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BIOTECHNOLOGY METHODS

Diatomaceous earth as a protective vehicle for bacteria applied for self-healing concrete

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Abstract Crack repair is crucial since cracks are the main cause for the decreased service life of concrete structures. An original and promising way to repair cracks is to pre-incorporate healing agents inside the concrete matrix to heal cracks the moment they appear. Thus, the concrete obtains self-healing properties. The goal of our research is to apply bacterially precipitated $CaCO_3$ to heal cracks in concrete since the microbial calcium carbonate is more compatible with the concrete matrix and more environmentally friendly relative to the normally used polymeric materials. Diatomaceous earth (DE) was used in this study to protect bacteria from the high-pH environment of concrete. The experimental results showed that DE had a very good protective effect for bacteria. DE immobilized bacteria had much higher ureolytic activity (12–17 g/l urea was decomposed within 3 days) than that of un-immobilized bacteria (less than 1 g/l urea was decomposed within the same time span) in cement slurry. The optimal concentration of DE for immobilization was 60% (w/v, weight of DE/volume of bacterial suspension). Self-healing in cracked specimens was visualized under light microscopy. The images showed that cracks with a width ranging from 0.15 to 0.17 mm in the specimens containing DE immobilized bacteria were completely filled by the precipitation. Scanning electron microscopy (SEM) and energy dispersive spectrometry (EDS) were used to characterize the precipitation around the crack wall, which was confirmed to be calcium carbonate. The result from a capillary water absorption test showed that the specimens with DE immobilized bacteria had the lowest water absorption (30% of the reference ones), which indicated that the precipitation inside the cracks increased the water penetration resistance of the cracked specimens.

Keywords *Bacillus sphaericus* · Ureolytic activity · Microbial $CaCO_3$ · Carrier · Crack repair

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Introduction

Biomineralization is a ubiquitous complex phenomenon in natural environments. It integrates biologically induced precipitation in local microenvironments created by organisms under certain conditions that allow visible extracellular chemical precipitation of mineral phases [10]. Bacterially induced carbonate precipitation is also related to biomineralization. Some bacteria can produce or induce bio-minerals during their growth and metabolism. Under suitable conditions, most bacteria are capable of inducing carbonate precipitation [2, 11, 21, 23]. Nowadays, microbial carbonate precipitation is being widely investigated in the field of civil engineering, such as for surface protection [4], cementation of sand [27], and concrete crack repair [5, 19].

Concrete is the most widely used building material. However, its inherent heterogeneous nature, low tensile

strength, and non-ideal service environments make it susceptible to cracking. In principle, there are two ways to repair cracks, i.e., a passive way and an active way. The passive way follows the procedure of detecting, monitoring, and repairing. The repair work will be performed after the cracks are detected. Healing agents are applied from the outside and have to penetrate inside the cracks. It is a method that operates from the outside to the inside. The active way is that the repair work will be done by the concrete itself when cracks appear, through a self-healing process. Cracks will be healed by certain kinds of healing agents released from the concrete when cracking happens. It is a method from the inside to the outside. Recently, self-healing concrete has become an important research topic because it holds the potential to be more economical and convenient [24]. Self-healing properties in concrete may be obtained by secondary hydration of unhydrated cement [12], encapsulation of polymers [7, 8], addition of expansive agents [16], and so on. Another alternative self-healing method is microbial carbonation precipitation. Use of microbial carbonation to repair concrete cracks was first reported by Bang et al. [1]. They first immobilized bacteria into polyurethane foam and then put the strips of PU foam inside the simulated cracks on mortar specimens. Subsequently, the specimens were immersed into a medium containing urea and $CaCl_2$. Compressive strength was increased about 10% in the cracked specimens with PU-immobilized bacteria compared to the ones without PU-immobilized bacteria. However, in this method, the active agents are applied from the outside. Hence, it cannot be considered as self-healing. The research on self-healing concrete by implementing bacterially precipitated carbonation started in recent years [14, 20, 26].

Bacillus sphaericus was used in our study, which is a ureolytic strain that is able to precipitate calcium carbonate ($CaCO_3$) in its microenvironment by the decomposition of urea ($CO(NH_2)_2$) into ammonium (NH_4^+) and carbonate (CO_3^{2-}). The biogenic $CaCO_3$ has a good potential to be used to heal concrete cracks because it is natural, environmentally friendly, and compatible with the concrete matrix. For the aim of self-healing concrete cracks, the bacteria should be added to concrete during the process of mixing. When cracks appear, bacteria inside the concrete would be activated by water and oxygen penetrating inside the cracks, and then precipitate $CaCO_3$ to fill the cracks. However, bacterial cells could not be added to concrete directly based on previous research. On the one hand, bacterial activity will be greatly decreased when they are added to an environment with pH higher than 12; on the other hand, bacterial cells might be destroyed during the process of hydration because the micropores become smaller and smaller during hydration, and this might squeeze the bacteria inside the pores [15]. In this work, diatomaceous earth (DE) was used as a carrier for the bacteria.

DE is a natural soft siliceous sedimentation. It has a particle size ranging from less than 1 μm to more than 1 mm, but is typically from 10 to 200 μm. DE consists of fossilized remains of diatoms. The diatoms skeletons are highly porous, light in weight, and chemically stable and inert. DE has been mainly used as a filtration agent and functional fillers for paints and plastics. Meanwhile, it can also be used in fireproof cement, insulation materials, and as an absorbent in explosives manufacture because of its great resistance to heat and chemical action [3].

