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Bacterial carbonate precipitation improves the durability of cementitious materials

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ABSTRACT

Shortcomings of conventional surface treatments have drawn the attention to alternative techniques for the improvement of the durability of concrete. This paper reports the effects of bacterial carbonate precipitation (biodeposition) on the durability of mortar specimens with different porosity. Durability was assessed from the permeation properties and resistance towards degradation processes. The surface deposition of calcium carbonate crystals decreased the water absorption with 65 to 90% depending on the porosity of the specimens. As a consequence, the carbonation rate and chloride migration decreased by about 25–30% and 10–40% respectively. An increased resistance towards freezing and thawing was also noticed. The results obtained with the biodeposition treatment were similar as those obtained with conventional surface treatments.

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1. Introduction

Nowadays a broad range of products is available on the market for the protection of concrete surfaces [1–3]. Several of these products are organic coatings consisting of volatile organic compounds. The air-polluting effect of these compounds during manufacturing and coating has lead to the development of new formulations such as inorganic coating materials. Traditional inorganic coatings consist of calcium-silicate compounds, which exhibit a composition similar to cement [4]. Promising results of innovative techniques based on microbial mineral precipitation have lead to several investigations on the use of bacteria in concrete. Different microorganisms have been used to increase the compressive strength of cement mortar and for the remediation of cracks in concrete [5–7].

Considerable research on carbonate precipitation by bacteria has been performed using ureolytic bacteria [8–10]. These bacteria are able to influence the precipitation of calcium carbonate by the production of a urease enzyme. This enzyme catalyzes the hydrolysis of urea to CO_2 and ammonia, resulting in an increase of the pH and carbonate concentration in the bacterial environment [10]. Precipitation of calcium carbonate crystals occurs by heterogeneous nucleation on the bacterial cell wall once supersaturation is achieved. The fact that hydrolysis of urea is a straightforward common microbial process and that a wide variety of microorganisms produce the urease enzyme makes it ideally suited for biotechnological applications [11].

Research has indicated that a concrete which has a low permeability lasts longer without exhibiting signs of distress and deterioration [12]. Therefore, the permeation properties have been used principally for the comparison of the effectiveness of different surface treatments enhancing the durability of concrete. Results from preliminary research have shown that a surface treatment based upon microbial carbonate precipitation (biodeposition) with pure cultures of *Bacillus sphaericus* was more effective in decreasing the permeation properties of mortar and concrete compared to a treatment with ureolytic mixed cultures [13,14].

In the current study, the effectiveness of the biodeposition treatment with pure cultures was investigated by evaluating the resistance of mortar specimens towards degradation processes. Furthermore, the influence of the surface porosity on the efficiency of the treatment was also investigated. Mortar cubes of varying water–cement ratio, were subjected to accelerated carbonation, chloride migration and freezing and thawing. To gain a better insight into the efficiency of the bacterial treatments, results were compared with experimental data and published results of conventional surface treatments.

2. Materials and methods

All experiments were performed in triplicate ($n=3$), unless otherwise stated.

2.1. Mortar mixture proportions and dimensions of specimens

The materials used for the preparation of the test specimens were mortar mixtures with a Portland cement (CEM I 52.5 N):sand 0/4 ratio

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of 1:3 and w/c ratios of 0.5, 0.6 and 0.7. The higher w/c ratios were chosen to simulate the more porous outer layers of concrete as a result of the wall effect or low quality (or weathered) concrete that needs repair or protection. For each mixture, a number of slabs were cast and cured for one day at 20±2 °C and at a relative humidity (R.H.) of 90±10%. The slabs were demolded after 24 h and then stored in water for 27 days prior to the drilling of the specimens for the different tests. Afterwards the specimens were stored at 60±10% R.H. and 20±2 °C until application of the surface treatment at the age of 1–2 months. Surface treated specimens were then stored according to the procedure for the tests under investigation (Table 1). Average compressive strength of each mixture was obtained from the measurements (according to the Belgian standard NBN B15-220) on three cubes ($100\times100\times100$ mm^3) after 28 days of water curing and amounted to 50.3±2.29 MPa, 48.5±1.96 MPa and 41.6±2.26 MPa for w/c of 0.5, 0.6 and 0.7 respectively.

2.2. Microorganisms and growth conditions

B. sphaericus LMG 225 57 (Belgian co-ordinated collections of microorganisms, Ghent) was used for this study. Selection of this spore forming strain was based upon earlier work [8,15]. This strain showed a high urease activity, a continuous formation of dense calcium carbonate crystals and a very negative zeta-potential. *B. sphaericus* is unlikely to cause human disease. No measurable health effects were seen in laboratory animals that were exposed to large concentrations of *B. sphaericus* by multiple routes of exposure. Cases involving human health effects following exposure to this organism are extremely rare. Mild eye and skin irritation may occur in humans following contact with *B. sphaericus* [16,17].

Liquid culture media consisted of 3 g/L nutrient broth (Oxoid N.V., Drongen, Belgium), 2.12 g/L $NaHCO_3$, 10 g/L ammonium chloride and 10 g/L urea (VWR International, Leuven, Belgium). Liquid media were sterilized by autoclaving for 20 min at 120 °C. Urea was added after autoclaving by means of filtration through a sterile 0.22 µm Millipore filter. Cultures were incubated at 28 °C on a shaker at 100 rpm for 24 h.

Table 1
Overview of the different tests, treatment and storage procedures

Test	Specimen[a]	Treatment procedure[b]	Storage[c]
Characterization of the biodeposition treatment			
SEM analyses	□ 10	/	Oven at 95 °C, Gold coating, vacuum chamber
Thin sections	□ 40	/	20 °C, 90% R.H., epoxy impregnation
Quantification of spores at varying depths	○ 50/100	/	20 °C, 90% R.H.
Weight increase	□ 10	/	Oven at 95 °C (before and after treatment)
Transport processes			
Water absorption	□ 40	Coating[d] at 4 sides adjacent to treated side	Oven at 45 °C
Gas permeability	○ 50/150	/	Oven at 60 °C, subsequent sealed with 2 layers of aluminium foil and stored for 1 day at 20 °C, 60% R.H.
Degradation processes			
Carbonation	□ 100	Coating[d] at all non-treated sides	20 °C, 60% R.H.
Chloride migration	○ 50/100	/	Vacuum saturated with $Ca(OH)_2$ for 18 h
Freezing and thawing	□ 100	/	Immersed in water for 168 h

[a] Dimensions in mm: □ cube: side ○ cylinder: height/diameter.
[b] After application of the surface treatment, /: no extra treatment.
[c] After the treatment procedure, specimens were stored establishing a mass equilibrium of less than 0.1% between 2 measurements at 24 h intervals unless otherwise stated.
[d] Coating consisted of 2 layers of polysiloxane and one layer of silicon paint.

