Microbiologically induced cementation of $CaCO_3$ nanoparticles for limestone conservation

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ABSTRACT

Consolidation of limestone by means of microbiologically induced carbonate precipitation (MICP) requires substantial amounts of $CaCO_3$ precursors, some of which may lead to salt formation and damage to the stone in the long term. In this study, we proposed the use of $CaCO_3$ nanoparticles as a strategy to decrease the amount of $CaCO_3$ precursors required for strengthening of the stone. For this purpose, we have evaluated the performance of biodeposition treatments in which 0, 25 and 50% of the calcium chloride and urea were replaced with $CaCO_3$ nanoparticles. Treatments were applied on Maastricht limestone. The protective and consolidation effect of the biodeposition treatments were evaluated by means of capillary water absorption measurements and hardness profiles obtained by means of drilling resistance measurements (DRMS), respectively. The nanoparticles did not exert a significant consolidation effect when applied as such. The biodeposition treatments resulted in a significant increase in strength, the extent to which being dependent on the application procedure (immersion or pouring) and dosage of $CaCO_3$ precursors (30-120 kg $CaCO_3$ per cubic meter of limestone). The strength increase (132%) for the biodeposition treatment applied by immersion was limited to a superficial layer of 5 mm depth. By pouring $CaCO_3$ precursors on the surface at a concentration of 90 $kg.m^{-3}$, for the first time ever, consolidation by biodeposition was achieved at depths up to 30 mm and more. The overall strength increase in the consolidated zone reached 375%. The sole presence of nanoparticles resulted in a decreased sorptivity of about 66%. The biodeposition treatment applied by immersion resulted in a 29% decreased sorptivity, while in case of application by pouring, the decrease in water uptake rate was about 47-58%, depending on the amount of $CaCO_3$ precursors. From this study, it appears that up to 50% of the calcium chloride and urea could be replaced by $CaCO_3$ nanoparticles without affecting the overall performance of the biodeposition treatment.

Keywords: consolidation, biomineralization, calcification, bacteria

1. Introduction

Biogenic carbonate surface treatments, known as biodeposition treatments, have been investigated by several research groups for the conservation of ornamental stone [1-6]. The protective and consolidation effects of this treatment both rely on the microbiologically induced formation of calcium carbonate. These biogenic crystals may form a protective layer on the surface, decreasing the uptake of water and noxious components, and act as cementing layer between the grains of the stone, increasing its cohesion [7, 8].

Microorganisms can induce the precipitation of calcium carbonate in a variety of ways, including the formation of metabolic products, affecting the saturation state of the solution, and/or the production of nucleation sites, catalyzing the nucleation reaction [9, 10]. The latter can be attributed to the physical and chemical characteristics of the cell wall. Microorganisms can increase the saturation state of a given system by means of an increase of the concentration of dissolved inorganic carbon (DIC), an increase of the pH or a combination thereof. An example of such a process is the hydrolysis of urea. This process presents several advantages over the other carbonate generating processes, as it can be easily controlled and it has the potential to produce high amounts of carbonate within a short period of time [8].

For all types of surface treatments, the depth of penetration depends on a variety of parameters. Apart from climatic conditions (temperature and relative humidity (R.H.)), it is influenced by the viscosity and surface tension of the product, its rate of deposition, the application procedure and the rate of solvent evaporation. Moreover, since liquid transport also depends on the pore structure of a stone [11], the latter will also affect the penetration depth of a surface treatment. For biodeposition treatments, the depth of penetration not only depends on transport of liquid within the stone, but also that of bacteria. The transport of bacteria within a porous material depends both on the pore structure of the stone and the adsorption of the bacteria on the mineral matrix. Transport of bacteria occurs in pores of which the diameter is at least two times that of bacteria [12]. To date, only limited penetration depths have been reported for biodeposition treatments, ranging from several µm (100 µm for the Calcite Bioconcept treatment [6], 500 µm for a biodeposition treatment with *Myxococcus xanthus* [7]) to a few mm (2 mm in our previous study [13]).

Results from our previous studies revealed that the effectiveness of a ureolytic biodeposition treatment is very dependent on the dosage of $CaCO_3$ precursors [8] and the porosity of the stone [13], since both parameters affect the amount of carbonate produced. An increased amount of biogenic crystals, and hence, a higher performance, was observed for increasing amounts of $CaCO_3$ precursors (urea and calcium chloride) and macropores of the stone. From these studies, it was concluded that for the consolidation of degraded and very porous stone, substantial amounts of $CaCO_3$ precursors are required. With the current biodeposition procedures, this would result in elevated levels of ammonium and chloride in the stone. Since these salts may cause damage to the stone in the long-term (e.g. acidification upon nitrification), the amount of precursors should be kept as low as possible. Therefore, we proposed the use of $CaCO_3$ nanoparticles as a strategy to decrease the amount of $CaCO_3$ precursors required to obtain sufficient binder material for a microbiologically induced consolidation of limestone.

