AN EXPERIMENTAL INVESTIGATION ON THE STRENGTH AND DURABILITY OF SELF-HEALING BACTERIAL CONCRETE

S. Bhaskar[1], M. Lachemi[1], K. M. A. Hossain[1], G. Wolfaardt[2], M. Kroukamp[2]

[1]Department of Civil Engineering, [2]Department of Chemistry and Biology, Ryerson University, 350 Victoria street, Toronto M5B 2K3, Canada

Abstract: Crack repair in concrete is crucial since cracks are the main cause of 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. By incorporating bacteria and nutrients as a two component healing agent in concrete matrix, the process of bacterially mediated calcium carbonate deposition was triggered upon crack formation and self-healing of cracks can be expected. This research is aimed at investigating the potential of the mineral producing bacteria on their long-term viability, their incorporation in the concrete matrix and their self-healing ability for autonomous crack repair. Zeolite is used as a carrier material in this study to protect bacteria from the high-pH environment of concrete. In order to develop a self-healing bacterial concrete, it is crucial to understand how the introduction of the mineral producing bacteria and affect of nutrients on the properties of concrete, giving emphasis on closure of cracks. The long term durability of concrete is affected to a large extend by its permeability. Hence, the permeation properties are the important factors to study in relation to concrete durability. Therefore, this paper presents the results of an experimental investigation carried out to evaluate the influence of bacteria on compressive strength and permeation properties.

1 INTRODUCTION

Concrete is presently the most used construction material worldwide (Emmons and Sordyl 2006). Concrete structures often suffer from cracking that leads to deterioration and shortening of their service life. Cracks can occur at any stage of the service life due to volume instabilities within concrete or external factors such as extreme loading, harsh environmental exposure, poor construction procedures or design error. Micro-cracks permit the penetration of water and other impurities such as chloride and sulphate ions in to the concrete matrix leading to premature matrix degradation, corrosion of embedded reinforcement etc. which in turn hinders structural integrity. Generally, huge expenses are incurred in maintenance and repair of concrete structures. It is estimated that damage due to corrosion of concrete reinforcement in the US is $276 billion (Koch et al. 2001) with annual cost in repairs to be $18 – 21 billion per year (Emmons and Sordyl 2006). Moreover, indirect costs due to traffic jams and associated loss of productivity due to reparation are even 10 times higher than the direct costs of maintenance and repair (Freyermuth 2001). Therefore, it is imperative that crack propagation in concrete must be minimized to extend the longevity thus reducing maintenance. There is a compelling economic incentive to develop a concrete that can treat and repair the damage all by itself.

Concrete has the ability to self-heal, known as autogenous healing, which is similar to the self-healing abilities of living organisms to small bodily damage. This autogenous healing has been observed for many years (Neville 2002, Li and Yang 2007). It has been noticed that the micro cracks in old structures were self-healed by the recrystallization of calcite (Edvardsen 1999). This reveals that under the right environment concrete is able to seal the cracks by itself with the augmentation of some chemical and/or biological additives and with the presence of moisture. In general, under the right environment, carbon dioxide in the air is dissolved in water, and this carbon dioxide reacts with the calcium ions in the concrete to produce calcium carbonate crystals. The calcium carbonate crystallization made in this way is attached and grown on the crack surface. This leads to the reduction in crack width and eventual repair of the whole crack (Edvardsen 1999). However, depending on this natural process alone, only a limited crack with width up to 100µm can be repaired (Neville 2002, Li and Yang 2007). However, to repair larger

cracks with better healing consistency, the addition of both chemical and biological amendments might be needed. Previous studies by various researchers have concluded that self-healing behaviour can be achieved by the introduction of bacteria into the concrete matrix (Jonkers and Schlangen 2009, Wang et al. 2012). In brief, it was hypothesized that once moisture enters through freshly formed cracks, dormant but viable bacterial spores immobilized in the concrete matrix becomes metabolically active. Then, these cracks will be healed through microbial calcite precipitation, impeding further ingression of water and other chemicals.

The research aims at investigating the potential of the mineral producing bacteria on their self-healing ability for autonomous crack repair. This paper has summarised the results of the efficiency of zeolite as a bacterial carrier to be used in the high-pH concrete environment for self-healing cracks in mortar. In this paper, both ureolytic activity and self-healing efficiency (in terms of compressive strength and permeation properties) of zeolite immobilized bacteria are investigated.