Table 2
Overview of the different treatments ranked according to mechanism and composition

Group	Subgroup	Composition	Abbreviation
Untreated	–	–	Ref.
Biodeposition[a]	*Bacillus sphaericus*	No calcium source	Bac. sph. No Ca
		Calcium chloride	Bac. sph. $CaCl_2$
		Calcium acetate	Bac. sph. CaAc
Coatings	Acrylates	Water based, dispersion	Acryl. A1
		Water based, dispersion	Acryl. A2
Water repellents	Silanes	Water based, gel-like	Sil.
	Silane/siloxane mix.	Siloxane/alkoxysilane	Sil./Silox.
	Silicones	Silicones	Silic.
Inorganic	Silicates	Sodium silicates in water	NaSil

[a] Nutrient components in media of biomineralisation experiments had the following concentration: (basic medium:) urea, 10 g/L; sodium bicarbonate, 2.12 g/L; ammonium chloride, 10 g/L; nutrient broth, 3 g/L; (additions:) calcium chloride, 25 g/L and/or calcium acetate, 26 g/L.

2.3. Treatment procedure

All different groups of treatments were applied on the side opposite to the troweled face of the mortar specimens. As the specimens for the freezing and thawing tests remained completely submerged during the test, these specimens were treated on all sides. For the biodeposition treatment, mortar specimens were immersed for 24 h (10±5 mm depth) in a one day old stock culture of *B. sphaericus* (fully grown culture contains ca. 10^7 cells/mL). After this inoculation, specimens were wiped with a paper towel to remove excess liquid from the surface. In this way ureolytic activity primarily resulted from bacteria on the surface and inside the specimens. Following this wiping, specimens were immersed (10±5 mm depth) in solutions of varying composition in order to investigate the effects of the external calcium source (Table 2). The specimens were removed from the solution after 3 days, and left to dry for a week at room temperature.

Conventional surface treatments were applied with gentle brushing (following technical data instructions). Choice of treatments was based upon commercial availability and care was taken to cover a wide range of treatments acting according to different mechanisms (Table 2).

2.4. Characterization of the biodeposition treatment

2.4.1. Thin section and SEM analyses

The influence of the w/c ratio and the calcium source on the thickness of the carbonate layer was determined by means of thin section and SEM analyses. Mortar cubes (Table 1) were treated with bacteria and a different calcium source as described above. For the SEM analysis ($n=2$), however, mortar cubes were fully immersed. Fluorescent epoxy impregnated thin sections ($n=2$) were prepared following the procedures described in [18]. Thin sections were analysed by means of a Leica DMLP microscope connected with a Canon S50 camera to a desktop. Mortar samples for the SEM analysis were coated with a Baltec SCD005 Sputter Coater. The thin Au layer was applied by operating the equipment at a pressure of 0.1 mbar for 60 s with a current of 40 mA. The samples were subsequently studied in a FEI XL30 SEM microscope equipped with a tungsten filament.

2.4.2. Quantification of bacteria at varying depths

The influence of the w/c ratio on the absorption of bacteria was measured by means of quantification of the amount of spores inside

w/c 0.5 and 0.7 specimens (Table 1) after treatment with bacteria without a calcium source. Ground mortar from different depths (in mm: 0–0.5, 0.5–1, 1–1.5, 2–3, 3–5 and 5–10) was obtained by means of the PF-1000 Profile Grinder (Germann instruments). One gram of mortar from each depth was then added to glass tubes containing 10 mL of physiological solution to which 50 mM L-alanine was added to stimulate the germination of spores [19]. Subsequently, the tubes were vortexed to bring the bacteria into solution. In order to prevent the possible outgrowth of bacteria as a result of contamination during storage and handling of the specimens, glass tubes containing the vortexed solution were then sterilized by means of heating in a water bath for 15 min at 80 °C. Quantification of spores was done by plate counting on agar plates (same composition as liquid medium except 3 g/L nutrient broth was replaced by 20 g/L nutrient agar).

2.4.3. Weight increase

The influence of the w/c ratio and calcium source on the amount of carbonate deposited was measured by means of the weight increase of w/c 0.5 and 0.7 specimens (Table 1) after treatment with bacteria and different nutrient media (n=4). Treatment of cubes was similar as described above, except for the fact that the specimens were completely immersed in a plastic tube containing 10 mL of the respective medium.

2.5. Transport processes

2.5.1. Water absorption

To determine the increase in resistance towards water penetration a sorptivity test, based on the RILEM 25 PEM (II-6), was carried out. The preconditioned specimens (Table 1) were exposed to 10±1 mm of water, with the treated side facing downwards (water level about 2 mm above the base of the specimen) in an atmosphere of 20±2 °C and R.H. of 60±10%. The water level was kept constant through addition of tap water. At regular time intervals (15 min, 30 min; 1 h, 1.5 h, 3 h, 5 h, 8 h, 24 h, 72 h, 96 h, 120 h, 144 h and 168 h) the specimens were removed from the water and weighed, after drying the surface with a wet towel. Immediately after the measurement the test specimens were submerged again. The sorptivity coefficient, k [cm $s^{-1/2}$], was obtained by using the following expression:

$$\frac{Q}{A} = k\sqrt{t} \tag{1}$$

where Q is the amount of water absorbed [cm^3]; A is the cross section of the specimen that was in contact with water [cm^2]; t is the time [s], Q/A was plotted against the square root of time, then k was calculated from the slope of the linear relation between the former.

2.5.2. Gas permeability

Measurement of permeability towards gas was performed according to the RILEM-CEMBUREAU method [20–22]. From the Hagen–Poiseuille relationship, the specific permeability coefficient, K [m^2], can be calculated as follows:

$$K_{\text{oxygen}} = \frac{4.04 \cdot p_2 \cdot Q \cdot L \cdot 10^{-16}}{A \cdot (p_1^2 - p_2^2)} \tag{2}$$

where p_2 is the atmospheric pressure [bar], Q is the volumetric gas flow rate [mL/s], L is the thickness of the specimen [m], A is the cross section of the specimen [m^2] and p_1 is the applied oxygen pressure [bar]. Measurement of the oxygen flow rate was performed using a Martin Sommer oxygen permeability apparatus (Schmidtheim, Germany). K_{oxygen} values were obtained at the pressure stage of 2 bar. Measurements were performed on 3 preconditioned cylinders (Table 1) at the same time.

2.6. Degradation processes

2.6.1. Accelerated carbonation test

The accelerated carbonation tests were performed in a CO_2-closet at a temperature of 20±3 °C, a R.H. of 70±10% and a CO_2 concentration of 10%. After two weeks of carbonation, specimens were removed from the closet. A slice (10 mm) perpendicular to the treated surface was cut off and sprayed with phenolphthalein solution for the determination of the carbonation depth. The remaining mortar specimen was coated and put back in the CO_2-closet. Carbonation depth was determined after 2, 4 and 6 weeks. Resistance towards carbonation is expressed as the carbonation rate constant (K) [mm $s^{-1/2}$]. This parameter can be obtained as follows:

$$x = K \cdot \sqrt{t} \tag{3}$$

where x is the mean carbonation depth [mm] after a certain time [years] [23].

