

## Article

# Cascade System for Biomineralization in Cement: Project, Construction and Operationalization to Enhance Building Energy Efficiency

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**Abstract:** Anthropogenic and natural actions cause internal and external fractures in concrete. To recover these structures, bio-concretes have been developed with bacteria of the genus *Bacillus*. These microorganisms consume calcium lactate, synthesize calcium carbonate and biomineralize  $\text{CaCO}_3$  crystals within the structures of concrete. The aim of the present study was to construct equipment, denominated “Cascade System for Biomineralization in Cement” (CSBC), to determine the limiting velocity of the biomineralization of  $\text{CaCO}_3$ . The construction of the equipment took into consideration chemical and biochemical phenomena responsible for biomineralization. Parts made with 3D printing and a circuit with Arduino UNO R3 board were used in the assembly of the system. The prototype proved to be stable and can be considered a promising tool for future application in research of the regeneration of reinforced concrete in a practical, fast and economical way, especially to the energy sector.

**Keywords:** *Bacillus*; calcium carbonate; limiting velocity; 3D printing; Arduino UNO R3

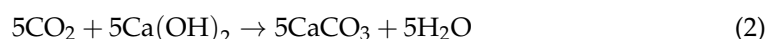
## 1. Introduction

Cement is the most widely used material throughout the world due to the continual need for construction to meet the demands of globalization. When water is added to cement, a chemical reaction occurs, forming hydrated cement, which is combined with fine and coarse aggregate to form concrete [1]. Rain, wind, solar radiation and lixiviation cause considerable surface and structural wear in concrete. Internal and external fractures can alter macroscopic aspects, such as tensile, compressive and flexion strength, as well as the porosity, of concrete. Such harm can render a building unviable, mainly due to safety reasons [2].

The recovery of these structures normally occurs by means of so-called healing agents, which internally regenerate the worn pores. Such healing agents may be chemical or biochemical. Chemical agents include the compounds magnesium oxide, sodium silicate and methyl methacrylate. Among biochemical agents, bacteria of the genus *Bacillus* predominate, but some species of yeast are also used. The main advantage of a biochemical agent is the capacity of bacteria and yeasts to reproduce, with biotechnological healing potential [3].

The bioprocess by which bacteria are capable of transforming an organic compound into an inorganic one is called biomineralization. This phenomenon is common as a defense mechanism found in crustaceans, such as the production of an exoskeleton or the transformation of a parasite into a pearl [4].

Calcium carbonate production is one of the aims of biomineralization for concrete healing, which can occur through the following three main metabolic pathways: (I) ureolytic, (II) denitrification and (III) calcium lactate. The first two pathways have the disadvantage of producing nitrogenated compounds as byproducts, which acidify concrete and can damage civil engineering structures [5]. Therefore, the lactate pathway is the most suitable, as it does not produce nitrogen compounds, as shown in the equations:



The use of chemical and biochemical systems (bio-mineralizers) in the self-regeneration of concrete has been described. There are pressurized liquid or gaseous systems with curing agents in the concrete, systems with gelling agents to be mixed with curing bioagents and systems with the use of surfactants to favor percolation in the concrete. As regards lifetime, bacteria can be inactivated for 50 years until the need for self-regeneration of concrete through microencapsulation [6]. However, none of these systems is intended to determine the rate-limiting process, between crystallization and chemical or biochemical reaction, which occurs simultaneously. Therefore, a prototype that allows identification of the rate which is the limiting rate is essential.

A component of cement that has antibacterial activity is iron ore, but as cement has different kinds of components the mixture can either accelerate bacterial viability (synergistic effect) or can reduce bacterial viability (antagonistic effect). Probably the resulting effect of the mixture on cement compounds is synergistic, as bacteria can indeed produce calcium carbonate. Bacterial viability was already recorded with iron oxide solutions, but was not assessed with cement mixtures [7,8].

In addition to the production of  $\text{CaCO}_3$ , the second aim is the allocation of this inorganic compound into the concrete pores. The phenomenon responsible for sealing fractures is crystallization, which can be thought of as the inverse of solubilization. Hence, the determination of the solubility curve is essential so that the thermodynamic conditions necessary for the formation of crystals can be achieved [9].

Solubility curves need to adjust the thermodynamic conditions of crystallization to those of bacterial biosynthesis. In other words, a study needs to be conducted within a similar temperature range for both the biochemical reaction and crystallization, bearing in mind that very high temperatures can inactivate, or even kill, the bacteria involved in the biochemical reaction [10].

According to Equation (1), the presence of oxygen, when aeration is used, accelerates the biochemical reaction and favors the evaporation of water through forced convection. The higher the water vapor content in the air, the greater the relative humidity, which is an output variable in both Equations (1) and (2) [11].

