Sporosarcina pasteurii induced carbonate formation for repairing and preventing damage in existing stone masonry structures

Magdalini Theodoridou[1], Oana Adriana Cuzman[2], Piero Tiano[2], Brunella Perito[3], Michael Harbottle[4]

1: Hub for Biotechnology in the Built Environment, School of Engineering, Newcastle University, Newcastle Upon Tyne NE1 7RU, UK

2: Institute for Heritage Science, National Council of Research–ISPC-CNR, Via Madonna del Piano 10, Sesto Fiorentino 50019, Italy

3: Department of Biology, University of Florence, Via Madonna del Piano 6, Sesto Fiorentino 50019, Italy

4: School of Engineering, Cardiff University, Cardiff CF24 3AA, UK

Abstract

Natural stone is one of the most widely used geological construction materials. Although stone masonry structures have the potential to survive over centuries, they may be subject to significant damage and deterioration. Various conservation treatments have been explored for modifying the characteristics of stone, often in the layer closer to the surface. However, treatments may limit the breathability of the material triggering further damage.

This work studies microbially induced carbonate precipitation by bacteria as a breathable alternative for the protection of building stone from deterioration. The mineralogical composition and pore structure of most stone types used in construction are favourable for the growth of bacterial communities, while calcium carbonate as the healing product is highly compatible with the substrate.

A protocol for the application and assessment of biological healing was determined, taking into consideration the specific needs of bulk materials and existing structures. *Sporosarcina pasteurii*, an aerobic, ureolytic bacterium, was applied to two different types of stone: i) a massive calcitic chalk from Cyprus, popularly called Lympia stone, ii) a dolomitic limestone from Italy, popularly known as Pietra d'Angera. The healing effect of the newly formed minerals was determined and compared to reference samples by recording changes in water absorption and drilling resistance, as well as by means of SEM/EDS and confocal microscopy on calcein stained samples. The results demonstrated that *Sporosarcina pasteurii* induced sufficient cementation in the near surface region of the specimens to an extent that could be considered protective, yet compatible with the natural properties of the materials.

Introduction

Geological materials comprise most cultural heritage building materials worldwide. However, they are subject to damage and deterioration [e.g., 1-2]. Surface treatments [3] may reduce the 'breathability' of the material and can be limited to specific stones [4].

Biological methods of strengthening a range of materials can be found in the literature [e.g., 5-9]. However, self-healing concepts have yet to be explored for building stones in construction. This work investigates the potential for providing building stone with a system that could heal damage and prevent further deterioration. Taking the advantage of the stones' bioreceptivity and suitability for biomineralisation, the system developed in this study is based on the use of naturally occurring biological mechanisms, which can induce calcium carbonate precipitation. *Sporosarcina pasteurii,* one of the most commonly used microorganisms in biocementation of particulate media [10-12], has been selected for this study. This paper discusses the potential of biological systems to change the microstructure of porous natural stone in order to increase resilience to deterioration.

Materials and methods

Lympia stone, a massive chalk from Cyprus (>99% calcite), was chosen as the model rock for this study due to its relatively homogeneous microstructure and mineral composition [13]. Angera stone, a dolomitic limestone from Italy [14], was also added the investigation as a substrate of different mineralogical composition (>98% dolomite) compared to the healing products ($CaCO_3$).

Lympia stone specimens (2×2×2 cm) were divided in three groups (i.e., A, B, C), while one more group (D) was added in the study of Angera specimens (Table 1); group A was treated in aseptic conditions with a *Sporosarcina pasteurii* bacteria solution (NCIMB 8221, UK) and cementation medium (per litre of water: 3 g nutrient broth CM0001-Oxoid, UK, 10 g NH_4Cl, 2.12 g $NaHCO_3$, 22.053 g $CaCl_2 \cdot 2H_2O$, 20 g urea), group B was treated only with cementation medium (aseptic), group C only with water and group D with dead bacteria and cementation medium (aseptic). Groups A, B and D were treated two more times with cementation medium, while group C was only supplied with water. The specimens were stored at 30 °C during all healing stages.

Table 1: Groups of specimens

group	Bacteria	Cementation medium	Water
A	+ (living cells)	+	-
B	-	+	-
C	-	-	+
D	+ (dead cells)	+	-

The efficiency of the treatments was assessed through the implementation of various tests and analysis; here we present indicative results/observations from (i) water absorption measurements by capillary action (i.e., mass changes at different time intervals), (ii) micro-drilling resistance measurements (DRMS by SINT Technology) [15], (iii) the use of SEM/EDS (GAIA 3 TESCAN), (iv) the use of confocal microscopy (LEICA TCS SP5) on calcein stained specimens, (v) the use of colourimetry (MINOLTA CM 700D) and the determination of colour changes according to the CIELAB 1976 system.

