**X-Ray Tomography evaluation of Microbially Induced Calcite Precipitation (MICP) in mortar cubes**

Diana Tamayo-Figueroa1\*, Henry Omar-Meneses2, Pedro Brandão3

*1 Doctorado Biotecnología, Instituto de Biotecnología (IBUN), Facultad de Ciencias, Universidad Nacional de Colombia, Bogotá, Colombia; 2 Clínica para Pequeños Animales, Departamento de Salud Animal, Facultad de Medicina, Veterinaria y Zootectnia , Universidad Nacional de Colombia, Bogotá, Colombia; 3 Grupo de Estudios para la Remediación y Mitigación de Impactos Negativos al Ambiente (G.E.R.M.I.N.A.), Laboratorio de Microbiología Ambiental y Aplicada, Departamento de Química, Facultad de Ciencias, Universidad Nacional de Colombia, Bogotá, Colombia.*

*\*Corresponding author:* [*dptamayof@unal.edu.co*](mailto:dptamayof@unal.edu.co)

**Highlights**

* A non-destructive methodology to follow crack repair by MICP is reported.
* MICP treatment showed a higher CaCO3 filling effect when compared to control.
* MICP activity allowed 18-27% of CaCO3 filling of mortar cube holes after 9 weeks.

**1. Introduction**

Cement based materials are widely used for construction but their durability is affected by cracking, a common phenomenon in this type of materials [1]. The use of microorganisms that induce calcite (calcium carbonate) precipitation (MICP) has been proposed as an alternative crack repair technology [2]. In this process microbial metabolism increases environmental alkalinity that favours CaCO3 precipitation [3]. This phenomenon is an extremely strain-specific (bio)chemical reaction and depends on urease diversity, ion strength, cell density, and medium pH [4]. Current methodologies used to track crack repairs do not provide a clear picture of the system behaviour while the repair occurs, especially in internal cracks, and usually it is necessary to destroy test specimens. This work reports the use of X-ray tomography to evaluate calcium carbonate filling of different size holes in mortar cubes, due to crystals precipitation by *Arthrobacter crystallopoietes* KNUC403, a bacteria previously isolated from a concrete structure [5].

**2. Methods**

*Mortar cubes*: For each treatment, a standard mortar mixture was prepared. A ratio of 1:2.75 (cement:sand) was used maintaining a water:cement ratio of 0.52. Curing time was 7 d. Four holes with different diameter sizes (in cm: 0.476, 0.635, 0.794, and 0.952) were made in the mortar cubes. Three replicas were made for each hole size.

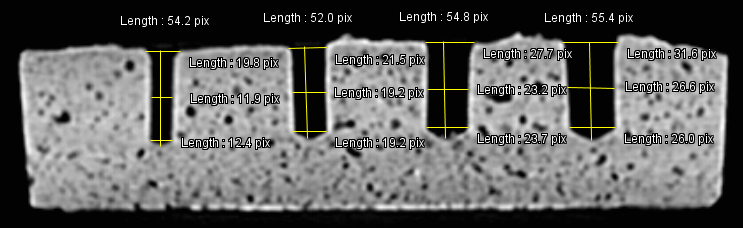
*Biological treatment of mortar cubes:* Two treatments with (B) or without cells (C) were carried out to assess CaCO3 production by *A. crystallopoietes* KNUC403 using X-ray tomography. Treatment B consisted in the addition of bacterial biomass + Urea-Ca(NO3)2 media to each hole, while control treatment C consisted in the addition of Urea-Ca(NO3)2 only to each hole. The addition of culture medium, with or without microorganisms, to the mortar cubes was performed every seven days. The mortar cubes were incubated at 30 °C.

*X-ray tomography analysis*: Mortar cubes holes depth variation was evaluated regularly in a Philips Helical tomograph (Tmoscan AV) by simple cuts with 2 mm thickness, a tube rotation of 2 s, a kilovoltage at 120 Kv, 140 MA, a window level of 3500 and a window width of 1100.

**3. Results and discussion**

X-ray tomography allowed to monitor the difference in the mortar cubes hole filling processes between treatments, a situation related to the production of CaCO3 by MICP. This technique allowed to measure the change in the holes depth without destroying the sample or altering its mechanical properties (Figure 1), which could be useful to track the repair of material failures. After 9 weeks, the calcium carbonate filling percentage of the different hole sizes evaluated showed differences between the two treatments (Table 1). It is evident that after the experimental time period treatment B showed filling values between 18 and 27% compared to control treatment C with values lower than 8%, reaffirming the feasibility of using X-ray tomography as a strategy to monitor and control crack repair in cement-based materials. The fact that the filling percentage for the different hole sizes is similar for treatment B suggests that microbial behaviour has been affected by the available oxygen, a factor that may influence the amount of precipitated CaCO3. This should be addressed in future studies. Mechanical and permeability tests will be performed to the mortar cubes of both treatments when a filling percentage close to 50% is obtained.

**Figure 1.** Image obtained by X-ray tomography showing the measurements of width and depth of different holes in mortar cube. Hole diameter sizes: 1 - 0.476 cm; 2 - 0.635 cm; 3 - 0.794 cm; 4 - 0.952 cm.

**Table 1.** Calcium carbonate filling percentage of four different hole sizes in mortar cubes without (C) or with (B) bacterial (*A. crystallopoietes* KNUC403) biomass treatment, after 9 weeks of incubation. Hole diameter sizes: 1 - 0.476 cm; 2 - 0.635 cm; 3 - 0.794 cm; 4 - 0.952 cm.

|  |  |  |
| --- | --- | --- |
| **Hole size** | **Treatment C**  **[Urea-Ca(NO3)2 media only]** | **Treatment B**  **[*A. crystallopoietes KNUC403* + Urea-Ca(NO3)2 media]** |
| 1  **1**  **2**  **3**  **4** | 2,31% | 19,95% |
| 2 | 3,73% | 18,29% |
| 3 | 2,33% | 27,37% |
| 4 | 7,91% | 19,36% |

**4. Conclusions**

X-ray tomography showed a positive effect of using *A. crystallopoietes* KNUC403 on the CaCO3 filling of holes in mortar cubes as compared to control without cells. The technique allows to track the filling of holes in cement-based materials without the need to alter the mechanical properties of the material. This permits to follow-up the repair of superficial and internal cracks on this type of materials using MICP.

**References**

1. American Concrete Institute. Control of cracking in concrete: state of the art. Technical document 2006.
2. W. De Muynck, D. Debrouwer, N. De Belie, W. Verstraete, Cement Concrete Res. 38 (2008) 1005–1014.
3. W. De Muynck, N. De Belie, W. Verstraete, Ecol. Eng. 36 (2010) 118–136.
4. F. Hammes, N. Boon, J. de Villiers, W. Verstraete, S.D. Siciliano, Appl. Environ. Microbiol. 69 (2003) 4901–4909.
5. S.J. Park, Y.M. Park, W.Y. Chun, W.J. Kim, S.Y. Ghim, J. Microbiol. Biotechnol. 20 (2010) 782–788.