Furthermore, DE can be used as a bacterial carrier. Immobilization of *Pseudomonas* species on DE resulted in an increased efficiency of removing 3,5,6-trichloro-2-pyridinol from industrial wastewater [9]. The oxidation rate of ferrous ions by DE supported *Thiobacillus ferrooxidans* was increased by about 20% (10–15 h shorter than that of free cells) and the bacterial specific growth rate also increased 1.2 times from 0.077 to 0.09 h^{-1} [28]. DE is also used as a carrier of non-pathogenic microbes inside a system for in situ bioremediation of contaminated soil and ground water [22]. The porous cells of DE pellets can provide a home both for microbes and for oxygen, water, and nutrients to help sustain the life of the augmented colonies of microbes introduced in the pellets.

The aim of this study was to use DE as a carrier or a protective vehicle for the bacteria, which were added into the high-pH concrete environment as a self-healing agent. First, the experiments were performed in a mimicked concrete environment (a kind of cement slurry, made of cement and water, 20 g/l). In a second series of experiments, the DE-immobilized bacteria were incorporated into mortar specimens, in which realistic cracks were made, to investigate the potential use of DE-immobilized bacteria in the self-healing of cracks. Crack filling was visualized by means of a microscopy and the healing effect was evaluated by testing the capillary water absorption.

Materials and methods

For each series of experiments, there were three replicates.

Bacteria and growth conditions

Bacillus sphaericus LMG 22557 (Belgian Co-ordinated Collections of Micro-organisms, Ghent) was used in the study based on previous research [6]. The medium used to grow *B. sphaericus* consisted of yeast extract and urea (VWR International, Belgium). The yeast extract medium was first autoclaved for 20 min at 120°C and then the

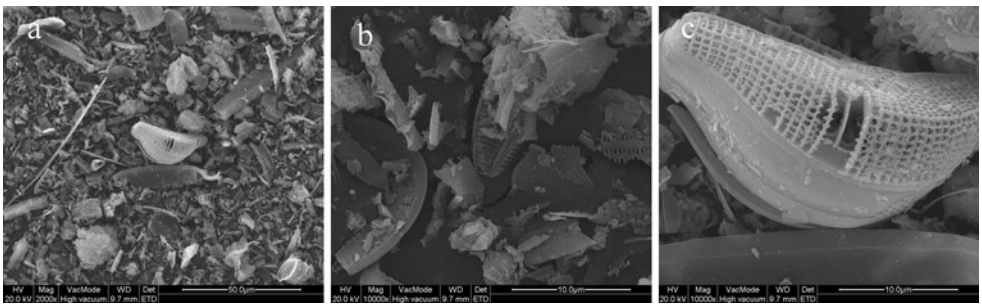

Fig. 1 Morphology of the DE powders

sterilized urea solution was added, which was obtained by means of filtration through a sterile 0.22-μm Millipore filter (Millipore, USA). The final concentrations of yeast extract and urea in the growth medium were both 20 g/l. For all experiments, *B. sphaericus* cultures were obtained after two times subsequent culturing (1% inoculums) from a −80°C stock culture. Cultures were incubated at 28°C on a shaker at 100 rpm for 24 h. Bacterial cells were harvested by centrifuging the 24-h old grown culture (5,000 g, 7 min) and were re-suspended in a physiological solution (NaCl, 8.5 g/l). The concentration of bacterial cells in the suspension was 10^9 cells/ml.

Diatomaceous earth (DE)

The DE used in the experiments was provided by the company Dicalite Europe NV, Belgium. The specific surface area of DE was 29 m^2/g, determined by nitrogen gas adsorption (BELSPORP-Mini II). The morphology of DE was observed by a scanning electron microscope (FEI QUANTA 200F SEM), shown in Fig. 1. The DE particles used in the experiments were raw materials without any treatment. They had different kinds of irregular shapes (Fig. 1a). The particles size distribution was not homogenous, ranging from 4 to 20 μm (Fig. 1b). A large amount of tiny surface pores are present on the DE particles. The size of these pores ranged from 0.1 to 0.5 μm. Some particles had hollow inner structures (Fig. 1c).

Activity of immobilized bacteria under different pH conditions

The ureolytic activity of the bacteria with and without immobilization onto DE was examined in different kinds of pH environments. Bacterial suspension (BS, 10^9 cells/ml) obtained from the same procedure as described in "Bacteria and growth conditions" was mixed with sterile DE powders in a 50-ml falcon tube (20 ml of BS was mixed with 4 g DE in each falcon tube). Subsequently, the falcon tube was put on a shaker at 100 rpm for 1 h. After 1 h, the 20-ml mixture (pre-mixture) containing DE immobilized bacteria in the falcon tube was transferred to 80 ml of sterile urea medium, which consisted of urea and yeast extract. The medium was then put on the shaker (100 rpm) again for 3 days. As a control, 20 ml BS not mixed with DE was added to the same urea medium. The initial concentrations were 20 g/l or 40 g/l for urea and 1 g/l for yeast extract, respectively.

The urea media with different pH values were made as follows. The urea medium with a neutral pH was obtained by adjusting the pH of the medium to 7.0 using a 1-M NaOH solution. The urea medium with a moderate alkalinity was obtained by autoclaving. A small amount of urea (1 g/l) was decomposed into ammonia and carbonate ions during the process of autoclaving, through which the pH of the medium increased to about 9.1.

To mimic a high-pH concrete environment, a cement suspension was made by adding cement powder (Ordinary Portland Cement CEM I 52.5 N, 20 g/l) to the urea medium. The cement suspension was first put on the shaker (100 rpm) for 1 day to make sure that the cement powder reacted with water completely and the pH reached a stable value (about 12.5). After 1 day, the pre-mixture was transferred to the cement suspension. The mixture containing pre-mixture (DE-immobilized bacteria) and cement suspension is referred to as cement slurry. The cement slurry was also put on the same shaker for 3 days. The detailed information about the experimental arrangement can be seen in Table 1.