In the operationalization of any process, meters, supports, equipment and other instruments need to be integrated, or even created. Three-dimensional (3D) printing is a tool that has been gaining ground due to its broad applicability. In simple terms, practically any object that can be constructed digitally using computational software can also be printed three-dimensionally (within size constraints). Advantages, such as different types of materials (plastics, glass and even chocolate) and printing technology (filament, resin, etc.) have made 3D printing a valuable tool in scientific research [12].

For a prototype, a greater degree of automation in the operations translates to a lower occurrence of human errors and also enables the determination of necessary adjustments. Therefore, a control board is another tool that accelerates the development of research. For instance, the Arduino UNO R3 microcontroller is a low-cost board for hardware and

sensors, which, when combined with 3D printing, enables the construction of effective, low-cost prototypes for the analysis of processes [13,14].

Cracks in concrete form empty spaces in buildings, possibly causing less heat conduction, i.e., greater thermal resistance. The more cracks and the larger their distribution, the higher the temperature gradient, thus affecting heat distribution. Air, despite being a good thermal insulator (low heat conductor), under uncontrolled conditions can create hotspots in factories, making temperature conditions unhealthy for employees and reducing the energy efficiency of the structure. On the other hand, in bridges these temperature differences can cause thermal dilation in the structure, which makes their periodic maintenance necessary [15].

Therefore, the aim of the present study was to construct a prototype called Cascade System for Biomineralization in Cement, dedicated to improving the energy efficiency of buildings. An Arduino UNO R3 microcontroller and 3D printing were used to ensure the efficiency and low cost of the process.

## 2. Materials and Methods

### 2.1. Determination of Calcite Solubility Curve

The behavior of the solubility curve of calcium carbonate was studied using 60 pairs of solubility and temperature data for calcite (99%) in demineralized water, which is the most stable polymorph of  $\text{CaCO}_3$  [16]. The selected data were between temperatures of 5 and 35 °C, considering that the molar mass of  $\text{CaCO}_3$  is 100 mg·mmol<sup>−1</sup>. The aim of the curve was to predict the maximum saturation limit at each temperature in order to work under conditions from the non-crystallization of water (above freezing) to the upper limit of the working temperature (35 °C) for bacteria of the genus *Bacillus*. No microorganism was used during data selection, and the only microbial parameter used was the optimum temperature for microorganism growth.

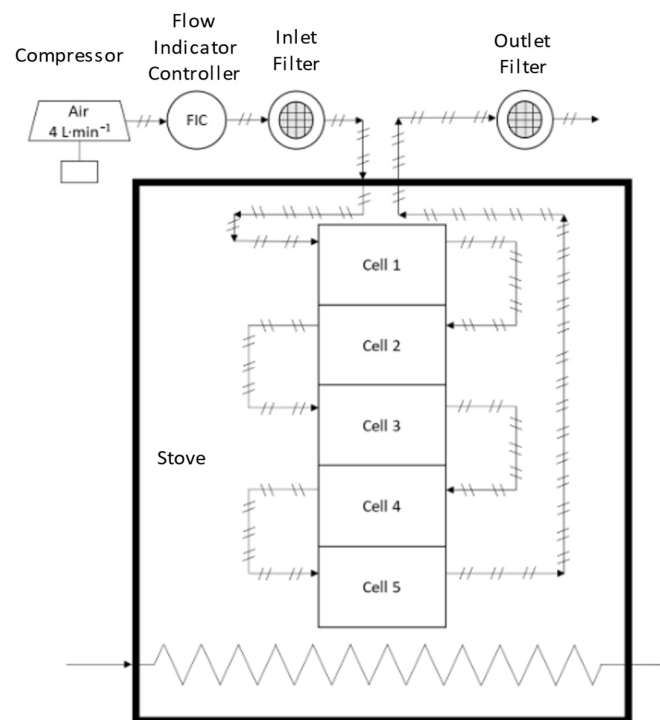
### 2.2. Cascade System for Biomineralization in Cement (CSBC)

The data from the calcite solubility curve and optimal temperature of bacteria of the genus *Bacillus* enabled the construction of a system to investigate microbial production at 35 °C and the crystallization of calcium carbonate separately. The Cascade System for Biomineralization in Cement (CSBC) was based on the calcium lactate regeneration pathway (Equations (1) and (2)), stemming mainly from the need to regenerate concrete without inducing corrosion in reinforced concrete. The construction of this system was divided into two steps: (I) process engineering step and (II) electrical and programming step.

