Coventry University and
The University of Wisconsin Milwaukee Centre for By-products Utilization,
Second International Conference on Sustainable Construction Materials and Technologies
June 28 - June 30, 2010, Università Politecnica delle Marche, Ancona, Italy.
Main Proceedings ed. J Zachar, P Claisse, T R Naik, E Ganjian. ISBN 978-1-4507-1490-7
http://www.claisse.info/Proceedings.htm

Potential of Applying Bacteria to Heal Cracks in Concrete

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ABSTRACT

Concrete is a construction material that is used world-wide because of its first-rate properties. However, the drawback of this material is that it easily cracks due to its low tensile strength. As large costs are involved in crack repair, the potential of self-healing of these cracks by means of calcium carbonate ($CaCO_3$) precipitating bacteria was investigated in this study. First, the survival of the bacteria was tested. Next, the optimal concentrations of bacterial cells, urea and Ca^{2+} were determined in order to obtain a maximum amount of $CaCO_3$ precipitation. Finally, self-healing of cracks in mortar specimens, by means of bacteria, was investigated. Glass tubes, containing the healing agent were provided inside the mortar matrix. Upon crack occurrence, the tubes break and the healing agent, consisting of a filler material and bacteria, is released into the crack and can cause crack repair. Strength regain up to 60% was thus observed due to self-healing.

INTRODUCTION

Cracks often occur in concrete because of the low tensile strength of this material. Rapid crack-healing is necessary since it is easier for aggressive substances to ingress into concrete through cracks than through the concrete matrix. It is known that it is costly to inspect, monitor and repair cracks. Moreover, some of the repair methods currently used are not so sustainable [Neville 1996]. Therefore, it would be desirable if concrete cracks could be healed autonomously by releasing healing agents inside the matrix when cracks appear. In this research, an environment-friendly and autonomous crack repair technique is explored. Previous research has shown that *Bacillus sphaericus* bacteria are able to precipitate calcium carbonate ($CaCO_3$) on their cell constituents and in their micro-environment by conversion of urea ($CO(NH_2)_2$) into ammonium (NH_4^+) and carbonate (CO_3^{2-}). The bacterial degradation of urea locally increases the pH and promotes the microbial deposition of calcium carbonate

in a calcium rich environment. Through this process, the bacterial cell is coated with a layer of calcium carbonate [Dick et al. 2006].

The aim of our research is to use this bio-precipitated $CaCO_3$ to heal cracks in concrete. A calculation showed that precipitation of $CaCO_3$ is not enough to fill wide concrete cracks completely. Therefore, a filling material is needed. In previous research, on manual healing of cracks in concrete by means of bacteria, silica gel was used as filling material [Van Tittelboom et al. 2010b]. Bacteria were mixed into the gel and both were injected into the crack. However, as the gel matrix showed some tiny cracks due to shrinkage and had no strength on its own, polyurethane (PU) was used as filling material in this research. PU with immobilized bacteria has already been reported to be used to repair concrete cracks [Bang et al. 2001]. Bacterial cells were first immobilized into the PU foam. Then, the PU foam was cut into equal-sized pieces. Afterwards, PU foam strips were placed into simulated cracks of mortar specimens. The specimens were then incubated in a urea-$CaCl_2$ medium at room temperature. As a result of $CaCO_3$ precipitation, the regain of compressive strength of the specimens was reported to be obtained.
Different from the method described above, in which PU foam with immobilized bacteria was pre-formed and placed into the cracks manually, in our research, PU foam was formed in the crack automatically upon crack appearance. Furthermore, at the same time bacteria were incorporated inside the foam. Bacteria and PU were encapsulated in glass tubes with three compartments which were embedded inside mortar specimens. One compartment of the tubes contained the first component of the PU. The second compartment was filled with the nutrients for bacterial growth, a calcium source and the second component of the PU. The last compartment was filled with the bacterial cells. When a crack appears in the mortar matrix, the glass tubes break and all components mentioned above can flow into the crack and mix together. First, polymerization of the filling agent (polyurethane) is initiated and through this process strength regain is obtained. In a second step, the bacteria, which are dispersed through the filling material can precipitate $CaCO_3$ crystals in the pores of the PU and through this, an additional regain in strength may be obtained.

