Biological Durability of Bamboo Bio-Concretes

Vanessa Maria Andreola[1](✉), Rayane de Lima Moura Paiva[1], Beatriz Palermo Lepine[1], Daniele Oliveira Justo dos Santos[1], Keyna Proença[2], Bruno Menezes da Cunha Gomes[1], Aurea Moraes[2], Simone Quinelato[2], Nicole Pagan Hasparyk[3], and Romildo Dias Toledo Filho[1]

[1] Federal University of Rio de Janeiro, Rio de Janeiro, RJ 21941-972, Brazil
{vanessa.andreola,daniele.justo}@numats.coc.ufrj.br, {rayane, toledo}@coc.ufrj.br, bpalermolepine@poli.ufrj.br

[2] Oswaldo Cruz Institute, Rio de Janeiro, RJ 21040-900, Brazil
{aurea,simone.quinelato}@ioc.fiocruz.br

[3] Eletrobras FURNAS, Goiânia, GO 74993-600, Brazil
nicole@furnas.com.br

Abstract. Studies on bio-based building materials are promising due to their insulating characteristics, low density and good hygrothermal performance. However, range of factors can influence its durability, including biological processes. For that reason, this study investigates the susceptibility to the growth of microorganisms in bamboo bio-concretes (BBC) with different finishes: (i) natural samples and (ii) samples waterproofed. The bio-concretes, after curing until 28 days (23 °C; 55% RH), were sterilized for 24 h in an oven (100 °C) and the hydrogenion potential (pH) determined. Subsequently, the samples were sprayed with suspensions of conidia of the fungus *Aspergillus niger* (IOC 0207) and a control suspension without fungi. After, the bio-concretes were conditioned in two controlled conditions of temperature (T) and relative humidity (RH), simulating environments of possible uses: (1) without water blade (23 ± 2 °C; 74 ± 2% RH) and (2) with water blade (23 ± 2 °C; 98 ± 2% RH). Biological durability was evaluated according to visual analyses by photographs and optical microscopy at 0, 7, 14, and 28 days. For conditioning 1, it was not possible to observe fungal proliferation on the surfaces of any sample during the test. For conditioning 2, the high RH environment and the presence of the water layer caused a color change, saturation and growth of microorganisms. The results show that the higher the RH, the greater the susceptibility to the growth of microorganisms and changes in appearance. Despite this type of study being introductory, bio-concrete is a promising material, with potential to be used in civil construction, showing durability to the growth of the fungus *A. niger.*

Keywords: Durability · Bio-degradation · Bio-concrete · *Aspergillus niger*

S. Amziane et al. (Eds.): ICBBM 2023, RILEM Bookseries 45, pp. 716–728, 2023.
https://doi.org/10.1007/978-3-031-33465-8_55

1 Introduction

Currently, the quest for sustainability is a universal challenge, both in industrial technology to automate processes and equipment and in different sectors of research [1, 2]. Civil construction, especially the concrete industry, aims to create strategies to increase sustainability and reduce impacts on the built environment [3–5].

The use of plants residues, generated in the industry, which can be used in developing new product, is a strategy that has been adopted [6, 7]. From this, researches haves been developed to explore the potential of these innovative materials [8–14] obtained from the use of unconventional constituents in mortars and concrete [15, 16, 18].

Bio-concrete stands out for its versatility, enabling production with different plant sources, such as bamboo waste, wood shavings, rice husks [2, 10, 19, 20], corn stalk, peach peel and damask [8, 14, 21]. Normally, the residues are chosen according to the availability of vegetal residues in each region/country. In the case of bamboo, residues can be obtained during the primary processing of the culms to laminates, which, depending on the species, can generate 65% to 85% of leftovers and pieces [20, 23].