2 EXPERIMENTAL INVESTIGATION

2.1 Bacterial strains and cultivation

Sporosarcina pasteurii of genus *Bacillus* was selected for the study and was purchased from German Collection of Microorganisms and Cell Cultures, Braunschweig, Germany. This is an aerobic alkali-resistant spore forming strain, with a high urease activity (Bang et al. 2001, Ramakrishnan et al. 2005, Achal et al. 2011) and ability to produce $CaCO_3$ in a controllable way (Bang et al. 2001, Ramakrishnan et al. 2005). The medium used to grow Sporosarcina pasteurii consisted of yeast extract and urea. The yeast extract medium was first autoclaved for 20 min at 120^{o}C and then the sterilized urea solution was added, which was obtained by means of filtration through a sterile 0.2-µm Millipore filter. The final concentrations of yeast extract and urea in the growth medium were both 20g/L. The culture was incubated aerobically at 30°C for 24 h with shaking at 250 rpm. The growth and sporulation yield of bacteria was checked regularly by light microscopic analysis. The culture was streaked on nutrient agar plates and kept at room temperature. The pure culture is maintained in liquid, on nutrient agar plate, and cryopreserved in 20% glycerol at -80°C.

2.2 Urease activity of immobilised bacteria under different pH conditions

The bacterial cells could not be added to concrete directly (Jonkers and Thijssen 2010) in order to protect it from the harsh environment. Therefore, in this research, potential use of zeolite as protective vehicle for bacteria was studied. The ureolytic activity of *Sporosarcina pasteurii* with and without immobilization into zeolite was examined in various pH environments. Urease positive strain *Sporosarcina pasteurii* was grown by the same method as described in “Bacteria strains and cultivation” section. Bacterial cells were harvested by centrifuging the 24 h old grown cultures (5000 g, 5 min) and were re-suspended in a physiological solution (NaCl, 9g/L). The concentration of bacterial cells in the suspension was 10^{8}cells/ml. Bacterial suspension obtained was mixed with the sterile zeolite granules, in a 50 ml falcon tube. In each falcon tube, 30 ml of bacterial suspension was mixed with carrier material and was put on a shaker for 2h.

Urea medium with neutral pH was obtained by adjusting the pH by using a 1M NaOH solution. High pH concrete environment was created by adding cement powder to the urea medium. In order to make sure that the cement powder reacted completely with water, the cement suspension was placed on the shaker for one day and the pH was measured as 12.5. Then the zeolite or pumice immobilised bacteria was transferred to this cement suspension and this mixture is referred to as cement slurry. The cement slurry was put on the shaker for 5 days. Un-immobilised bacterial cells were also added to the cement suspension in order to compare the ureolytic activity of immobilised and un-immobilised bacterial cells. The ureolytic activity of the bacteria was indicated by the amount of urea decomposed by the bacteria, which was determined by the total ammonium nitrogen in the urea media (Figurovskaya et al. 2005). One mole of urea produces 2 mol of NH_4^+ and hence the amount of NH_4^+ can show the amount of urea

decomposed. Amount of urea decompose in 1, 3 and 5days was measured calorimetrically by the method of Nessler (Figurovskaya et al. 2005).

2.3 Investigation of self-healing behaviour of bacterial concrete

The experimental program which consists of the self-healing behaviour investigation of bacterial concrete involves two parts of investigation. The first part is focused on the influence of healing agent additions on compressive strength. The second part investigates the effect of bacteria on the permeation properties of cracked specimens.

2.3.1 Effect of self-healing on compressive strength

2.3.1.1 Microbial healing agent preparation

The selected bacteria *Sporosarcina pasteurii* was grown by the same method as described in "Bacteria strains and cultivation" section. For removing the bacterial cells from medium residues, 30ml of the bacterial culture were put in separate 50ml falcon tubes and the bacterial cells were harvested by centrifuging each falcon tube containing the grown cultures (5000 g, 5 min). The harvested cells were re-suspended in a physiological solution (NaCl, 9g/L). The obtained clean bacterial suspension was subsequently diluted to obtain different final cell densities. Three different bacterial cell concentrations such as 10^4, 10^6 and 10^8cells/ml were selected to investigate the optimum bacterial cell concentration which gives the maximum strength. These three different concentrations of bacterial suspension were mixed with sterile zeolite powders in a 50-ml falcon tube. Subsequently, the falcon tube was put on a shaker at 100 rpm for 1 h (Wang et al. 2005).

