BIOMANUFACTURING OF ARCHITECTURAL PROTOTYPES WITH CYANOBACTERIA

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Abstract. Cement and concrete production are responsible for nearly 8% of the world's annual emissions of greenhouse gas carbon dioxide. Biodesign can potentially address this challenge in architecture by integrating living materials in design processes and enhancing the ecological performance of materials. As part of an interdisciplinary approach between architecture and microbiology, this research outlines a systematic workflow consisting of pre-fabrication, fabrication, and post-fabrication phases. The workflow leverages additive processes based on biological data and utilizes cyanobacteria's output capabilities towards architectural production. Cyanobacteria through their photosynthetic process are able to absorb CO2 and induce calcium carbonate (CaCO3) precipitation, the main ingredient in limestone and cement. This paper focuses on the pre-fabrication phase and develops material protocols for designers. It examines the compatibility of two bacterial strains in order to formulate a biomixture suitable for integration in an additive biomanufacturing process.

Keywords. Biodesign, Additive Manufacturing, Biofabrication, Sustainability, Cyanobacteria, Carbon Dioxide fixation.

1. Introduction

Building materials and their production play a significant role in increasing industrial waste, inefficient use of energy and contribute to emissions of greenhouse gases (GHG). Subsequently, the Architecture, Engineering and Construction (AEC) industry, is placing major emphasis on developing alternative sustainable design approaches to address such environmental urgencies (Yu al., 2022). In this context, the emerging field of biodesign incorporates living organisms in the fabrication processes of construction materials in order to develop sustainable, functional, and degradable applications (Myers, 2012). Such materials present biological properties that can enhance the performance of building materials as they inherit new abilities such as carbon dioxide fixation, degradability, recyclability, and adaptability to the environment (Qiu et al., 2021).

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New emerging opportunities of utilizing biological processes to modify the properties of surfaces are studied within the fields of microbiology, geochemistry and engineering (Dejong et al.,2010). Microbial carbonate precipitation (MCP) is one of the biological processes that are investigated in relation to soil improvement. Recent studies have shifted their focus towards examining MCP in mortar and sand surfaces, and has proved to enhance solidity and stiffness (Dejong et al.,2010). Within architecture, recent precedents have tried to utilize the effect of MCP on sand and integrate living bacteria within casting processes in order to develop new living building materials (Qiu et al., 2021). It is our aim to expand on this approach towards a co-fabrication workflow that integrates living bacteria within an additive biomanufacturing process. By constructing a workflow for harnessing living organisms' biological data as an input in the architectural co-fabrication process, we could potentially bridge the gap between microbiological and architectural processes to produce printed biological blocks (PBB). Developing such a systematic design approach entails understanding and designing the biomixture for printing on the two scales. Biologically (micro), as a habitat for the bacteria which provides a biocompatible microenvironment. Architecturally (macro), as a printing medium suitable for relatively large extrusion fabrication processes and capable of maintaining form integrity.

In this paper we focus on the development of maintenance protocols and material adaptation of the biomixture at the biological scale towards integration in an architectural co-fabrication workflow (See figure 1). The developed biomixture includes living cells of the photosynthetic cyanobacteria, agar medium enriched with nutritional solution and sand.

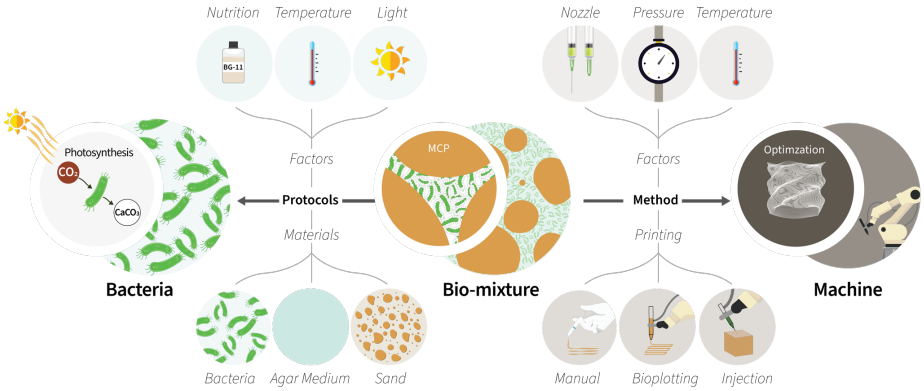

Fig. 1. Diagram, Research workflow for adapting the biomixture to both the bacteria and the machine through environmental, material and fabrication parameters.

2. Cyanobacteria

Recent research has demonstrated diverse implementation of living organisms such as algae and mycelium in design applications, and similar developments are currently being examined with living bacteria. Owing to their diverse metabolism, bacteria demonstrate a diverse array of activities and biological products (Myers, 2012).