2.6.2. Accelerated chloride migration coefficient

The resistance towards chloride diffusion was investigated by means of the CTH rapid test according to the NT Build 492 Nordtest method. Results are expressed as the non-steady-state migration coefficient. This parameter is obtained as follows:

$$D_{\text{nssm}} = \frac{0.0239 \cdot (273+T) \cdot L}{(U-2) \cdot t} \cdot \left(x_d - 0.0238\sqrt{\frac{(273+T) \cdot L \cdot x_d}{U-2}} \right) \tag{4}$$

where D_{nssm} is the non-steady-state migration coefficient [m^2/s], U is the absolute value of applied voltage [V], T is the average value of the initial and final temperatures in the anolyte solution [K], L is the thickness of the specimen (Table 1) [m] and x_d is the average value of the chloride penetration depth [m] [24].

2.6.3. Freezing and thawing

To determine the increase in resistance towards freezing and thawing a test based on the Belgian standard NBN B-15 231 was carried out. After the storage period (Table 1), the specimens were subjected to freezing and thawing. Each cycle lasted for 24 h (7 h freezing and thawing periods between 10 °C and −15 °C; 10 h period at −15 °C). Deterioration was assessed by visual observation and sonic measurements. After 21 cycles, the tensile strength of the specimens was measured by means of splitting tests.

2.7. Statistical analysis

Experiments were performed in triplicate. Error bars on graphs and values in tables present the standard error. Comparison of mean values was done by using one-way ANOVA analysis. Statistical Software SPSS 12.0 was used for this purpose. Grouping of treatments based on significant differences in mean values was done according Student Newman Keuls or Dunnet T3 tests (0.05 level of confidence), depending on homoscedasticity results of the Levene test.

3. Results and discussion

3.1. Characterization of the biodeposition treatment

3.1.1. Absorption of bacteria

The immersion of mortar specimens in a fully grown culture of *B. sphaericus* (ca. 10^7 cells/mL) resulted in the absorption of bacteria in the porous matrix throughout the complete immersion depth (Table 3). With the given test procedure it was not possible to observe any differences between the number of spores retained per g of mortar for specimens with a different w/c (w/c 0.5 and 0.7) for any

Table 3
Quantification of the amount of bacterial spores at different depths after treatment of mortar cubes (n=3) with bacteria

w/c	10^3 spores/g mortar at different depths (mm)						
	0–0.5	0.5–1	1–1.5	1.5–2	2–3	3–5	5–10
0.5	1.22±0.41	0.62±0.36	0.07±0.07	1.23±0.73	0.80±0.42	0.50±0.29	0.30±0.25
0.7	3.18±1.48	0.45±0.30	0.67±0.33	0.68±0.34	2.45±1.60	0.17±0.17	2.20±1.86

given depth up to 10 mm. The amount of bacteria found in untreated samples was negligible (data not shown).

3.1.2. Precipitation of carbonate crystals

Treatment of mortar specimens with bacteria and a calcium source resulted in the presence of carbonate crystals on the surface (Fig. 1). Thin section and SEM analysis of biodeposition treated mortar specimens revealed large variations in the thickness of the carbonate layer across the surface, and to a limited extent areas where no crystals were present. From the thin sections it was observed that the majority of the surface was covered with a layer of crystals within the range of 10–40 μm, in which often larger crystals (up to 110 μm) could be found. These large crystals show evidence of the bacterial mediation of the carbonate precipitation, as a large amount of small rods (1–2 μm) could be observed inside the crystalline structure (Fig. 1.3). The water–cement ratio had no influence on the amount of crystals that was precipitated on the surface as could be observed from the SEM pictures. The influence of the calcium source was limited to the morphology of the crystals. The presence of chloride ions resulted in rhombohedral crystals, while the presence of acetate ions resulted in spherical ions, which is in accordance with our previous findings [13].

3.1.3. Weight increase

Absorption of bacteria and precipitation of carbonate crystals resulted in a weight increase of the mortar specimens (Table 4). For a given w/c, the lowest weight gain was observed for the treatment with bacteria in the absence of a calcium source. This weight gain was attributable to the occurrence of bacterial cells and spores on the surface and inside the porous matrix of the mortar specimens. Furthermore, a part of this weight gain could be attributed to the precipitation of calcium ions from the pore solution, as carbonate ions were already present in the fully grown culture. In the presence of an external calcium source, a significant higher weight gain could be observed as a result of the additional precipitation of $CaCO_3$ crystals. No significant differences in relative weight gain could be observed between the biodeposition treatments with a different calcium source (while Table 4 presents the absolute weight gain as a result of the treatment, comparison of different treatments for a given w/c was done based upon relative weight gain, in order to include variations in sample size due to cutting). From Table 4 it is clear that a second treatment resulted in an additional weight increase. This weight increase was however higher than that of the first treatment. This could be attributed to the fact that the presence of carbonate crystals on the surface, acting as template for crystallization enhanced the amount of carbonate precipitation on the surface.

For the nutrient solution without a calcium source, the weight gain increased with increasing w/c. As the specimens had a similar outer surface area, the increase of biomass absorption with increasing w/c is probably attributable to the increasing porosity. Consequently a larger urease activity and a larger amount of nucleation sites were obtained. This resulted however not in an increased amount of $CaCO_3$ precipitation with increasing w/c. The latter can be attributed to the fact that from a certain number of bacteria there is sufficient urease activity to hydrolyze all the urea, and as a result precipitate all the calcium ions that are present.

3.1.4. Biodeposition versus natural carbonation

Compared to natural carbonation of concrete, biodeposition is a relatively quick process. Natural carbonation occurs from the dissolution of atmospheric CO_2 in the pore solution and formation of $CaCO_3$ from CSH or portlandite. In the biodeposition treatment however, calcium ions are also provided by an externally added calcium source, while the carbonate ions result from the microbiological hydrolysis of urea. As a result of the rapid hydrolysis of urea (measured specific urease activity: ca. 3 mM urea OD^{-1} min^{-1} (OD: optical density) under optimal conditions), the majority of the calcium ions added to the specimens are precipitated within a couple of days.

Furthermore, while natural carbonation also occurs in subsurface layers, biological precipitation of calcium carbonate mainly occurs at the outer surface of the specimens. The latter is due to the limited penetration of the bacterial cells in the cementitious matrix. Samonin and Elikova [25] reported that for a maximum absorption of microbial cells, the absorbent pores must be 2–5 times greater than the cells. Spore forming microorganisms, such as *B. sphaericus*, however, are absorbed most when the pore size is about 1–4 times the spore size. Cementitious materials have pores with diameters in the nano- (gel pores and small capillaries) and micrometer range (big capillary pores: 0.05 to 10 μm) [26]. Since cells and spores of *B. sphaericus* have dimensions (*L*: length, *D*: diameter) of about 1–4 μm (*L*), 1 μm (*D*) respectively 1.07±0.1 μm (*L*) and 0.85±0.06 μm (*D*) [27], bacteria will be mainly absorbed in the large capillary pores. Given that an increased quantity of pores larger than 1 μm is observed with increasing w/c [28], an increased amount of biomass absorption could be expected with increasing w/c. This was clearly noticed from the weight increase measurements.

