An autonomous system to bio-bricks production by biomineralization activity

Um sistema autônomo para produção de biotijolos pela atividade de biomineralização

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ABSTRACT
Urbanization causes a significant increase in the consumption of building materials, with negative environmental effects. Thus, it is a priority to develop alternative applications that promote infrastructure progress and reduction in the environmental impacts derived from civil construction activities. One example is microbial-induced calcium carbonate precipitation (MICP), a process by which ureolytic bacteria, such as *Sporosarcina pasteurii*, induce calcium carbonate (CaCO₃) precipitation by urea hydrolysis. The shells are a by-product that has great potential for various uses and applications in industry. In the search for a more sustainable process, this study seeks to evaluate CaCO₃ - shell powder as an aggregate to replace Portland cement with bio cement synthesized by *S. pasteurii*. Based on the autonomous mini-controlled irrigation system, was possible to maintain *S. pasteurii* activity that aggregates the particles via a biomineralization process.

Keywords: *Sporosarcina pasteurii*, CaCO₃, precipitation by urea hydrolysis, anomalocardia flexuosa.

RESUMO
A urbanização provoca um aumento significativo no consumo de materiais de construção, com efeitos ambientais negativos. Assim, é prioritário desenvolver aplicações alternativas que promovam o progresso da infraestrutura e a redução dos impactos ambientais decorrentes das atividades de construção civil. Um exemplo é a precipitação de carbonato de cálcio induzida por organismos microbianos (MICP), um processo pelo qual bactérias ureolíticas, como *Sporosarcina pasteurii*, induzem a precipitação de carbonato de cálcio (CaCO₃) por hidrólise de uréia. As conchas de marisco são um subproduto que apresenta grande potencial para diversos usos e aplicações na indústria. Na busca por um processo mais sustentável, este estudo busca avaliar o CaCO₃ - pó de concha de marisco como agregado para substituição do cimento Portland pelo biocimento sintetizado por *S.
1 INTRODUCTION

The growth of urbanization has caused a significant increase in the consumption of building materials, with negative environmental effects. The civil construction sector consumes up to 40% of global energy and emits up to 20% of greenhouse gases (ACHAL et al., 2015; REIS et al., 2017). The production of conventional kiln brick to construction material is one of the most energy-intensive processes that contribute to anthropogenic greenhouse gas emissions. The manufacturing of conventional bricks requires a high temperature with a carbon emission of nearly 800 million tonnes per year, which results in pollution of the free environment (KUMARAPPAN, et al., 2018).

It is urgent to develop alternative applications that promote both infrastructure progress and a reduction in the environmental impacts derived from civil construction. An environmentally friendly biobased Biobrick material is a special type of brick and it is an innovative construction material (Henze & Randall, 2018). The elaboration of biobricks is based on natural processes that perform some microorganisms in the formation of aggregates or cementation of soil particles by biochemical action.

An example is microbial-induced calcium carbonate precipitation (MICP), a process by which ureolytic bacteria, such as Sporosarcina pasteurii, induce calcium carbonate (CaCO₃) precipitation by urea hydrolysis (Dhami et al., 2012; Raut et al., 2014). S. pasteurii is a common soil bacterium that is non-pathogenic (Anbu et al., 2016). This bacterium can fuse loose grains of sand, soil, fibers, basalt, glass beads, recycled glass foam, fly ash composite, soil, and small stones loose, by inducing the formation of cement. S. pasteurii degraded the urea to form carbonate ions which then combined with the calcium ions produce calcium carbonate. This calcium carbonate glues loose sand particles together in the shape of a brick (Henze & Randall, 2018). Because of these advantages, biobricks has the potential to play a significant role in the next generation of sustainable construction materials.

The bivalve Anomalocardia flexuosa, is an important fishing resource that is extracted by coastal communities in the estuary of the Paraíba River in Brazil. Of the
volumes of shellfish extracted, only 10% of the total biomass is used (Santos et al., 2013). The remainder shells are discarded in mangrove environments as a landfill or on the natural banks of the same river. Thus, about 3 tons of shells are deposited daily only by an extractive local community, causing silting up of the river channel, decomposition of organic matter, and eutrophication in the deposition area (Silva et al., 2013).

The shells are constituted of 93% of calcium carbonate - $\text{CaCO}_3$ (Lee et al., 2008), a by-product that has great potential for several uses and applications in the industry (Bezerra et al, 2011). Application tests of $\text{CaCO}_3$ as an aggregate for the production pavements after being crushed were promissory for application in civil construction (Santos et al, 2017). However, in the search for a more sustainable process, this study seeks to evaluate shell powder as an aggregate to replace Portland cement with bio cement synthesized by the $S. \text{ pasteurii}$. Thus, the construction of biobricks represents a new production process that might be considered more sustainable than current methods as it does not utilize energy-intensive production processes such as kiln firing or unsustainable materials such as cement. This study aims to test the viability of using shell powder as an aggregate by bio cement synthesized by the $S. \text{ pasteurii}$.