The ureolytic activity of the bacteria was indicated by the amount of urea decomposed by bacteria in the urea media, which was determined by the total ammonium nitrogen (TAN) in the urea media. One mole of urea ($CO(NH_2)_2$) produces 2 mol of NH_4^+. The amount of NH_4^+ can hence indicate the amount of urea decomposed. TAN concentrations were measured calorimetrically by the method of Nessler [13]. The amount of urea decomposed after 1 day and 3 days was calculated based on the TAN values measured in the urea media.

Optimization of immobilization methodology

Different concentrations of DE (w/v), 4 g DE + 20 ml BS (20%), 8 g DE + 20 ml BS (40%), 10 g DE + 20 ml BS

Table 1 Composition of urea media with different pH values

Series	Pre-mixture (20 ml)		Urea medium (80 ml)			Initial pH of the final mixture
	DE (g)	BS (ml)	Urea (g)	Yeast (g)	Cement (g)	
Free cells, U20, 7	0	20	2	0.1	0	7
Free cells, U40, 7	0	20	4	0.1	0	7
DE+BS, U20, 7	4	20	2	0.1	0	7
DE+BS, U40, 7	4	20	4	0.1	0	7
Free cells, U20, 9.1	0	20	2	0.1	0	9.1
Free cells, U40, 9.1	0	20	4	0.1	0	9.1
DE+BS, U20, 9.1	4	20	2	0.1	0	9.1
DE+BS, U40, 9.1	4	20	4	0.1	0	9.1
Free cells, U20, 12.5	0	20	2	0.1	2	12.5
Free cells, U40, 12.5	0	20	4	0.1	2	12.5
DE+BS, U20, 12.5	4	20	2	0.1	2	12.5
DE+BS, U40, 12.5	4	20	4	0.1	2	12.5

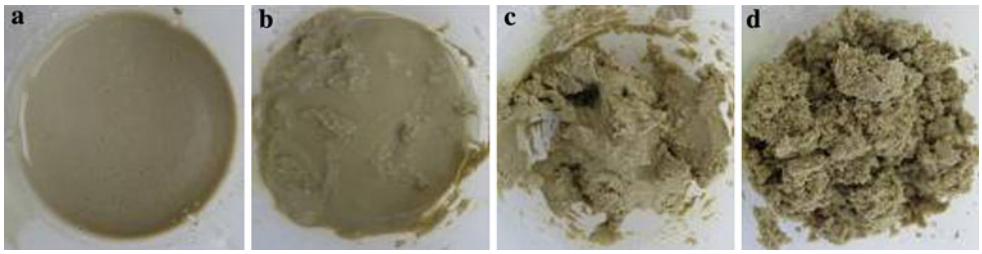

Fig. 2 Digital photos of the mixture of DE and BS at different concentrations of DE (DE concentration in **a**, **b**, **c** and **d** were 40, 50, 60, and 70%, respectively)

(50%), 12 g DE + 20 ml BS (60%), 14 g DE + 20 ml BS (70%), were used in the pre-mixtures to investigate the protective effect of DE for bacteria in the high-pH cement slurry. When the concentration of DE was higher than 50%, the workability of the pre-mixtures decreased significantly (Fig. 2). Therefore, they were not put on the shaker but were kept still for 1 h at the same temperature of the shaker. After 1 h, the pre-mixtures were added to the cement suspensions, which were made by the same method as described in "Activity of immobilized bacteria under different pH conditions". The concentrations of urea and yeast extract in cement slurry were 20 and 1 g/l, respectively.

Effect of nutrient

The influence of the nutrient (yeast extract) on bacterial ureolytic activity in the cement suspension was also investigated. The detailed experimental arrangement is shown in Table 2. At a certain DE concentration (40, 50, and 60%), during the process of immobilization, 0 or 10 g/l yeast extract was used. The concentration of yeast extract in cement slurry was 1 or 10 g/l. There were three replicates in each series. The initial concentration of urea in cement slurry was 20 g/l.

DE-immobilized bacteria applied in mortar specimens

Bacteria immobilized into DE were added to mortar specimens during the process of mixing to examine their effect on self-healing of cracks. Two series of mortar specimens (40 × 40 × 160 mm) were made with a water-to-cement ratio of 0.5 and a sand-to-cement ratio of 3. The components of the specimens are shown in Table 3.

During the process of mixing, nutrients (yeast extract, urea, and $Ca(NO_3)_2.4H_2O$) were firstly dissolved in the water. The nutrients solution was mixed with cement, sand, and DE. When making specimens with DE-immobilized bacteria, the pre-mixture was first made by adding 45 g DE to 225 ml BS. Then the pre-mixture was put on the shaker (100 rpm, 28°C) for 1 h. Subsequently, the pre-mixture containing DE-immobilized bacteria were mixed with cement, sand, and the nutrient solution. The BS used in the specimens was the same as in "Activity of immobilized bacteria under different pH conditions" and "Optimization of immobilization methodology".

