Research Directions: Biotechnology Design

A 3d printable Biopolymer Composite incorporating Kombucha SCOBY:

Towards a locally adaptive architecture using living biomaterials

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Abstract

In this paper we investigate the integration of living bacteria into a 3D printable biopolymer composite for architectural applications. We specifically focus on incorporating cellulose-producing bacteria to grow 3D bacterial cellulose in-situ as a localised protective skin on a printed geometry. To produce and test large-scale samples, the research relaxes controlled lab conditions and pursues a more resilient culturing method, a kombucha culture or SCOBY.

This paper reports exploratory experiments that assess the compatibility of the living bacteria with the biopolymer composite matrix, the printability of this new material, and the ability to reactivate the bacteria post-printing to grow 3D bacterial cellulose films on the samples. Our findings show successful bacterial reactivation and significant bacterial cellulose growth. These findings contribute to the field by demonstrating a novel approach to creating printable engineered living materials, and by identifying their potential to enable localised adaptation in large scale architectural elements.

Introduction

In Architecture and the construction industry, efforts to shift to a circular bioeconomy for the built environment are increasing interest in regenerative bio-based materials. Beyond their potential to substitute out more carbon intensive materials, bio-based materials offer an opportunity to re-diversify the fundamental materiality of buildings by introducing new unique functionalities not possible with other architectural materials. 3D printing with bio-based materials to create architectural elements connects these opportunities to a large geometrical design space and architectural performative drivers.

In this research we incorporate living cellulose-producing bacteria into a 3D printable biopolymer composite material. Bacterial Cellulose (BC) is a natural and biodegradable biomaterial which shares many characteristics with plant cellulose while offering additional benefits such as good tensile strength, high water-holding capacity, and responsive growth. The research aims towards growing 3D BC in-situ as a localised protective skin on a printed geometry. The living bacteria is integrated into the paste material and stages of dormancy and reactivation are evoked.

Our research sits within a growing interest in additive manufacturing of biopolymer composites (Nicholas et al. 2023a, Ramsgaard Thomsen et al. 2022, Dritsas et al. 2020), and appreciation for the new modes of repair and adaptation they might embed (Nicholas et al. 2023b, Nicholas et al 2024). Our research goes beyond state of the art by incorporating living organisms into the printed material, adding biologically responsive properties to achieve locally responsive material adaptation, and responding to the developing field of Engineered Living Materials (ELM)(Nguyen et al. 2018).

In this paper, we report on exploratory experiments that 1) test the compatibility of the living bacteria against each constituent material of the base biopolymer composite, 2) test the printability of this new material in an architectural scale element, and 3) reactivate the bacteria and evaluate the growth of bacterial cellulose films at the surface of the samples.