In this context, bio-concrete and bio-mortar produced with a cement-based matrix and a lime-based matrix stand out, mainly because those composites use vegetal residues in the form of bio-aggregates. In the literature, studies are found with bio-concrete between 15% [22] and 80% [12], but most of the researches test volumes of bio-aggregates over than 50% [10, 15, 19, 20]. These materials improve thermal characteristics and reduce energy consumption when applied in buildings [4, 5, 14]. They are promising materials due to their insulating characteristics [9, 10, 16], low density [11, 14], good mechanical properties [2, 8, 10] and good hygrothermal performance [17, 22].

The high volume of plant residues used in bio-concrete production can make them sensitive to the growth of microorganisms, as they are lignocellulosic materials, made up of chemical substances [13, 16, 24]. According to the researches available in the literature [14, 25, 26], many factors can influence durability and performance, and one of the main issues related to the use of bio-concrete is their biological durability, including processes that can directly affect human health [26, 27]. Therefore, before being made available on the market, it was particularly important to evaluate biological durability.

In this context, the present study analyzed the susceptibility to the growth of microorganisms in bamboo bio-concretes (BBC) in natural samples and waterproofed with a water-repellent agent. The samples were conditioned in two conditions of temperature (T) and relative humidity (RH), simulating environments of possible uses of bio-concrete: (1) without water depth (23 ± 2 °C; 74 ± 2% RH) and a critical environment: (2) with water depth (23 ± 2 °C; 98 ± 2% RH). The biological evaluation was analyzed according to visual analysis by photographs (0, 7, 14 and 28 days) and optical microscopy at 28 days.

2 Materials and Methods

2.1 Bio-Aggregates: Raw Material

The bamboo bio-aggregates used in this study were acquired from the municipality of Petrópolis (Rio de Janeiro, Brazil). First, the culms were cut with a table saw to reduce their length from 15 cm. After the cut, the segments were processed in an industrial crusher (twice) to obtain the particles with lengths between 1.18 to 5.60 mm. Then, the residues were submitted to mechanical sieving using sieves with openings of 5.60 mm and 1.18 mm. All material retained between the sieves was used (Fig. 1). After sieving, the bio-aggregates were treated by immersion in an alkaline calcium hydroxide solution ($Ca(OH)_2$) at a concentration of 1.85 g/L. The proportion, by mass, of water/bamboo used was 1:7. The bio-aggregates were immersed in the solution for 1 h, washed with water and air-dried until reaching to constant mass. The apparent density and moisture content were 580 kg/m^3 and 12.3%, respectively, according to the standards [28, 29]. Water absorption was 65%, considering the mixing conditions during bio-concrete production [2].

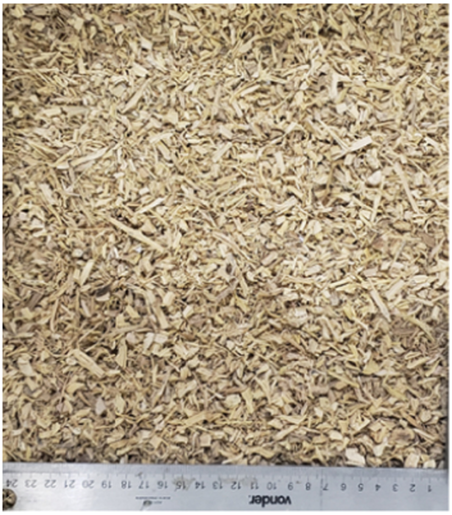


Fig. 1. Bamboo bio-aggregates used in this study.

2.2 Fabrication and Waterproofing of Bamboo Bio-Concrete (BBC)

The bio-concretes were produced with a matrix composed of 45% (CP II-F-40 Brazilian cement), 25% rice husk ash (RHA) and 30% fly ash (FA). A volumetric fraction of bio-aggregates of 45% was used, and the water-to-blinder ratio was set at 0.30. In addition, was used 2% of calcium chloride ($CaCl_2$) in relation to the mass of the binders. $CaCl_2$ was used to accelerate cement setting and minimize the inhibitory effect of bio-aggregates, prior to the action of extractives in the mixture [30]. The proportion of the mixtures produced in this work is summarized in Table 1.