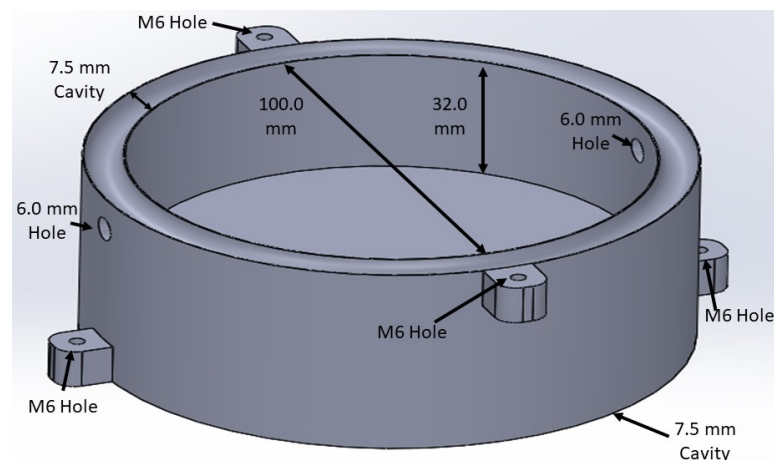
#### 2.2.1. Process Engineering Step

Figure 1 displays the flowchart of CSBC engineering, which involved the following items:

- RS-A98 compressor (RS Electrical) with four outputs for the injection of air into the environment at a total flow rate of 12 L·min<sup>−1</sup>;
- LZT M-6 air rotameter with flow measurement between 0.5 and 4.0 L·min<sup>−1</sup> (Flow Indicator and Controller [FIC]);
- Two Millipore Millex-GV hydrophilic filters with 0.22 µm pore diameter;
- Tecnal TE-392/93L bacteriological heat chamber with temperature measurement between 35.0 and 60.0 °C and error of 0.1 °C;
- Five reaction cells (Figure 2) made with glycol-modified polyethylene terephthalate (PETG) by a Furling Yan printer (layer height: 0.2 mm), unit volume (each reactor) of 250 mL and coated with IBEX crystal polyester resin 67; and
- Five rubber gaskets for reactors.



**Figure 1.** Flow diagram of process engineering of the Cascade System for Biomineralization in Cement.



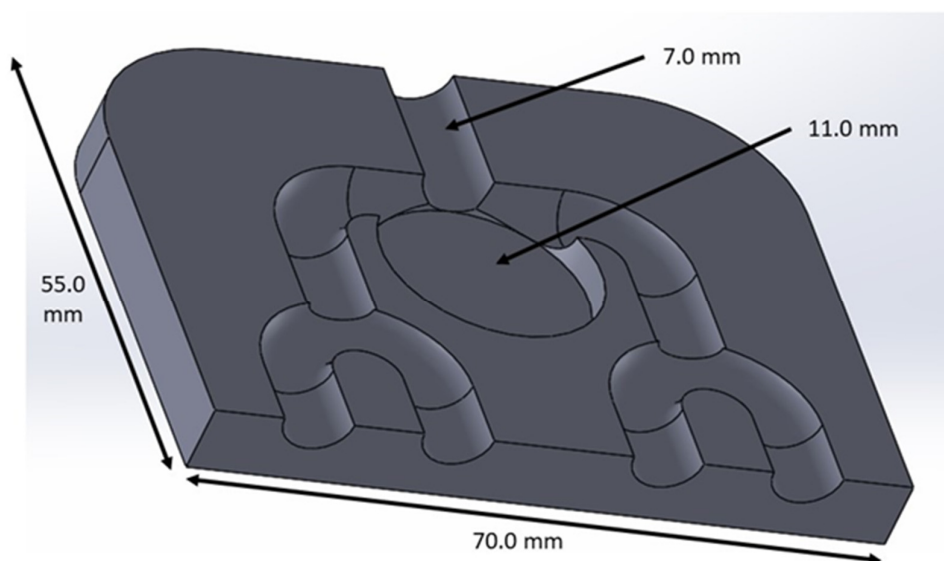
**Figure 2.** Illustration of Cell of the Cascade System for Biomineralization in Cement.

The five reaction cells were built in order to be applied in a Factorial Design, by the repetition of experiments under the same air flow rate conditions. Due to the high number of experiments, it was necessary to increase the number of reaction cells to reduce the total experimental time. In other words, the number of five reaction cells was not used to amplify the contact area as in a continuous reactor, but to apply different conditions, excepting air flow, in a set of assays, ordered according to an increasing concentration of reagents.

Compared to a batch reactor, the CSBC has controlled aeration and temperature, as well as facilitating the filtering of microbial cells at the inlet and outlet of the system. Filters were placed at the inlet to prevent indigenous microorganisms from entering the reaction medium via the compressor, thereby avoiding calcium lactate degradation by other species. Filters placed at the outlet were used to prevent inoculated microbial cells from contaminating the external environment.

