DIFFERENCES IN PROTECTIVE AND CONSOLIDATION EFFECT OF TWO TYPES OF BIODEPOSITION TREATMENTS

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Abstract

Evidence of microbial involvement in mineral precipitation has led to the exploration of this process in the conservation sector. One of the first patented applications concerned the use of microbially induced carbonate precipitation (MICP) for the protection of ornamental stone, a process known as biodeposition. The promising results of the patented Calcite Bioconcept (CB) treatment have stimulated different research groups to develop similar approaches. While many authors commented on its limitations, to date, the CB treatment remains the only biodeposition technique that is commercially available. So far, however, its consolidation effect has never been reported, neither has it been compared to other biodeposition treatments. The aim of this study was to bridge this gap and to justify comments on the CB treatment stated by other research groups. For this purpose, the protective and consolidation effect of this type of MICP treatment were evaluated and compared with a biodeposition treatment developed at Ghent University (GU). The selected substrate is Maastricht limestone. The protective effect of the treatment was evaluated by means of capillary water absorption measurements while the consolidation effect was assessed by means of hardness profiles obtained from drilling resistance measurements (DRMS). While both biodeposition treatments resulted in a decreased water absorption, a significant strengthening effect could only be observed on the stone treated according to the biodeposition procedure developed at GU. For the latter, a strengthening was achieved up to 30 mm to an extent depending on the concentration of calcium used. In case of the treatment that aimed to precipitate 90 kg of $CaCO_3$ per cubic meter limestone, an overall strength increase in the consolidated zone up to 375% was reached. While the the CB treatment exerts mainly a protective function, the GU procedure offers both a protective and consolidation effect, which makes it more suited for applications in practice where both properties are required.

Keywords: limestone, surface treatment, consolidant, biomineralization

1. Introduction

Biogenic carbonate surface treatments, known as biodeposition treatments, have been investigated by several research groups for the conservation of ornamental stone (Jimenez-Lopez et al. 2008; Le Metayer-Levrel et al. 1999; Rodriguez-Navarro et al. 2003; Tiano et al. 1999). The protective and consolidation effects of this treatment both rely on the microbiologically induced precipitation of calcium carbonate (MICP). These biogenic crystals may form a protective layer on the surface, decreasing the uptake of water and noxious compounds and act as cementing layer between the grains of the stone, increasing its cohesion (De Muynck et al. 2010b; Rodriguez-Navarro et al. 2003).

Adolphe et al. (1990) were among the first to consider the use of MICP for the protection of ornamental stone, which resulted in the patented Calcite Bioconcept biodeposition method. Although results from large scale applications demonstrated a sustainable and effective protective effect (Le Metayer-Levrel et al. 1999), some authors commented on potential limitations of this technique: (1) ineffectiveness for in-depth consolidation, (2) formation of a superficial film consisting of a mixture of biological remains and (3) possibility of uncontrolled bacterial growth and biofilm formation (Rodriguez-Navarro et al. 2003; Tiano et al. 1999). Consequently, alternative methods were developed which rely on different bacterial strains and metabolic pathways as a way to overcome the aforementioned limitations (De Muynck et al. 2010a). At Ghent University, a biodeposition procedure was developed based upon the hydrolysis of urea (Dick et al. 2006). This process presents several advantages over the other carbonate generating processes, as it can be easily controlled and has the potential to produce high amounts of carbonate within a short period of time [8].