The rational dosage of bio-concrete was used for the production of mixtures [2]. Initially, the cementitious materials were homogenized (using a 20 L mixer - 136 rpm), followed by the gradual addition of 90% of the total water with the calcium chloride solution. After 1 min, the bio-aggregates were gradually inserted into the cementitious paste and mixed for 1:30 min. After which, the process was completed by adding the remaining water until reaching a total of 7 min of mixing.

Table 1. Mix proportion of BBC, with materials by mass (kg/m^3).

Bamboo	CP II	RHA	FA	*H_w	**W_c	$CaCl_2$
232.00	341.43	151.75	265.56	227.62	150.80	19.12

* H_w = hydration water; ** W_c = Water of compensation.

The material was cast in prismatic molds with 400 × 100 × 30 mm (length × width × thickness) and mechanically vibrated on a vibrating table (68 Hz) for 15s. After casting, the samples were protected from moisture loss and demoulded after 24 h. Subsequently, with the aid of a circular saw, the samples were cut into dimensions of 100 mm × 100 mm × 25 mm (length × width × thickness), leaving the bio-aggregates scraped and visible on all sides. After cutting, the BBC was dry cure (21 ± 2 °C; 55 ± 5% RH), until reaching 28 days of age.

After complete curing, a surface application (using a brush) of a waterproofing agent with a concentration of 5% silane/siloxane was performed on the bio-concrete samples. The surface protector was applied in two application coats, totaling a consumption of 13.7mL/m^2. The waterproofing agent has a density of 1.01kg/L, is non-flammable and has low emission of volatiles.

2.3 Biological Durability and Characterization Tests

Sample Preparation. After curing for 28 days (21 °C; 55% RH), the BBC was sterilized for 24 h in an oven (100 ± 2 °C), in order to sterilize the surface of the samples in terms of the presence of fungi [13, 17, 30].

Determination of the Hydrogenation Potential (pH). After 28 days of curing, the bio-concretes were milled, macerated and diluted in distilled water (in triplicates). From manual homogenization, the samples solution were sealed and conditioned in a controlled environment (21 °C; 55% RH) for 24 h. The solutions were analyzed using a benchtop pH meter (Nova Instruments, model NI PHM).

The Aqueous Suspensions Used in the Tests. For the biological analysis, the objective was to analyze the susceptibility of bioconcretes to colonization by a fungus species. The samples were sprayed with a high concentration of conidia. After that, they were kept under controlled conditions of temperature and humidity. Thus, the fungus *Aspergillus niger* was selected because it is anemophilous and widely distributed in indoor and outdoor environments, with excellent growth at 30 °C [11, 13, 27]. The fungus strain *A. niger* (IOC 0207) was acquired from the Filamentous Fungi Culture Collection (CCFF) IOC/FIOCRUZ. Two types of aqueous suspensions were prepared for the tests. A control suspension containing only water and 0.1% Tween 80 and a conidial suspension containing water, 0.1% Tween 80 and conidia of the fungus *A. niger* at a concentration of 1.5 × 10^7 conidia.mL^{-1}.

Biological Durability by Spraying Method. For the analysis, the methodology of Andreola [10] and Paiva [17] was followed. The procedure consisted of measuring the suspensions using a beaker and transferring the material to a sterile spray bottle. For samples, equal volumes of conidial suspension and control suspension were used.

Sample Incubation Conditions. After spraying the aqueous suspensions, the samples were kept in glass containers, inside the laboratory environment and the temperature and relative humidity inside the container were controlled by an automatic sensor, which continuously acquired the data (sensor model Impac), as follows:

1) *Without water blade (23 ± 2 °C; 74 ± 2% RH).* Simulated high humidity. The method consisted of keeping the samples with the conidial suspension/control suspension in a container without water. The samples remained in the same condition shown in Fig. 2, with the only difference being that the container was kept dry throughout the test.
2) *With water blade (23 ± 2 °C; 98 ± 2% RH).* Simulated extreme humidity. The method consisted of keeping the samples with the conidial suspension/control suspension in a container that simulated an aquarium. For this, a glass container was used (Fig. 2) which had a plastic mesh at the base. The samples were placed on the mesh, allowing free access to water to their bases. The water level remained constant (5 mm) above the base of the sample. After placing the water slide, the top of the container was sealed with plastic film and small openings in the plastic were made.