The number of spores retained in the porous matrix remained however low compared to the amount of cells applied (10^7 mL^{-1}) as could be observed from Table 3. Given a bulk density of 2.16±0.02 g mL^{-1}, 2.08±0.01 g mL^{-1} and a porosity of 18.45±0.93%, 20.69±1.19% for w/c 0.5 respectively w/c 0.7 specimens (determined from vacuum water absorption and drying at 105 °C) it follows that the theoretical maximum absorption amounted to about 10^6 cells g^{-1} ground mortar. The amount of spores found however, was on average only about 10^3 spores g^{-1}. Furthermore, as the dry weight of one bacterial spore amounts to about 10^{-12} g [29], it thus follows that about 10^{-9} g spores was absorbed per gram of ground mortar. The mass increase (1.6–3.1 mg) of the specimens treated with bacteria could therefore mainly be attributed to the presence of a superficial biofilm.

From a technical point of view, the biodeposition treatment might be considered as a 2-component coating system with pore blocking characteristics. Application of bacteria resulted in the plugging of pores (as could be noticed from the water absorption tests) and formation of a biofilm on the surface. This biofilm acted as a primer for the carbonate coating, as bacteria inside the biofilm attract positively charged metal ions from their surroundings and act as nucleation sites due to the negative charge respectively the composition of their cell wall [11]. Upon addition of the nutrients and the calcium ions, the bacterial activity resulted in the supersaturation of the liquid phase in relation to calcium carbonate. This resulted in the heterogeneous precipitation of calcium carbonate crystals on the biofilm. As experiments were performed in solution, heterogeneous precipitation of

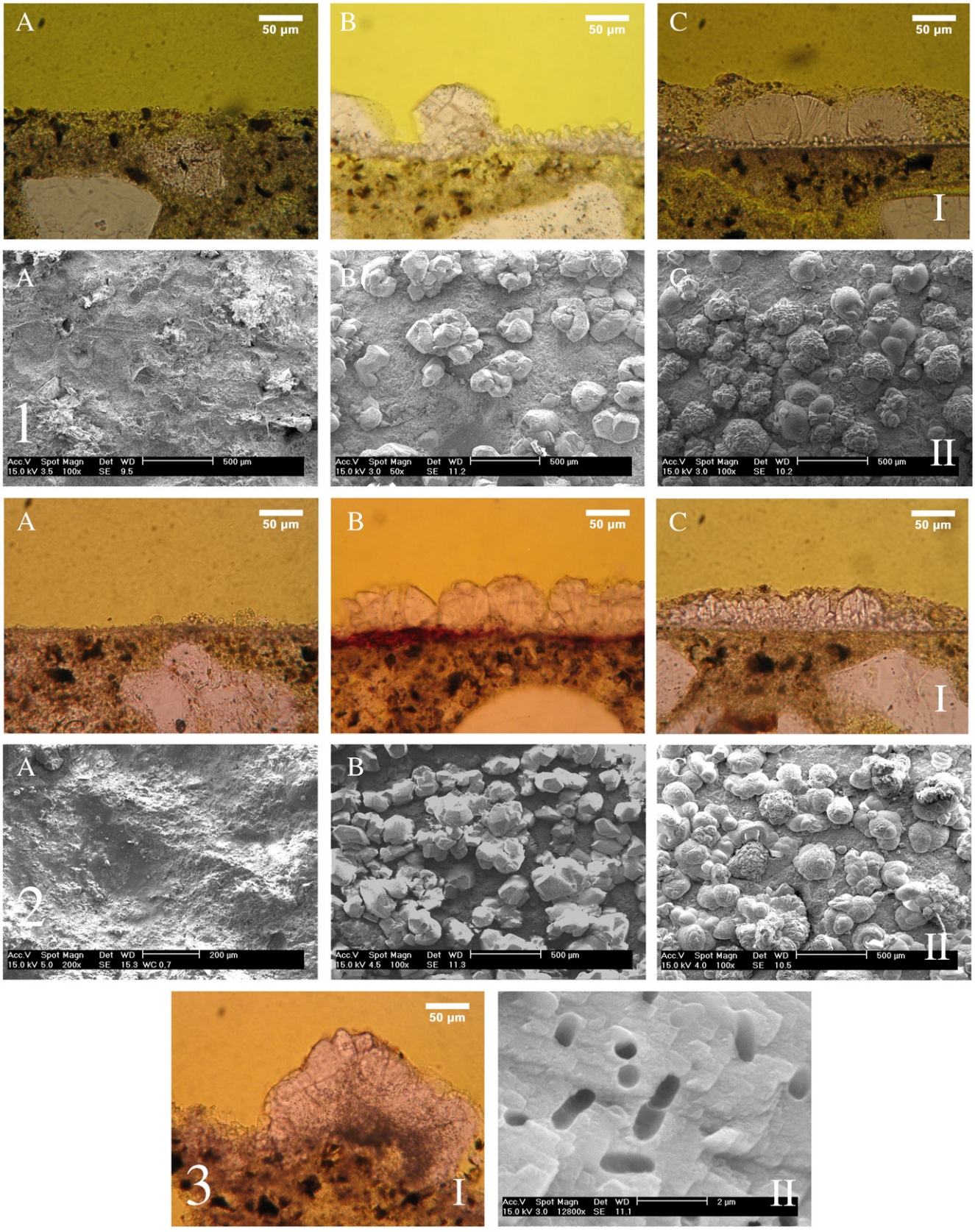

Fig. 1. Thin sections (I) and SEM pictures (II) revealing the presence of a layer of carbonate crystals on the surface of w/c 0.5 (1) and w/c 0.7 (2) specimens treated with bacteria and calcium chloride (B) or bacteria and calcium acetate (C). 1A and 2A present an untreated surface. In B and C crystals with size up to 100 μm are clearly present. 3 indicates the bacterial mediation of carbonate precipitation, as black spots (I) and small rods (II) are clearly visible inside the crystal structure.

Table 4
Weight increase (mg) of small mortar cubes (n=4) treated with bacteria and different media

w/c	Medium			
	No calcium	Calcium chloride		Calcium acetate
		After 1 treatment	After 2 treatments	
0.5	1.6±0.3	65.8±5.0	161.3±1.9	61.4±6.9
0.6	3.0±0.4	77.1±3.8	191.5±4.5	81.8±1.4
0.7	3.9±0.2	70.8±1.8	162.6±3.6	80.3±2.6

For the calcium chloride series, two treatments were performed, including the addition of bacteria and a calcium source.

$CaCO_3$ was also likely to occur inside the pores that were not covered by a biofilm. From the weight increase due to $CaCO_3$ precipitation it was possible to calculate a theoretical carbonate layer thickness, assuming: (1) a density of calcium carbonate of 2.71 g/cm^3 (http://www.kimyaevi.org/d01/102059.pdf) and (2) that precipitation only occurred on the surface (6 cm^2) of the cube. From these calculations, the layer thickness was found to be in the range of 35 to 50 μm, which corresponds with the measurements from the thin section analyses. For all w/c, the weight increase of the specimens due to carbonate precipitation amounted to about 50% of the theoretical maximum compared to the amount of calcium ions that were initially present in solution. The latter is attributable to the fact that heterogeneous precipitation also occurred on the surface of the plastic tubes containing the mortar specimens.

The presence of biomass and carbonate crystals on the surface as well as inside the porous matrix resulted in a decreased permeability of mortar specimens as could be observed from the transport and degradation processes, as follows.