Calcium lactate ($CaC_6H_{10}O_6$) was used as calcium carbonate precursor. In addition, urea as urease enzyme source and yeast extract was added as nutritional carbon and nitrogen source for bacteria. Individual ingredients were autoclaved separately and mixed afterwards to avoid precipitation. The final pH of the media was adjusted to 9 in order to avoid possible chemical precipitation of calcium carbonate.

These bacterial species immobilised in zeolite together with the nutrient solution constitutes the healing agent.

2.3.1.2 Mortar specimen preparation

Since bacteria and nutrients should be incorporated in mortar matrix to achieve self-healing, the compatibility between mortar and components of healing agent must be evaluated by determining the compressive strength in advance. A series of tests were performed in order to determine the potential effects of the addition of bacteria and organic compounds on strength characteristics of cement mortar. In order to determine the effects of healing agent additions on strength property, mortar cube specimens were prepared with and without (control) incorporating bacteria. During the process of mixing, nutrients (yeast extract, urea, and calcium lactate) were firstly dissolved in part of the mixing water and part of the mixing water was replaced by zeolite immobilised bacterial suspension. The mixture containing zeolite immobilized bacteria were mixed with cement, sand, and the nutrient solution.

2.3.1.3 Compressive strength

Ordinary Portland cement with water to cement ratio of 0.5 and cement to sand ratio of 0.333 was used to prepare the cement mortar cubes with dimensions of 50.8 mm x 50.8 mm x 50.8 mm. Minimum of three replicate mortar cubes were prepared for each cell concentration, mineral substrate and carrier material. Control specimens were also prepared in a similar way without adding bacterial cells. All specimens were de-moulded at the age of 24 h, and then cured at the air conditioned room until testing. Compressive

strength of cement mortar cubes at 7, 14, 28 days were determined in order to investigate the effect of healing agent addition on the strength with age.

2.3.2 Effect of self-healing on permeation properties

The self-healing efficiency of bacteria incorporated mortar specimens was evaluated by Sorptivity test and Rapid Chloride Permeability tests (RCPT). Two different types of mortar mixes were prepared for the RCPT and sorptivity tests; normal mortar with holes and fibre reinforced mortar. Normal mortar was prepared with ordinary portland cement with water to cement ratio of 0.5 and cement to sand ratio of 0.333. Holes were created by using fishing line of diameter 0.25mm which was inserted into the moulds during casting and were removed after 24 hrs. Both for rapid chloride penetration test (RCPT) and sorptivity test, 6 cylinder specimens were prepared for each mixture with diameter and thickness of 100 mm and 50 mm, respectively. Originally, cylindrical specimen of diameter 100mm and height 200 mm were prepared from which three 50 mm thick discs were extracted by using a diamond blade saw from the central portion of the cylinder specimen. After 7 days curing, three specimens are kept as control while three other specimens of fibre reinforced mortar were pre-loaded by tensile splitting test so as to produce cracks. Before the test, crack width of all cracked specimens was measured using crack scope. For all the mixtures, first round of both tests were conducted after 7 days curing and then the specimens were cured in water for 120 days before conducting the second round of test.

2.3.2.1 Sorptivity test

The sorptivity test was conducted based on ASTM C1585. The increase in mass of a cylindrical specimen (100x50mm) at given intervals of time when permitted to absorb water by capillary suction was registered. The specimens were dried in an oven at 50^{o}C for 3 days before each test. Only one surface of the specimen was allowed to be in contact with water, with the depth of water between 1 and 3 mm. The sides of the specimen were sealed with an epoxy coating in order to guarantee one directional flow through the specimen. Measurement was taken at regular intervals of 1, 5, 10, 15, 20, 30 min; 1, 2, 3, 4, 5 and 6 hrs; 1, 2, 3, 4, 5, 6 and 7 days. Immediately after the measurement, the test specimens were re-submerged. The test was performed in triplicate. The rate of absorption (mm^3/mm^2), defined as the change in mass (g) divided by the cross sectional area of the test specimen (mm^2) and the density of water at the recorded temperature (g/mm^3), was plotted against the square root of time ($sec^{1/2}$). The slope of the resulting curve defines the sorptivity of the specimen during the period of testing.