MATERIALS AND METHODS

Bacterial strain

Bacillus sphaericus LMG 22557 (Belgian coordinated collection of microorganisms, Ghent) was used based on previous research. This strain has a high urease activity and can produce $CaCO_3$ in a simple and controlled way [De Muynck et.al, 2009].
The growth medium for *Bacillus sphaericus* was composed of yeast extract and urea. (The growth medium was used to grow bacterial cells). The yeast extract medium was first autoclaved for 20 min at 120°C and then added to the filter sterilized urea solution. The final concentrations of yeast extract and urea were 20g/L. Cultures were incubated at 28°C on a shaker at 120 rpm for 24 h. Bacterial cells were harvested by centrifuging the 24 h-old grown culture and were resuspended in saline solution (NaCl, 8.5 g/L).

Determination of the optimal conditions for bio-precipitation

The more $CaCO_3$ precipitates, the better the self-healing effect will be. The concentrations of bacteria, urea and Ca^{2+} will greatly affect the amount of precipitated $CaCO_3$. A series of tests were performed to investigate the optimal concentrations of bacteria, urea and Ca^{2+}.

Bacterial concentration. Different amounts (0.1, 1 and 5 mL) of bacterial solutions (10^8cells/mL) were added into solutions with different concentrations of urea (90, 120, 150 and 180 g/L). The final volume of all urea solutions (after addition of the bacterial solutions) was 100 mL. The final bacterial concentration in the different urea solutions was $1.0x10^5$, $1.0x10^6$ and $5.0x10^6$ cells/mL, respectively. Conductivity of the urea solutions was measured to indicate the amount of urea decomposed by the bacteria (urea hydrolyzed (mM) = conductivity ($mS.cm^{-1}$) ×9.6) [De Muynck et al. 2009].

Concentration of urea and Ca^{2+}. The deposition medium (the deposition medium was used for bacteria to precipitate $CaCO_3$) was made up of urea, $Ca(NO_3)_2$ and yeast extract. Yeast extract (20 g/L) was used to serve as nutrient for the bacteria. The composition of the deposition medium is shown in Table 1. Each medium with inoculated bacteria (group A, B, C and D) had three replicates and for each composition of the deposition medium also a control series (medium with no bacteria added) was used. Concentrations of urea and Ca^{2+} were equimolar, with 1M urea corresponding to 60 g/L and 1M Ca^{2+} corresponding to 236 g/L $Ca(NO_3)_2.4H_2O$. All media were put on a shaker (28°C, 120rpm). After three days, Total Ammonium Nitrogen (TAN) of the media was measured colorimetric ally by the method of Nessler (Ivanov et al., 2005). The obtained results were transformed into the amount of urea hydrolyzed (mM) in the deposition medium, which can be used as an indication of the amount of precipitated $CaCO_3$.

Table 1. Concentration of Urea, $Ca(NO_3)_2$ and Bacteria in the Deposition Media

	Concentration of Urea/Ca^{2+} (M/M)	Concentration of bacteria (cells/mL)
Control 1	0.5/0.5	0
Control 2	1/1	0
Group A	0.5/0.5	1.7×10^7
Group B	1/1	1.7×10^7
Group C	0.5/0.5	1.7×10^8
Group D	1/1	1.7×10^8

Survival test of bacteria

In this experiment it was tested how long the bacteria can remain viable and sustain high urease activity in a concrete environment. Batches of 2 mL bacterial solution (10^8 cells/mL) were added into a tube. The tubes were then closed tightly and put in the incubator at 28°C. At specific time intervals (every two weeks), six tubes were taken from the incubator. For three of those tubes, bacteria were inoculated into sterile growth medium (yeast extract 20 g/L and urea 20 g/L). For the remaining three tubes, bacteria were inoculated into sterile deposition medium (yeast extract 20 g/L, urea 20 g/L and $Ca(NO_3)_2.4H_2O$ 79 g/L). All media were then put on the shaker (28°C,

120 rpm). Changes of optical density (OD), which can be used as an indication of the bacterial concentration, conductivity and pH of the growth media were measured to see whether the bacteria were still alive after storage for some period. The amount of $CaCO_3$ precipitated was obtained by measuring the TAN value of the deposition media after three days.