The aim of this research was to investigate the effect of the addition of $CaCO_3$ nanoparticles on the protective and consolidating effect of biodeposition treatments. For this purpose, the treatments were evaluated by means of water absorption tests and a drilling resistance measurement tests, respectively. To our knowledge, this is the first study in which the spatial distribution of the strengthening effect of a biodeposition treatment is investigated.

2. Experimental study

2.1. Materials

2.1.1. Limestone

Maastricht stone is a soft limestone with a total porosity up to 47% and a low compressive strength (3-5 $N.mm^{-2}$). Its softness enables a clear evaluation of a strengthening effect. Prior to the experiments, cubes of 10 cm side were dried at 80°C until constant weight (a weight change less than 0.1% between two measurements at 24 h intervals). Then, all sides were covered with aluminum foil, except the one to be treated, to ensure that evaporation of water could only occur through the treated side. In case the treatments were applied by pouring, the foil was applied in such a way that it reached 2 cm above the surface that had to be treated. As such, loss of liquid during pouring was prevented.

2.1.2. Bacteria

Bacillus sphaericus LMG 22557 (BCCM, Ghent) was used as bacterium for this study. Selection of this spore-forming strain was based on results obtained in previous work [2]. The liquid culture media for the immersion experiment consisted of 20 $g.l^{-1}$ yeast extract and 20 $g.l^{-1}$ urea. Liquid media were sterilized by autoclaving for 20 min at 120°C. Urea was added after autoclaving by means of filtration through a sterile 0.33 µm Millipore filter (Millipore, USA). Culture media for the pouring experiments consisted of 13 $g.l^{-1}$ nutrient broth, 10 $g.l^{-1}$ ammonium chloride, brought to a pH of about 8.5 by the addition of sodium hydroxide before autoclaving. For all experiments, *B. sphaericus* cultures were obtained after subsequent culturing (two times and 1% inoculum) from a stock culture conserved at -80°C. Cultures were incubated for 24 h at 28°C on a shaker at 100 rpm. Culturing was performed under sterile conditions.

2.1.3. $CaCO_3$ nanoparticles

The procedure employed for the synthesis of citrate-stabilized $CaCO_3$ nanoparticles was based on the decomposition of urea at temperatures above 60°C. The fabrication of the nanoparticles was carried out in batches. For each batch, an aqueous solution of citric acid (5 g) was neutralized with aqueous ammonia solution. Calcium nitrate (4.72 g) and urea (12 g) were dissolved in distilled water. All the starting materials were of analytical grade and used without further purification. All the solutions were placed in a tightly closed round bottom flask. The total volume of the reaction solution was 500 ml. The reaction mixture was stirred continuously for 3 h at 90 °C and then allowed to cool to room temperature. The resulting nanoparticles were separated from the suspension by centrifugation. The powder was washed several times with ethanol and left to dry over night at 60°C. Afterwards, the particles were resuspended in distilled water to obtain a concentration of 30 $g.l^{-1}$. Modifying the surface of $CaCO_3$ particles *in situ* with citric acid and NH_4OH in aqueous solution resulted in irregular stick shaped particles with a diameter of 20-30 nm and a length of 100-300 nm.

2.2. Biodeposition treatment procedure

All treatments were applied in triplicate (n=3).

2.2.1. Immersion

The biodeposition procedure was performed in two steps, similar to the procedure described in [13], i.e. at 28°C, one day immersion in a bacterial culture followed by four days immersion in a 0.3 M $CaCO_3$ precursor (i.e. urea and calcium chloride dihydrate) solution.

2.2.2. Pouring

Similar to the immersion treatment, the treatment by pouring was applied in two steps. First, 125 ml of a one day old culture of *B. sphaericus* was poured on the surface. After one hour, an equal amount of a solution containing urea and calcium chloride was applied to the surface. The concentrations of urea and calcium chloride (equimolar) inside the solution used in the second step are presented in Table 1. In order to obtain the concentrations present inside the stone, these values need to be divided by two. The total amount of liquid applied (250 ml) corresponds to a theoretical penetration depth of about 5 cm for the stone used in this study, supposing a complete filling of the pores in the treated zone. The dosages ($kg.m^{-3}$) mentioned in Table 1 correspond to the amount of $CaCO_3$ to be precipitated in this first 5 cm. Treatments and conditioning were carried out in a climatized room at 20°C and 65% R.H. For the 90 and 120 $kg.m^{-3}$ treatments, the time between successive applications was 1 week.