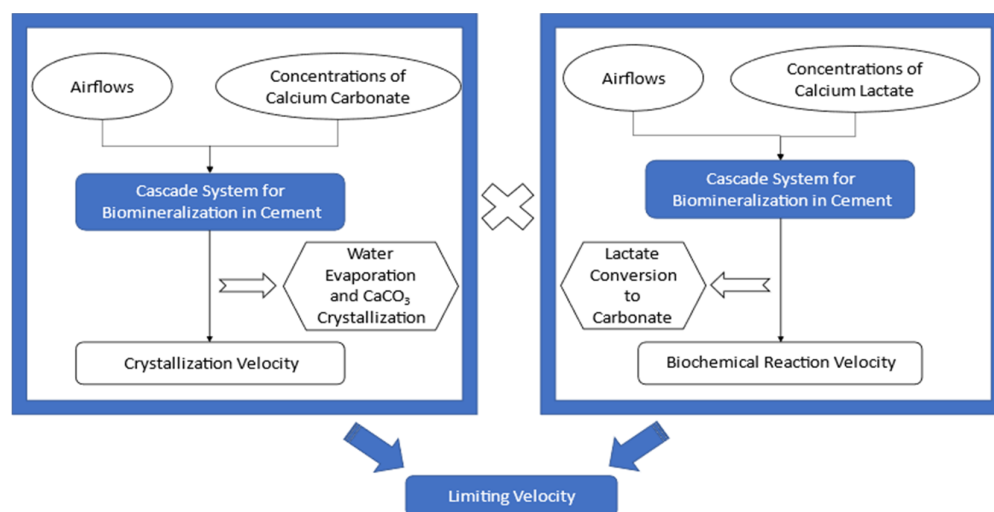
The reaction cells consisted of Petri dishes adapted with lateral holes for the inlet and outlet of air and were made of a material more resistant than glass (PETG). A space was designed in each cell to house rubber gaskets for 100 mm PVC tubes. Four side connectors were also designed to join each reaction cell to the other cells stacked on top of each another to enable work in series. The system could also have been built up in parallel, but would have required greater flow control in each reaction cell; therefore, the cascade option was the best choice for the process.

In the compressor, it was necessary to combine the four outlets into one, so a lung was designed and built with the 3D printer. This lung was printed in two halves to enable post-processing with resin waterproofing of the entire inner surface to avoid leakages. The two halves were then glued together, and the outer surface was coated with two layers of resin (Figure 3). The maximum air flow rate was  $4 \text{ L} \cdot \text{min}^{-1}$ .



**Figure 3.** Illustration of one of the halves of the lung for the Cascade System for Biomineralization in Cement.

The CSBC construction had the following two main objectives: (i) to determine the phenomenon limiting the process and (ii) to determine the best possible environment for the formation of calcium carbonate crystals in cement or concrete. The first objective can be translated into a comparison between the rates of the biochemical reaction and that of crystallization. The slower phenomenon of either conversion of ions into crystals or conversion of lactate into carbonate, would be the rate limiting the process. This is the innovation of the work and the motivation for the construction of the prototype. The process diagram of further experiments is illustrated in Figure 4. The second objective was achieved with the precise control of air flow rate and temperature. The forced convection of air inside the reaction cells removed water molecules by evaporation, while oxygen, accounting for about 21% ( $v/v$ ) of atmospheric air, reacted with lactate, according to Equation (1), producing water, which was also evaporated by the continuous convection. Therefore, it was necessary to know the inner and outer humidity (environment condition), which required the inclusion of a supervisory control in the CSBC.



**Figure 4.** Process diagram of further experiments for the Cascade System for Biomineralization in Cement.

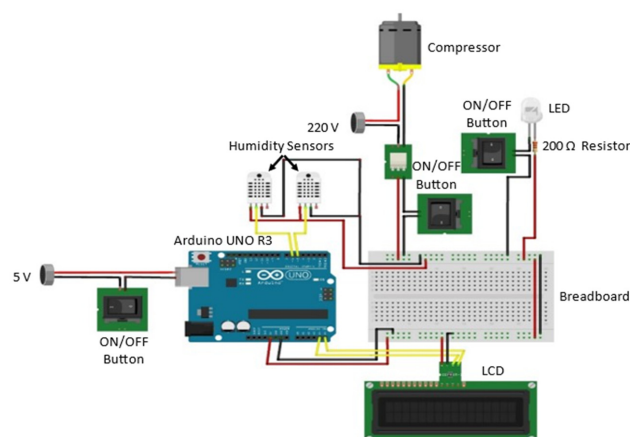
### 2.2.2. Instrument, Electrical and Programming Step

After the process, the Arduino UNO R3 hardware was used to measure the humidity inside and outside the CSBC, as well as to activate a compressor (providing aeration for the reaction cells) and an LED (lighting the interior of the heat chamber).