Self-healing by the use of PU-immobilized bacteria

Preparation of mortar specimens. The optimal concentrations obtained from the above experiments were used in the following experiments. Polyurethane (MEYCO MP 355 1K, represented by PU) was used as filling material. Glass tubes with a length of 50 mm and an inner diameter of 3mm were used to carry the healing agent (shown in Figure.1). First, three glass tubes were glued together and one of their ends was sealed. Then the healing agent was injected into each tube from the other end, which was sealed afterwards. For the test samples belonging to group A, one compartment of the tubes was filled with the first component of the PU. The second one was filled with the second component of the PU together with the deposition medium. The third compartment was filled with active bacterial cells (10^8 cells/mL, represented by BS). For samples in group B, the first two compartments were filled with the same components as in the case of group A. The third compartment was filled with autoclaved (dead) bacterial cells (represented by BSA). In both groups (A and B), the volume ratio of the first component of the PU to the deposition medium was 1:2. The deposition medium was composed of urea (20 g/L), $Ca(NO_3)_2.4H_2O$ (79 g/L) and yeast extract (20 g/L).

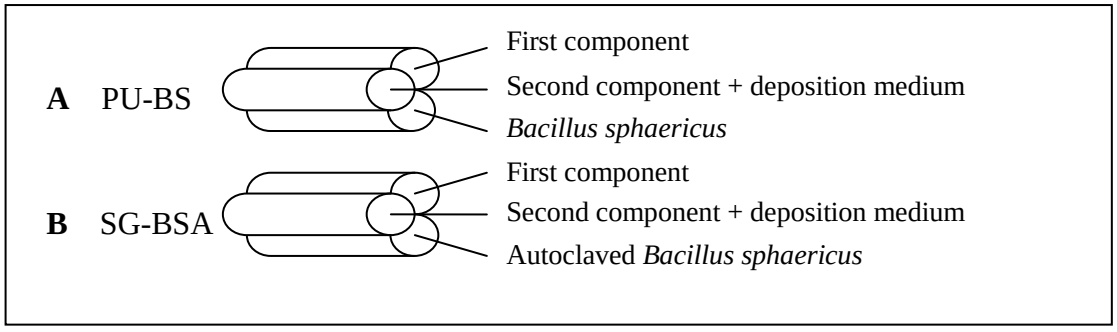

Fig. 1. Glass Tubes with the Self-Healing Agents

Mortar specimens (60 mm x 60 mm x 220 mm) with a water to cement ratio of 0.5 and a sand to cement ratio of 3 were made by using ordinary Portland cement CEM I 52.5N. First, a 10 mm mortar layer was brought into the moulds. After this layer was compacted by means of vibration, two reinforcement bars were placed on top of it. Meanwhile, two compartmented tubes, consisting of three glass tubes glued next to each other, were also put on the top of the layer. Afterwards, the moulds were completely filled with mortar and vibrated. Three replicates were prepared with each type of tubes (groups A and B). Also one reference specimen, without glass tubes was prepared. All moulds were put in an air-conditioned room with a temperature of 20°C and a relative humidity of more than 90% for 24h. After demoulding, the mortar specimens were placed in the same room for two weeks.

Creation of cracks. After two weeks, the mortar specimens were taken out of the curing room

and cracks were created by means of a crack width controlled three-point bending test, as shown in Figure. 2. The reference specimens were loaded after 1 week and reloaded one day later. Crack width was measured by means of a linear variable differential transformer (LVDT) which was attached at the bottom of the specimens [Van Tittelboom et al. 2010a]. The crack width was increased with a velocity of 0.0005 mm/sec until a crack of 0.5 mm was obtained. After unloading, the remaining crack width was about 0.3 mm.

Regain in mechanical properties. After performance of the bending test, all specimens were placed again into the curing room. One week later, strength regain was measured by reloading the specimens. The self-healing efficiency was evaluated by comparing the peak load obtained during the first loading cycle and the reloading cycle.