In literature, only few studies are available that focus both on the protective and consolidation action of biodeposition treatments (De Muynck et al. 2010a). Moreover, the use of different types of substrates, diverse application and evaluation methods hamper any objective comparison between the different types of treatments. So far, evaluation of the penetration depth was mainly based on microscopic observations of the distribution of biogenic crystals inside the stone rather than a spatial quantification of the strengthening effect. From Scanning Electron Microscopy (SEM) analyses on cross sections of biodeposition treated stone samples, precipitation of calcium carbonate was observed at depths of about 100 μm for the Calcite Bioconcept treatment (De Muynck et al. 2010a) while up to at least 500 μm for the Granada University treatment (Rodriguez-Navarro et al. 2003). The penetration depth of our biodeposition treatment has been visualized by means of micro-tomographic analyses on small cores of limestone (i.e. diameter of 5 mm) (De Muynck et al. 2011). From that study, it was concluded that the penetration depth and the effectiveness of a biodeposition treatment are highly dependent on the porosity of the stone. Since macro-pores favor the transport of bacteria, and hence, crystal precipitation at greater depths, macroporous stones exhibit a more pronounced increase of the resistance towards water related degradation phenomena compared to microporous ones. Recently, we demonstrated for the first time that consolidation can be achieved up to 30 mm in macroporous stone (De Muynck et al. 2012). The strengthening effect was evaluated by means of hardness profiles obtained by drilling resistance measurements.

The aim of the current study was to compare the performance of our biodeposition treatment with that of the commercially available Calcite Bioconcept and to verify whether the comments on the consolidation effects of the latter are justified. For that purpose, experiments were performed on a macroporous stone that due to its softness allows a clear evaluation of the strengthening action.

2. Materials and methods
2.1 Stone
Maastricht stone is a soft limestone with a total porosity up to 47 vol.% 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.

2.2 Calcite Bioconcept treatment (CB)
The treatment was applied over 4 days according to the procedure suggested by the supplier (Amonit – Calcite Bioconcept). In the first day, stones were treated with an overnight grown bacterial suspension obtained by adding lyophilized *Bacillus cereus* cells (Biocal) and nutrients (Nutrical that also includes a calcium source) to tap water at a concentration of 5 and 25 $g.L^{-1}$, respectively. In the subsequent 3 days, the bacteria were fed every morning with a nutritional medium (Nutrical) designed to stimulate the production of carbonate by means of nitrogen cycle metabolic pathways (Le Metayer-Levrel et al. 1999). The Nutrical solution ($25g.L^{-1}$) was also applied to the stone in the afternoon of the second day of the treatment, bringing the total number of nutrient applications to 4. The bacterial and nutrients solutions were applied to the stone by complete immersion of the stone in the respective solutions (n=1), capillary absorption during 20 seconds (n=3) or by pouring the respective liquid to the surface until the formation of a layer of water on the surface (n=3). For the capillary absorption treatment, the stones were placed in the solution to a depth of 1-2 mm.

2.3 Ghent University treatment (GU)
Table 1 gives an overview of the different treatment procedures that have been used in this study. A first series of treatments was based on the 4 day Calcite Bioconcept procedure in which the bacterial (1 x 50 mL) and nutrients (4 x 50 mL) solutions were applied by pouring, the nutritional composition being the only variable. A second series of treatments aimed to precipitate a given amount of calcium carbonate to a depth of about 5 cm. These treatments consisted of at least two parts. First, 125 ml of a one day old culture of *B. sphaericus* was poured on the surface. Second, after one hour, an equal amount of a solution containing urea and calcium chloride was applied to the surface. 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 concentration of nutrients and the number of applications

of bacteria and nutrients were modified according to the desired amount of precipitation (Table 1).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.

Table 1. Overview of the different types of treatments applied following the Ghent University (GU) biodeposition procedure.

	Bacteria		Nutrients			
	Vol. (mL)	Appl. Day	[CaCO$_3$ precursors] (M)*	[N. Broth] (g.L^{-1})	Vol. (mL)	Appl. day
CB-1	50	1	0.17	13	50	2 (2x), 3 and 4
CB-2			1.2	-		
CB-3			1.2	13		
30 kg.m^{-3}	150	1	1.2	-	150	1
60 kg.m^{-3}			2.4			
90 kg.m^{-3}	150	1 and 8	1.2 (1) /2.4 (8)	-	150	1 and 8
120 kg.m^{-3}			2.4 (1)/2.4 (8)			

*Number between brackets indicates day of application; CaCO$_3$ precursors are urea and CaCl$_2$.H$_2$O; N. Broth = nutrient broth (oxoid)

2.4 Weight increase due to biodeposition

The dry weight gain was calculated from the difference in weight before and after treatment, after drying at 80°C until constant weight (average weight of the samples before treatment was 1.3 kg). The wet weight gain is the sum of weight increases measured immediately after each application of bacterial and nutrient liquids.