It is important to know the difference between the humidity of the system and the environment, as not all experiments are performed at the same time or on the same day. Moreover, depending on the climatic conditions of the location of the experiment, the humidity of the surrounding environment can change quickly. The Arduino software enables simple arithmetic calculations so that the operator of the supervisory control can directly visualize the desired information (in this case, the difference in humidity). In the present study, the tests were performed in one hour, comparing the differences in humidity between the sensors over one hour in the city of Recife, PE, Brazil.

Tests were also carried out for drying 100 mL of demineralized water in a reaction cell of the CSBC system. The tests were performed in triplicate and with the compressor running at  $3 \text{ L}\cdot\text{s}^{-1}$  or  $5 \times 10^{-5} \text{ m}^3\cdot\text{s}^{-1}$ . When the difference in humidity reached the one-hour test limit of the sensors, the drying flow rate of the system was calculated as the total volume of liquid in the container over the total drying time.

A circuit board was put together (Figure 5) with the aid of Fritzing software to represent, in practice, how the supervisory control with the Arduino UNO R3 microcontroller was configured.



**Figure 5.** Schematic of electrical circuit of the Cascade System for Biomineralization in Cement.



The components of the circuit were the following:

- Arduino UNO R3 microcontroller;
- 220 V line filter;
- 5 V source;
- On/off buttons;
- Breadboard;
- Blue LCD screen, 16 × 2 digits, with I2C module;
- Relay module for activation of up to 5 V;
- Two DHT22 humidity sensor modules;
- White LED;
- 200  $\Omega$  resistor;
- RS-A98 compressor (RS Electrical); and
- Wires.

Besides the electrical and electronic components, a circuit-breaker box and wire organizer spirals were used to house the circuit and offer protection and stability to the parts and instruments. The supervisory control was connected to a 220 V supply with a current of 10 A. A 5 V source converted the 220 V to the 5 V of supply for the Arduino. A 220 V link was connected directly to the open relay module to be activated only with the supervisory control command.

The total power of the CSBC was also calculated as the sum of powers of the stove, the LED, the two humidity sensors and the Arduino UNO R3 microcontroller.

### 3. Results

#### 3.1. Determination of Calcite Solubility Curve

Based on data described by Visscher and Vanderdeelen [16], 60 pairs of data were collected at temperatures in the selected range (5–35 °C), and the calcite solubility curve was plotted (Figure S1). The first point to discuss regards the reliability of the data source. The other point deals with the logarithmic solubility curve with a determination coefficient ( $R^2$ ) of 0.954, indicating that the 60 pairs of data were in good agreement with the curve.

#### 3.2. Cascade System for Biomineralization in Cement (CSBC)

As mentioned earlier, the CSBC was constructed in the following two steps: (I) process engineering and (II) instrument, electrical and programming step.

##### 3.2.1. Process Engineering Step

Figure 6 shows the CSBC with its supervisory control, stove, humidity chamber and humidity sensors. The equipment had an aeration system from the supervisory control to the stove and from the stove to the humidity chamber. Air was taken from the environment and returned to the same environment.

The reaction cells are shown in Figure 7, with a unit printing time for each cell of 15 h and nine minutes, totaling 75 h and 45 min for the whole system. The cover took approximately two hours and there was the time required to apply and dry the resin.

In the control of aeration, the flow was from top to bottom to ensure the least possible loss, since aeration was low. The gaskets and hardened resin ensured that the reaction cells did not leak. The lung of the compressor used to join the four outlets is shown in Figure 8A after being printed and in Figure 8B after being coated with resin. Each half of the lung was produced by the 3D printer in one hour and 47 min.