In each series, six prisms were made. Reinforcements were added to the mortar specimens to control the crack width. To make specimens with reinforcements, a 10-mm mortar layer was firstly added into the molds. After this layer was compacted by means of vibration, two

Table 2 Experimental arrangement for investigation of the effect of the nutrient

Series	DE used (w/v) [%]	Concentration of yeast extract (g/l) in pre-mixture (20 ml)	Concentration of yeast extract (g/l) in cement suspension (80 ml)	Total concentration of yeast extract in cement slurry (100 ml)
Y0, Y1.25	40	0	1.25	1
Y0, Y12.5	40	0	12.5	10
Y10, Y1.25	40	10	1.25	3
Y10, Y12.5	40	10	12.5	11
Y0, Y1.25	50	0	1.25	1
Y0, Y12.5	50	0	12.5	10
Y10, Y1.25	50	10	1.25	3
Y10, Y12.5	50	10	12.5	11
Y0, Y1.25	60	0	1.25	1
Y0, Y12.5	60	0	12.5	10
Y10, Y1.25	60	10	1.25	3
Y10, Y12.5	60	10	12.5	11

Table 3 Components of mortar specimens in each series

Series	Cement (g)	Sand (g)	Water (g)	DE (g)	BS (ml)	Nutrients (g)
DE	900	2,700	450	45	0	69.75
DE+BS	900	2,700	225	45	225	69.75

Nutrients included 2.25 g yeast extract, 22.5 g urea and 45 g $Ca(NO_3)_2{\cdot}4H_2O$

reinforcement bars ($D = 2$ mm, $L = 14$ cm) were placed on top of it (Fig. 3a). Afterwards, the molds were completely filled with mortar and vibrated. All of the molds were put in an air-conditioned room with a temperature of 20°C and a relative humidity of more than 90% for 24 h. After demolding, the mortar specimens were placed again in the air-conditioned room.

After 14 days, specimens were taken out of the air-conditioned room and cracks were created by means of a crack width controlled three-point bending test, as shown in Fig. 3b. Crack width was measured by means of a linear variable differential transformer (LVDT) that was attached at the bottom of the specimens. The crack width was increased with a velocity of 0.0005 mm/s until a crack of 0.3 mm was obtained. After unloading, the remaining crack width ranged from 0.15 to 0.17 mm. Afterwards, three of the cracked specimens were immersed into water and three were immersed into a deposition medium (made of urea and $Ca(NO_3)_2$, 0.2 M) for 40 days.

After 40 days, the specimens were taken out of the water or the deposition medium and were rinsed gently with tap water to remove the particles that were not firmly attached to the surface of the specimens. The specimens were then stored at room temperature for 3 days to let the surfaces dry.

Visualization of crack filling

After the surfaces became dry, the specimens were subjected to a light microscopy analysis. The microscope (Leica S8AP0) was connected to a camera (Leica DFC295) to examine the crack filling. The cracks were viewed and photographed under a specific magnification.

Capillary water absorption

A modified capillary water absorption test based on RILEM 25 PEM II-6 [18] was performed to measure the water penetration resistance of the cracked mortar

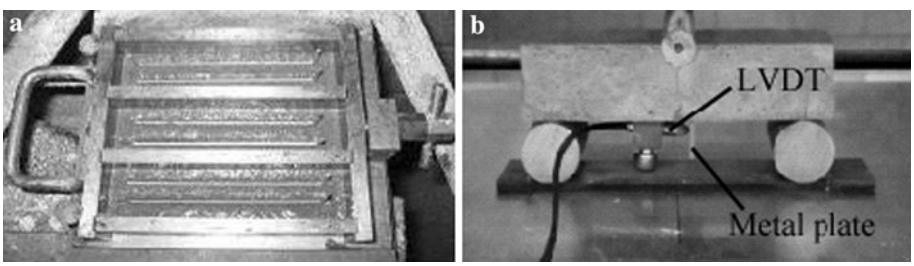

Fig. 3 Mortar specimens and the methodology for making a realistic crack. **a** Mold and reinforcement used for making prisms. **b** Setup of three-point bending test to create cracks in the mortar prisms

specimens with or without bacteria. The obtained water absorption coefficient was used to compare the crack-healing efficiency between the test series. The specimens were dried at 40°C in an oven until weight changes came to less than 0.1% at 24-h intervals. Before the test, the specimens were coated with a waterproof paint (SikaCor® 277) at four sides adjacent to the bottom surface where the crack existed. There was only one crack on the bottom surface. In order to protrude the effect of the crack filling and decrease the influence of other surface flaws on the capillary water absorption, part of the bottom surface was also coated with the paint (Fig. 4). An area with 40 × 20 mm around the crack was kept uncoated. The coated specimens were weighed before their exposure to a water bath (initial weight) and then were immersed to a depth of 5 ± 1 mm of tap water with the bottom surface facing downwards. The test was carried out in an atmosphere of 20°C and relative humidity of 60%. At regular time intervals (1, 2, 4, 8, 12, 24, 48, 72, 120, 240, and 360 h), the specimens were taken out of the water and weighed after wiping the surface with a wet towel. After the weighing, the specimens were immediately put back into the water. The water absorption coefficient K (g cm^{-2} h$^{-1/2}$) was determined by Eq. (1).

$$\frac{Q}{S} = K\sqrt{t} \quad (1)$$

in which Q is the weight of water absorbed at different time intervals (g); S is the uncoated area of the bottom facing to the water (cm^2); t is the time (h). K can be obtained from the slope of a plot of water absorbed per cm^2 against the square root of time.

Characterization of precipitation

After the water-absorption test, the specimens with obvious precipitation in cracks were broken along the original cracks (also by means of a three-point bending) and samples were taken from the fracture surface to characterize the precipitation along the crack.

The morphology of the precipitation was studied in a FEI QUANTA 200F SEM at accelerating voltage of 20 kV. Secondary electron imaging (SEI) was used for electron micrography. Samples were completely dried in an oven at 40°C for 2 days and were gold coated by a Baltec SCD030 Sputter Coater before examination. An energy dispersive spectrometer (EDAX, America) connected with SEM was used simultaneously to detect the components of the precipitation.