Cyanobacteria, a robust and abundant photosynthetic microorganism, are considered to be a part of the most influential organisms in the biosphere since they have important roles in the global carbon cycle and oxygen production (Mehdizadeh et al., 2022). Along with other microphytic communities, such as algae, cyanobacteria are also a critical component in the desert ecosystem worldwide since they modify the surfaces they occupy as part of the biological soil crust (BSC) formation. Experiments have shown that soil stability and carbon fixation can be increased as a result of bacterial growth and a higher biomass (Rozenstein and Karniele, 2015). Moreover, Examining MCP within bio-mediated soils, has suggested that the formation of calcite precipitation reduces the pore space between the sand particles and binds the particles together which results in the increase of the solid content (Dejong et al., 2010). Cyanobacteria are able to produce carbonate minerals in shape of calcite-crystals (CaCO3) by capturing and converting CO2 through the process of photosynthesis (Kamennaya et al., 2012).

Therefore, based on the substantiated relation between cyanobacterial activity, soil stability and carbon fixation, two strains of two genera of cyanobacteria (Synechococcus sp. strain PCC 7002 and Synechocystis sp. strain PCC 6803) were selected for experimentation in our research. Both bacterial strains of the selected genera are of biosafety level 1 and therefore, they do not pose danger to their surroundings. For this reason, these microorganisms are not only suitable for our research aim but also safe for implementation in human environments.

3. Co-Fabricating with The Living

Designing sustainably through additive manufacturing can manifest in many advantages such as decreasing wasteful processes and reducing energy consumption. In addition, it enables designing with new materials and designing with the ability to reuse, repair and remanufacture (Mehrpouya et al., 2021).

Within architectural research, designers are adopting new developments in software and fabrication workflows, such as Computer-aided design to Computer-aided manufacturing (CAD to CAM), to better interact with the potential of material properties, optimize geometries and generate complex properties (Cohen and Barath, 2023; Weissenböck, 2015; Mehrpouya et al., 2021).

Biofabrication methods are also emerging and are being applied in a diverse spectrum of fields such as regenerative medicine, tissue engineering (Harley et al., 2021), and construction industries have also recently started adopting such processes (Andréen and Goidea, 2022). Biofabrication relies on three main disciplines; Biology, Mechanical engineering, and Material science (Mironov et al., 2009). It emphasizes the importance of environmental conditions (such as habitat environments) and addresses the living materials properties and spatial organization (Kalantari et al., 2017). In relation to bacteria, habitat geometries play a significant role in increasing/decreasing the bacterial growth and biomass as recent research concluded that sharp angled channels limited the bacteria's mobility and reduced the bacterial biomass (Arellano-Caicedo et al., 2021).

Recent developments concerning the built environment include; using bacteria in the production of architectural responsive panels (Birch et al., 2021), utilizing bacteria

in the creation of probiotic tiles (Beckett, 2021) and living building materials (Qiu et al., 2021) and, harnessing sprayed living cells for the solidification of a textile column (Beyer et al., 2019). With that, few have succeeded in breaking through the limitations of the current fabrication workflows (Goidea et al., 2022).

In our research we address this gap by harnessing cyanobacteria's photosynthesis mechanism (calcium deposition) in the formulation of biocement through a direct relation to surface geometry. Understanding that light is a critical factor in the metabolism of cyanobacteria, we are able to design specific geometries taking into consideration the surface area and light orientation of the habitat microenvironment in order to regulate light penetration. This potentially allows control over the cell's distribution and biomass inside the habitat geometry which could result in an increase of MCP. However, as the biological and architectural processes perform at different scales, multiple effects of different factors need to be addressed as part of an integrated fabrication workflow. For example, on the micro scale, bacterial growth speed, biological production of metabolites and environmental conditions are crucial. On the macro scale, shape fidelity, stability and scale are important (Goidea et al., 2022).

3.1. GAPS IN FABRICATION METHODS

Designing with living materials introduces new constraints to architecture. The most notable are the specific environmental conditions of the living organism and maintenance of viability. We therefore address this by studying the gaps between architectural and biological processes in relation to additive methods on the environmental, material and mechanical levels.

Environmental - By learning the behavior and growth of bacteria we can better engineer living materials and develop analogs based on their growth patterns, adaptations, and development (Kalantari et al., 2017). Contrary to the construction world, bacteria are normally treated in supervised lab conditions through specific processes that aim to provide safety for the living cells from toxic substances. When incorporating living cells into fabrication processes, taking them out of the lab environment, many challenges arise due to toxic materials and limiting conditions such as high temperatures and the lack of needed nutrition (Schaffner et al., 2017). Therefore, such integrations may require custom-made fabrication setups that cater not only to the fabrication process but also rely on the environmental needs and behavior of the living material and, cater to its biological properties.