2.2.3. Application of nanoparticles

The nanoparticles suspensions were applied at 20°C and 65% R.H. by pouring prior to the bacteria and $CaCO_3$ precursors. From preliminary experiments, it was noticed that in case the concentration of nanoparticles was higher than 2.5 $g.l^{-1}$ rapid clogging of the surface was obtained preventing further uptake. Every Monday and Friday, 250 ml of a 2.5 $g.l^{-1}$ solution of nanoparticles was applied to the surface until the desired content was reached (up to 6 weeks). After each application of the nanoparticles, the specimens were dried at 47°C to speed up the evaporation of water. One week after the last application of the nanoparticles, the biodeposition treatment was applied as described in 2.2.2. The concentrations of urea and calcium chloride were adjusted in accordance to the amount of nanoparticles applied to the surface.

2.3. Weight increase due to biodeposition

The weight gain was calculated from the difference in weight before and after treatment, after drying at 80°C until constant weight (average weight before treatment was 1.3 kg).

2.4. Capillary water absorption

The protective effect of the biodeposition treatment was investigated by means of a sorptivity test. Determination of the water absorption by capillarity was performed on two specimens per type of treatment according to EN 1925:1999. Prior to the test, the stones were dried in an oven at 80°C, until a constant weight was obtained, The sorptivity (water uptake rate) coefficient was calculated from the slope of the linear curve presenting the amount of water absorbed per unit of surface and the square root of time.

2.5. Drilling resistance measurements

The strengthening effect was measured by means of the drilling resistance measurement system (DRMS Cordless SINT Technology, Italy). The system is equipped with a software program allowing the continuous recording and monitoring of the drilling resistance in relation to the advancement of the drill bit. For this study, a rotation speed of 600 rpm and a penetration speed of 40 $mm.min^{-1}$ were used. DRMS tests were performed using drill bits with 4.8 mm diameter allowing a maximum penetration depth around 3.5 cm. The results of the DRMS measurements are expressed as differential hardness profiles, obtained by subtracting the drilling forces measured after treatment with those measured before treatment (i.e. an untreated stone). For each type of treatment, 3 drilling measurements were carried out on one stone from which the average hardness profile was calculated.

3. Results and discussion

3.1. Weight increase due to biodeposition

The biodeposition treatment resulted in a weight gain of all stones (Table 1). With exception of the 120(4) kg.m^{-3} treatment, the weight increase was proportional to the increased concentration of $CaCO_3$ precursors applied. The smaller weight increase observed for the 120(4) kg.m^{-3} treatment compared to the 120(2) kg.m^{-3} treatment can be attributed to the fact that the stones were almost completely saturated after two weeks of treatment and that evaporation was rather limited during the tests. As such, only limited amounts of bacteria and $CaCO_3$ precursors were taken up by the stone in the third and fourth week (100 g instead of 250 g of liquid absorbed). This explains why the treatment performed in 4 runs (120(4) kg.m^{-3}) resulted in a weight increase similar to that of the 90-1 kg.m^{-3} treatment. The weight increase can be attributed to the presence of bacteria, biogenic carbonate and other compounds such as nutrients that have not been metabolized as well as salts that have been formed as a result of the hydrolysis of urea, e.g. ammonium chloride.

Table 1. Influence of the application procedure and dosage of $CaCO_3$ precursors on the weight gain, decrease in sorptivity (S) and strength increase of biodeposition treated Maastricht limestone

Treatment*	**Concentration of precursors applied (M)**				**Weight gain (g)**	**S↓ (%)**	**Increase of resistance (%)**				
	Week 1	*Week 2*	*Week 3*	*Week 4*			*0-5 mm*	*5-10 mm*	*10-20 mm*	*20-30 mm*	***0-30 mm***
Immersion	0.3				n.d.	29	132	17	19	43	**46**
30 kg.m^{-3}	1.2				25	47	612	52	14	41	**126**
60 kg.m^{-3}	2.4				45	52	774	0	38	139	**192**
90 kg.m^{-3}	1.2	2.4			62	52	245	145	361	542	**375**
120(2) kg.m^{-3}	2.4	2.4			84	53	296	62	265	1008	**506**
120(4) kg.m^{-3}	1.2	1.2	1.2	1.2	60	58	1169	387	191	203	**374**

*(2) or (4) indicates the number of applications of precursors.