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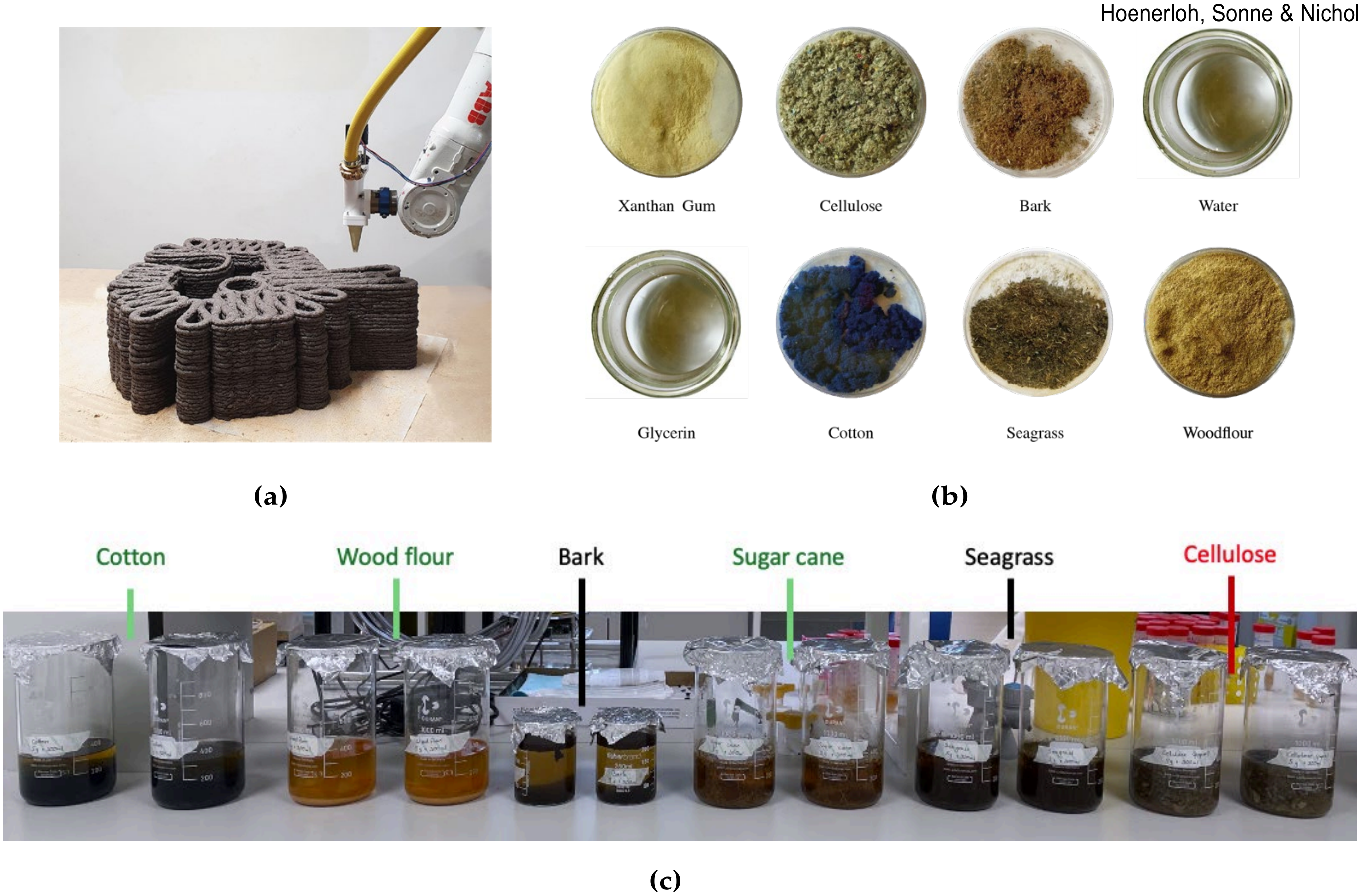

Figure 1. (a) Robotic printing of paste material, (b) Paste ingredients in their raw form, (c) testing the compatibility of each paste material with the BC kombucha culture (Image credits: authors).

Material and Methods

Material

The biopolymer composite combines a binder mixed into a solvent and reinforced by fibres and fillers sourced from side and waste streams materials to create a composite. This recipe is fully biodegradable under natural conditions and producible at large scale, making it an attractive architectural material (Rech et al. 2022). We use a xanthan gum biopolymer, a bio-based plasticizer glycerol, and a calcium chloride crosslinker. Multiple and differing fillers can be added, enabling a flexible recipe that can be tailored to locally available material resources. We have developed variations of this recipe including the following constituent materials (Figure 1 (b)):

- A flocked cellulose insulation derived from recycled paper, consisting of an inhomogeneous mixture of paper pieces and fibres from 1 µm to 2 cm,
- A fine 0.5mm wood flour,
- A recycled cotton milled to 0.2 mm
- Beach-cast seagrass milled to 0.2mm
- Tree bark milled to 0.2mm

In addition to the materials used to create the based biopolymer paste, living bacteria in the form of hydrated Bacterial Cellulose (BC) are added. BC is a biopolymer that is produced as by-product of a fermentation process of certain aerobic bacteria, particularly those belonging to the genus *Gluconacetobacter*. Beneficial characteristics, such as high tensile-strength, good water-holding capacity, as well as biodegradability and compatibility (Wang et al. 2019), have sparked a wide interest in the use of this living biomaterial. BC grown in controlled lab conditions from a single bacterium strain can offer very high purity and fine crystallinity, which makes it of particular interest in the medical field (Portela et al. 2019). This method of pure culturing, however, requires a sterile work environment as well as specific lab-produced nutrient media to feed the bacteria. For architectural application, alternative approaches are required. In this research we investigate a kombucha culture or SCOBY, traditionally used to brew a fermented tea beverage. SCOBY is composed of multiple bacteria and yeasts which produce BC through symbiotic processes. The diversity of microorganisms within the SCOBY makes this culturing method more resilient towards possible contamination, allows a large-format production of BC (thr34d5 2019), and broadens the spectrum of possible nutrient sources from household items to industrial waste streams (Tsouko et al. 2015).