3.2. Transport processes

3.2.1. Water absorption

Mortar cubes treated with bacteria and a calcium source showed significantly less water absorption compared to untreated specimens (Fig. 2). This is in agreement with the findings of Tiano et al. [30], who noticed a decrease of the water absorption after the application of calcinogenic bacteria on limestone. These authors indicated that about 50% of this decrease was attributable to the presence of biological matter, which physically hindered the movement of water. Similar findings were observed from Fig. 2. No significant differences could be observed between the different w/c specimens regarding the contribution of the biomass to the overall decrease in sorptivity coefficient.

In contrast with conventional surface coatings that showed a similar sorptivity coefficient regardless the water–cement ratio, w/c 0.7 specimens treated with bacteria and a calcium source showed a higher sorptivity coefficient than the w/c 0.5 and 0.6 biodeposition treated specimens. This could be due to an incomplete coating of the surface, as could be observed from the SEM and thin section analysis. Due to the larger porosity and connectivity of the pores with increasing w/c, the absence of a coating will have a larger effect on the water absorption of higher w/c specimens than in the case of lower w/c specimens. Increasing the calcium concentration or additional treatments could result in an additional decrease of the permeability, as the presence of additional carbonate crystals will result in a more complete plugging of the superficial pores. Nemati and Voordouw [31] and Whiffin [32] noticed an additional decrease of the permeability of sandstone cores after injecting $CaCO_3$ forming reactants for a second time. From the weight increase measurements (Table 4) it was clear that a second application of bacteria and calcium chloride resulted in an additional weight increase of the mortar specimens due to the carbonate precipitation. The influence of this second application of bacteria and a calcium source on the permeation properties needs however further exploration.

Fig. 3 shows the influence of the surface treatment on the water absorption rate for mortar cubes with a w/c 0.6. Over a period of 168 h the cubes treated with bacteria and calcium chloride absorbed nearly five times less water than the control cubes (Fig. 3). The water absorption rate in this period was similar to that of the silanes and acrylates, which are reported to be very effective in reducing the rate of water penetration [1,12,33].

3.2.2. Gas permeability

The effects of the water–cement ratio and the surface treatment on the gas permeability of mortar are presented in Fig. 4. The presence of a surface treatment resulted in a decreased gas permeability for all w/c specimens. The presence of biomass contributed to a large extent in the overall decrease of the gas permeability. In general, similar observations and explanations could be made as in the case of the water absorption experiments. The results of the silanes are in contrast with those found in literature. While silanes are reported to have no influence on air permeability, coatings can reduce the breathability by more than 50% [1]. The differences in gas permeability between the

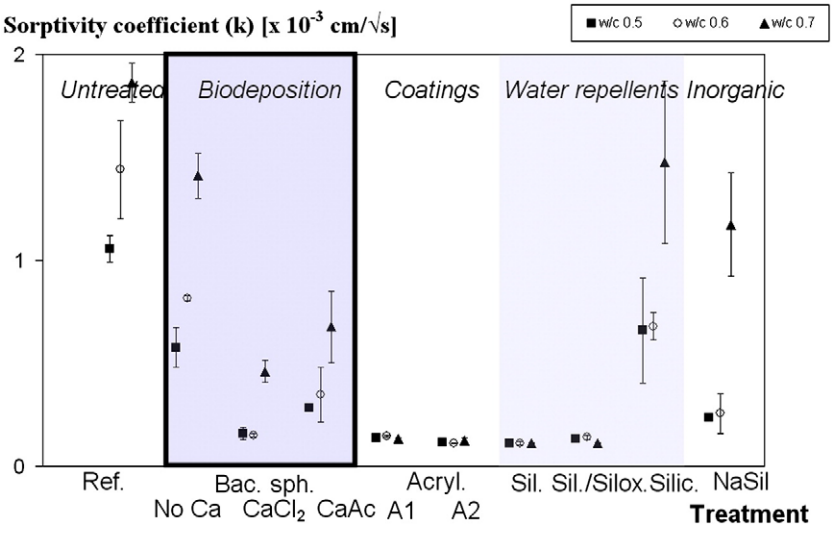

Fig. 2. Sorptivity coefficients, k, for different grades of mortar applied with different types of surface treatments.

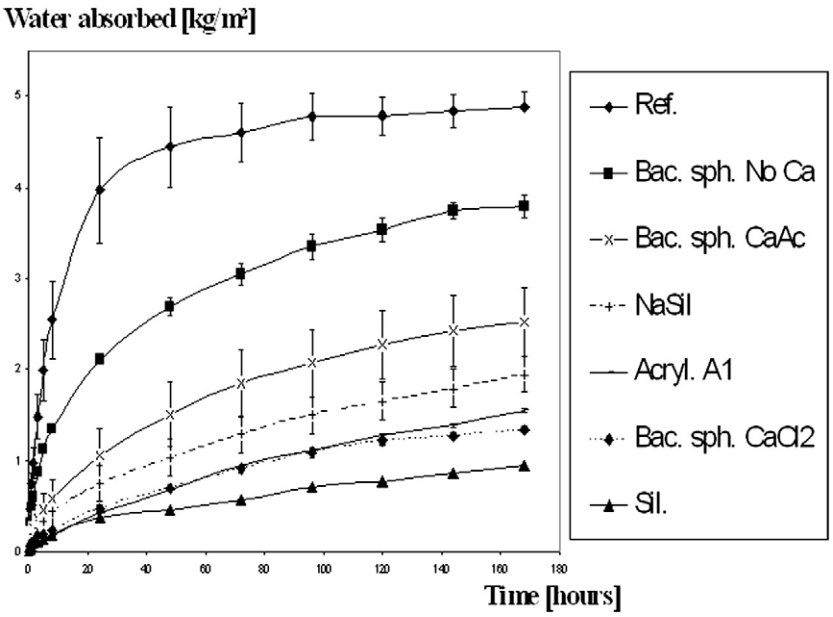

Fig. 3. The influence of the surface treatment on the rate of water absorption versus time for w/c 0.6 mortar cubes.

biodeposition treatments and coatings on the one hand and the water repellents on the other hand were however noticeable from the results of carbonation tests as follows.

3.3. Degradation processes

3.3.1. Carbonation

The decrease in gas permeability due to the biodeposition treatments resulted in an increased resistance towards carbonation (Fig. 5). Except for the water repellents, similar tendencies were observed between the gas permeability and carbonation rate results. The rate of carbonation and the performance of the surface treatment were correlated to the water–cement ratio. Several authors have demonstrated an increase in the carbonation rate in concrete with increasing water–cement ratio [23]. Carbonation was shown to be related to the nature and connectivity of the pores, with larger pores giving rise to higher carbonation depths. Significant differences in carbonation depth between treated and untreated specimens were already noticeable after 2 weeks of accelerated carbonation (results not shown). Except for the most porous specimens (w/c 0.7) the increased resistance towards carbonation of cubes treated with biodeposition was similar to that of the A1 acrylic coating. Several authors have reported on the effectiveness of acrylic coatings as anti-carbonation treatment [1,34].