Biodeposition treatments in which a part of the $CaCO_3$ precursors had been replaced with $CaCO_3$ nanoparticles exhibit a similar or lower weight gain compared to biodeposition treatments without nanoparticles (Table 2). The fact that the weight gain observed for the 15 kg.m^{-3} treatment (100% nanoparticles, Table 2) was much lower compared to the 30 kg.m^{-3} treatments (Table 1 and 2) can be attributed to several reasons. Firstly, a part of the nanoparticles that had been deposited on the surface was removed during drying in the oven because of their weak attachment to the surface. Secondly, while the weight gain of the 15 kg.m^{-3} treatment can be completely attributed to $CaCO_3$, less than 50% of the weight gain obtained with the biodeposition treatments can be attributed to $CaCO_3$. The other part can be attributed to the presence of ammonium chloride and other compounds (see above), e.g. for a desired dosage of 30 kg $CaCO_3$ per cubic meter of limestone, about 51 kg.m^{-3} precursors is required. For a given concentration of $CaCO_3$ to be precipitated inside the stone, less $CaCO_3$ precursors are required with increasing concentrations of nanoparticles added. As such, compounds other than $CaCO_3$ will be introduced in the stone to a lesser extent. This explains for the lower weight gain of the 30 kg.m^{-3} series in which 50% of the precursors had been replaced with nanoparticles compared to the 30 kg.m^{-3} series in which 25% of the precursors was replaced.

Table 2. Influence of the replacement level of $CaCO_3$ precursors by $CaCO_3$ nanoparticles on the weight gain, decrease in sorptivity (S) and strength increase of biodeposition treated specimens.

Treatment	Replacement level (%)	Weight gain (g)	S↓ (%)	Increase of resistance (%)				
				0-5 mm	*5-10 mm*	*10-20 mm*	*20-30 mm*	***0-30 mm***
15 kg.m^{-3}	100	2	66	69	6	11	84	**44**
30 kg.m^{-3}	25	29	58	251	110	131	193	**168**
	50	18	65	411	84	64	128	**146**
60 kg.m^{-3}	25	51	70	246	136	123	186	**167**

3.2. Protective action

The biodeposition treatment resulted in a decreased rate of water uptake (Fig. 1 and Table 1). As indicated in our previous study, the decrease in water uptake could be attributed to the presence of biogenic carbonate crystals, since no decrease in water absorption was observed for control series without bacteria and/or a calcium source [8]. Furthermore, from that study, it was observed that treatments characterized by a higher amount of carbonate precipitation showed a more pronounced decrease in water absorption. Similar to the previous investigation, the results from the current study revealed the highest decrease in water absorption for the 90 kg.m^{-3} and the 120 kg.m^{-3} treatments.

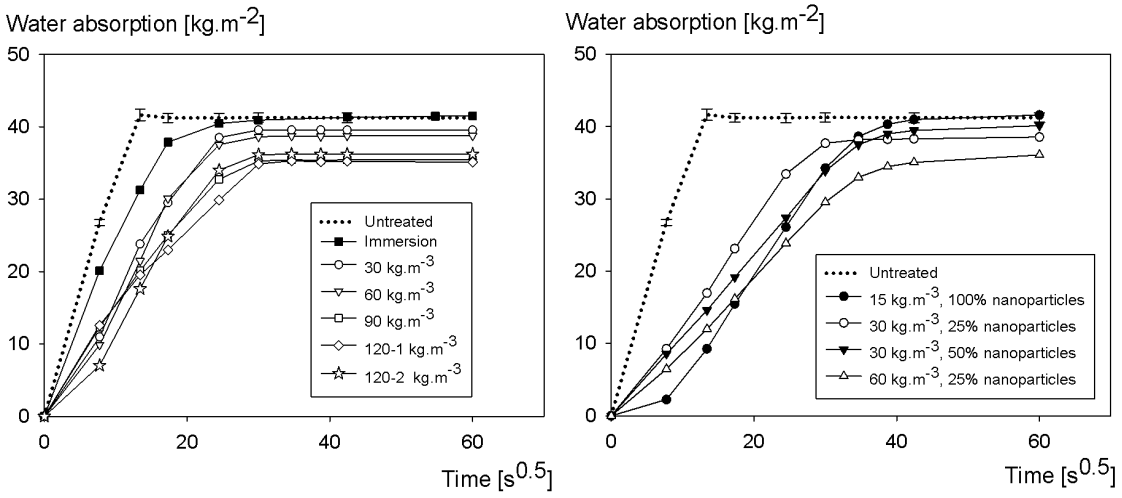

Fig. 1. Influence of the application procedure, dosage of $CaCO_3$ precursors (left) and $CaCO_3$ nanoparticles (right) on the water absorption of biodeposition treated Maastricht limestone.