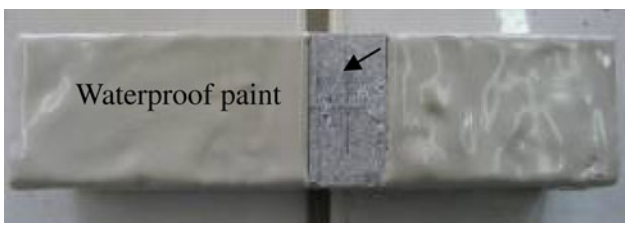

Fig. 4 Part of the bottom surface of the prism coated by the waterproof paint (**a** crack was in the uncoated area, at the position of the *arrow*)

Results

Bacterial ureolytic activity at different pH conditions

As shown in Fig. 5, both free bacterial cells and immobilized cells showed a high ureolytic activity in neutral pH and moderate alkaline environment, in which about 95% (in 20 g/l urea media) and 90% (in 40 g/l urea media) of urea was decomposed. There was no difference in ureolytic activity between un-immobilized and immobilized bacteria. However, the amount of urea decomposed by the free bacterial cells in the high pH cement slurry was greatly decreased, from 95% to less than 5%. The immobilized bacteria kept a much higher ureolytic activity than free bacterial cells. About 60% of the urea was decomposed by DE-immobilized bacteria in the cement slurry. The values of decomposed urea measured on the third day were slightly lower than the values after 1 day; this might be due to volatilization losses.

Influence of the DE concentration

It can be seen from Fig. 6 that upon increasing the amount of DE from 20 to 70%, the amount of urea decomposed by bacteria in cement slurry with initial urea concentration of 20 g/l was increased from about 60% (12 g/l) to 85% (17 g/l). The highest bacterial ureolytic activity was obtained by using 60 or 70% DE. There was an obvious increase in the amount of decomposed urea when the amount of DE was increased from 20 to 50%: the amount of decomposed urea was increased from about 60% (12 g/l) to 80% (16 g/l), but there was no significant difference between using 60% DE and 70% DE. A maximum of about 85% of the urea was decomposed in these two series. The values of decomposed urea measured on the third day were also slightly lower than the values on the first day due to ammonia losses.

Influence of nutrient

The nutrient used (yeast extract) had a positive effect on the bacterial ureolytic activity during immobilization. As shown in Fig. 7, in the series with a concentration of DE equal to 40%, there was an increase in the amount of decomposed urea

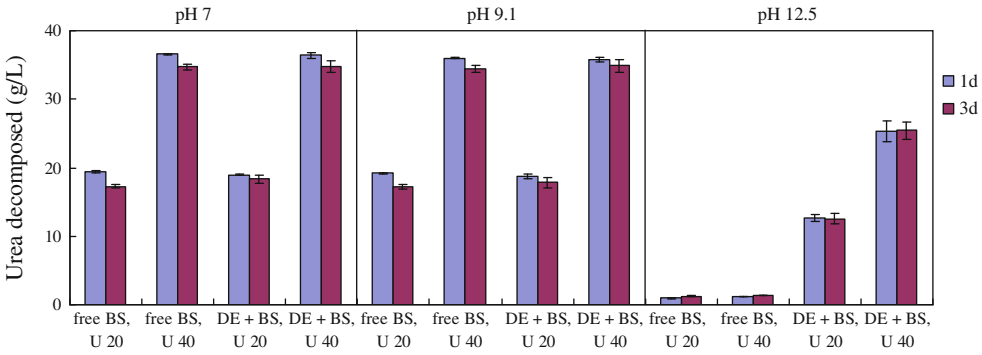

Fig. 5 Ureolytic activity of un-immobilized (free BS) and DE-immobilized bacteria (DE+BS) at different urea concentration and pH [codes such as U20 refer to urea concentration (e.g., 20 g/l)]

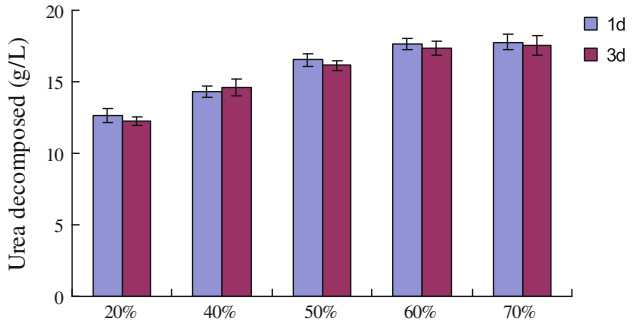

Fig. 6 Ureolytic activity of immobilized bacteria in high-pH cement slurry when using different concentrations of DE

in the cement slurries that contained more yeast extract (by comparing series 40%Y0Y1.25 and 40%Y0Y12.5, and series 40%Y10Y1.25 and 40%Y10Y12.5). However, no obvious difference in the amount of urea decomposed was observed between series 40%Y0Y1.25 and 40%Y10Y1.25, 40%Y0Y12.5 and 40%Y10Y12.5. In the series where the concentration of DE was 50 and 60%, more urea was decomposed when using yeast extract during immobilization. The overall trend in Fig. 7 is that the amount of decomposed urea increased as the concentration of DE increased, which confirmed the results in "Influence of the DE concentration".