The protective effect of the biodeposition treatment towards carbonation could be improved by additional treatments with bacteria and a calcium source or an increased concentration of calcium ions. Basheer et al. [1] reported that for film forming coatings and sealants to be effective against carbonation, the thickness of the treatment should be at least 200 μm. The mean thickness of the carbonate layer was about 30–50 μm, nevertheless an improved resistance towards carbonation was already noticed.

3.3.2. Chloride migration coefficient

Resistance towards chloride penetration of biodeposition treated samples was measured with the use of an accelerated migration test.

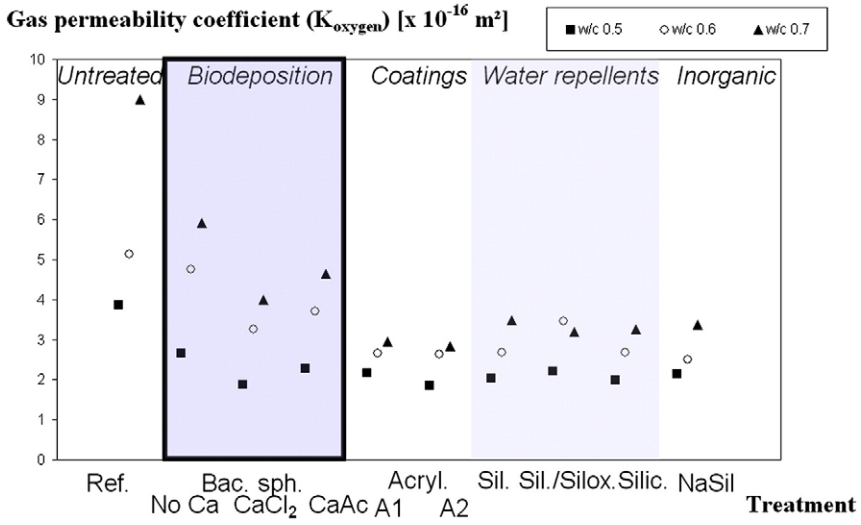

Fig. 4. Gas permeability coefficients, K_{oxygen} (measured at 2 bar), for different grades of mortar applied with different types of surface treatments.

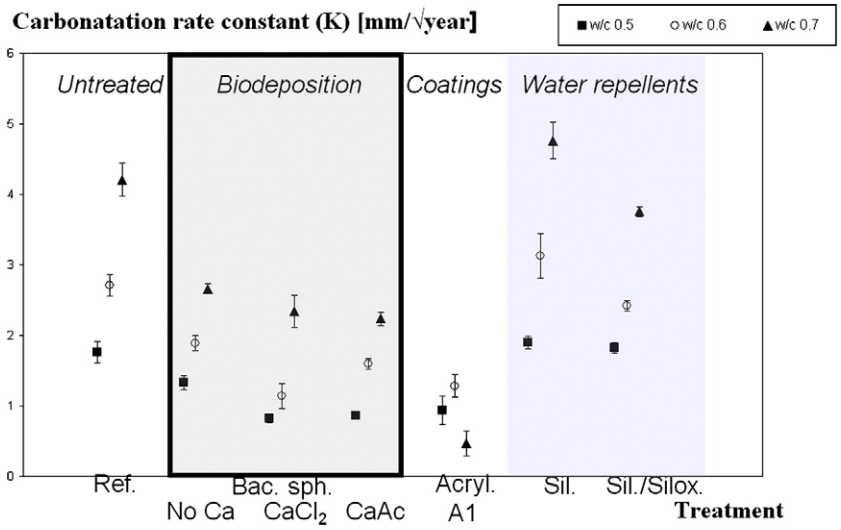

Fig. 5. Carbonation rate constants, *K*, for different grades of mortar applied with different types of surface treatments after 6 weeks of accelerated carbonation tests.

Published work on the chloride resistance of surface treated concrete however, is mainly based on the results of diffusion tests [1,35]. The long duration of this method however, has encouraged some researchers to investigate the use of more rapid tests [2,36].

The non-steady chloride migration coefficient (D_{nssm}) and the effectiveness of the surface treatment are affected by the w/c ratio (Fig. 6). Untreated specimens with a higher w/c showed a higher D_{nssm} which confirms the results of Yang [37]; who reported on the influence of the capillary pore volume and connectivity of the pores on the chloride migration coefficient. The biodeposition treatments resulted in a decrease of the D_{nssm} for each w/c. This is probably attributable to a reduced surface porosity as could be seen from the water absorption experiments (Fig. 2). The combination of the biomass and a calcium source resulted in significant lower chloride migration coefficients compared to untreated specimens. There were no significant distinctions in the D_{nssm} between treatments with a different calcium source (acetate or chloride). The presence of 12 L katholytic 1% NaCl solution in the test set-up for the determination of the D_{nssm} masked the effect of the chloride ions present inside the specimens treated with bacteria and calcium chloride.

The increased resistance towards the migration of chlorides of cubes treated with biodeposition was similar to that of the acrylic coating and the water repellent silanes and silicones and larger than in the case of the silanes/siloxanes mixture, which were all reported to be effective in decreasing the rate of reinforcement corrosion [1,2].

A lot of research on biodeposition has been conducted with $CaCl_2$ as the calcium source [9,38,39]. As chloride ions are detrimental for the reinforcement, the use of calcium acetate [40,41] as an alternative calcium source was investigated. Results show that a similar protective performance was obtained with the biodeposition treatment in the presence of calcium chloride or calcium acetate. In order to avoid possible adverse effects of the calcium chloride on concrete, future research will be done by using calcium acetate as calcium source.

3.3.3. Freezing and thawing test

Visual observations and ultrasonic measurements were unable to demonstrate the effectiveness of the different surface treatments towards freezing and thawing within the time frame of this research. No visual signs of deterioration could be seen after 21 cycles. From the ultrasonic measurements, it could be seen that the velocity of the sonic pulses increased with decreasing water–cement ratio as could be expected. For each treatment, there were however no significant changes in pulse velocity before and after 21 cycles of freezing and

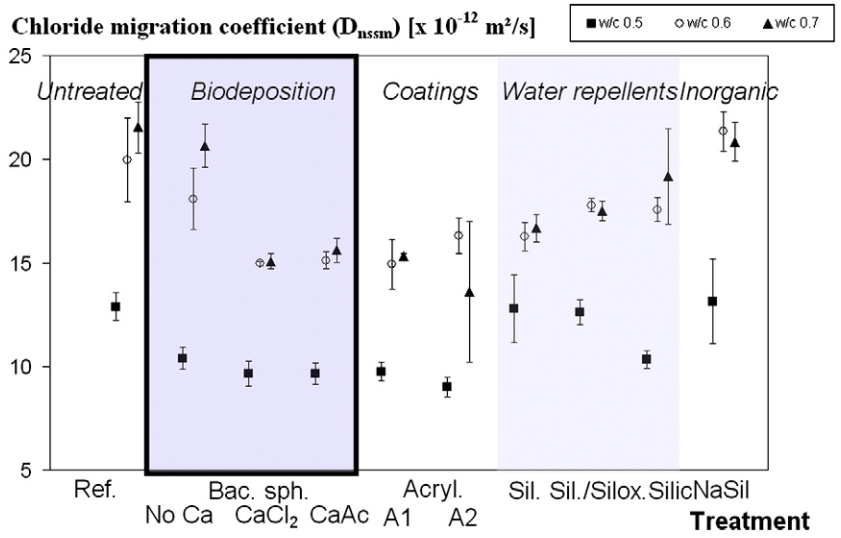

Fig. 6. Chloride migration coefficients, D_{nssm}, for different grades of applied with different types of surface treatments.