Without external interference, BC grows in flat sheets on the surface of a nutrient medium to a thickness of up to a few centimetres. This is referred to as static culturing. Once dried, the material changes from an opaque and gelatinous to a more transparent and leather-like appearance.

Current research on BC as material on a macro scale predominantly focuses on post-processing it into the desired form or application after its living growth phase. Examples of this can be found in the fashion and food industry (MakeGrowLab 2019; Ng & Wang 2016; Domskiene et al.

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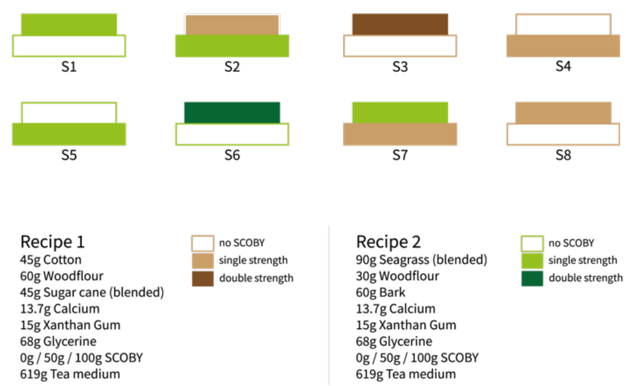
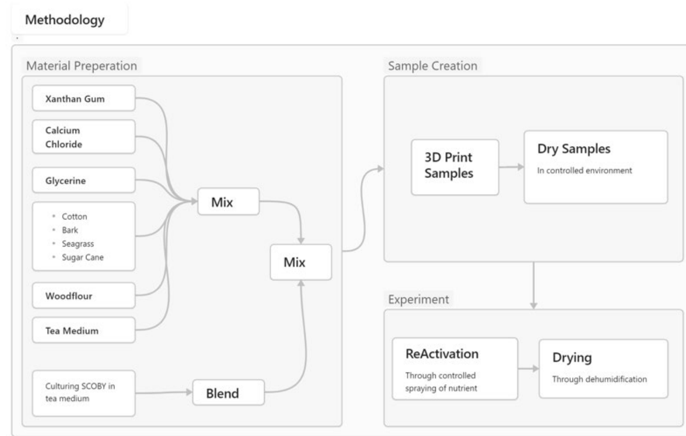


(a)



(b)

Figure 2. (a) Layer combinations of paste materials, (b) Diagrammatic visualisation of methodology

2019). BC has also been explored as composite material, for example as a food source (Elsacker 2021) or as living component (Hoenerloh et al. 2022). While first steps have been made to test the viability of BC as architectural element of a façade (Morrow et al. 2015), this early-stage research is one of few.

Material Compatibility

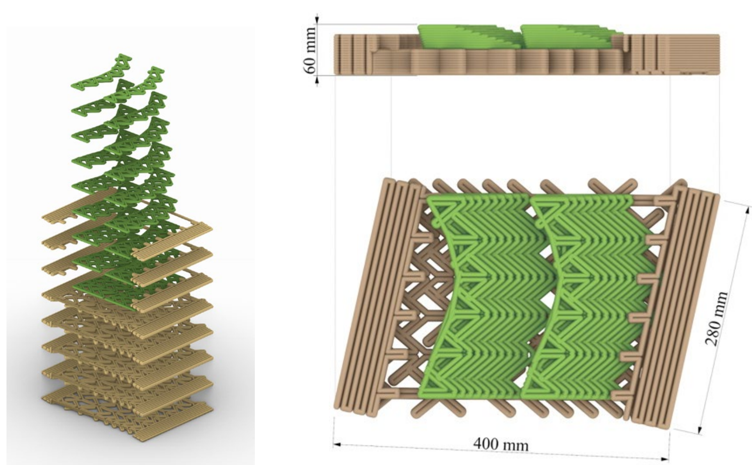
An initial experimentation with potential paste materials and SCOBY was set up to test their compatibility. The aim of the experiment was to determine whether BC would grow in the presence of the paste material and whether there were any changes in the growth behaviour compared to a control sample.