Visualization of cracking filling

The specimens in the same series showed similar crack-filling states. Therefore, one representative image of each series is shown in Fig. 8. There was almost no precipitation formed in the cracks of the specimens with DE that were immersed in water (Fig. 8a). It was noticed that small amounts of white crystals were formed at the surface and in the cracks of the specimens with DE that were immersed in the deposition medium (Fig. 8b), yet the specimens with DE-immobilized bacteria had a quite different appearance. Cracks in the ones immersed in water were partly filled by the precipitation (Fig. 8c). Much more white precipitation formed in cracks of the specimens immersed in deposition medium. For those specimens, the cracks were completely filled (Fig. 8d). Similar to Fig. 8b, there were also some white deposits at the surface, but the amounts were much higher in Fig. 8d. Some pores in the surface were entirely filled by the white precipitation.

Capillary water absorption

The precipitation in cracks profoundly decreased the water absorption of the cracked specimens, especially in the first 24 h. It can be seen from Fig. 9 that the speed of water absorption in the specimens without bacteria was much faster than in the ones with bacteria. The sequence was DEBS M < DEBS W < DE M and DE W. After 24 h, the speed of water absorption gradually decreased. Cracks of the specimens with DE-immobilized bacteria immersed in the deposition medium were completely filled by the precipitation and showed the lowest water absorption. The ones with partly filled cracks showed more water absorption, but less water absorption than those without bacteria. Statistically, there was no significant difference among the specimens only with DE (without bacteria). Whether they were immersed in water or in the deposition medium, they all had higher water absorption than the specimens that were added with DE-immobilized bacteria. The water absorption in DEBS M and DEBS W was about one-third and 50% of that in DE W, respectively.

Morphology of the precipitation in cracks

Precipitation from different series of specimens showed different morphology. Figure 10a provides the image of the precipitation in the crack of the specimen with bacteria, which was immersed in water. Small particles with a size of about 5 μm agglomerated into flower-shape grains (15–20 μm). These grains were attached to the crack wall. The result of the element analysis from EDS confirmed that these particles were calcium carbonate. Similarly, a large amount of particles was found in the crack of the specimen with bacteria which had been immersed in the deposition medium. The particles

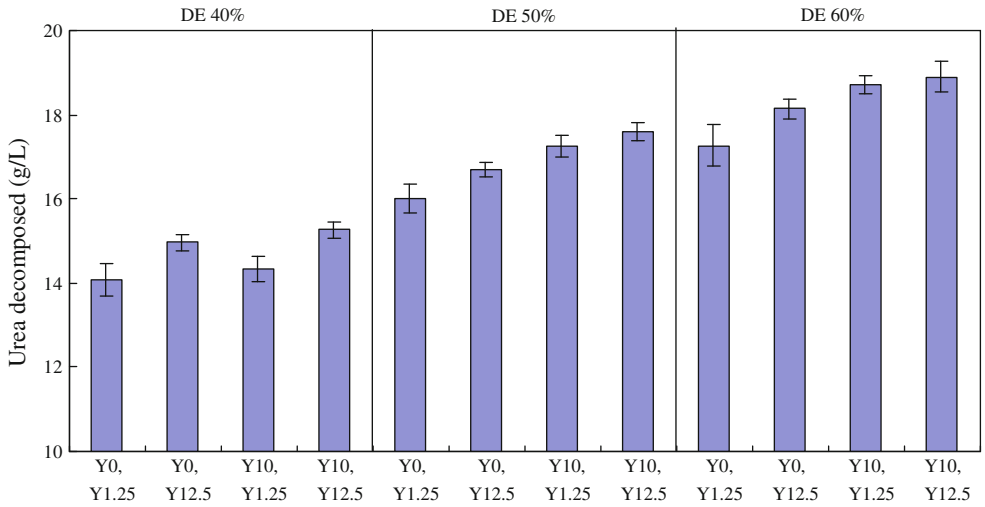

Fig. 7 Ureolytic activity of immobilized bacteria (with different concentrations of DE) under different concentrations of yeast extract

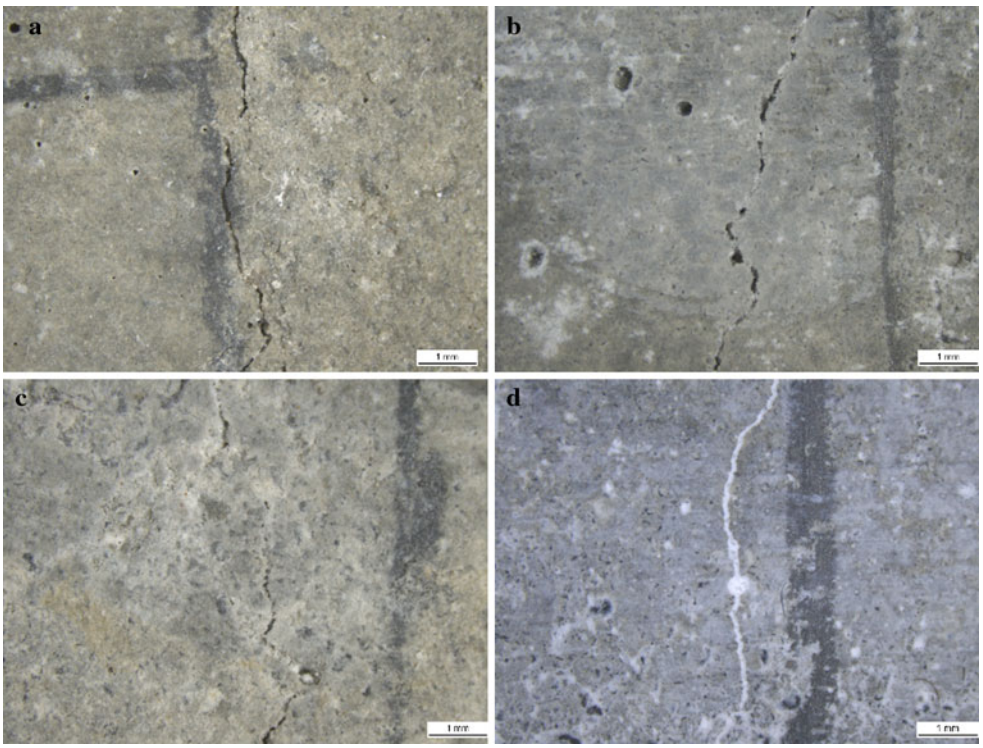

Fig. 8 Light microscopy images of the cracks in different specimens (**a**, **b**: specimen only with DE, immersed in water, and in the deposition medium, respectively; **c**, **d**: specimens with DE-immobilized bacteria, immersed in water, and in the deposition medium, respectively)