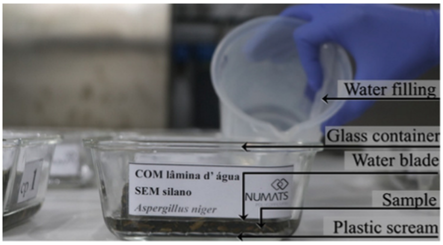


Fig. 2. Water blade testes of the BBC samples.

Visual Analysis by Photographs. The procedure was performed using pictures taken at the ages of 0, 7, 14 and 28 days after suspension spray. A camera attached to a fixed experimental bench was used, with three points of light [10, 15, 17]. The photographs were always taken with the same camera (EOS 80D, Canon) and the base of the bench was configured to receive the glass container, avoiding handling the sample (Fig. 3). The images obtained are always compared with the control samples, which are the photographs taken after spraying the suspensions (day 0).

Analysis by Optical Microscopy (OM). The analysis was carried out to observe the proliferation of microorganisms on the surface of the BBC, at 28 days of the start of the tests. Magnifications of 10x and 100x were adopted using a stereoscopic microscope, with reflected light (Model SMZ-800, Nikon) in order to obtain the images, with a parallel optical zoom system. In the microscope, a wooden base with a glass container fitting was used (Fig. 4). This base served as a support to move the set (container and

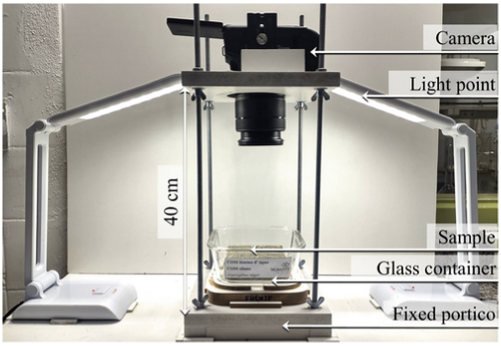


Fig. 3. Photo recording on experimental bench.

sample), without handling the BBC. In the sites where microorganisms were observed (with the naked eye), two points of light were directed for microscopic visualization.

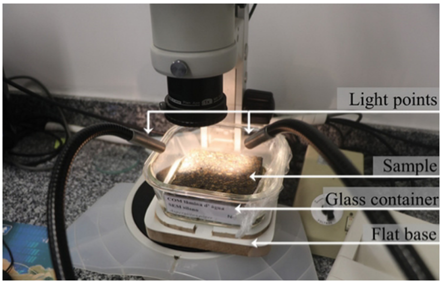


Fig. 4. Microscopic analysis setup: Sample on flat base, using two light points.

3 Results and Discussion

The following abbreviations were used to facilities the results presentation:

– For identify the type of aqueous suspension: (CS) control aqueous suspension and (FS) fungal aqueous suspension.
– For the incubation conditions: (1) Without water blade or (2) With water blade.
– For the finish used in bio-concretes: (N) Natural or (W) Waterproofed.