For the setup, 300ml of tea-based medium (930ml /L water, 70ml/L apple cider vinegar, 80g/L sugar, and 1 black tea bag (Kretzer, n.d) were mixed with 5g of dry paste material (cotton, wood flour, bark, sugar cane, seagrass, or flocked cellulose) and incubated with 15g SCOBY and 15ml liquid inoculum inside 1000ml Duran glass beakers. These static cultures were left to grow for 10 days and documented daily via photography (Figure 1 (c)).

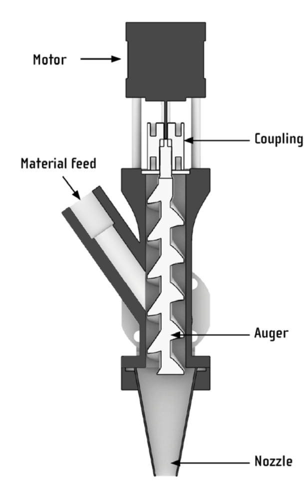
Apart from the cellulose floc, which was sourced from an insulation product that included borax, all paste materials showed good compatibility with the SCOBY and BC growth occurred on the surface. The cotton, woodflour, and sugar cane samples showed no inhibition of BC growth, while the bark and seagrass grew contamination after 8 and 7 days respectively.

Paste Preparation

The paste materials were prepared on a sterilised benchtop in a biodesign lab. The bark and seagrass were autoclaved for 15 minutes at 121 degrees Celsius before being added to the mixtures to reduce the risk of contamination. To prepare the material, a xanthan gum biopolymer was mixed with a glycerine plasticiser and specific fillers, chosen from the results of the compatibility test with the kombucha SCOBY. Recipe 1 used cotton, woodflour, and sugar cane, while recipe 2 used a combination of seaweed, woodflour, and bark (Figure 2 (a)). Three versions of each recipe were prepared: one with 50g of SCOBY (single strength), 100g of SCOBY (double strength), and no SCOBY. Before adding the SCOBY to the paste, it was blended with 200g of tea medium until it formed a smooth paste, making it easier to distribute



(a)



(b)

Figure 3. (a) Dimensions and toolpath of 3D-printed test geometry with two different paste materials, (b) section of extrusion printhead and nozzle

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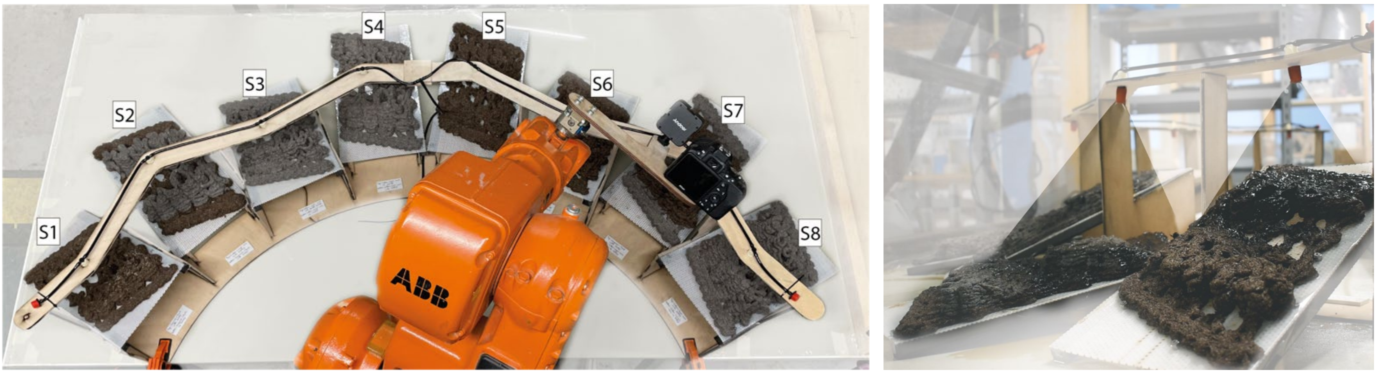

(a) **(b)**

Figure 4. (a) Top view of dried samples in reactivation setup with positioning of the camera through the robot arm, (b) angle of nutrient spraying (Image credits: authors).

uniformly throughout the paste. A total of 6 material recipes, 2 base materials with 3 versions of each, were produced and used to print the 8 samples.