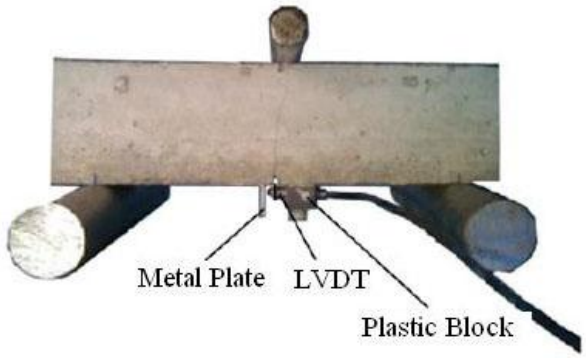

Fig. 2. Crack Formation by Means of a Three-Point Bending Test

Determination of the amount of $CaCO_3$ precipitation.
Thermogravimetric analysis (TGA) was performed on the crack repair material in order to see whether there were any $CaCO_3$ crystals formed in the PU foam. Samples were obtained by scraping off some PU foam, adhering to the crack wall, after specimens were completely broken. To determine whether autoclaved bacteria can precipitate $CaCO_3$ in PU foam, glass tubes filled with the same healing agents as used in the self-healing experiments were broken manually and the healing agents were poured into a beaker. In the beaker, PU foam formed and $CaCO_3$ precipitated inside the foam. The beaker was put in a closed jar with 100% relative humidity and the temperature inside was 28ºC. One week later, samples were taken from the PU foam and TGA measurements were performed on the material.

RESULTS AND DISCUSSION

Optimal conditions

Bacterial concentration. It can be seen from Figure. 3 that a higher concentration of bacterial cells can decompose more urea. When the bacterial concentrations were 10^5 and 10^6 cells/mL, the amount of urea decomposed decreased as the concentration of urea increased. When using a higher concentration of bacterial cells, the amount of urea decomposed increased with increasing concentrations of urea.

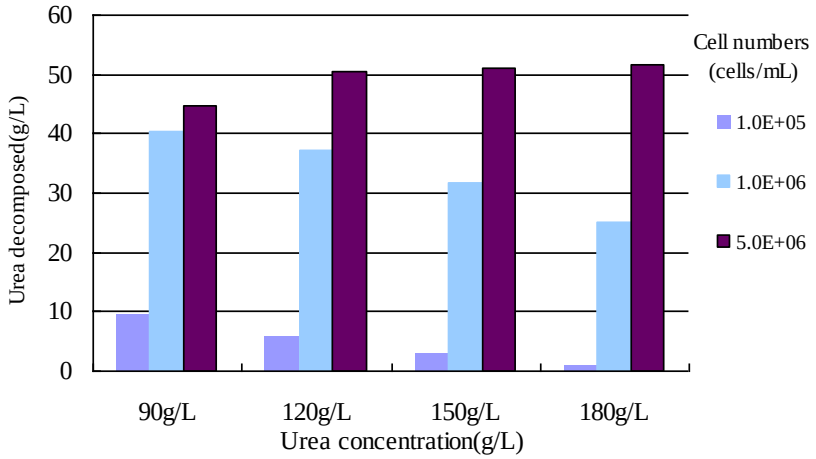

Fig. 3. Influence of the Concentration of Urea and Bacteria on the Amount of Urea Decomposed

Concentration of urea and Ca^{2+}. The amount of urea decomposed in each group was measured every day (as shown in Figure. 4). It can be seen that for the control series, no urea was decomposed. The more bacteria used, the higher the amount of urea hydrolyzed. When using a higher bacterial concentration (in group C and D), more urea was decomposed in the deposition medium containing higher concentration of Ca^{2+} (1M). However, when using a lower bacterial concentration (in group A and B), more urea was decomposed in the deposition medium containing lower concentration of Ca^{2+} (0.5M). The reason could be that bacterial activity is greatly affected by some metal ions such as Ca^{2+} [Yang et al. 2007; Qian et al.2009]. Ca^{2+} is necessary for bacteria during the growth and metabolisation. However, bacterial urease activity decreased when the concentration of Ca^{2+} was higher than 0.5M. Though the activity of each bacterial cell decreased a lot due to high amount of Ca^{2+}, the summation of urea decomposed by all bacteria could still be high, given the larger amount of bacterial cells. So the final amount of urea decomposed in group D was higher than that in group C. However, the percentage of urea decomposed in group D (67%) was lower than that in group C (80%).