2 MATERIALS AND METHODS

To induce the biomineralization process, the shells were pulverized and enclosed in forms made by a 3D printer. The forms were exposed to a nutrient solution, and inoculum, and fed by an autonomous recirculation system to induce the biomineralization process.

3 HYDROLYSIS OF UREA

To induce bio cement production the inoculum used was $\text{Sporosarcina pasteurii}$ (ATCC 11859), provided by Andre Tosello Technological Research Foundation. The culture medium consisted of 20g of yeast extract, 10g of sulphate of ammonium, and 0.13 M of prepared Tris (pH = 9.0), for one litter of solution, according to Choi et al. (2017). Subsequently, the medium was autoclaved at 121°C for 15 minutes. The inoculum was kept in an electric oven with a shaker (130 rpm) at 30 °C for two days. Cell growth was monitored by measuring the optical density (OD) of the broth at 600nm. When ready to use, the UPB solution had the OD600 range of 1.0-1.6.
4 PREPARATION OF CALCIUM ION SOLUTION

The calcium ion solution (Ca\(^{2+}\)) was produced by diluting 100 g of CaCO\(_3\) in 800 ml of acetic acid (C\(_2\)H\(_4\)O\(_2\)), according to Chou et al. (2017). After five days at room temperature (\(\sim 27^\circ\text{C}\)) the Ca\(^{2+}\) ion concentration and pH of the solution were measured. To adjust the ion concentration to 0.3 M, distilled water was added and the pH of the solution to 7.0-7.5 by adding pellets of sodium hydroxide (NaOH). The solution was centrifuged at 4,000 rpm for 20 min to obtain the supernatant, which constituted the final calcium ion solution for the cementation tests.

Figure 1. biomineralization autonomous system
5 DEVELOPMENT OF THE MICP-BASED BIOCEMENTATION PROCESS IN FREE SOLUTION TESTS

After cultivation for two days with a density of OD600 =0.8-1.2, the UPB culture broth (300 ml) was mixed with 0.3 M urea (300 ml) in proportions of 1:1 (v/v). The pH 7.0–7.5 solution was stored in a beaker for one day and then added to the calcium solution (300 ml). This final solution was stored in a flat-bottomed flask.
6 SPRINKLER SYSTEM FOR UREOLYTIC BACTERIA

The bacterial solution sprinkling system consisted of a laminar flow-NFT technique, controlled by an Arduino microcontroller, which, watered mini-brick molds with CaCO₃ powder from crushed shellfish shells (Fig. 1). A relay and display were connected to the humidity sensor and the mini recirculation pump installed on the outer surface of the incubation chamber. The sprinkler system was activated according to a humidity sensor when the aggregate reaches below 70%, which through a relay activates a minipump that collects and sprinkles the solution drained by the system itself, in a reuse cycle (Fig. 1).

The bacteria, urea, and calcium solution were placed in a flat-bottomed flask, on the stirring surface of the oven at a constant temperature of 30°C. In the upper part of the greenhouse, an aluminium table system was placed, containing the block's CaCO₃ aggregate and covered with a sponge to prevent erosion (Fig. 1). The sprinkler system worked to keep the bacteria active in urease synthesis and was activated with the reduction of humidity in the internal environment of the mini-block molds (Fig. 2).

Figure 2. Mini biobloks molds 3D print to implement irrigation system for biomineralization

7 RESULTS AND DISCUSSION

Based on the autonomous mini-controlled system of irrigation was possible to maintain the bacterial activity that aggregates the particles. S. pasteurii irrigation system was functional and evidence of the ureolytic activity of the bacteria for the biomineralization process. After six days of irrigation, the adhesion process of the shellfish powder aggregates was observed. Even observing the adhesion of the particles, the bio bricks did not show high resistance to pressure and disassembly of the forms (Fig. 2). It is possible the solution did not penetrate the aggregate in uniform cementation (Dosier, 2016).
The size of the calcium carbonate particle influences the cementation and can only precipitate on and around sand grains that are in contact with bacteria (Mukhari, 2018). The arrangement of the precipitate in the MICP process can indicate the strength of a solid produced. The pore volume between grains is critical to such a process. If the pore volumes are small, less calcium carbonate will precipitate, and if the pores are too small, it may inhibit the movement of bacteria which promotes cementation (DeJong, Fritzges & Nüsslein, 2006). Conversely, if pore volumes are too large then the compressive strength is compromised (Chen, Wu & Zhou, 2013).

It was also noticeable that for production on a larger scale, a laboratory equipped with a system of agitation and control of the external temperature to the culture system is necessary. Reagents and energy consumption to maintain the culture medium recirculation system was also factors that limit the production of bio bricks. Another aspect is related to the high cost of bacterial cultivation due to the conventional use of laboratory-grade culture media for MICP studies. Some alternatives proposed for the induction of urease synthesis by S. pasteurii, even though it is the more sustainable alternative given the reduction in greenhouse gas emissions caused by conventional processes, still demand energy consumption and reagents that limit its large-scale application.

Studies based on obtaining urea from human urine bring the possibility of reducing production costs and, through the process, obtaining nitrogenous compounds and phosphates as part of fertilizers (Lambert, 2019).

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