The lower water absorption rate observed for the treatments with nanoparticles may be attributed to the presence of a layer of nanoparticles on top of the surface. This layer blocks the pores, resulting in a decreased rate of water uptake. Furthermore, the presence of nanoparticles inside the pores below the surface also attributes to a decreased rate of water uptake. It should be mentioned, however, that the superficial layer was gradually removed during the water absorption tests. Because of the detachment of this layer, when immersed under water, nearly all white deposits had been removed at the end of the test.

3.3. Consolidation action

The intensity and depth of the strengthening effect of the biodeposition treatment was dependent on the application procedure and the dosage of $CaCO_3$ precursors applied (Fig. 2). The lowest strength increase was observed for the treatment applied by immersion. For this type of treatment, the strengthening effect was limited to a depth of about 5 mm (Table 1). Biodeposition treatments that were applied by means of pouring exhibited a higher consolidation effect. Treatments with a lower amount of calcium precursors resulted in the formation of a very hard superficial layer (Fig. 2), i.e. an increase in the drilling resistance of about 612% and 774% up to a depth of 5 mm for the 30 $kg.m^{-3}$ and the 60 $kg.m^{-3}$ series, respectively (Table 1). On the contrary, the consolidation action of biodeposition treatments with a higher amount of calcium precursors, that were applied over two weeks, was more pronounced at higher depths (Fig. 2), i.e. an increase in the drilling resistance of about 542% and 1008% at depths between 20 and 30 mm for the 90 $kg.m^{-3}$ and 120(2) $kg.m^{-3}$ series, respectively. For the biodeposition treatment that was applied over 4 weeks (120(4) $kg.m^{-3}$ series), the strengthening effect was again more pronounced at the first 5 mm (Fig. 2).

The large differences in strengthening observed between biodeposition treatments applied by means of immersion or pouring are in agreement with the findings of Ferreira Pinto et al. [14], who observed that different consolidation methods not only lead to differences in the absorbed amount of products but also to different strengthening properties in terms of intensity and spatial distribution [14, 15]. For the biodeposition treatment, the spatial distribution relates to the distribution of the biogenic carbonate inside the stone, which is governed by the transport of the calcite precursors. As such, differences in the transport mechanisms of the calcite precursors between the immersion and pouring treatments may account for the observed differences in the spatial strengthening distribution. In case of the biodeposition treatment applied by immersion, stones were fully saturated with the bacterial culture liquid after the first step of the treatment. Therefore, migration of calcite precursors during the second step was mainly diffusion controlled [13] while it was driven by capillary action in case of a treatment applied by pouring, allowing a greater penetration depth. Subsequently, migration also occurred by means of diffusion. Differences in the intensity of strengthening effect observed between the immersion and the pouring treatments could be attributed to the dosage of calcite precursors [8] and the fact that precipitation of calcium carbonate was not restricted to the pore volume of the stone for the treatment applied by immersion, i.e. precipitation could also occur in the bulk solution. The latter resulted in lower amounts of calcium carbonate precipitated inside the stone.

The addition of $CaCO_3$ nanoparticles at a concentration of 15 kg per cubic meter of limestone resulted in a very limited strength increase (6-84%). The fact that consolidation was limited can be mainly attributed to the tendency of the nanoparticles to move back to and to aggregate on the surface of the limestone. This resulted in the formation of a white layer on top of the surface which inhibited further penetration of the nanoparticles, and hence, a consolidation action at greater depths. In addition, the cementing behavior of these nanoparticles is considered to be very low. The latter could be clearly observed upon drying, where a substantial part of the surface layer could be easily removed by gentle rubbing on the surface.