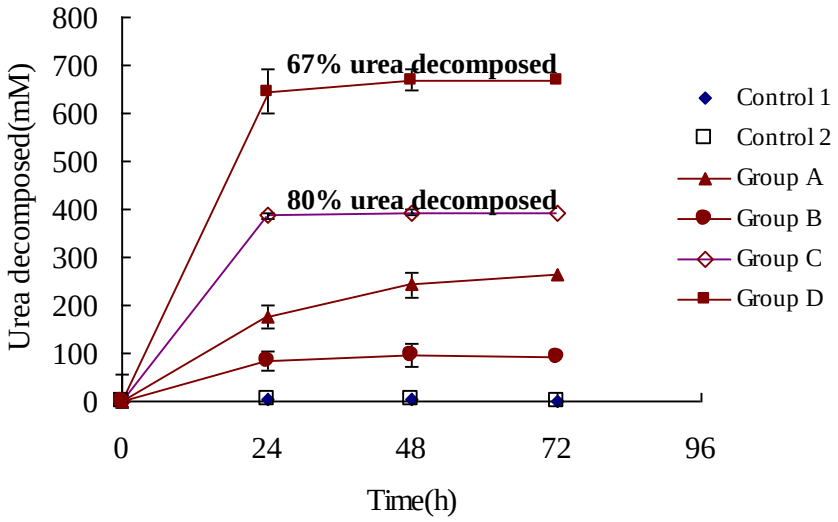

Fig. 4. Influence of the Concentration of Bacteria, Urea and Ca^{2+} on the Amount of Urea Decomposed in the Deposition Media

In order to obtain a maximum amount of precipitated $CaCO_3$, high concentrations of bacteria should be used. However, the concentrations of urea and Ca^{2+} cannot be too high, because the percentage of urea decomposed decreased as the concentration of urea increased. This means that there will be more urea left in the concrete matrix. Surface scaling can occur where urea crystallizes on concrete surfaces. However, some research indicated that urea can enhance concrete durability [Sadegzadeh et al. 1993; Shaaban et al. 1997]. So the remaining urea may have an unknown effect on the concrete matrix. Meanwhile, since a high concentration of Ca^{2+} will also decrease bacterial activity, the suitable concentration of urea and Ca^{2+} in the deposition medium might be 0.5M.

Survival of the bacterial cells

The amount of $CaCO_3$ precipitated by 10 weeks old bacteria cultures (stored at 28°C) was similar as that observed with 24 hours old bacterial cultures, as shown in Figure. 5. From this survival test it may be concluded that this kind of bacteria can remain viable and maintain high activity for more than 10 weeks when stored at 28°C. Further research will focus on using bacterial spores since they can survive a longer time and resist a much harsher environment.

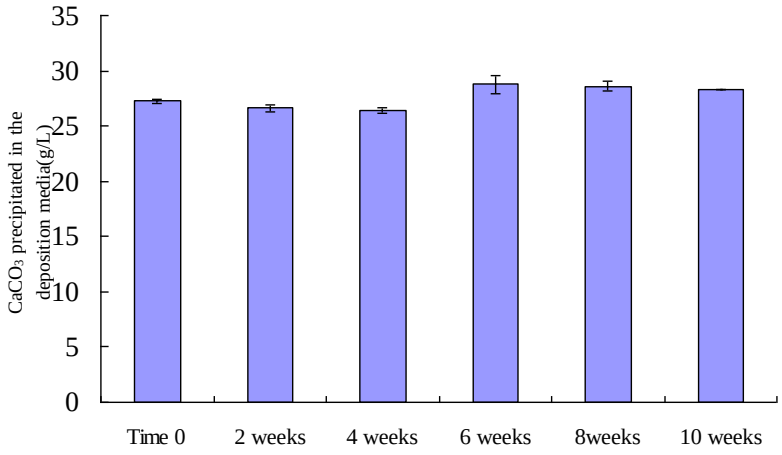

Fig. 5. Amount of $CaCO_3$ Precipitated in the Deposition Media Inoculated with the Bacteria after Different Times of Storage

Regain in mechanical properties

Figure. 6 indicates that no strength regain was obtained for the reference specimens with no healing agents inside.