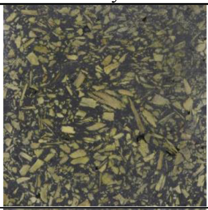
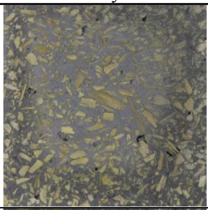
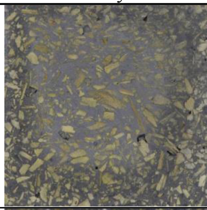
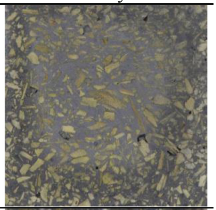
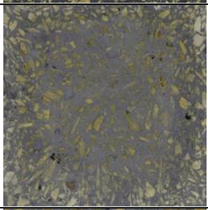
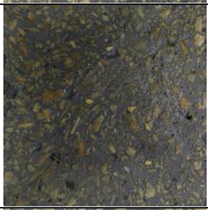
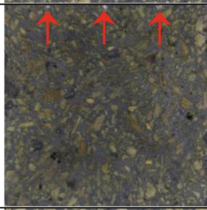
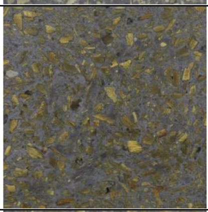
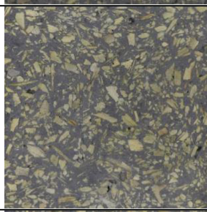
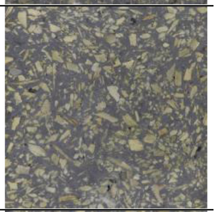
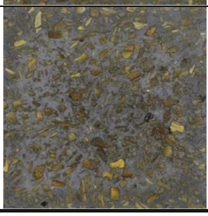
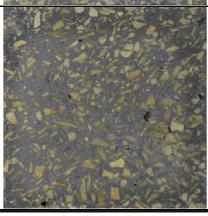
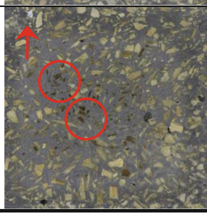
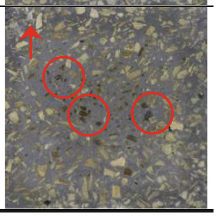
3.1 Determination of pH

The pH values obtained were 11.5 ± 0.04 and 11.3 ± 0.01 for natural and waterproofed bio-concrete, respectively, i.e.; both samples have an alkaline pH. The high pH values found are directly related to the properties of the binders used, mainly the presence of cement, whose pH varies between 12 and 13.5. The presence of the waterproofing agent used did not significantly alter the pH value of the evaluated BBC.

3.2 Visual Analysis by Photographs

The images obtained for the samples with control aqueous suspension (CS) and fungal aqueous suspension (FS) are presented in the Table 2 and Table 3, respectively.

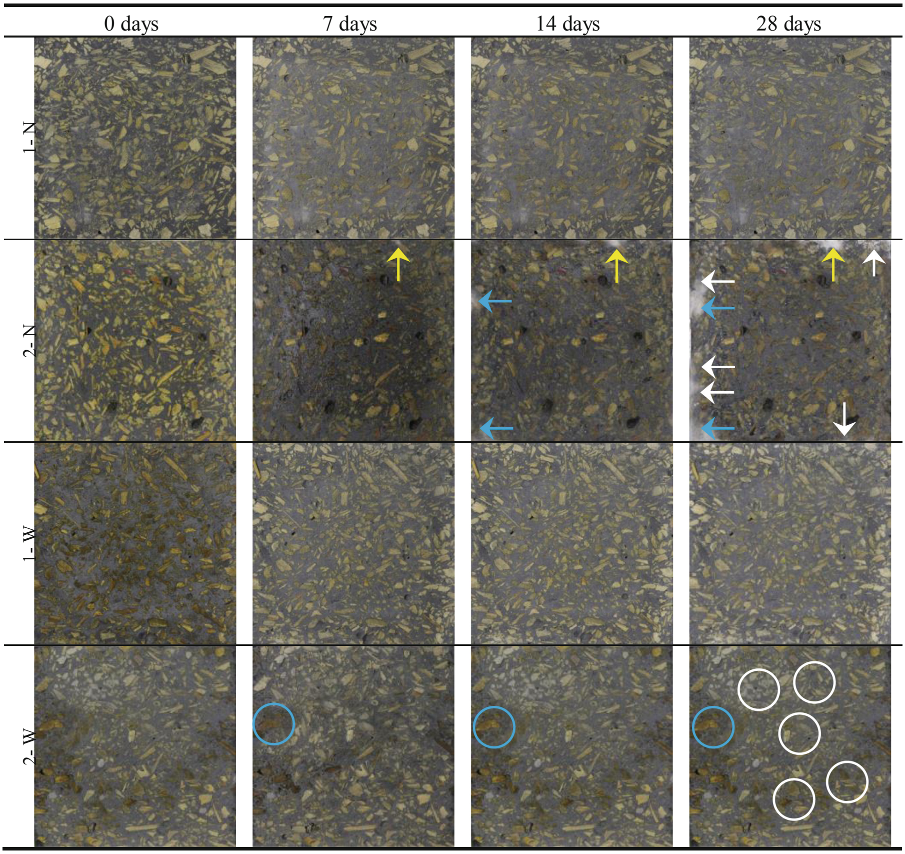
Table 2. Visual analysis by photographs of the control aqueous suspension (CS).