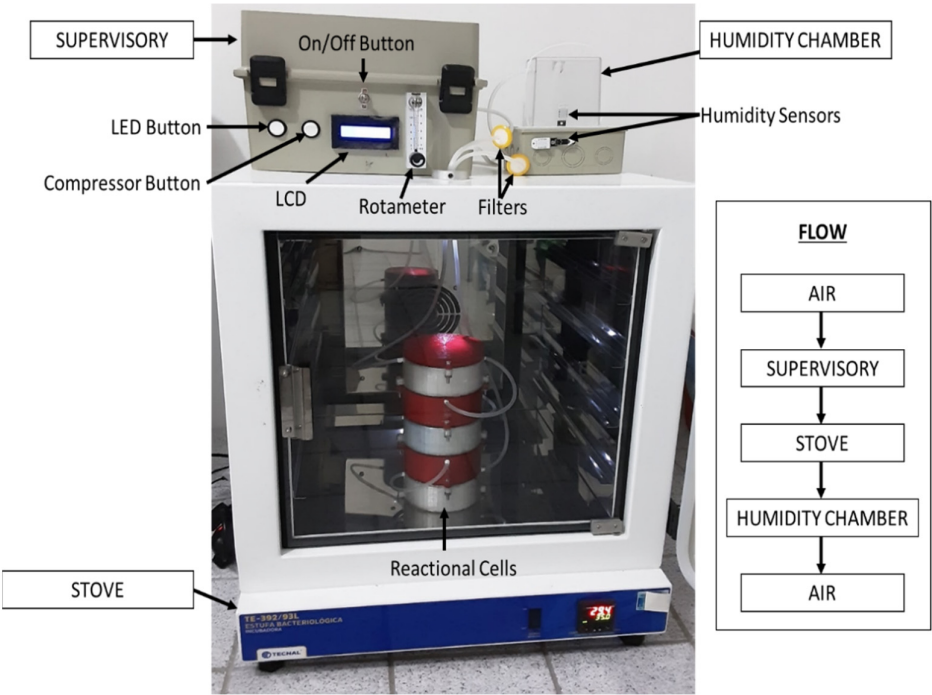
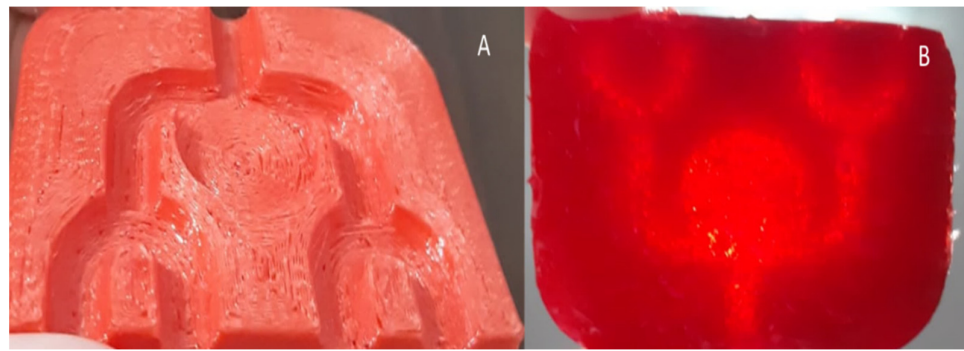


Figure 6. Overall Cascade System for Biomineralization in Cement.



Figure 7. Reaction cells of the Cascade System for Biomineralization in Cement.



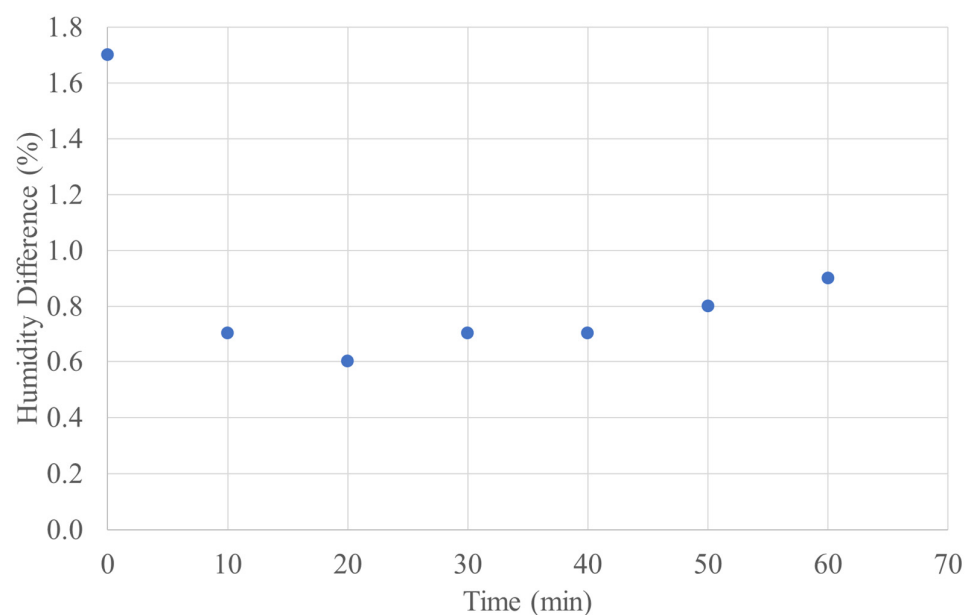


**Figure 8.** Lung (A) after being printed and (B) after being coated with resin.

### 3.2.2. Instrument, Electrical and Programming Step

The Arduino UNO R3 microcontroller enabled connections with the different components of the CSBC, such as the compressor, relay module, LED and humidity sensors. The sensors were placed in two different locations. The first location was in the humidity chamber. Water vapor from the reaction cells reached a sensor placed under a transparent dome to enable the reading of the internal humidity of the CSBC. The other location was away from the injection of water vapor of the reaction cells.