Once prepared, the composite slurry was transferred into a sterilised 2L reservoir which was fitted to the print setup. render the bacterial cellulose dormant after printing, they were placed in an enclosed chamber with a dehumidifier for 10 days. After the drying process, the samples are hardened and handleable.

Methods

The experimentation is divided into three stages of paste development, sample creation, and the reactivation phase (Figure 2(b)). All samples are printed with the same geometry of dimension 400mm x 280mm and a height of 60mm. This equates to 5 'base' layers and 7 'top' layers (Figure 3, (a)).

The underlying geometric logic aims to make a porous sample that dries quickly and maximises surface area. Additionally, the lattice geometry of the base is designed to create pockets that retain nutrient and minimise runoff, while the geometry of the top is sloped to create areas that are occluded from direct nutrient spray. On the base, the lattice geometry varies in density in both x and y directions. This aims to test the ability for the bacterial cellulose skin to create bridges during the reactivation and growth phase. Different materials are assigned to the base and the top from the recipe matrix (Figure 2 (a)).

During the drying process water evaporates from the samples, which shrink by approximately 25% in height and lose approximately 60% of their weight. Tracking and analysis of this behaviour, performed on the base bio-polymer composite only, is reported in Rossi et al. 2021.

The samples are printed using a custom nozzle and 2L polycarbonate reservoir attached to a Universal Robot UR16e. From the reservoir the material is fed pneumatically to the printhead under 1.5 bar pressure and into a lead screw that controls the flow of material through the nozzle. The lead screw also allows the print to start and stop as required. A nozzle diameter of 5mm is used to avoid clogging and to increase overall exposed surface area (Figure 3, (b)). The samples are printed onto a perforated acrylic bed to increase airflow and prevent moulding during the drying process, as well as allow for easy sterilisation in between prints.

Reactivation Setup

After drying, the samples are arrayed under a spraying mechanism (Figure 4 (a)). They are automatically sprayed at 30min intervals for 2 seconds with a tea-based nutrient mixture. The spraying period lasted 42 days, and over each 24-hour period the collected samples received 2L of nutrient.

To vary the area of samples that experienced direct nutrient spray or nutrient run-off, the samples were positioned at two different angles of incidence to the spray nozzle. The first angle of 25 degrees resulted in a spray zone covering approximately 50% of the upper surface of each sample, and a zone experiencing run-off of approximately 50%. The second angle of 10 degrees resulted in a spray zone covering approximately 70% of the upper surface of each sample, and a run-off zone covering approximately 30% (Figure 4 (b)). During the experiment, intermittent clogging of the spray

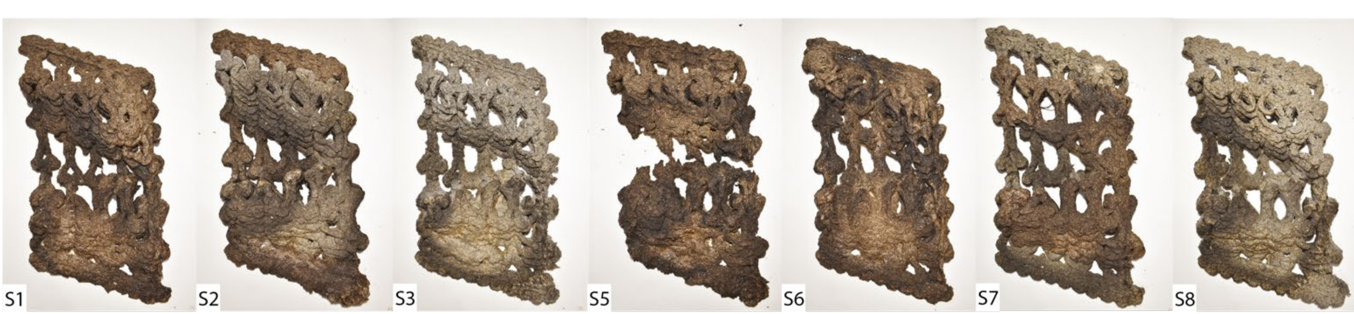

Figure 5. All samples after the reactivation phased and with fully dried bacterial cellulose. Sample 4 was removed due to contamination (Image credits: authors).