	0 days	7 days	14 days	28 days
1- N				
2- N				
1 W				
2- W				

As shown in Table 2 and Table 3, the appearance of the bio-concretes was not changed for the mixtures without a water blade (1), both for the control suspension and for the fungal suspension. The difference in color and saturation is only observed on the day of spraying (0 day), because the suspensions are aqueous, which humidify the sample. The saturated sample showed greater vividness of color, especially the bio-aggregate. This tendency was not observed for samples kept in a water blade, which was expected due to the higher humidity in which the bio-concrete was kept.

The 2-N (CS) samples showed surface saturation on day 0, a more saturated (soaked) appearance on day 7 and remained saturated on days 14 and 28. By day 7 these samples showed fungal growth on the surface, expanding growth points as the assay time

Table 3. Visual analysis by photographs of the fungal aqueous suspension (FS).



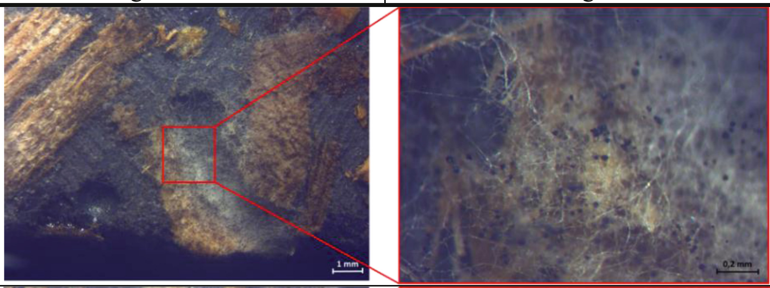
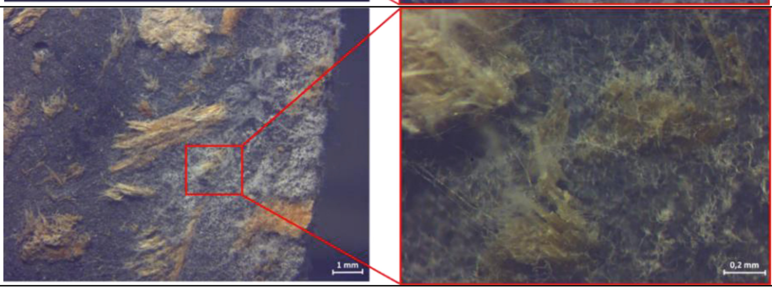
increased (see Table 2, as indications in red). However, the greatest growth of microorganisms was observed in the 2-N (FS) sample and, even on day 7, fungal growth was already observed (see Table 3, the identification in yellow) surrounding a particle of bamboo. From 7 to 14 days it was observed, in all the ends of the sample (see Table 3, the indications in blue), growth of filamentous fungi. From the 14th to the 28^{th} day of testing, it was noted that the growth region increased, spreading to the central region of the specimen (see Table 3, the white arrows).