2.3.2.2 Rapid chloride permeability test

Rapid chloride permeability test (RCPT) has been developed as a quick test able to measure the rate of transport of chloride ions in concrete. Rapid Chloride Permeability Test (RCPT) was conducted according to two very similar standards AASHTO T 277 and ASTM C 1202. Specimens were placed in the vacuum desiccator's bowl and the vacuum was maintained in the desiccator's bowl for 3 h. Then the distilled water was allowed to flow into the desiccator, so that it completely covers the specimens and no air was allowed to enter. Again the vacuum was maintained for another 1 h. Subsequently, the specimens were left to soak in the container water for another 18 h. The specimens were removed from the desiccator, dried and placed in gasket. One side of the container is filled with 3% sodium chloride solution (that side of the cell will be connected to the cathode terminal of the power supply) and other side sodium hydroxide solution (0.3 N) was poured and connected to anode terminal. The total charge that passed through the samples was determined (expressed in terms of coulombs) at the end of 6 h. Chloride penetrability is directly proportional to the charge passed. The interpretation is that the larger the Coulomb number or the charge transferred during the test, the greater the permeability of the sample. Test was conducted at 60 V for normal mortar and at 30 V for fibre reinforced mortar.

3 RESULTS AND DISCUSSION

3.1 Bacterial ureolytic activity

In neutral pH environment, a very high ureolytic activity (more than 95% urea was decomposed) was observed both for *Sporosarcina pasteurii*. As shown in figure 2 (left), there was not much difference in ureolytic activity between un-immobilised and immobilized bacterial cells. However, in high pH cement slurry, the amount of urea decomposed by the un-immobilised bacterial cells was around 4%. About 70% of the urea was decomposed by the zeolite immobilized bacteria. Slightly lower values observed on the measured decomposed urea in the 3rd and 5th day might be due to volatization losses. Figure 2 (right) shows the ureolytic activity of un-immobilised and immobilised *Sporosarcina pasteurii* in high pH cement slurry. In the figure 2 and figure 3 given below, BS stands for bacterial solution and zeo stands for zeolite.

It was shown that *Sporosarcina pasteurii* has high ureolytic activity in neutral pH. However, this activity decreases in the high pH cement slurry, which demands some kinds of protection for the bacteria. Zeolite immobilized bacteria showed a profound protective effect on the bacteria in the high pH cement slurry, which was made to mimic the real high pH environment inside the concrete.

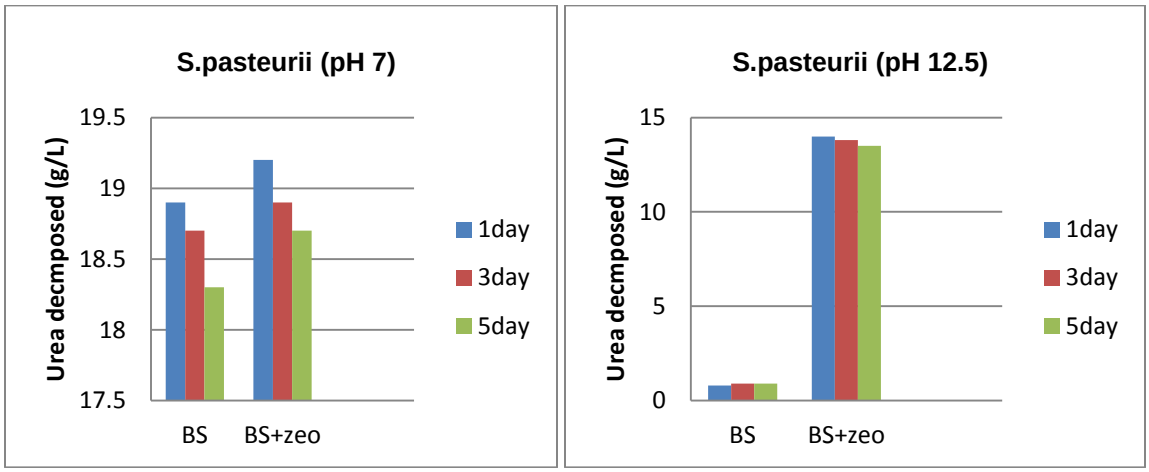

Figure 2: Ureolytic activity of unimmobilised and immobilised *Sporosarcina pasteurii* in pH 7 (left) and pH 12.5 (right)

3.2 Effect of self-healing on compressive strength

Figure 3 shows the 7, 14 and 28 days compressive strength of mortar specimen without bacteria but with varying concentration of calcium lactate while keeping the concentration of other nutrients constant. It can be seen from the figure that the addition of these nutrients, slightly reduced the compressive strength of mortar cubes compared to the control normal mortar specimen. Even though the variations are minor, out of the three different concentration of calcium lactate, 2% gave the maximum strength. Therefore, 2% was considered as the optimum concentration for further study. However, it was found that more than 4% of calcium lactate greatly affected the properties of concrete.

