



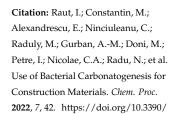
Abstract

## Use of Bacterial Carbonatogenesis for Construction Materials †

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Concrete is the most used construction material, but its industrial production from lime consumes 2–3% of the global energy demand, generating 0.73–0.99 t CO<sub>2</sub>/t of cement produced [1]. At the same time, concrete structures are susceptible to physical, chemical and biological factors, which affect their mechanical and durability properties. A viable alternative to reduce cost and environmental impact is considered as the incorporation in the cement matrix of bacteria capable to precipitate calcium carbonate [2,3]. Microbially induced calcium carbonate precipitation (MICP) is possible through the following metabolic pathways: urea hydrolysis, ammonification of amino acids, denitrification, sulfate reduction and photosynthesis. Among them, urea hydrolytic metabolism is the most studied [4]. The aim of this study was to evaluate the capability of several *Bacillus* strains to precipitate calcium carbonate. Each bacterial strain was cultivated on a urea-CaCl<sub>2</sub> medium of different concentrations and CaCO<sub>3</sub> precipitation was evaluated.

The experimental study was carried out with bacterial strains from the Microbial Collection of ICECHIM, as follows: *Bacillus amyloliquefaciens*, *B. licheniformis* and *B. subtilis*. The bacteria were cultured on media with different compositions: (i) tryptic soy broth (TSB); (ii) nutrient broth (NB), with different concentrations of urea and Ca<sup>+2</sup>. The cultures were incubated and then centrifuged, with the obtained pellets being analyzed with different techniques (FTIR-ATR, SEM and TGA).

SEM investigations presented several morphological aspects of the pellets. The bacterial pellets from cultures were characterized by TGA analysis. The results indicated that the pellets from the media with calcium and urea featured two main weight loss steps, 17–24%, (138–145 °C) and 19–20% (343–350 °C), respectively. The residue at 700 °C corresponded to a significant weight loss of 45–50%. The behavior of the control samples from the medium, without urea and calcium, was quite different: loss of 65–68% (248–281 °C) and 27–31% residue. This aspect indicated that on the medium with urea and Ca<sup>+2</sup> ions, bacterial strains were able to precipitate calcium carbonate. FTIR spectra of pellets presented the following distinguishable regions: 3000–2800 cm<sup>-1</sup> for cell membrane fatty acids and carbohydrates; 1700–1500 cm<sup>-1</sup> for amide I; band around 1401–1416 cm<sup>-1</sup> assigned to CO<sub>2</sub>; 1200–900 cm<sup>-1</sup> polysaccharides or carbohydrates of microbial cell walls.

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The corroboration of the present results with those obtained previously, regarding the secretion of urease [5], indicated *Bacillus subtilis* to be a microorganism with great potential for application in cementitious materials. Further investigation regarding the immersion of bacterial cells in cement will be carried out.

**Author Contributions:** L.J., I.R. and M.D. conceived and designed the experiments; M.C., I.R., A.-M.G., I.P., E.A., C.N., G.V., M.R. and C.A.N. performed the experiments; L.J., M.D., N.R. and A.-M.G. analyzed the data; L.J., I.R., M.C. and M.D. wrote the paper; L.J., A.-M.G. and N.R. reviewed and edited. All authors have read and agreed to the published version of the manuscript.

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