Material - Most of the fabrication processes involve one or more steps that are deadly for the living cells (Lim and Thomsen, 2021). Recently researchers have tried to address these issues and offer different approaches to fabricating with bacteria. For example, some suggest embedding the bacteria in hydrogels which provides the cells with an ideal microenvironment that keeps them alive (González et al., 2020). Using hydrogels as a medium to create a spatial organization of living cells is a commonly applied biomanufacturing method that allows scientists to create 3D functional scaffold with customized properties (Persaud et al., 2022). This introduces new materials with new rheological properties that are far more sensitive to the fabrication factors than materials normally used in architectural fabrication processes, such as concrete and cement. Moreover, it introduces additional procedures such as adapting the gel biocompatibility to the cells, developing protocols to reach a suitable viscosity or

possibly needing a physical or chemical crosslinking throughout or after 3D printing (Persaud et al., 2022).

Mechanical – even when embedded in a safe environment, cells are still exposed to some deadly fabrication factors. Friction, shear stress caused by extrusion forces, compression shear stress caused by the nozzle's shear field and using a screw driven 3D bioprinter which could decrease cell viability. Therefore, the 3D printer and the fabrication set up should take into consideration mechanical parameters and provide certain features such as: enabling control over the printing head and printing bed temperature, maintaining the temperature throughout the printing process, compatibility of the printing head with different nozzles and an easy sterilization procedure (Persaud et al., 2022).

Understanding the challenges and requirements for the implementation of living cells in an additive fabrication process we formulated a fabrication framework with a clear aim to potentially upscale the biological activity to facilitate the fabrication of architectural components.

4. Suggested Co-fabrication Framework

Adopting a design approach that works in a plurality of scales (biological and architectural) and addresses material, environmental and mechanical considerations, we constructed an integrated workflow consisting of three phases, pre-fabrication, fabrication and post-fabrication (See figure 2).

The pre-fabrication phase focuses on the micro scale as its main aim is to ensure bacterial activity within different media. It includes all processes needed for maintaining and preparing the biomixture. Much like lab procedures and biofabrication processes it works in a linear way that allows growing the bacteria, optimal acclimation to the growth environment conditions and embedding of the living cells into a new biocompatible micro-environment. In addition, this phase includes designing the printing tool path while strengthening the relation between geometrical properties (porosity, density, patterns) and needed environmental conditions for enhancing cell growth. For example, in aim to prolong cell viability we could design architectural geometries with increased surface area for optimized light exposure.

The second phase, the fabrication, examines the interrelation of design features, the viability of cells and their behavior. It observes the effect of the macro scale on the micro scale through mechanical (flow rate, pressure, printing speed) and printing environmental parameters (light exposure, temperature). The post-fabrication phase demonstrates bacterial activity on both the micro and macro scale simultaneously. It includes the processes needed for maintaining structural integrity and spatial bacterial growth such as incubation. In this phase the intention to bridge the gap between

architectural and biological processes is manifested and tested.

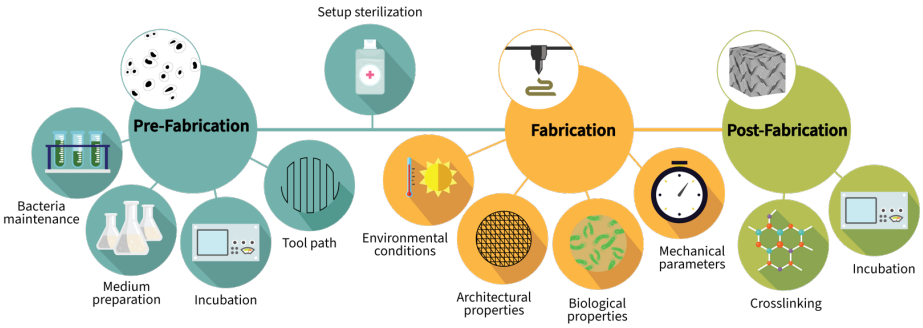

Fig. 2. Diagram, Suggested co-fabrication workflow for an integrated biological architectural fabrication process with cyanobacteria.

5. Developing the Biomixture

Experimentations on the biomixture, the optimal conditions to formulate biocement, and an initial prototyping of architectural habitat geometries were conducted in a Design Biolab set up and equipped for conducting biological experiments (i.e., growth medium, growth chamber targeted to meet with microbial requirements, basic lab equipment and sand).

5.1. MAINTENANCE PROTOCOLS

For the purpose of growing the photosynthetic bacteria an incubator with adjustable fluorescent light, temperature features and sufficient air-circulation was selected, providing ideal conditions for the cyanobacteria. Moreover, we developed protocols for growing and proliferating the cells that could potentially ease the appliance of biological maintenance procedures for designers.

