

# The feasibility of encapsulation of self-healing bacterium with ethyl cellulose

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## ABSTRACT

Recently, the application of bacterial-based self-healing technology, which is based on microbial induced calcium carbonate precipitation, has been drawing much attention in the repair of concrete crack. However, due to the high internal pH, relative dryness and the small size of pores inside concrete, it is necessary to set up a protection strategy for self-healing bacteria before their introduction into concrete. In this work, ethyl cellulose (EC), a pH sensitive material, was used to fabricate microcapsule to encapsulate an alkaliphilic spore-forming bacterium. The technical feasibility of encapsulated spores, the influence of pH value and four kinds of organic solvents for the fabrication of microcapsule were studied by calcium precipitation activity (CPA) of the bacterium. Further, the CPA of broken/unbroken microcapsules was evaluated, and the micro-morphology of the precipitation produced by the bacterium was investigated. The results showed that compared with unbroken microcapsules, broken microcapsule displayed higher CPA by releasing the bacterium, thus, suggesting that microcapsule can provide a good protection for the

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encapsulated spores. Four organic solvents showed deleterious influence on the spores, while ethanol was relatively less harmful. Production of calcium carbonate was confirmed.

## **1. INTRODUCTION**

Nowadays, the application of bacterial-based self-healing technology, which is based on microbially induced calcium carbonate precipitation, has been drawing much attention by researchers. It can be utilized for enhancement of the durability of building materials, consolidation of sand columns, and repair of limestone monuments, as well as healing of concrete cracks [1-3].

However, due to the high internal pH, relative dryness and the small size of pores inside the concrete, it is necessary to set up a protection strategy for self-healing bacteria before their introduction into concrete. For a microcapsule-based microbial self-healing system in the cementitious material, a fundamental issue is to find a suitable microcapsule system, concerning both the material selection and manufacture process [4]. In this study, a pH sensitive material was used for the shell of microcapsule, and an alkaliphilic spore-forming bacterium was encapsulated as the healing agent inside the microcapsule.

## **2. MATERIALS AND METHODS**

### **2.1 Preparation of microcapsules**

Microcapsules were fabricated via extrusion- spheronisation and spray drying method by using a pH sensitive material, Ethyl Cellulose (EC), and an alkaliphilic spore-forming bacterium, *Bacillus pseudofirmus* DSM8715.

First of all, the influence factors on DSM8715 spore production and activity, such as the optimum pH value to induce mineralization, the optimum germination agent, the optimum buffer, and four kinds of organic solvents (methylbenzene, ethanol, acetone and ethyl acetate), which were used for the fabrication of microcapsule, were studied by monitoring the calcium precipitation activity (CPA) of the bacterium. In addition, microcrystalline cellulose (MCC) which was used as filling skeleton was mixed with spores to prepare the core material. In order to obtain a suitable solution for the shell of microcapsule, ethyl cellulose was dissolved by ethanol. Spray drying process was carried out to form the outer coating of the microcapsules.

### **2.2 Characterization of the microcapsules**

The micro-morphology of microcapsule and the interface between microcapsule and cement paste was studied by Scanning Electron Microscopy (SEM). The morphology of the precipitation produced by the bacterium was

investigated through X-ray Diffraction (XRD), and X-ray energy dispersive spectroscopy (EDS). In addition, an alkaline pH condition was designed to characterize the releasing performance of the microcapsule under a simulated concrete environment. The release feature of capsule wall at different pH gradients is monitored by means of the micro-plate spectrophotometer. Further, the CPA of broken/unbroken microcapsules was evaluated by high-throughput assay which based on the detection of free  $\text{Ca}^{2+}$  concentration via O-Cresolphthalein Complexone(OCPC) method.