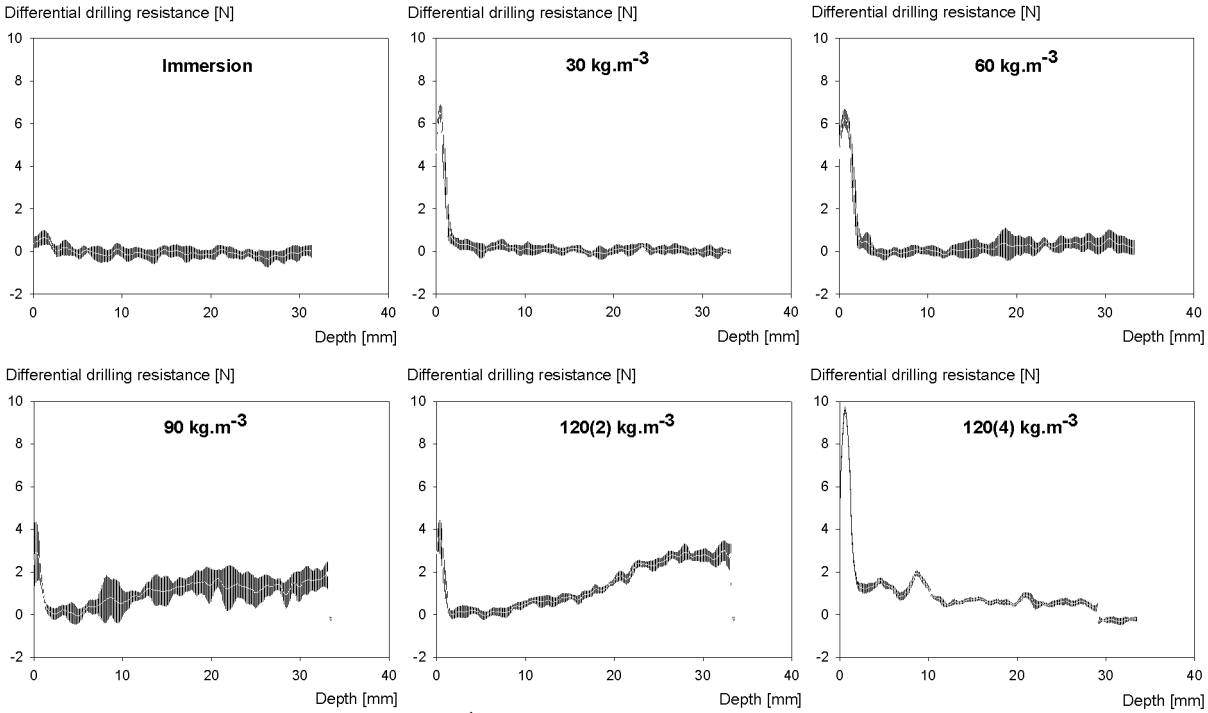

Fig. 2. Differential hardness profiles[1] of biodeposition treatments on Maastricht limestone as a function of application procedure and dosage of $CaCO_3$ precursors.

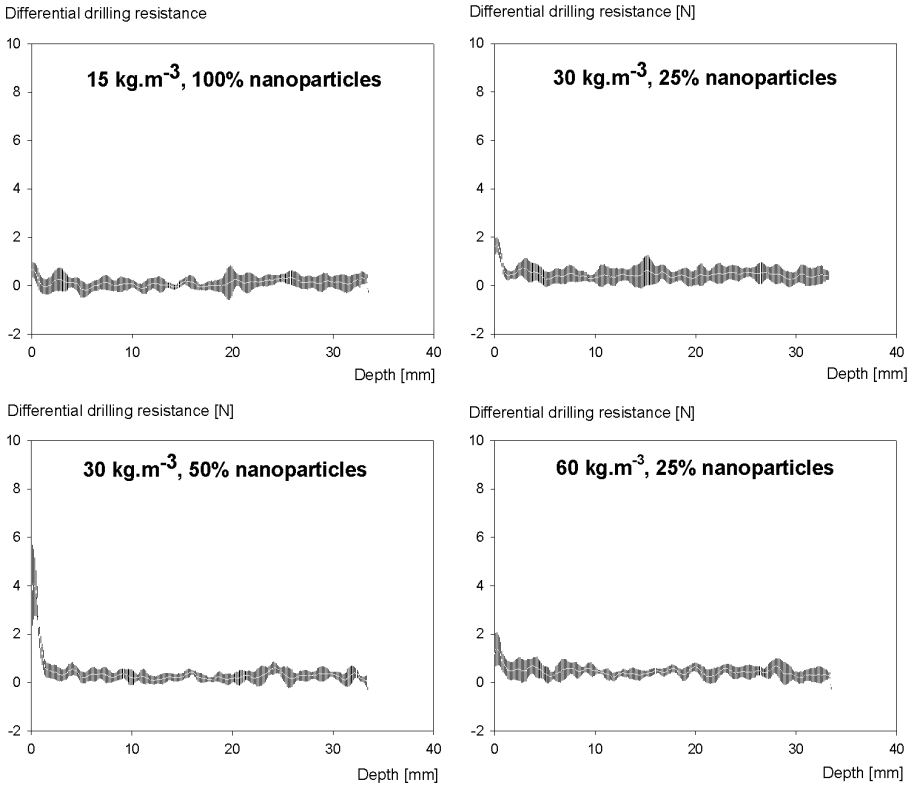

Fig. 3. Differential hardness profiles[1] of biodeposition treatments on Maastricht limestone as a function of the replacement of $CaCO_3$ precursors by $CaCO_3$ nanoparticles.

[1]The profiles consist of the differential drilling resistance values (white line), i.e. the difference in average drilling force observed between biodeposition treated and untreated limestone. The standard deviation is indicated by the gray area.