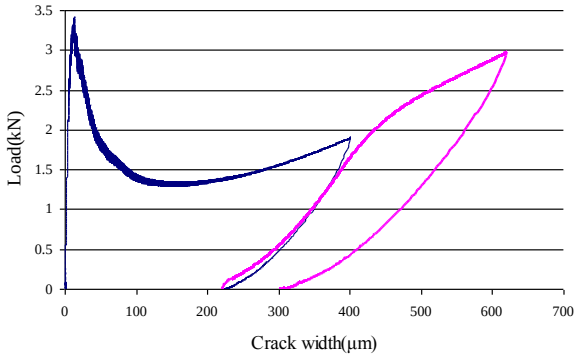

Fig. 6. Loading Cycles of the Specimen with No Healing Agents Inside

However, considerable strength regain was obtained in the case of specimens with incorporated healing agents (Figure. 7 and Figure. 8). The amount of strength regain varies from 42% to 88%. The average strength regain was about 60% for both groups. There was no obvious difference in strength regain between the two groups, the one with living bacteria and the other with dead bacteria, as shown in Figure. 7 and Figure. 8.

The results of TGA measurements are shown in Figure. 9. It is seen that $CaCO_3$ crystals were present in the cracks of specimens with PU and living cells and also in the cracks of samples with PU and autoclaved bacteria.

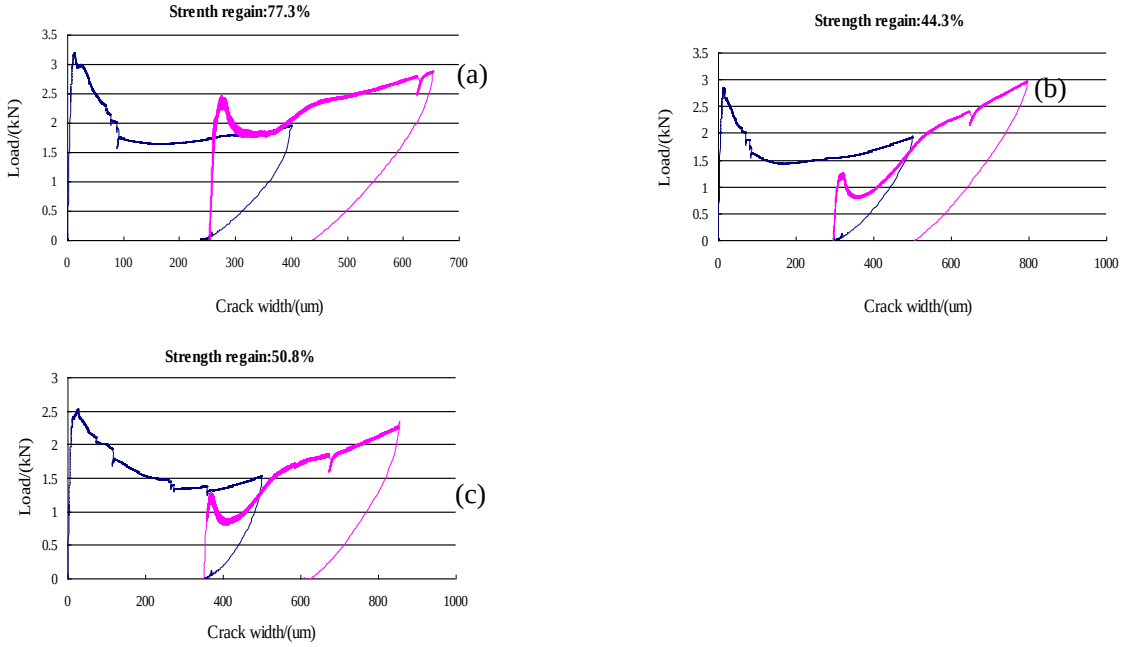

Fig. 7. Loading Cycles of the Specimens with PU and Living Bacteria (a, b, c are three replicates)