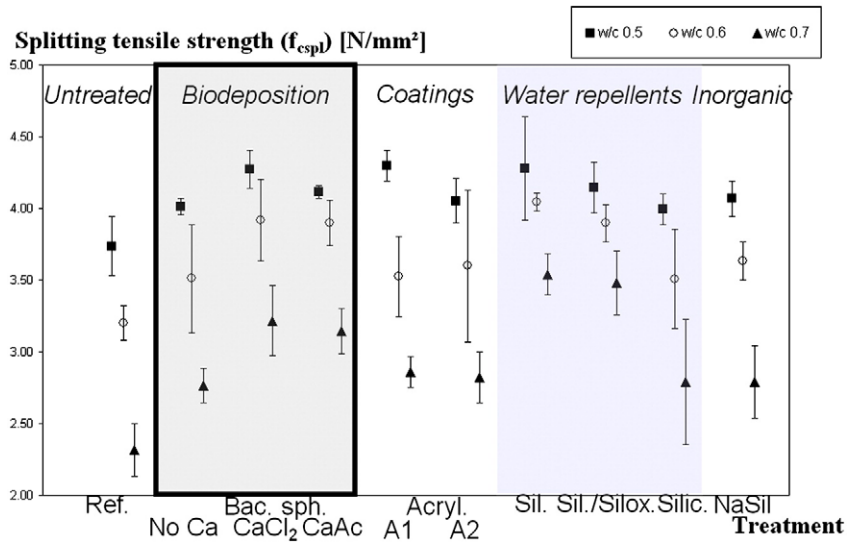

Fig. 7. Tensile strength after 21 cycles of freezing and thawing, for different grades of mortar applied with different types of surface treatments.

thawing. Only in the case of the w/c 0.7 specimens the presence of a surface treatment resulted in a significantly higher splitting tensile strength after 21 cycles of freezing and thawing compared to untreated specimens (Fig. 7). The latter could be attributed to the influence of the amount of freezable water within the pore system. Frost damage is likely to occur once the degree of saturation exceeds a critical value ranging between 80 and 90% [42]. Due to an increasing porosity and pore connectivity, specimens with a higher w/c obtain the critical degree of saturation much faster than lower w/c specimens. The specimens for the freeze–thaw tests were submerged in water for 168 h prior to testing. For the same time period, untreated specimens in the capillary water absorption experiments already showed a saturation degree of 57±2%, 65±4% and 74±7% for a w/c of 0.5, 0.6 respectively 0.7. Specimens with a surface treatment however showed saturation degrees below 60%. As this saturation degree was the result of the capillary water absorption from one surface, a higher degree of saturation was likely to be obtained from the complete immersion of the specimen. The reported effectiveness of the surface treatments for the highest w/c specimens could therefore be attributed to the fact that in the absence of a surface treatment, a critical degree of saturation was only obtained for the higher w/c specimens. The presence of the surface treatment however, prevented a critical saturation to be reached within the given time period. The high amount of freezable water in the untreated w/c 0.7 specimens resulted in internal cracking and a decreased splitting tensile strength.

For the w/c 0.7 specimens the effectiveness of the biodeposition treatment with a calcium source was similar to the coatings, silanes and silicates under investigation. Silanes and silicates are reported to be effective in improving the freeze–thaw durability of concrete respectively mortar [3,4].

3.4. Future research

One of the factors to be considered when selecting coatings is the durability of the coating. As calcium carbonate is dissolved in acidic environments, there is a need to investigate the effect of acidic rain on the durability of the biodeposition treatment. Bacterially induced calcite crystals however, are assumed to be more resistant to dissolution since it has been experimentally demonstrated that biologically deposited calcite is less soluble than inorganically precipitated calcite [43]. In future research the durability of the calcite layer under varying conditions will be investigated.

4. Conclusions

The current study demonstrated that the biodeposition treatment resulted in an increased resistance of mortar specimens towards carbonation, chloride penetration and freezing and thawing. In the case of cementitious materials, the biodeposition treatment might be regarded as a coating system, as the carbonate precipitation was mainly a surface phenomenon due to the limited penetration of the bacteria in the porous matrix. The results from this research confirm the interrelationship that exists between transport and degradation mechanisms occurring in concrete. The transport mechanisms in concrete are influenced by the surface treatment with the ureolytic bacteria and a calcium salt. The presence of a layer of calcium carbonate and microbial biomass resulted in a decrease of the permeation properties of cementitious materials. As a result, an increased resistance towards carbonation, chloride migration and freezing and thawing was noticed. The biodeposition treatment showed a similar protection towards degradation processes as some of the conventional surface treatments under investigation. From the above, it is clear that the presence of a layer of carbonate crystals on the surface has the potential to improve the resistance of cementitious materials towards degradation processes.

Further research however is warranted to investigate the durability of the treatment under environmental conditions.

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References

[1] P.A.M. Basheer, L. Basheer, D.J. Cleland, A.E. Long, Surface treatments for concrete: assessment methods and reported performance, Construction and Building Materials 11 (7–8) (1997) 413–429.
[2] M. Ibrahim, A.S. Al-Gahtani, M. Maslehuddin, A.A. Almusallam, Effectiveness of concrete surface treatment materials in reducing chloride-induced reinforcement corrosion, Construction and Building Materials 11 (7–8) (1997) 443–451.
[3] L. Basheer, D.J. Cleland, Freeze–thaw resistance of concretes treated with pore liners, Construction and Building Materials 20 (10) (2006) 990–998.