had irregular round shapes and most of them had a size of 5–10 μm. A few grains had a larger size of 20–25 μm. These particles were also calcium carbonate based on the result of EDS. Except for irregular round particles, some kind of long needle-like materials were also observed in the cracks. They were distributed around the calcium carbonate particles (indicated by the black arrow in Fig. 10b). It could be that they were crystals of un-reacted urea or $Ca(NO_3)_2$ along the crack wall. This needs to be further explored.

Discussion

In this study, it was shown that DE powders had a profound protective effect on the bacteria, particularly in the high-pH

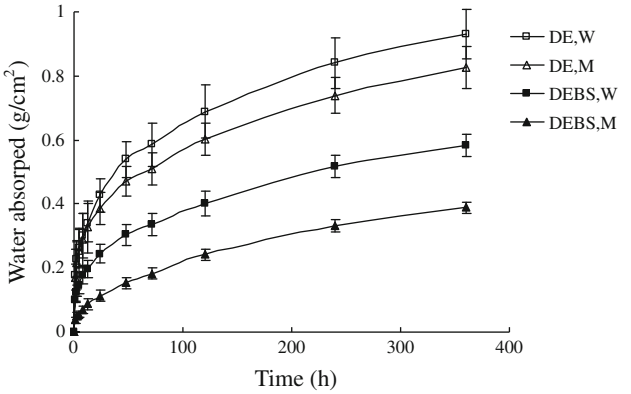

Fig. 9 Capillary water absorption of the specimens (in different series) as a function of time

cement slurry, which was made to mimic the really high pH environment inside concrete. The more DE was used, the higher the protective effect. DE particles have a porous structure. Most of the pores are at the nano-scale. That is the reason why DE has such a high specific surface area. Only some particles have hollow inner structures (Fig. 1c), which can shelter the bacteria that may be absorbed inside. Most of the pores are only 0.1–0.5 μm, but the size of the bacteria is about 1–2 μm. Therefore, bacterial cells were mainly sorbed on the surface of the particles. As shown in Fig. 5, free bacterial cells had a high ureolytic activity in neutral and moderate alkaline environment. Yet, this activity decreased rapidly in the high-pH cement slurry, which means that the unprotected bacteria can only show ureolytic activity in the environments of moderate pH. Therefore, the possible reason why DE immobilized bacteria could still keep a certain degree of ureolytic activity in the extremely high environment is that DE particles provided a kind of microenvironment for bacteria, in which the local pH around the bacteria was not as high as that in the cement slurry [25]. A higher ureolytic activity (indicated by more urea decomposed in cement slurry) was obtained when using a higher amount of DE. This is due to the fact that the same amount of bacteria was surrounded by more DE particles, which could form a thicker protective layer for the microenvironment and the bacteria.

The nutrient (yeast extract) had a positive effect on bacterial activity during the stage of immobilization and in cement suspension. The more nutrient used, the more urea was decomposed. At lower concentrations of DE (20 and 40%), the nutrient in the cement suspension had more of an effect on bacterial ureolytic activity than in the pre-mixture during immobilization. The situation was different when using a higher concentration of DE. It can be seen that more urea was decomposed in series 50%Y10Y1.25 and 60%Y10Y1.25, though the total concentration of yeast extract of these series (3 g/l) was lower than that in series 50%Y0Y12.5 and 60%Y0Y12.5 (10 g/l). In this case, the nutrient had a more obvious impact on the process of immobilization than in the cement suspension. The pre-mixture made of DE and BS was a kind of easily flowable suspension when using low concentrations of DE and the nutrient was distributed homogeneously in the pre-mixture. At higher DE concentrations (60 and 70%), the pre-mixture became un-flowable paste (Fig. 2c, d). The nutrient was incorporated inside the paste together with the bacterial cells. Therefore, the bacteria could get more nutrients around them from the microenvironment in the un-flowable paste than in the flowable suspension. After the pre-mixtures were added to the cement suspensions, the amount of nutrients in the cement suspension had less of an impact on the bacteria in the paste than in the flowable suspension, where the bacteria did not get enough nutrients from the microenvironment during the process of immobilization.

In view of the crack filling in different kinds of specimens, it can be concluded that there was a self-healing behavior in the specimens with DE-immobilized bacteria. The healing was more effective when the specimens were immersed in the medium with urea and Ca^{2+}. Bacteria can not only decompose urea from the crack wall of the specimens (incorporated during casting), but also use the urea and Ca^{2+} from the deposition medium to produce more calcium carbonate. That is why more precipitation formed and completely filled the cracks in the specimens (with bacteria) immersed in the medium. Based on the SEM images and EDS results of the precipitation, it can be seen that the precipitation in the partly filled cracks (in the specimens with bacteria that were immersed in water) was calcium carbonate, but the precipitation in the completely filled crack contained both calcium carbonate and some other kind of material, which had a needle shape. Since the only difference between the specimens DEBS W and DEBS M was the immersion media, the extra long needle particles were mainly due to the re-crystallization of un-reacted urea or $Ca(NO_3)_2$ from the deposition medium. From the result of capillary water absorption, it can be seen that the specimens with completely filled cracks had the highest water penetration resistance due to the precipitation inside the cracks.