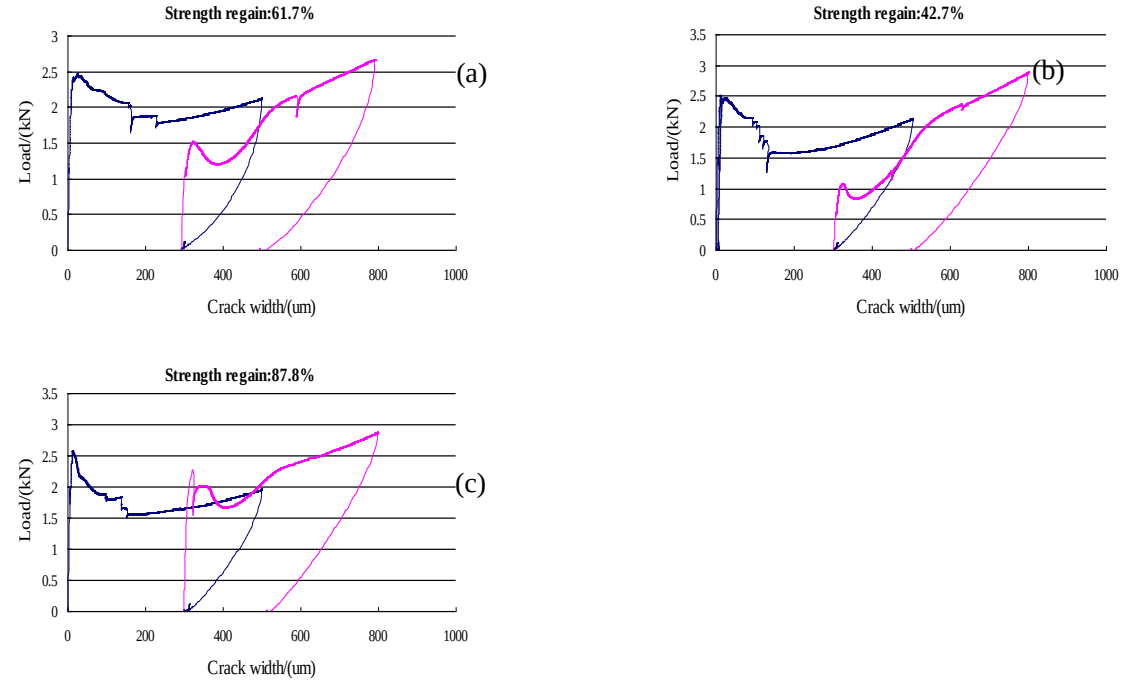

Fig.8 Loading Cycles of the Specimens with PU and Autoclaved Bacteria (a, b, c are three replicates)

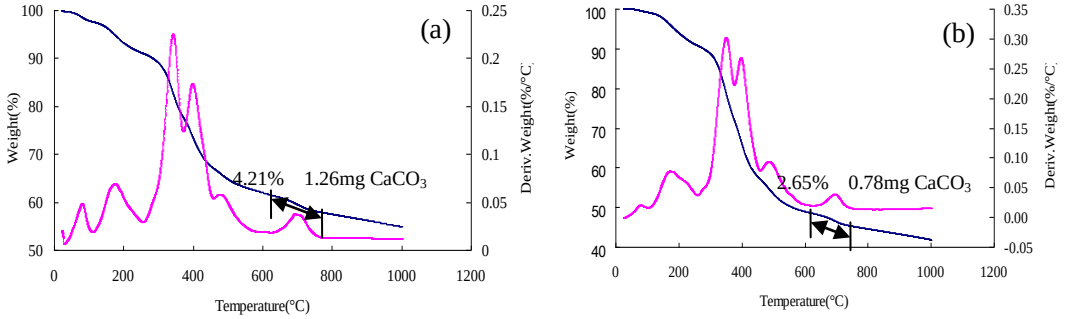

Fig. 9. TGA Results of the Material Scraped from the Crack Wall of the Mortar Samples, (a) PU with Living Bacteria (PU BS) and (b) PU with Autoclaved Bacteria(PU BSA)

The TGA results of the PU foam samples from the beakers are shown in Figure.10. It can be seen that no $CaCO_3$ was formed when there were no bacteria in the PU foam or when autoclaved bacteria were embedded inside the foam (Figure. 10 (a) and(b)). An obvious peak around 700°C existed when PU was mixed with living bacteria. This peak suggests the presence of $CaCO_3$ precipitation inside the foam.