2.5 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 area and the square root of time (kg.m^{-2}.s$^{-0.5}$).

2.6 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 (ϕ 4.8 mm). For this study, a rotation speed of 600 rpm and a penetration speed of 40 mm.min^{-1} were used. The maximum penetration depth is about 3.5 cm. The results of the DRMS measurements are expressed as differential hardness profiles, obtained by subtracting the drilling forces measured after treatment from the reference values obtained on the corresponding untreated stone. For each type of treatment, 4 drilling measurements were carried out on each sample from which the average hardness profile was calculation.

3. Results and discussion

3.1 Weight increase due to biodeposition

All types of biodeposition treatments resulted in a weight increase of Maastricht limestone (Table 2). The extent to which samples gained weight depends on the amount of absorbed liquid and the concentration of nutrients and $CaCO_3$ precursors applied. The smallest weight increase (3 and 5 g) is obtained for samples that received the lowest amount of liquid (177 and 178 g), i.e. samples treated according to the CB by means of pouring and capillary absorption during 20 sec, respectively. Despite the higher amount of liquid absorbed, samples on which a CB treatment was applied by means of immersion exhibited a lower weight gain (11 g) compared to the ones on which the GU CB-2 (36 g) and CB-3 (42 g) treatments were applied. This lower weight gain can be attributed to the low concentration of $CaCO_3$ precursors used in the CB treatment, since the total amount of calcium applied is 10 times smaller compared to the GU CB-2 and 3 treatments (personal communication with Calcite Bioconcept).The effect of the concentration of calcium precursors on the weight gain can also be observed in the first series of GU treatments, where the weight gain of the GU CB-1 treatment (7 g) was much lower compared to the CB-2 and CB-3 treatments. For the second series of GU treatments (30 to 120 kg.m^{-3}) the weight increase was proportional to the amount of calcium precursors used, which is in accordance with our previous studies on Euville limestone (De Muynck et al. 2010b).

Table 2. Influence of the application procedure and dosage of $CaCO_3$ precursors on the weight gain, decrease in sorptivity (S) and strength increase of Maastricht limestone samples treated according to the biodeposition procedure developed at GU.

Treatment		Weight gain (g)		S↓	Increase of hardness(%)				
		Wet	Dry		*0-5 mm*	*5-10 mm*	*10-20 mm*	*20-30 mm*	*0-30 mm*
CB	Immersion	305	11	n.d.	29	-18	-17	3	**-8**
	20 sec	178	5	46	90	-11	-17	26	**10**
	Pouring	177	3	48	98	48	2	35	**37**
GU	CB-1	248	7	36	378	69	69	79	**118**
	CB-2	268	36	38	187	55	56	115	**99**
	CB-3	268	42	42	2036	484	362	267	**602**
	30 kg.m^{-3}	254	25	47	612	52	14	41	**126**
	60 kg.m^{-3}	265	45	52	774	0	38	139	**192**
	90 kg.m^{-3}	485	62	52	245	145	361	542	**375**
	120 kg.m^{-3}	503	84	53	296	62	265	1008	**506**