About the bio-concrete 2-W (CS), the growth of microorganisms was also observed after the first week of the test. On the 14th day, it was observed that the bio-aggregates were saturated, with punctual water droplets around their geometry (see Table 2, circles in red). In addition, spots began to appear in the upper left corner of these samples (Table 2, the red arrows). From 14 to 28 days, there was a greater concentration of these spots, while the water droplets expanded to other bio-aggregates. The 2-W (FS) showed a similar trend (droplet concentration), however, more intensely than the reference. Even on the 7th test day, it was possible to observe the presence of fungal structures on the bio-aggregate (Table 3, the blue circles). From 7 to 14 days after the contamination, the saturated particles became stained. On the 28th day, dark spots appeared at different points in the sample (Table 3, the white circles).

3.3 Microscopy Analysis

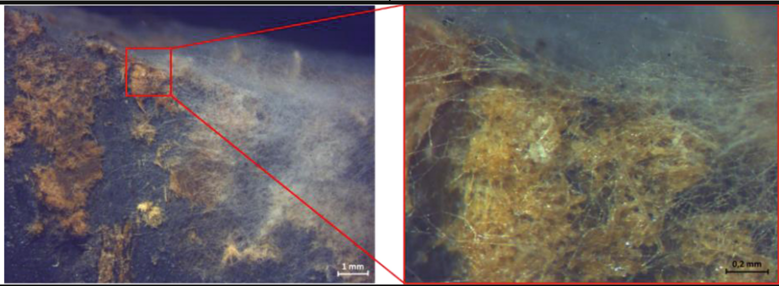
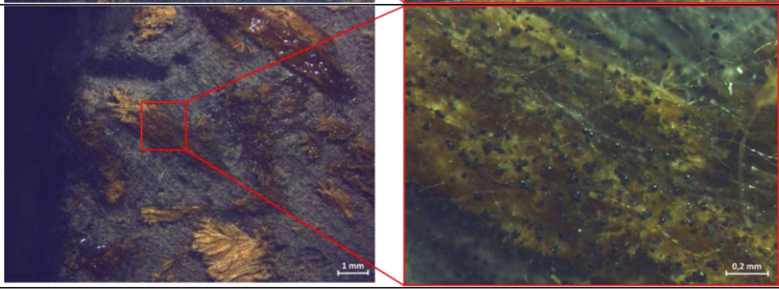
Table 4and Table 5, show the MO images of the bio-concrete samples after 28 days of conditioning under exposure of incubation with water depth (2) (23 ± 2 °C; 98 ± 2% RH) with aqueous control suspension (CS) and the aqueous suspension of the fungus *Aspergillus niger* (FS), respectively. This analysis was carried out only for this age because the surfaces of the samples showed greater susceptibility to fungal proliferation, as previously evidenced in the visual analysis by photographs.

Table 4. Visual analysis by optical microscopy (OM) of the aqueous control suspension (CS).

	10x magnification	100x magnification
2-N		
2-W		

From the simplified MO images, it was possible to verify the presence of the fungus in all tested samples, and observation of its morphological structures, which was evidenced through hyphae, structures in the form of elongated filaments in white color and conidial heads, spherical structures in dark color. This pattern was observed mainly on the bamboo bio-aggregates that are exposed on the surface of the samples and at the interface between the bio-aggregates and the cementitious material. The high susceptibility to fungal patterns observed at these points is directly correlated to: (i) the chemical composition of the bio-aggregates, which is rich in Carbon (C), thus favoring a greater supply of nutrients, (ii) the high hygroscopic characteristic of the BBC due to the multi-scale porosity which favors the a moisture concentration and also (iii) the roughness at

Table 5. Visual analysis by optical microscopy (OM) of the fungal aqueous suspension (FS).

	10x magnification	100x magnification
2-N		
2-W		

the ends of the specimens caused during the process of stripping the samples prepared for the test.