The next objective is to test the effect of the addition of bacteria together with nutrients on the compressive strength of concrete. The effects of the bacteria with three different cell concentrations on the 28-day compressive strength is given in Figure 4. It is evident that compressive strength is increased with increase in bacterial cell concentration up to 10^6 cells/ml, and then there was reduction in the

strength at 10^8 cells/ml. Maximum increase in compressive strengths was achieved at 10^6 cells/ml. Figure 5 summarises comparison of the compressive strength of bacterial specimen with a cell concentration of 10^6 cells/ml with control specimen. It can be observed that the compressive strength had significantly increased for the mortar cubes that contained microbial cells compared to that of control specimen.

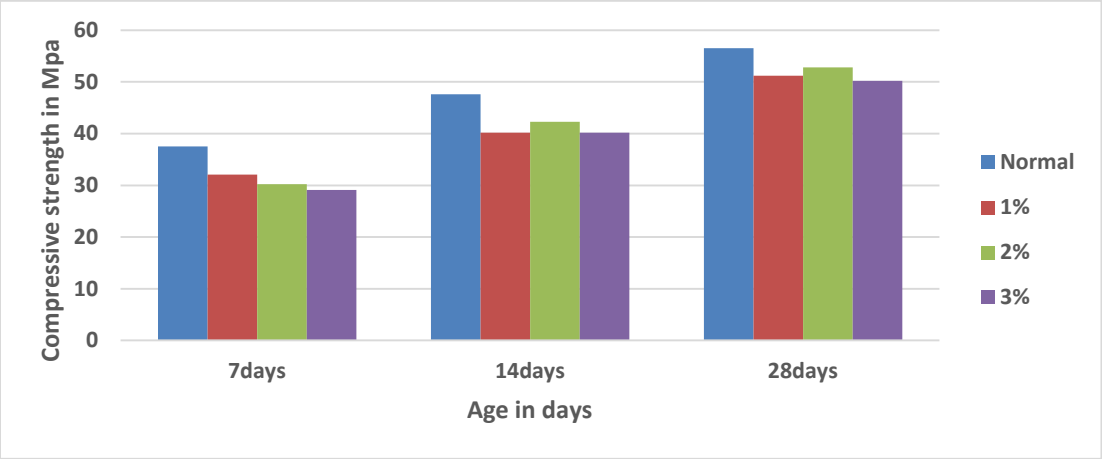

Figure 3: Compressive strength of mortar specimen without bacteria but with varying concentration of calcium lactate

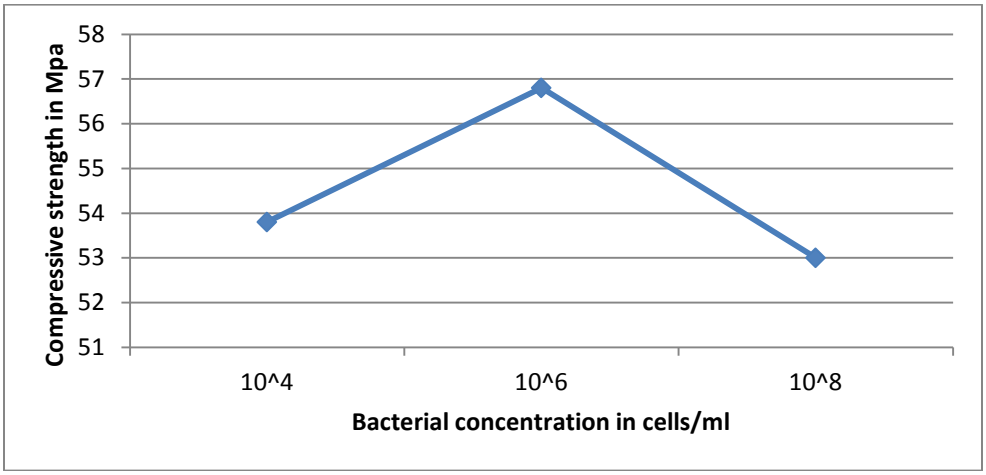

Figure 4: 28-day compressive strength of mortar specimens treated with different concentration of *Sporosarcina pasteurii*

In this research work, the improvement in compressive strength by the selected bacteria was probably due to deposition of $CaCO_3$ on the microorganism cell surfaces and within the pores of the mortar. These results have showed that with the aid of bacteria, concrete with enhanced strength and low-permeability could be produced. The increase in the matrix strength for concrete made with bacterial cells would have eventually increased the overall durability performance of the concrete. Therefore, increase in compressive strengths is mainly due to deposition of microbiologically induced calcium carbonate precipitation within the pores of the cement mortar cubes.