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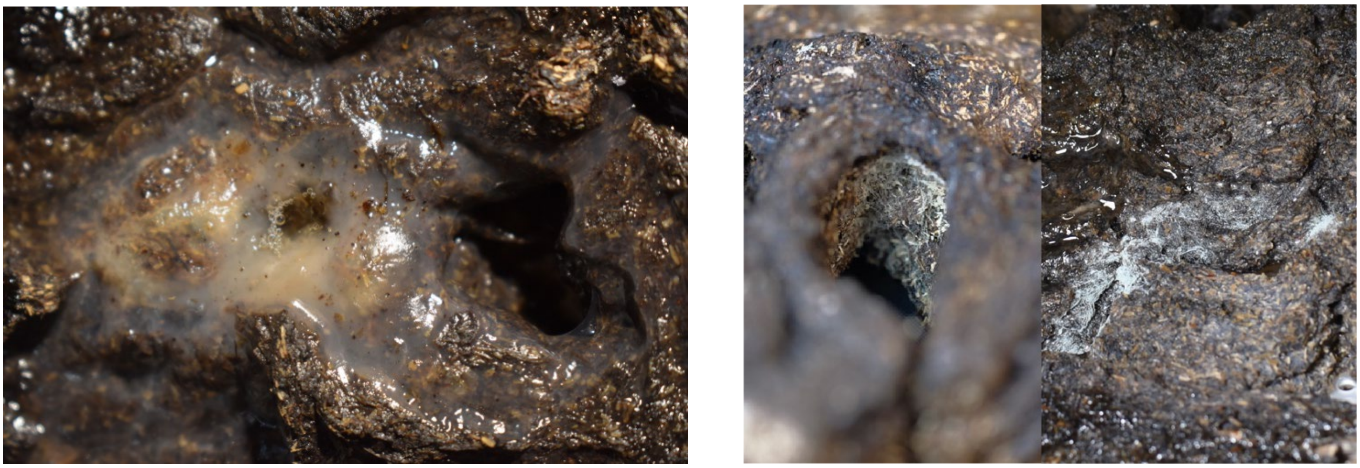


(a) (b)