For the 30 kg.m^{-3} and 60 kg.m^{-3} biodeposition treatments, a 25% replacement of $CaCO_3$ precursors by $CaCO_3$ nanoparticles resulted in the disappearance of the superficial strength peak (Fig. 3). Instead, a more homogeneous strengthening was obtained with much higher strength increases between 5-30 mm (110-193%, Table 2) compared to treatments without nanoparticles (0-139%, Table 1). This may indicate that the $CaCO_3$ nanoparticles may act as a filler and additionally enhance the strengthening effect of the biogenic carbonate that binds the nanoparticles and the loose limestone grains. In case 50% of the $CaCO_3$ precursors was replaced by nanoparticles for the 30 kg.m^{-3} treatment, a superficial strength peak could again be observed. However, the strength of this peak was 2.4 times lower compared to that obtained in the absence of the nanoparticles. Similar as for the 15 kg.m^{-3} treatment, the 30 and 60 kg.m^{-3} biodeposition series with nanoparticles exhibited a white layer on top of the surface. Differences in structure (density and thickness) between the layers of nanoparticles of the 30 and 60 kg.m^{-3} series may account for differences in the spatial strength distribution. The latter can be attributed to the fact that such layers may affect the penetration depth of the bacteria, the diffusion, and hence, the availability of oxygen and the rate of water evaporation, which are parameters that affect the penetration depth of the treatment. Due to pore plugging, increasing concentrations of nanoparticles resulted in a decrease of the amount of bacteria that deeply penetrated the stone. For the 50% replacement, penetration of bacteria, and hence, urease activity was mainly located in the outer 5 mm. The presence of a layer of nanoparticles for the 25% replacement series did not significantly affect the penetration of bacteria, although it probably decreased the oxygen availability and evaporation rate, resulting in more homogeneous strengthening conditions throughout the stone, and hence, a more homogeneous strength distribution.

Ferreira Pinto et al. [14] indicated that the formation of superficial crusts is highly probable in very porous stones. With regard to biodeposition, the occurrence of the strength peaks can be related both to physicochemical and biological processes: (1) upon contact of the precursor solution with the bacterial culture liquid (pH 8.5), chemically induced crystal formation can occur. Initially, this will occur at the interface between the two solutions, i.e. the outer surface of the stone; (2) since *B. sphaericus* is a facultative anaerobic microorganism, its activity is higher in the presence of oxygen. This may account for the higher amount of carbonate precipitation near the surface. The formation of hard superficial layers, however, is unwanted since they are potentially harmful [14]. Therefore, the current application procedures for the 30, 60 and 120(4) kg.m^{-3} appear to be less suited for in practice. Currently, the most promising application procedure appears to be the 90 kg.m^{-3} treatment, since this treatment resulted in the most homogeneous strengthening effect. Furthermore, strengths obtained with this treatment (375%) were higher compared to the reported strengths of ethyl silicate based surface treatments (125 – 225%) on Maastricht stone [16]. It should be mentioned that the ethylsilicates were applied two or three times by capillary absorption during 20 seconds, the time between successive applications being 1 week.

3.4. General remarks

Despite the good performance that was obtained for biodeposition treatments in which part of the $CaCO_3$ precursors were replaced by $CaCO_3$ nanoparticles, such treatments are currently not feasible for *in situ* applications. The latter can be attributed to the characteristics of the $CaCO_3$ nanoparticles used in this preliminary study. $CaCO_3$ nanoparticles of which the surface had been modified by means of the addition of citric acid and ammonium hydroxide exhibited dispersibility in water that was three times higher than for unmodified particles. Despite the surface modification, the particles showed a tendency to aggregate after standing in solution for a couple of days. Moreover, this tendency for aggregation resulted in the unwanted formation of white layers on the surface of this pale yellow stone and prevented the

use of concentrations higher than 2.5 $g.l^{-1}$. From the above, it is clear that more research is necessary to optimize the consolidation method through modification of the nanoparticles so that white haze formation can be prevented and that the number of applications can be drastically decreased. Due to the limited amount of $CaCO_3$ nanoparticles that could be produced within the time frame of this study, replacement of the $CaCO_3$ precursors of the 90 and 120 $kg.m^{-3}$ treatments could not be investigated. Since these series, however, exhibited the best performance and suffer from the highest salt burden, they are most suitable for the application of $CaCO_3$ nanoparticles. This will be investigated in the near future.