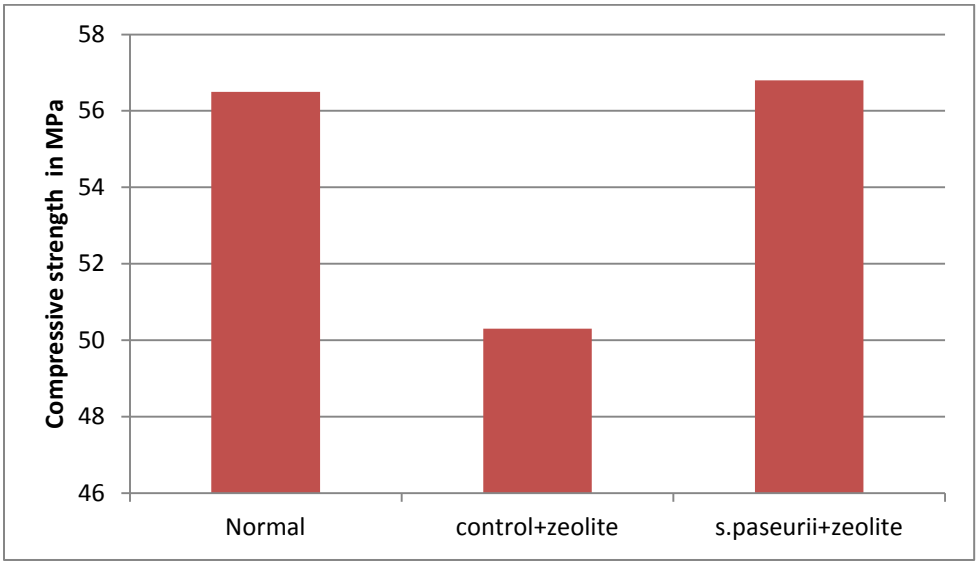

Figure 5: Comparison of compressive strength of bacterial specimen with a cell concentration of 10^6cells/ml with control specimen

3.3 Effect of self-healing on sorptivity

Figure 6 and 7 respectively shows the influence of bacteria on the water absorption of normal mortar specimens with holes and cracked fibre reinforced mortar after 4 months of healing. It can be observed that the microbial induced calcite precipitation in cracks greatly decreased the water absorption rate of the cracked specimens. The speed of water absorption in the specimens without bacteria was much faster than in the ones with bacteria. Specimens without bacteria but with nutrients showed slightly lower rate of water absorption compared to the control sample. The reason for this is probably be due to the presence of calcium lactate which can precipitate calcium carbonate upon reaction with carbonate ions. However, the bacteria specimen that contained nutrients showed a much lesser rate of water absorption compared to the specimens without bacteria but with nutrients. This concludes that the specimen with the highest precipitation has lowest rates of water absorption.