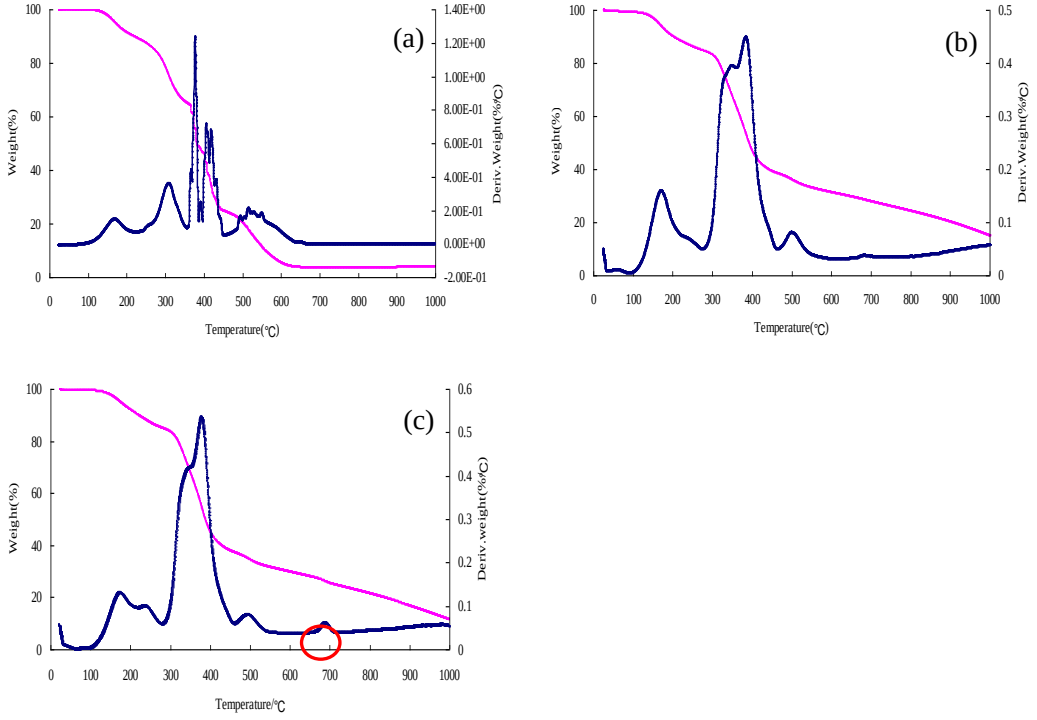

Fig. 10. TGA Graphs of the Manually Mixed Solutions, (a) PU Foam without Bacteria, (b) PU Foam Mixed with Autoclaved Bacteria and (c) PU Mixed with Living Bacteria

Therefore, the amount of $CaCO_3$ detected in Figure. 9(b) came from the mortar specimen itself when scraping the PU foam from the crack wall. From Figure. 10(c) it can be seen that some bio-$CaCO_3$ can be formed in PU foam. However, when testing strength regain due to crack healing it was seen that the healing effect was mostly caused by the PU foam and not by the bacterially precipitated $CaCO_3$, because the amount of precipitation was quite limited.
It was seen that bacteria can precipitate a large amount of $CaCO_3$ in an aqueous environment (the deposition medium). However, in PU foam, when the same concentrations of bacteria, urea and Ca^{2+} were used, only a limited quantity of $CaCO_3$ was formed. The main reason might be that there is no sufficient water for microbial activity inside PU foam. This will hinder the contact among bacteria, urea and Ca^{2+}. Future research will focus on increasing the amount of bio-$CaCO_3$ precipitation in the PU foam. Based on the results from the initial experiments, the theoretical maximum amount of $CaCO_3$ precipitation is 20mg/mL tube, which means that 20 mg $CaCO_3$ could be obtained in per 1 cm^3 volume of the tube. Since each specimen, with the geometry described, contained 2 sets of 3 tubes, (each tube having a volume of 353 mm^3), a maximum amount of about 40 mg $CaCO_3$ can be precipitated in the crack. An additional point for future research will be the evaluation of the decrease in water permeability for specimens with PU healing agent with and without immobilized bacteria.

CONCLUSIONS

The following conclusions can be drawn from the research provided in this paper:

- The higher the concentration of the bacteria, the more $CaCO_3$ precipitated.
- A suitable concentration of urea and Ca^{2+} for self-healing is 0.5M.
- The activity of the bacteria stored at 28°C did not decrease even after 70 days.
- Considerable strength regain was obtained when using polyurethane and living or dead bacteria. Bacteria can precipitate $CaCO_3$ inside the PU foam, but the amount was not enough to improve the regain in strength. The strength regain which was observed was mainly caused by the release of PU foam inside the crack.

ACKNOWLEDGEMENTS

Financial support from the Research Foundation Flanders (FWO-Vlaanderen) for this study (Project No. G.0157.08) is gratefully acknowledged.

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