It can be seen from Figs. 5 and 6 that the amount of urea decomposed at the first day was almost the same as that at the third day, which indicated that bacterial ureolytic activity under this extremely high pH might not last beyond 1 day. However, the DE-immobilized bacteria showed a different behavior after being added into real cement specimens. They still hold ureolytic activity after 2 weeks because the cracks, made after 2 weeks, were completely or partly filled by the precipitation produced by the bacteria. The reason could be due to the difference between the cement slurry and real cement specimens although they

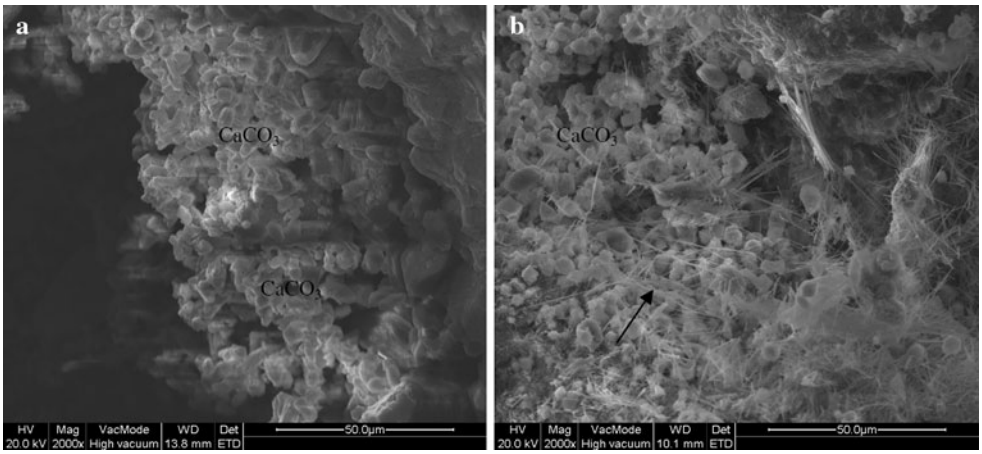

Fig. 10 Morphology of the precipitation in cracks of the specimens with DE-immobilized bacteria (the *black arrow* in **b** indicated the long needle-like material). **a** Precipitation in the crack of the specimen with DE-immobilized bacteria immersed in water. **b** Precipitation in the crack of the specimen with DE-immobilized bacteria immersed in the deposition medium

almost had the same pH. The cement slurry was kept being stirred on a shaker (100 rpm, 28°C) during the whole period of testing, which means more severe exposure to bacteria in the slurry. In mortar specimens, the mixing only lasts 2 min before casting. After that, there was no continuous mixing in the specimens since they started to solidify. The DE-immobilized bacteria might transfer to the dormant condition under the environment with high pH and no oxygen. It has been reported in US Patent 3898132 [17] that DE was used to obtain reversible dormancy of active microorganisms by mixing a high amount of DE with microorganisms in a closed environment. After cracking occurred in the specimens, the bacteria around the crack were activated by the oxygen and water and then showed the activity to precipitate $CaCO_3$.

It is noted that although the optimal concentration of DE for immobilizing bacteria was 60%, practical difficulties were encountered during the casting of the specimens. DE powders have a strong capacity to absorb water because of the high specific surface area. If the addition of DE is more than 5% of the cement by mass, the mortar paste would become very dry and the workability decreases a lot. For the latter application, if a larger amount of DE is needed to improve the protective effect and obtain more precipitation, some amount of sands could be considered to be replaced by DE at the prerequisite that the strength of the specimens will not be decreased. Overall, taking the optimal combination of DE, BS, nutrients and mortar specimens, one comes to the equivalent weight of 11 kg DE empowered with 6 g bacterial dry mass, 5.5 kg urea, 11 kg $Ca(NO_3)_2$, and 0.55 kg yeast extract, i.e., a total of about 28 kg additives per 1,000 kg of plain mortar (made of cement, sand, and water). The costs of the concrete could be increased by 20% because of these supplements. However, in terms of the added value generated by the putative self-healing process, it is still very promising for the practical use since the costs for the later on maintenance would be completely saved or at least greatly decreased.

Further research needs to be done on how to supply more urea and Ca^{2+} from the inside of the concrete matrix for the bacteria to precipitate enough calcium carbonate to completely fill the cracks. The supply should come from the inside (for example by only immersing into water or sprayed with water) because this constitutes real self-healing.

Conclusions

Diatomaceous earth (DE) was found to have a protective effect for the bacteria in a high-pH cement environment. The possible mechanism is that DE particles have a strong capacity to sorb bacterial cells on the surfaces due to their high specific surface area. After sorption, DE provided a kind of microenvironment around the bacteria, in which the local pH was less aggressive than that in the whole cementitious environment and thus bacteria could still decompose urea. The more DE that was used, the more urea that was decomposed, which indicated a higher ureolytic activity. The optimal concentration of DE for immobilization was 60% (w/v). Cracks with a width of 0.15–0.17 mm in mortar specimens were partly or completely filled by the aid of DE-immobilized bacteria depending on the immersion media. The precipitation in the cracks was mainly composed of calcium carbonate with a small amount of excessive urea or $Ca(NO_3)_2$ crystals

(if the specimens were immersed in the deposition medium). The capillary water absorption in the specimens with bacteria was about 50% (cracks were partly filled) or 70% (cracks were completely filled) lower than in the specimens without bacteria.

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