Results and discussion

Indicative results of the capillary absorption tests are presented in Table 2, where a decrease of 5.68% in capillary absorption was observed for the Lympia specimens treated with *S. pasteurii* (group A), while no significant change is observed in the cases of group B and C. This result proves the potential of the treatment to change the microstructure of the specimens in a promising way which can prohibit deterioration related to water transport.

Table 2: Indicative decrease in capillary absorption (average values) after treatment on one of the stone types (Lympia stone). Negative values correspond to increase in capillary absorption. All measurements took place after 40 mins (ΔCA40 %) of partial immersion in deionised water.

group	Average ΔCA40 (%)	Standard deviation
	Lympia stone	
A	5.68	0.63
B	-0.14	0.22
C	-1.00	0.18

The drilling profiles of Lympia specimens showed a clear peak of higher resistance to drilling in the case of group A (data not shown). This agrees well with the capillary absorption results shown in Table 2 and it can be attributed to the effective consolidation of the material in the area close to the treated surface. No similar peak was recorded for the specimens of group B and C.

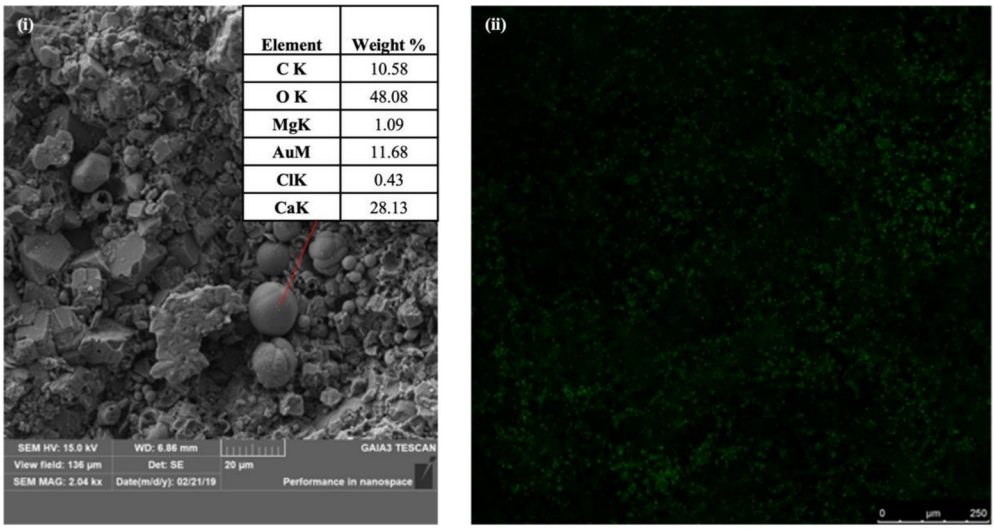

Figure 1: (i) SEM/EDS and (ii) confocal microscopy evidence of new $CaCO_3$ formation on a stone specimen (Angera type) treated with bacteria and nutrients (group A).

The determination of the changes in the chromatic components is reported in Table 3. The overall colour changes (ΔE^*_{ab}) of the specimens treated with bacteria and/or cementation medium compared to the relevant group C values, are equal to less than 3, which is the value under which the average human eye cannot detect colour differences (Tiano et al. 2006). Therefore, the biological treatments performed in this study may be considered that they do not cause any aesthetical change on the surface of the treated materials.

Table 3: Chromatic coordinates of the surface of all specimens after treatment. Δ values for groups A, B and D were calculated in comparison with the relevant groups C.

group	ΔL^*	Δa^*	Δb^*	ΔE^*_{ab}
		Lympia stone		
A	-0.73	0.26	1.93	2.07
B	0.08	0.79	1.07	1.33
		Angera stone		
A	-0.31	0.11	0.54	0.63
B	0.24	0.08	-0.05	0.25
D	0.11	-0.13	-1.01	1.02

Conclusion

This work presents the application and evaluation of biological treatments in natural building stone using the bacteria strain *Sporosarcina pasteurii*. *S. pasteurii* induced significant differences in the microstructure of laboratory stone specimens, which is proved by the decrease in capillary absorption, increase in drilling resistance, microscopic observations and elemental analyses. Sufficient supply of nutrients and the use of living cells was found to be the treatment that better promoted healing in both stone types. Dead cells with nutrients also

led to new formation of $CaCO_3$, however further research and optimisation of the treatment is needed. None of the biological treatments performed in this study was found to cause colour alterations that can be detected by the average human eye.