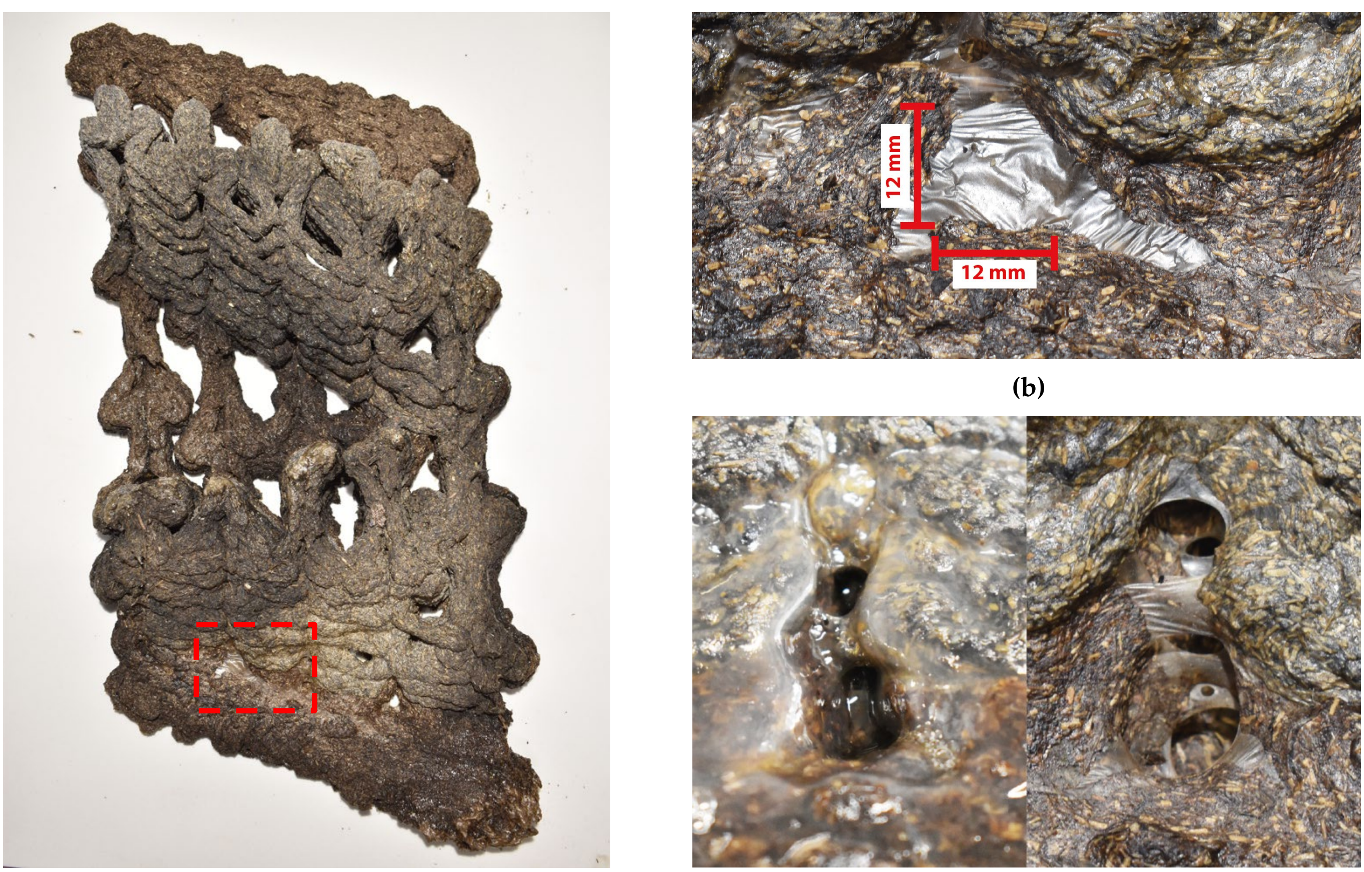
Figure 6. (a) BC growth on sample 5 after 42 days, (b) Examples of contamination occurring on samples no. 4 and 7 (Image credits: authors).

system by sugar led to inconsistent spraying, and a concentration of nutrient deposition more directly under the spray nozzle. The experimental period was stopped when it was felt sufficient growth had been attained.

During the experimental period an image-based dataset was generated, recording the ongoing state of each sample and the experimental results. This dataset can be accessed at https://doi.org/10.5281/zenodo.10118361.

Results and Discussion

A reactivation of the bacteria was possible in every printed geometry irrespective of paste ingredients and SCOBY strength. First signs of growth occurred after 10 days and were visible as thin translucent layer on the paste in direct proximity to the spraying nozzle. After 15 days, sample 5 showed concentrated opaque BC growth which continued to mature for the duration of the experiment (Figure 6 (a)). The



(a) (c)

Figure 7. (a) Top view of sample no.2 with highlighted area of close-ups, (b) largest area horizontally bridged in dried state, and (c) vertical bridging of BC in wet and dry state (Image credits: authors).

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(a) (b)

Figure 8. (a) Top view of sample no. 3 with highlighted area of close-up, (b) large area of print covered by BC in hydrated (top) and dried (bottom) state (Image credits: authors).

two main factors influencing the BC growth were the geometry of the print and the position of the spraying nozzle. Flat areas and indentations in the geometry allowed for the accumulation of sprayed liquid which created growth conditions similar to static cultures.

Samples 4 and 7 grew contamination in the form of white speckles, concentrated in areas of low airflow and liquid pooling (Figure 6 (b)). Sample 4 was removed after 10 days to reduce the contamination risk of the remaining samples. Small areas of contamination were wiped off sample 7 using ethanol. In addition to the BC growth, the formation of a sugar crust was observed in areas of the print further away from the spray nozzle.