In this case, as already highlighted in subtitle 3.2, the bio-concrete waterproofed showed less biological incidence when compared to natural samples, that is, the waterproofing agent delayed the proliferation of fungi on the surface of the samples exposed to incubation conditions 2 (with water blade), however, its use was not enough to completely inhibit its proliferation.

The results found in this study show the same pattern of behavior found by Laborel *et al.* [13].The research evaluated the biological growth under different environmental conditions in earth-based materials, with and without the addition of bio-aggregates. The inoculum used was *Aspergillus brasiliensis* and the samples were incubated for 12 weeks at 76, 84 and 93% RH and 30 °C or 20 °C. Results showed that earth-based materials were more sensitive to fungi when enriched with 3% plant aggregates. Fungal growth was observed in soil material containing bioaggregates after 4 weeks of exposure (93% RH and 30 °C). While for the raw earth material, under the same conditions, growth was observed after 8 weeks. It should be noted that, despite the samples having a pH of 7.8, in the present study a fraction of biomass equal to 45% was used, and an extreme incubation condition (with water depth) justifying the susceptibility to biological growth, even for waterproofed samples.

Santos *et al.* [32] evaluated the biological susceptibility of mortars and the pH values obtained were between 8.5 and 9.6. The authors found that the partial replacement of fine sand by PCM reduced the pH of the mortars, the addition of fibers did not provide relevant changes, while the addition of lime increased the pH of the mortars. In fact, regardless of the presence of fibers or PCM, the addition of lime and the consequent increase in pH prevented, although not completely, the colonization and development of fungi. In this research it can be seen that the presence of the waterproofing agent used did not significantly change the pH value of the evaluated BBC, this fact is related to the chemical characteristics of the waterproofing agent used and also to the volume used for the surface coating of the samples.

In addition to the analyzes presented here, colonies that grew on the surfaces of the samples were collected. The collection with a swab will allow identifying if there was growth of other fungi, in addition to the *A. niger.* Previously sprayed on the bio-concrete.

4 Conclusion

This work evaluated the resistance to the tolerance of bio-concretes to the susceptibility to colonization by a fungus species. The BBC was stored in two conditions: (1) High moisture (23 ± 2 °C; 74 ± 2% RH) and (2) Extreme moisture (23 ± 2 °C; 98 ± 2% RH). Based on the results, the following conclusions may accordingly be drawn:

(1) In the condition of high moisture, it was not possible to observe the proliferation of fungi on the sample surfaces, as well was no change in their appearance or color. This can be explained by the moisture and temperature levels used, which did not provide a favorable environment. Furthermore, the fact that the BBC has an alkaline pH (11.5 and 11.3), the proliferation of fungi on the surfaces is hindered.
(2) For the extreme moisture condition, the growth of microorganisms was favored, being intensified by the high volume of bio-aggregates used in the bio-concrete, which consists of a greater supply of nutrients for the proliferation of fungi. In addition, there is the hygroscopic capacity of the material as well as the greater roughness of samples due to the procedure used during preparation and cutting. Regarding appearance and color, the photographs showed that the natural BBC (1-N) indicated greater saturation, color change, loss of brightness, and greater growth of microorganisms when compared to samples with waterproofing (2-N). The extreme moisture condition (98 ± 2%) provoked the greatest proliferation of microorganisms due to growth at different locations on the surface of the sample (from 7 to 14 days as well as from 14 to 28 days).

Thus, the results of this research show the importance of evaluating the biological durability of bio-concretes in different exposure conditions and treatments. Despite the high volume of biomass in the composition of bio-concretes, those studies can prove that BBC is a versatile material that can be used safely and without harm to human health. The method proposal can be used to evaluate the biological resistance of other materials, since the choice of a standard and reliable method is a problem encountered by other authors, as reported in this article. With these advancements, it is possible to indicate in which uses and applications the bio-based materials are more appropriate. Tests are easily reproducible as they do not require advanced equipment.

It is important to highlight that the present study is in progress to evaluate the biological durability of bio-concrete over time, at advanced ages.

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