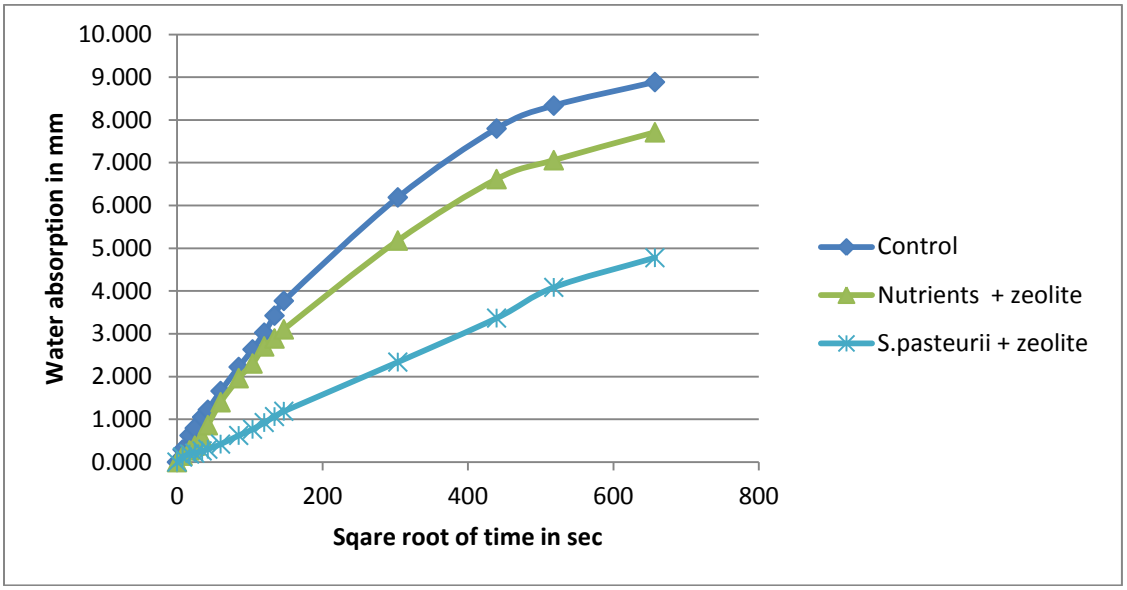

Figure 6: Normal mortar with holes after 4 months of healing with zeolite

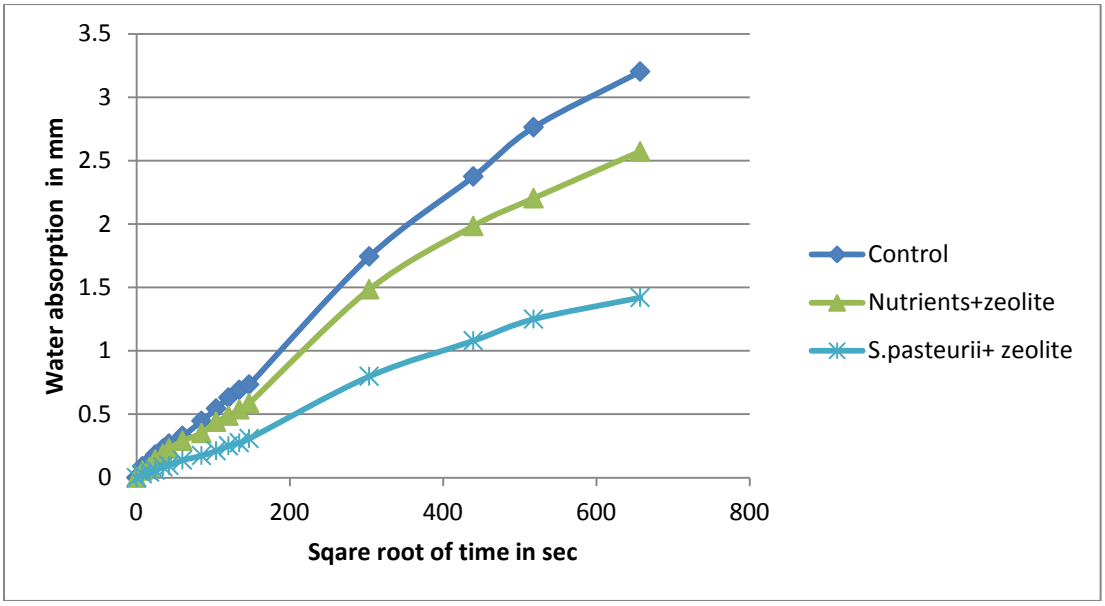

Figure 7: Cracked fibre reinforced mortar after 4 months of healing with zeolite

3.4 Effect of self-healing on rapid chloride permeability

Results of the effect of bacteria on the rapid chloride permeability of normal mortar and Fibre reinforced mortar, with and without the addition of bacteria, are given in Figure 8 and 9, respectively. It is evident from these figures that with the inclusion of bacteria, chloride ingress capacity of both the normal mortar and FRC are significantly decreased after 120 days of healing. It can be observed that the chloride ion permeability rate decreased as the age of all mixtures, with or without bacteria, increased. Furthermore, this decrease was higher for mixtures with bacteria than for mixtures without bacteria. The bacterial pore

blockage by the deposition of calcium carbonate precipitation resulted in an increase in resistance towards the chloride permeation.