Two other important aspects of the difference in humidity are the humidity measurement range and the accuracy of the sensors. Some sensors measure humidity from 20 to 90%; however, depending on climatic conditions and the systems used, humidity can be higher than 90%. The DHT22 sensor selected for the CSBC measured the complete range of humidity (0 to 100%). Moreover, sensors that measure humidity from 20 to 90% have accuracy of  $\pm 5\%$ , whereas the accuracy of the DHT22 sensor was  $\pm 2\%$ . This meant that, whereas the difference in humidity could have a propagation of error of up to 10% ( $2 \times 5\%$ ) when using sensors with less accuracy, the two DHT22 sensors used had a propagation of error of up to 4% ( $2 \times 2\%$ ). To test this accuracy, differences in humidity between the two DHT22 sensors were compared for 60 min at 10 min intervals, as shown in Figure 9. The errors did not exceed half of the propagated error, which demonstrated the reliability of the sensors.



**Figure 9.** Errors in humidity difference without activation of compressor in the Cascade System for Biom mineralization in Cement.

At the start, the process showed a minimal difference in humidity, which was considered merely an instrument error. From the moment water from the reaction cells evaporated, the difference in humidity increased until reaching a state of water saturation in the air. Evaporated water came mainly from the solutions in the reaction cells, but also from the reaction of bacterial nutrition by lactate (Equation (1)). When the difference in humidity began to decrease again, it meant that the humidity in the reaction cells was approaching that of the environment.

The total drying time of the 100 mL-volume was 99.83 h with a standard deviation of 0.05 h. Therefore, the drying flow rate was  $1.01 \text{ mL} \cdot \text{h}^{-1}$ , or  $3.10 \cdot 10^{-10} \text{ m}^3 \cdot \text{s}^{-1}$ . This meant that 0.0006% of the drying output flow came from demineralized water previously contained in the reaction cells and that the prototype was operational for about 100 h.

The programming code for the supervisory control (Code S1) assisted in three ways: in the measurement of humidity, in the determination of the difference in humidity, and in the transformation of this difference into the modulus of the value (always a positive value) such that it was physically compatible with the process.

Powers of the constituents of the operating CSBC were: stove—250 W, Arduino UNO R3—10 W, LED—0.01 W,  $2 \times$  humidity sensors— $2 \times 0.014 \text{ W}$ , and compressor—12 W. Thus, the total power of the CSBC could be estimated to be around 272 W.

## 4. Discussion

### 4.1. Process Engineering Step

Technological applications of 3D printing have been used in different fields of knowledge, such as health, industry, and art. The advantage of using graphic resources for the creation of parts is accessibility for the development of science.

According to Noor et al. [17], a small, vascularized heart was created using the processes of microencapsulation and 3D printing. This technology favors the future creation of artificial human organs, avoiding long waiting lists for organ transplants. In the case of the CSBC, 3D printing was fundamental to build reaction cells of the desired size. In the future, this 3D printing tool could even be used to adapt to different geometries based on factorial designs.

Krimpenis et al. [18] developed a robotic arm for situations of difficult positioning or access, which was made with 3D printing in PETG, due to the strength and durability of the filament. The reaction cells and lung in the present study were also printed with PETG filament, mainly due to the chemical resistance of the material, which was required to cope with potential damage caused by the resin in the absence of chemical protection.

Higueiras et al. [19] photographed a broken Hispano-Roman architectural ornament and reconstructed part of the piece with 3D printing. The authors created a mold for the reconstruction of the piece and filled it with acrylic resin. Building the CSBC did not require the same level of detail as restoring an architectural ornament, but 3D printing assisted in detailing the holes of reaction cells and couplings among reaction cells. Therefore, it is a tool that encourages the researcher's creativity, allowing the creation of different structures.

### 4.2. Instrument, Electrical and Programming Step

Kondaveeti et al. [14] described the advantages of using Arduino boards in prototypes, such as the low cost of the boards and Arduino kits, operational versatility of the Arduino software, the plurality of sensors, the low energy expenditure of the boards and the fast processing of information. The authors also cited some fields of knowledge in which Arduino is applied, such as residential automation, smart farming, education, security, etc. This versatility of applications and features of Arduino boards consolidates their use in engineering prototypes, such as the CSBC built in this work.