Acknowledgment

This project has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Sklodowska-Curie grant agreement No 745891, the Engineering and Physical Sciences Research Council (EPSRC), grant number EP/P02081X/1 and from Research England and The Hub for Biotechnology in the Built Environment (E3 Fund), Newcastle University. The help of Dr Giada Magni (IFAC-CNR Florence) is acknowledged for the use of confocal microscopy.

References

[1] Amoroso GG, Fassina V. 1983. *Stone decay and conservation*. Elsevier, Amsterdam 453p.
[2] Theodoridou M, Török Á. 2019. In situ investigation of stone heritage sites for conservation purposes: a case study of the Székesfehérvár Ruin Garden in Hungary. *Progress Earth Plan Sci* 6:15.
[3] Pinna D, Salvadori B, Porcinai S. 2011. Evaluation of the application conditions of artificial protection treatments on salt-laden limestones and marble. *Const Build Mater* 25: 2723-2732.
[4] Maravelaki-Kalaitzaki P, Kallithrakas-Kontos N, Agioutantis Z, Maurigiannakis S, Korakaki D. 2008. A comparative study of porous limestones treated with silicon-based strengthening agents. *Prog org Coat* 62:49-60.
[5] Perito B, Marvasi M, Barabesi C, Mastromei G, Bracci S, Vendrell M, Tiano P. 2014. A *Bacillus subtilis* cell fraction (BCF) inducing calcium carbonate precipitation: Biotechnological perspectives for monumental stone reinforcement. *J Cult Herit* 15: 345-351.
[6] Jroundi F, Gómez-Suaga P, Jimenez-Lopez C, González-Muñoz MT, Fernandez-Vivas MA. 2012. Stone-isolated carbonatogenic bacteria as inoculants in bioconsolidation treatments for historical limestone. *Sci Tot Envir* 425:89-98.
Tiano P, Cantisani E, Sutherland I, Paget JM. 2006. Biomediated reinforcement of weathered calcareous stones. *J Cult Herit* 7: 49-55.
[7] De Muynck W, De Belie N, Verstraete W. 2010. Microbial carbonate precipitation in construction materials: A review. *Ecol Eng* 36:118-136.
[8] Le Métayer-Levrela G, Castaniera G, Orial J, FLoubière J, Perthuisot P. 1999. Applications of bacterial carbonatogenesis to the protection and regeneration of limestones in buildings and historic patrimony. *Sediment Geol* 1 4:25-34.
[9[Van Tittelboom K, De Belie N, De Muynck W. 2010. Use of bacteria to repair cracks in concrete. *Cement Concrete Res* 40: 157-166.
[10] Botusharova S, Gardner D, Harbottle M. 2020. Augmenting Microbially Induced Carbonate Precipitation of Soil with the Capability to Self-Heal. *J Geotech Geoenviron Eng* 146(4): 04020010.
[11] Harbottle, M, Lam MT, Botusharova SP, Gardner DR. 2014. Self-healing soil: Biomimetic engineering of geotechnical structures to respond to damage. In: *Proc. 7th Int. Congress on Environmental Geotechnics,* edited by A Bouazza, S Yuen and B Brown, Melbourne, Australia: Engineers Australia, 1121–1128.
[12] Montoya BM, DeJong JT. 2013. Healing of biologically induced cemented sands. *Geotech Lett* 3 (3): 147–151.
Botusharova SP. 2017. *Self-healing geotechnical structures via microbial action*. PhD thesis, School of Engineering, Cardiff University.
[13] Modestou S, Theodoridou M, Fournari R, Ioannou I. 2016. Physico-mechanical properties and durability performance of natural building and decorative carbonate stones from Cyprus. In: *Sustainable Use of Traditional Geomaterials in Construction Practice*, edited by R Přikryl, Á Török, M Gómez-Heras, KnMiskovsky and & M Theodoridou, London: Geological Society, Special Publications, 416, pp. 145-163.
[14] Alessandrini G. et al. La Ca' Granda di Milano. L'intervento conservativo sul cortile richiniano. Milano, Silvana Editoriale, 1993.
[15] Theodoridou M, Dagrain F, Ioannou I. 2015. Micro-destructive cutting techniques for the characterization of natural limestone. *Int J Rock Mech Min Scie* 76: 98-103.
[16] Tiano P, Cantisani E, Sutherland I, Paget JM. 2006. Biomediated reinforcement of weathered calcareous stones. *J Cult Herit* 7: 49-55.