3.2 Protective action

All biodeposition treatments resulted in a decreased rate of water uptake (Fig. 1 and Table 2). Contrary to the CB treatments, the GU ones resulted in a decrease of the total amount of water absorbed. The highest decrease in water absorption rate and amount of water absorbed could be observed for the GU 90 and 120 kg.m^{-3} treatments. The latter is in accordance with our previous studies which have shown that treatments characterized by a higher amount of carbonate precipitation showed a more pronounced decrease in water absorption (De Muynck et al. 2010b). For the GU series, the decrease in water

uptake rate can be mainly attributed to the presence of biogenic crystals (De Muynck et al. 2010b).
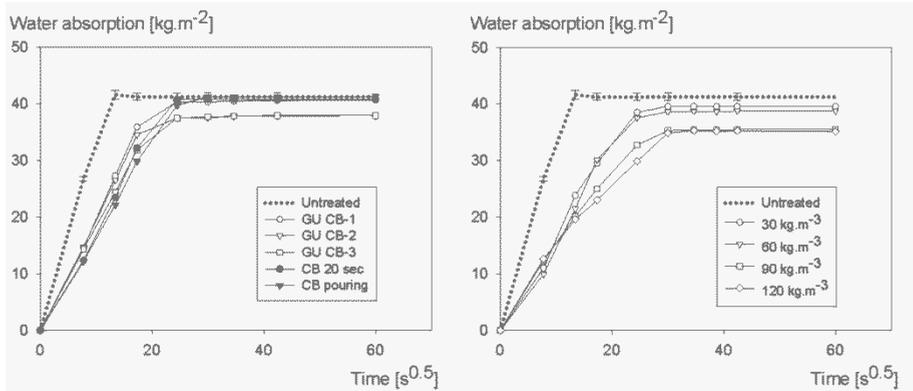

Figure 1. Influence of the application procedure (left) and dosage of $CaCO_3$ precursors (right) on the water absorption of Maastricht limestone samples untreated and treated according to several biodeposition methods.

3.3 Consolidation action

With exception of the CB immersion treatment, all biodeposition treatments resulted in an overall strength increase in the first 30 mm below the surface (Table 2). The intensity and depth of the strengthening effect of the biodeposition treatments depend on the application procedure and the dosage of $CaCO_3$ precursors applied (Table 2 and Fig. 2 and 3). The smallest strength increases were noticed for the CB treatments, for which the strengthening effect was mainly restricted to the upper 1-2 mm. The small decrease in strength observed for the CB immersion treatment might be attributed to interactions between the culture liquid and the carbonate matrix (dissolution and precipitation processes) and small variations between the hardness profiles of different samples.

Overall, the highest strength increase could be observed for the GU CB-3 treatment. Similar to many of the GU treatments, the strength increase of this treatment was most pronounced at the surface, resulting in the formation of a dense outer layer, i.e. a strength increase up to 2000% in the first 5 mm (Table 2 and Fig. 2).