4. Conclusions

From this study, it is clear that the application procedure (immersion or pouring) and the dosage of $CaCO_3$ precursors (30-120 kg $CaCO_3$ per cubic meter of limestone to be treated) has an important influence both on the protective and consolidation effects of a biodeposition treatment. Treatments for which lower amounts of $CaCO_3$ precursors were used resulted in limited strengthening (immersion) or undesired hard superficial layers (30 and 60 $kg.m^{-3}$ pouring treatments). By means of pouring $CaCO_3$ precursors on the surface at a concentration of 90 $kg.m^{-3}$, we demonstrated for the first time that consolidation by biodeposition can be achieved at depths up to 30 mm and more, which is much higher than values reported so far (i.e. 2 mm). Furthermore, from this study, it appears that up to 50% of the $CaCO_3$ precursors could be replaced by $CaCO_3$ nanoparticles without affecting the overall performance of the 30 and 60 $kg.m^{-3}$ biodeposition treatments. Moreover, this replacement resulted in a more homogeneous strengthening of the stone and lowered the salt burden of the biodeposition treatment.

Acknowledgments

Willem De Muynck works as a Postdoctoral Fellow of the Flemish agency for Innovation by Science and Technology (IWT Vlaanderen, Belgium).

References

1. Adolphe JP, Loubière JF, Paradas J, Soleilhavoup F. Procédé de traitement biologique d'une surface artificielle. 1990.
2. Dick J, De Windt W, De Graef B, Saveyn H, Van der Meeren P, De Belie N, Verstraete W. Bio-deposition of a calcium carbonate layer on degraded limestone by Bacillus species. Biodegradation 2006; 17(4): 357-367.
3. Jimenez-Lopez C, Rodriguez-Navarro C, Piñar G, Carrillo-Rosúa FJ, Rodriguez-Gallego M , Gonzalez-Muñoz MT. Consolidation of degraded ornamental porous limestone stone by calcium carbonate precipitation induced by the microbiota inhabiting the stone. Chemosphere 2007; 68(10): 1929-1936.
4. Le Metayer-Levrel G, Castanier S, Orial G, Loubiere JF, Perthuisot JP. Applications of bacterial carbonatogenesis to the protection and regeneration of limestones in buildings and historic patrimony. Sed Geol 1999; 126(1-4): 25-34.
5. Tiano P, Biagiotti L, Mastromei G. Bacterial bio-mediated $CaCO_3$ precipitation for monumental stones conservation: methods of evaluation. J Microbiol Meth 1999; 36(1-2): 139-145.
6. De Muynck W, De Belie N,Verstraete W. Microbial carbonate precipitation in construction materials: A review. Ecol Eng 2009; 36(2): 118-136.
7. Rodriguez-Navarro C, Rodriguez-Gallego M, Ben Chekroun K, Gonzalez-Munoz MT. Conservation of Ornamental Stone by *Myxococcus xanthus*-Induced Carbonate Biomineralization. Appl Environ Microbiol 2003; 69(4): 2182-2193.
8. De Muynck W, Verbeken K, De Belie N, Verstraete W. Influence of urea and calcium dosage on the effectiveness of bacterially induced carbonate precipitation on limestone. Ecol Eng 2009; 36(2): 99-111.
9. Castanier S, Le Metayer-Levrel G, Perthuisot JP. Ca-carbonates precipitation and limestone genesis -- the microbiogeologist point of view. Sed Geol 1999; 126(1-4): 9-23.
10. Hammes F, Verstraete W. Key roles of pH and calcium metabolism in microbial carbonate precipitation. Rev Env Sci Biotechnol 2002; 1: 3-7.
11. Cnudde V, Cnudde JP, Dupuis C, Jacobs PJS. X-ray micro-CT used for the localization of water repellents and consolidants inside natural building stones. Mater Charact 2004; 53(2-4): 259-271.
12. Vance, DB Particulate transport in groundwater part II - bacteria. Nat env J 1995; 5(1): 25-26.
13. De Muynck W, Leuridan S, Van Loo D, Verbeken K, Cnudde V, De Belie N, Verstraete W. Influence of Pore Structure on the Effectiveness of a Biogenic Carbonate Surface Treatment for Limestone Conservation. Appl Env Microbiol 2011; 77(19): 6808-6820.
14. Ferreira Pinto AP, Delgado Rodrigues J. Consolidation of carbonate stones: Influence of treatment procedures on the strengthening action of consolidants. J Cult Herit 2011; doi:10.1016/j.culher.2011.07.003
15. Ferreira Pinto AP, Delgado Rodrigues J. Stone consolidation: The role of treatment procedures. J Cult Herit 2008; 9(1): 38-53.
16. De Clercq H, De Zanche S, Biscontin G. Teos and time: the influence of application schedules on the effectiveness of ethyl silicate based consolidants. RBM 2007; 13(5): 305-318.