Poh et al. [20] built a portable UV-visible spectrophotometer using the Arduino Nano with a Bluetooth module as the microcontroller. The authors found that the replacement not only made the equipment cheaper, but also provided the portability aspect to the equipment, despite the limitations in certain wavelengths that could be optimized. The

authors also used 3D printing for the fabrication of the equipment, as performed for the CSBC in the present study.

Brasileiro et al. [21] constructed a microbubble generation and measurement unit using an Arduino UNO R3 as the microcontroller. The authors also constructed a sensor for low air flow rates, correlating air flow rate with the pressure of a water column, with an  $R^2$  of 0.98. Likewise, in the construction of the CSBC there was also detail on why the system was built, on how the instrument, electrical and programming steps were made and on what the next steps would be for the study of the response variables.

Sharath et al. [22] designed a “pick and place” robot with an Arduino Mega controller, which is used for projects with a larger number of sensors. The equipment was designed to separate a given object, based on its color, with an efficiency of over 90%. One of the aims of the project was to demonstrate that access to prototyping in homes and workplaces is possible, that more investment in research is needed for this equipment to reach a commercial level. Like the “pick and place” robot project, the CSBC prototype appears as an alternative to inspire scientists to develop their own prototypes using Arduino boards, allowing the delimiting of the variables that they want to work with.

To compare the power of the CSBC (272 W) with that of other electrical equipment in common use, it is noteworthy that a microcomputer requires 300 W to work. In other words, kinetic studies can be carried out at a lower cost than a connected microcomputer in order to increase the useful life of the concrete, making it even more resistant than the standard. According to Brasileiro et al. [6], 4 billion tons of cement are produced every year, which are responsible for 5 to 7% of the world's CO<sub>2</sub> emissions. With the implementation of optimized studies for bio-concrete, mainly with the use of CSBC, the intention is to reduce the consumption of the large amount of cement used both for repairing existing structural works and for new works, as well as improve energy efficiency of buildings.

This prototype was inspired by the experiments of Brasileiro et al. [23] on biomineralization in cement carried out in Petri dishes. In these experiments it was not possible to include, or to measure, the air flow easily due to the geometry of Petri dishes. So, this was the motivation to join 3D printing and the Arduino controller to create the CSBC and to analyze the two velocities.

## 5. Conclusions

The construction of the Cascade System for Biomineralization in Cement began with the filtering of data from the solubility curve between temperatures of 5 and 35 °C, which is the operational range of the biomineralization process. Considering the biochemical reactions of biomineralization and the control of aeration, the measurement of humidity inside and outside the system, and temperature were chosen as the three key parameters of the CSBC structure. The reaction cells and lung were created with 3D printing, which facilitated the fit of the parts to meet the needs of the project. The Arduino UNO R3 microcontroller assisted in the on/off operationalization of the compressor and LED. The humidity sensors enabled the online measurement of humidity inside and outside the CSBC, as well as the online determination of the difference in humidity. Furthermore, the instrument errors were quite low and acceptable for the work. Details, such as microbial concentration, analysis periods, the behavior of the crystallization curves and the biochemical reaction, will be provided in future studies with a greater focus on the analysis of the rate of the phenomenon limiting the process. The largest limitation of the study is the time required for many tests, but it can be overcome with factorial design. Future research intends to study these behaviors in common concrete systems and, later, in reinforced concrete. This work involved a combination of biotechnology, 3D printing and process control for the creation of the Cascade System for Biomineralization in Cement. The kinetic data that will be acquired will contribute to better control in the applicability of the restoration of concrete structures, thereby avoiding greater consumption of cement, maintaining structures resistant to natural and anthropogenic wear, and improving energy efficiency of buildings.

**Supplementary Materials:** The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/en15145262/s1>, Figure S1: Solubility Curve Obtained from Visscher and Vanderdeelen [16] for Variations in Calcite between 5 and 35 °C; Code S1: Arduino Code for Cascade System for Cement Biomineralization.

**Author Contributions:** Conceptualization, P.P.F.B. and M.B.; methodology, P.P.F.B. and M.B.; software, P.P.F.B.; validation, P.P.F.B.; formal analysis, P.P.F.B.; investigation, P.P.F.B. and B.A.C.R.; resources, P.P.F.B.; data curation, P.P.F.B.; writing—original draft preparation, P.P.F.B., A.A.C., A.C. and L.A.S.; writing—review and editing, P.P.F.B., A.A.C., A.C. and L.A.S.; visualization, P.P.F.B.; supervision, Y.B.B., L.A.S. and M.B.; project administration, Y.B.B. and M.B.; funding acquisition, A.A.C., A.C., L.A.S. and M.B. All authors have read and agreed to the published version of the manuscript.

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