Rivadeneyra, A. Ramos-Cormenzana, G. Delgado and R. Delgado, "Process of carbonate precipitation by Deleya halophila," Curr. Microbiol., 32(6), 308-313, 1996

[16] A. Talaiekhozan, M.A. Fulazzaky, A. Keyvanfar, R. Andalib, M.Z.A. Majid, M. Ponraj, R.B.M. Zin, C.T. Lee, A. Shafaghat and M.W.H. Ir, "Identification of Gaps to Conduct a Study on Biological Self-healing Concrete," J. Environ. Treat. Tech., 1(2), 62-68, 2013

[17] N. Chahal, A. Rajor and R. Siddique, "Calcium carbonate precipitation by different bacterial strains" Afr. J. Biotechnol, 10(42), 8359-8372, 2011

[18] P. Ghosh, S. Mandal, S. Pal, G. Bandyopadhyaya, and B.D. Chattopadhyay, "Development of bioconcrete material using an enrichment culture of novel thermophilic anaerobic bacteria," Indian J. Exp. Biol., 44, 336-339, 2006

[19]Y.N. Lee, "Calcite production by Bacillus amyloliquefaciens CMB01" Indian J. Exp. Biol., 41(4), 345-348, 2003

[20] K. Van Tittelboom, N. De Belie, W. De Muynck and W. Verstraete, "Use of bacteria to repair cracks in concrete," Cem. Concr. Res., 40(1), 157-166, 2010

[21] G. Willimsky, H. Bang, G. Fischer and M.A. Marahiel, "Characterization of cspB, a Bacillus subtilis inducible cold shock gene affecting cell viability at low temperatures," J. Bacteriol., 174(20), 6326-6335, 1992
[22] E.Y. Wuytack and C.W. Michiels, "A study on the effects of high pressure and heat on Bacillus subtilis spores at low pH," Int J. Food. Microbiol., 64(3), 333-341, 2011
[23] M. Luo, C.X. Qian and R.Y. Li, "Factors affecting crack repairing capacity of bacteria-based self-healing concrete," Constr. Build. Mater., 87, 1-7, 2015
[24] J.Y. Wang, H. Soens, W. Verstraete and N. De Belie, "Self-healing concrete by use of microencapsulated bacterial spores," Cem. Concr. Res., 56, 139-152, 2014
[25] J. Wang, K. Van Tittelboom, N. De Belie, and W. Verstraete, "Use of silica gel or polyurethane immobilized bacteria for self-healing concrete," Constr. Build. Mater., 26(1), 532-540, 2012
[26] H.M. Jonkers, "Bacteria-based self-healing concrete," Heron, 56 (1/2), 2011
[27] J.Y. Wang, D. Snoeck, S. Van Vlierberghe, W. Verstraete and N. De Belie, "Application of hydrogel encapsulated carbonate precipitating bacteria for approaching a realistic self-healing in concrete," Constr. Build. Mater., 68, 110-119, 2014
[28] J.Y. Wang, N. De Belie and W. Verstraete, Diatomaceous earth as a protective vehicle for bacteria applied for self-healing concrete. J. Ind. Microbiol. Biotechnol., 39(4), 567-577, 2012
[29] H.J. Kim, H.J. Eom, C. Park, J. Jung, B. Shin, W. Kim, N. Chung, I.G. Choi, W. Park, "Calcium carbonate precipitation by Bacillus and Sporosarcina strains isolated from concrete and analysis of the bacterial community of concrete," J. Microbiol. Biotechnol., 26, 540-548, 2016