[4] H.Y. Moon, D.G. Shin, D.S. Choi, Evaluation of the durability of mortar and concrete applied with inorganic coating material and surface treatment system, Construction and Building Materials 21 (1) (2007) 24–33.
[5] S.K. Ramakrishnan, R.K. Panchalan, S.S. Bang, Improvement of concrete durability by bacterial mineral precipitation, Proceedings of 11th International Conference on Fracture, Turin, Italy, 2001.
[6] S.K. Ramachandran, V. Ramakrishnan, S.S. Bang, Remediation of concrete using micro-organisms, ACI Materials journal 98 (2001) 3–9.
[7] P. Ghosh, S. Mandal, B.D. Chattopadhyay, S. Pal, Use of microorganism to improve the strength of cement mortar, Cement and Concrete Research 35 (10) (2005) 1980–1983.
[8] J. Dick, W. De Windt, B. De Graef, H. Saveyn, P. Van der Meeren, N. De Belie, W. Verstraete, Bio-deposition of a calcium carbonate layer on degraded limestone by *Bacillus* species, Biodegradation V17 (4) (2006) 357–367.
[9] S.S. Bang, J.K. Galinat, V. Ramakrishnan, Calcite precipitation induced by polyurethane-immobilized *Bacillus pasteurii*, Enzyme and Microbial Technology 28 (4–5) (2001) 404–409.
[10] S. Stocks-Fischer, J.K. Galinat, S.S. Bang, Microbiological precipitation of $CaCO_3$, Soil Biology and Biochemistry 31 (11) (1999) 1563–1571.
[11] F. Hammes, W. Verstraete, Key roles of pH and calcium metabolism in microbial carbonate precipitation, Reviews in environmental science and biotechnology 1 (2002) 3–7.
[12] E. Nolan, P.A.M. Basheer, A.E. Long, Effects of three durability enhancing products on some physical properties of near surface concrete, Construction and Building Materials 9 (5) (1995) 267–272.
[13] W. De Muynck, K. Cox, W. Verstraete, N. De Belie, Bacterial carbonate precipitation as an alternative surface treatment for concrete, Construction and Building Materials 22 (5) (2008) 875–885.
[14] W. De Muynck, J. Dick, B. De Graef, W. De Windt, W. Verstraete, N. De Belie, Microbial ureolytic calcium carbonate precipitation for remediation of concrete surfaces, in: M. Alexander, H.-D. Beushausen, F. Dehn, P. Moyo (Eds.), Proceedings of International Conference on Concrete Repair, Rehabilitation and Retrofitting, Cape Town, South Africa, 2005, pp. 296–297.
[15] F. Hammes, N. Boon, J. de Villiers, W. Verstraete, S.D. Siciliano, Strain-specific ureolytic microbial calcium carbonate precipitation, Applied and Environment Microbiology 69 (8) (2003) 4901–4909.
[16] Environmental protection agency. *Bacillus sphaericus*; Exemption from the requirement of a tolerance, http://www.epa.gov/fedrgstr/EPA-PEST/1998/September/Day-11/p24469.htm.
[17] Office of Environmental Health and Safety; Washington State Department of Health. Larvicide: *Bacillus sphaericus*, http://www.doh.wa.gov/ehp/ts/Zoo/WNV/larvicides/Bsphaericus.html#2.
[18] U.H. Jakobsen, D.R. Brown, Reproducibility of w/c ratio determination from fluorescent impregnated thin sections, Cement and Concrete Research 36 (8) (2006) 1567–1573.
[19] G.S. Stewart, K. Johnstone, E. Hagelberg, D.J. Ellar, Commitment of bacterial spores to germinate. A measure of the trigger reaction, Biochemical Journal 198 (1) (1981) 101–106.
[20] J.J. Kolek, The determination of the permeability of concrete to oxygen by the Cembureau method — a recommendation, Materials and Structures 22 (1989) 225–230.
[21] RILEM TC, 116-PCD, Permeability of concrete as a criterion of its durability, tests for gas permeability of concrete, Materials and Structures 32 (1999) 174–179.
[22] M. Carcassès, A. Abbes, J.-P. Ollivier, J. Verdier, An optimized preconditioning procedure for gas permeability measurement, Materials and Structures 35 (2002) 22–27.
[23] S.K. Roy, K.B. Poh, D.o. Northwood, Durability of concrete-accelerated carbonation and weathering studies, Building and Environment 34 (5) (1999) 597–606.
[24] Nord test method. Concrete, mortar and cement-based repair materials: chloride migration coefficient from non-steady state migration experiments. NT Build 492 4.
[25] V.V. Samonin, E.E. Elikova, A study of the adsorption of bacterial cells on porous materials, Microbiology 73 (6) (2004) 696–701.
[26] D. St John, A.W. Poole, I. Sims, Concrete Petrography, a Handbook of Investigative Techniques, Arnold Publishers, 1998.
[27] M. Carrera, R.O. Zandomeni, J. Fitzgibbon, J.L. Sagripanti, Difference between the spore sizes of *Bacillus anthracis* and other *Bacillus* species, Journal of Applied Microbiology 102 (2) (2007) 303–312.
[28] K. Tanaka, K. Kurumisawa, Development of technique for observing pores in hardened cement paste, Cement and Concrete Research 32 (9) (2002) 1435–1441.
[29] J.B. Cliff, K.H. Jarman, N.B. Valentine, S.L. Golledge, D.J. Gaspar, D.S. Wunschel, K.L. Wahl, Differentiation of spores of *Bacillus subtilis* grown in different media by elemental characterization using time-of-flight secondary ion mass spectrometry, Journal of Applied and Environmental Microbiology 71 (11) (2005) 6524–6530.
[30] P. Tiano, L. Biagiotti, G. Mastromei, Bacterial bio-mediated calcite precipitation for monumental stones conservation: methods of evaluation, Journal of Microbiological Methods 36 (1–2) (1999) 139–145.
[31] M. Nemati, G. Voordouw, Modification of porous media permeability, using calcium carbonate produced enzymatically in situ, Enzyme and Microbial Technology 33 (5) (2003) 635–642.
[32] V.S. Whiffin, Microbial $CaCO_3$ precipitation for the production of biocement, School of Biological Sciences and Biotechnology, Murdoch University, Perth, 2004, p. 155.
[33] A.A. Almusallam, F.M. Khan, S.U. Dulaijan, Al-Amoudi OSB. Effectiveness of surface coatings in improving concrete durability, Cement and Concrete Composites 25 (4–5) (2003) 473–481.
[34] A.M. Garcia, C. Alonso, C. Andrade, Evaluation of the resistance of concrete coatings against carbonatation and water penetration, Proceedings of International Conference, University of Dundee, Scotland, UK, 1990, pp. 233–245.
[35] N.R. Buenfeld, J.Z. Zhang, Chloride diffusion through surface-treated mortar specimens, Cement and Concrete Research 28 (5) (1998) 665–674.
[36] D. Whiting, In-situ measurement of the permeability of concrete to chloride Ions, In-situ Nondestructive Testing of Concrete, ACI SP-82, American Concrete Institute, 1984, pp. 501–524.
[37] C.C. Yang, On the relationship between pore structure and chloride diffusivity from accelerated chloride migration test in cement-based materials, Cement and Concrete Research 36 (2006) 1304–1311.
[38] Adolphe JM, Loubière JF, Paradas J, Soleilhavoup F.1990. Procédé de traitement biologique d'une surface artificielle. European patent 90400G97.0 (after French patent 8903517, 1989).
[39] Ferris FG, Stehmeier LG.1992. Bacteriogenic mineral plugging. USA Patent US5143155.
[40] P. Cacchio, C. Ercole, G. Cappuccio, A. Lepidi, Calcium carbonate precipitation by bacterial strains isolated from a limestone cave and from a loamy soil, Geomicrobiology Journal 20 (2) (2003) 85–98.
[41] C. Rodriguez-Navarro, M. Rodriguez-Gallego, K. Ben Chekroun, M.T. Gonzalez-Munoz, Conservation of ornamental stone by *Myxococcus xanthus*-induced carbonate biomineralization, Journal of Applied and Environmental Microbiology 69 (4) (2003) 2182–2193.
[42] G. Fagerlund, The international cooperative test of the critical degree of saturation method of assessing the freeze/thaw resistance of concrete, Materials and Structures 10 (58) (1977) 230–251.
[43] J. Morse (Ed.), The Kinetics of Calcium Carbonate Dissolution and Precipitation, Mineralogic Society of America, 1983.