Comparison of Hydrated and Dried BC

Sample no. 2, with top layer recipe 1 and bottom layer recipe 2 (Figure 7), showed BC growth in multiple areas within the spray perimeters. These ranged from a thin transparent film <1mm to opaque BC biofilms up to ca. 7mm bridging over indentations of the geometry. The largest BC bridge formed over an area of 12 x 12mm in a near static condition due to liquid pooling and, once dried, formed a suspended film over the 'hole' in the print design. The close-up image in Figure 7 (b) shows the close connection between the print paste and BC.

The bridging capabilities of the BC are not limited to horizontal growth as can be seen in Figure 7 (c) in a vertical cavity of the print. This type of multi-axis bridging formed through a combination of pooling and progressive layering of BC on either side through the repeated spraying. This layering behaviour of BC has previously been explored through manual dip-coating (Rühs et al., 2020) and during batch feeding inside a rotating disc bioreactor (Serafica 1997). Picture 7 (c, left) shows the BC in a wet state, with the opaquer colouring along the sides of the opening indicating more mature development of the biomaterial.

A different form of BC growth was observed in sample 8 and sample 3 (Figure 8). Directly underneath the spray nozzle an 80 x 50mm large patch of mature BC developed, with a thickness of up to 2mm. Figure 8 (b) shows the position of the BC predominantly in the creases between individual paste beads created through the additive layering of the robotic extrusion. During the hydrated state of the BC, the biomaterial flattens the surface of the print by filling in the creases. The opaque white colour indicates a higher level of BC maturity compared to areas with a transparent biofilm (Klemm et al 2001). During the drying process, the BC shrinks onto the paste beads which exposes the creases in the layered geometry once again. The area of the dried BC can easily be distinguished by the reflective coating it creates.

Future Vision

While biopolymer composites have significant potential for architectural interior and exterior applications, the biodegradability of this material class means that they are not as robust as conventional fossil-based materials. In particular, over-exposure to moisture can easily cause physical degradation leading to eventual failure. By upgrading this biopolymer composite into a living material through the inclusion of BC, it gains the ability to autonomously protect itself from damage by growing a responsive skin, demonstrating environmental responsiveness beyond the reach of existing synthetic materials. The embedding of self-adaptation and maintenance capabilities into biopolymer

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composites and 3d printed bio-based architectural elements would significantly increase their application possibilities within architecture, especially into semi-protected facade and panel elements (Figure 9), by reducing the costs of maintenance and repair while still maintaining the material's underlying attraction of full biodegradability.

The provision of nutrients re-awakens the BC within the biopolymer composite, which forms a localised biofilm that grows upon and bridges across the underlying material. The initially hydrophilic properties of the BC keep moisture near the surface of the material, and after drying the film reduces any subsequent moisture movement into the underlying 3D printed architectural element. The highly localised nature of this response transforms an initially undifferentiated architectural element, which can be designed and produced without knowledge of its future placement and orientation, into an element with highly specific and localised properties. In contrast to the vast majority of architectural materials, which are produced generically and engineered to minimise any variance in their properties with time, large scale 3D printed architectural elements produced using this material would have the inherent ability to adapt to local changes in their environment and to improve their situated performance over time, specific to their in-situ orientation and conditions of exposure.

To produce and test relatively large-scale samples, this research relaxes controlled lab conditions and pursues a more resilient culturing method. The environment of a robotics laboratory, where the samples were produced, dried and re-awakened, was clean but not controlled, and closely approximates the manufacturing environments associated with the higher volume and larger scale production required for architectural production. This concept of a 'dirty ELM' offers an alternative to the current paradigms for responsive materials, which require sterile lab conditions and microbiological tools to be fabricated. In demonstrating the viability of this idea for a 3d printable biopolymer composite incorporating living materials, the presented work therefore challenges the way in which we can think about the upscaling of functional living materials and the creation of hybrid bio-based architectures.

Figure 9. Envisioned potential application of the novel biohybrid material (Image credits: authors)

Author Contributions

The manuscript was written with the contribution of all authors. **All authors have approved the final version of the manuscript.**

Nicholas, P. Project conceptualization, methodology, writing – original draft, reviewing and editing, supervision, project management.

Sonne, K. Project conceptualization, methodology, experimental setup and data collection, writing – original draft, reviewing and editing.

Hoenerloh, A. Project conceptualization, methodology, materials testing and development, experimental work, writing – original draft, reviewing and editing.

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Competing interests. The authors have no conflicts of interest to declare for this publication.

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