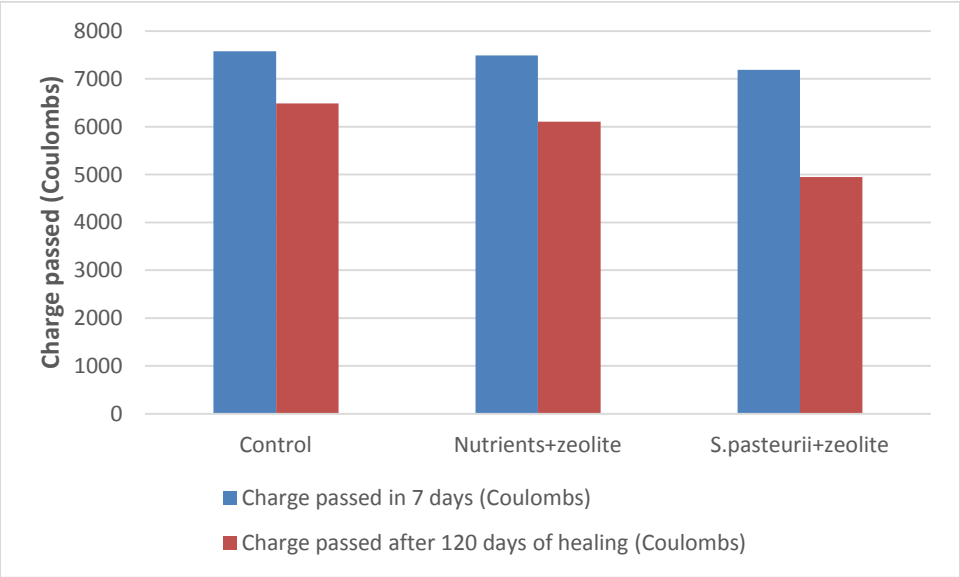

Figure 8: Rapid chloride permeability of normal mortar

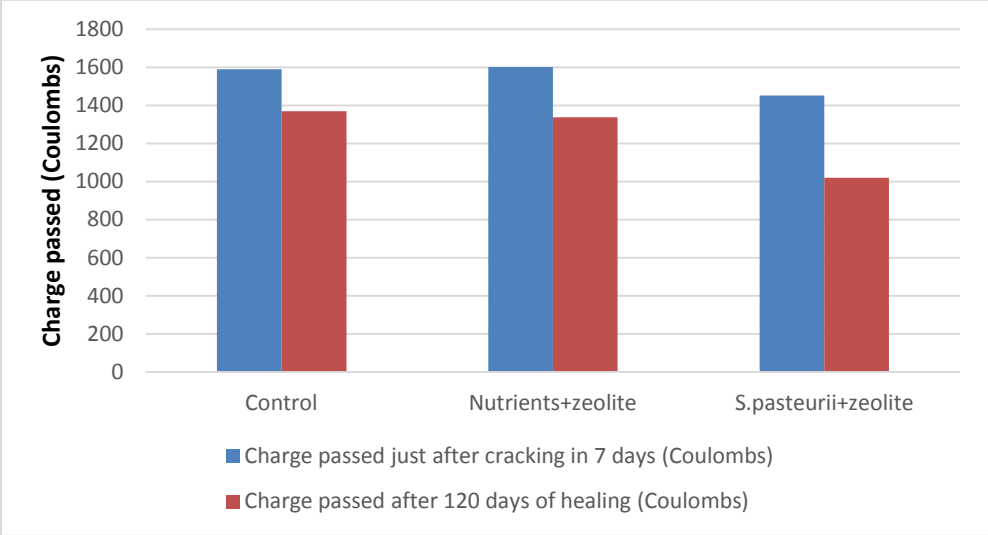

Figure 9: Rapid chloride permeability of fibre reinforced mortar

4 CONCLUSIONS AND RECOMMENDATIONS

Zeolite was found to have a protective effect for the bacteria Sporosarcina pasteurii in a high pH cement environment. The mechanism behind this might be that these materials have a strong capacity to adsorb bacterial cells on the surfaces. Then, these materials provided a kind of micro environment around the bacteria, in which the local pH was less than that in the cementitious environment.

Compressive strength was found to be increasing with bacterial addition and this increase is mainly due to deposition of microbial induced calcium carbonate precipitation in the pores. The presence of bacteria

resulted in a significant decrease in the rate of water uptake compared to control specimens. Normal mortar and fibre reinforced mortar containing bacteria along with nutrients showed good resistance towards rapid chloride penetration. It can be concluded from these results that the deposition of a layer of calcium carbonate on the surface and inside the pores of the mortar specimens resulted in a decrease of water absorption. When the pores are impeded by materials such as calcium carbonate, the passage for water, air and other pollutants is sealed. Consequently, it reduces the permeation of water and chloride in concrete. Microbial induced calcite precipitation can be regarded as a potential method to increase the durability of concrete. Use of bacteria in concrete may be highly desirable because the calcite precipitation induced by the metabolic activities is natural and pollution free.

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