### 3. RESULTS AND DISCUSSION

Given the alkaline environment inside the concrete, the bacterium used in the study was basophilic *Bacillus pseudofirmus* DSM 8715. Our experiment results showed that DSM8715 had a strong mineralization ability and grew fast at  $30^{\circ}\text{C}$ , pH 9.5-11. The influence factors on DSM8715 spore production were evaluated. The results showed that the optimum germination agent is inosine with the suitable concentration of 80mmol/L; The optimum buffer is N-cyclohexyl-3-amino propylsulfonic acid (CAPS); the optimum pH for DSM8715 to induce mineralization was 10 with spore mineralization activity being 86.8%(Figure1). Compared with unbroken microcapsules, a higher CPA was achieved by breaking the microcapsule to release the bacterium, suggesting good protection for the encapsulated spores(Figure 2). Four organic solvents showed harmful influence on the spores, while ethanol was relatively less harmful. Production of calcium carbonate was confirmed by SEM, XRD and EDS(Figure 3). Our results also found that releasing of the self healing bacterium from EC microcapsule could be controlled by pH values. With the increase of pH value, the releasing rate decreased greatly.

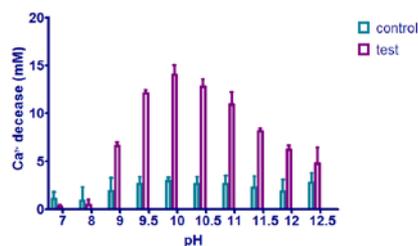


Figure1: The influence of pH on the bacterial mineralization.

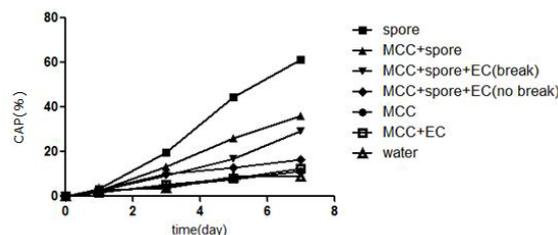


Figure2: Comparison of CPA of bacteria in different forms.

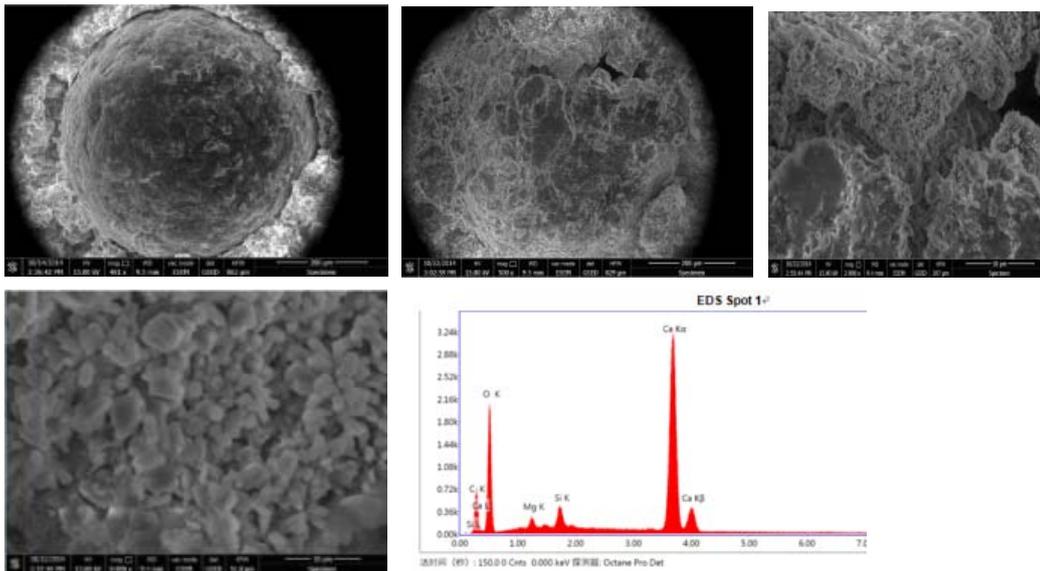


Figure 3: SEM image of microcapsule and EDS results of precipitation.

#### 4. CONCLUSION

In this study, a microcapsule-based microbial self-healing system with Ethyl Cellulose and an alkaliphilic spore-forming bacterium was designed and fabricated. The physical properties and morphology of spore and microcapsules were evaluated for self-healing system. In conclusion, all above discussed properties demonstrated that it is feasible to employ this adopted microcapsule in the self-healing cementitious material.

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