For the second series of GU treatments, an increase of the strength at higher depths (i.e. between 20 and 30 mm) could be observed with increasing amounts of $CaCO_3$ precursors applied (Table 2 and Fig. 3). The strength increase at these depths even exceeded 1000% for the GU 120 $kg.m^{-3}$ treatment. Additionally, the superficial hardness of the 90 and 120 $kg.m^{-3}$ treatments was much lower compared to the 30 and 60 $kg.m^{-3}$ treatments, i.e. 245 and 296% versus 612 and 774%, respectively (Table 2).
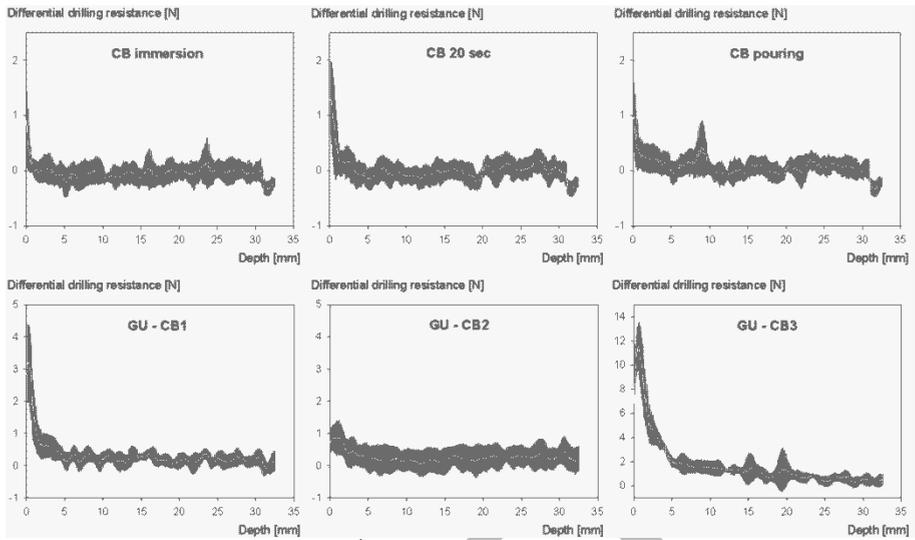
Figure 2. Differential hardness profiles[1] of Maastricht limestone samples treated according to several biodeposition methods. The upper and lower row show the hardness profiles of samples treated respectively with the Calcite Bioconcept method (CB) and the first series of Ghent University treatments (GU), i.e. modified CB treatments. Notice the difference in scale of the y-axes.
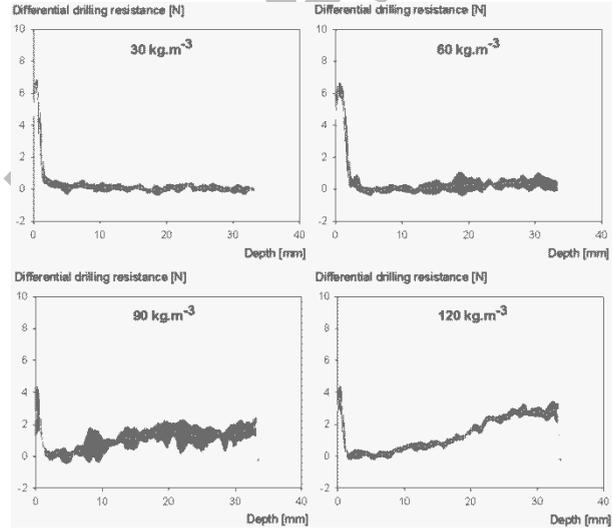
Figure 3. Differential hardness profiles of Maastricht limestone samples treated according to the biodeposition method as a function of the dosage of $CaCO_3$ precursors for the second series of GU treatments. [1]

Ferreira Pinto and Delgado Rodrigues (2012) indicated that the formation of superficial crusts is highly probable in very porous stones. With regard to the GU biodeposition treatments, the occurrence of the strength peaks can be related both to physicochemical and biological processes: (1) upon contact of the $CaCO_3$ 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 micro-organism, its activity is higher in the presence of oxygen. This might explain the higher amount of carbonate precipitation near the surface. The formation of hard superficial layers, however, is unwanted since they are potentially harmful (Ferreira Pinto and Delgado Rodrigues 2012). Therefore, the current procedures for the 30, 60 and 120(4) kg.m^{-3} appear to be less suited for applications 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, strength increases obtained with this treatment (375%) were higher compared to the reported strengths of ethyl silicate based surface treatments (125 – 225%) on Maastricht stone (De Clercq et al. 2007). It should be mentioned that the ethyl silicates were applied two or three times by capillary absorption during 20 seconds, the time between successive applications being 1 week.

4. Conclusions

This study revealed large differences between the performances of two types of biodeposition treatments. Whereas the commercially available Calcite Bioconcept treatment only offered a protective action to the stone, by decreasing the water absorption rate, our biodeposition method additionally offers a consolidation effect. The strengthening effect was very dependent on the application method and the amount of calcium carbonate precursors used. By means of pouring $CaCO_3$ precursors on the surface at a concentration of 90 kg.m^{-3}, 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). This study indicates the high potential of an ecological surface treatment based upon the calcinogenic activity of ureolytic bacteria.

Acknowledgements

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[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.

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