The protocols allowed us to gradually grow the bacteria in different methods, starting from growing cultures in nutritional solutions to embedding them in a new habitat, petri dishes containing sand. Following preliminary experiments, we found it more efficient to grow the cyanobacteria in tubes containing nutritional solutions. In order to maintain the bacterial growth, the re-culturing protocol was applied weekly. First, new tubes containing fresh nutritional solution of deionized waters (DIW) and BG-11 (provided by sigma) would be prepared in advance. Second, the bacterial cultures would be subjected to shaking to achieve a homogenous cell distribution within the solution. Third, the bacteria would be re-cultured within the new nutritional tubes in a concentration of ~10^6 CFU/mL of cyanobacteria at a ratio of 1:7 (cells: medium) and then incubated at a temperature of 22 +/- 1 °C.

5.2. MATERIAL STUDY EXPERIMENTATIONS

The medium is a nutritional and safe environment in which the bacteria grow (González et al., 2020) and therefore, it is inseparable from the design process. While gelatin is becoming an increasingly desirable medium for 3D bioprinting and was used

in a recent precedent for casting an LBM (Qiu et al., 2021), agar proved to be a more promising candidate for our research purpose. In relation to printability and cell growth, gelatin introduces numerous challenges such as rapid crosslinking leading to short duration of printing time and nozzle clogging, therefore, effecting cell growth and function as well (Tan et al., 2020). Agar demonstrates more suitable characteristics for the growth of cyanobacteria. Its porous structure eases the exchange of nutrition, oxygen and waste. It is highly biocompatible, biodegradable and enables the proliferation of cells (Salati et al., 2020).

Consequently, the biomixture we initiated our experiments with consisted of living bacterial cells of the Synechococcus sp. strain PCC 7002 and Synechocystis sp. strain PCC 6803, agar (growth medium) and three types of sand of different particles size. The thin sand particles range from 0.1-0.6 (mm), Quartz sand type 1 (QS1) particles range from 0.6-0.84 (mm) and type 2 (QS2) from 2.5-3.5 (mm). In order to tailor the biomixture for both the viability of the bacteria cells and the fabrication process we first examined the effect of different material ratios and environmental conditions on bacterial growth. For this purpose, the experiments were divided into two phases; the first examines the non-living materials ratios (i.e., agar and sand) in order to develop protocols for a printing mixture capable of form self-sustaining. The second examines the bacterial growth within different ratios of the biomixture.

5.2.1. Non-living mixture.

The non-living mixture refers to the mix of medium and sand only. First, the agar medium was prepared at a 1:1 agar DIW ratio. Afterward, different sand concentrations were added to the medium while it was still in a liquid state. The non-living mixtures at the ratios of 0.3:1, 0.5:1, 0.7:1 and 1:1 demonstrated different material behavior in terms of gelation time and sand particles distribution. The 1:1 ratio resulted in a more homogeneous mixture and formed a thin layer in which the sand particles were encapsulated. Based on the mixture's initial results we concluded that agar could potentially provide a temporary micro-environment that encapsulates the sand particles while enabling biological cell activity and adhesion of the sand particles through MCP (See figure 3).

5.2.2. Living biomixture.

Relying on the non-living experiments, we applied the developed protocol on a living biomixture consisting of agar, sand and cyanobacteria. In the experiments conducted simultaneously on both bacterial strains, we relied on their inherent green pigmentation, to examine and document their growth. Throughout the experiments, different ratios of bacteria, medium and sand were tested in order to define the effect of different environmental conditions such as distance from light source and temperature on the bacterial growth.

Biomixtures were prepared at two different bacteria concentrations, low concentration (LC) of ~10^6 CFU/mL and high concentration (HC) of ~2X10^6 CFU/mL. The samples were examined in two different sets of environmental conditions, incubation at 22 +/- 1 °C with fluorescent lights and growth at room temperature (25 °C) with natural light. The HC samples of both bacterial strains

demonstrated rapid growth after a week of incubation while all samples grown in room environmental conditions demonstrated a slow growth rate. After two weeks, the incubated HC samples demonstrated solidification of the biomixture through curvatures and deformations of the sand as a united surface. This indicates that the cyanobacteria were not only able to adjust to the new habitat but were also biologically active within the sand and bound the biomixture's particles through the precipitation of CaCO3. Moreover, the biomixtures demonstrated differences in the areas of bacterial growth within the different sand types. This indicates that the sand particles size considerably affected the distribution of cells within the samples. For example, the spatial cell distribution within the QS1 biomixture was better comparing to thin sand as the porosity of the QS1 biomixture enabled spatial bacterial mobility and green pigmentations were noticeable on both ends of the sample (See figure 3).

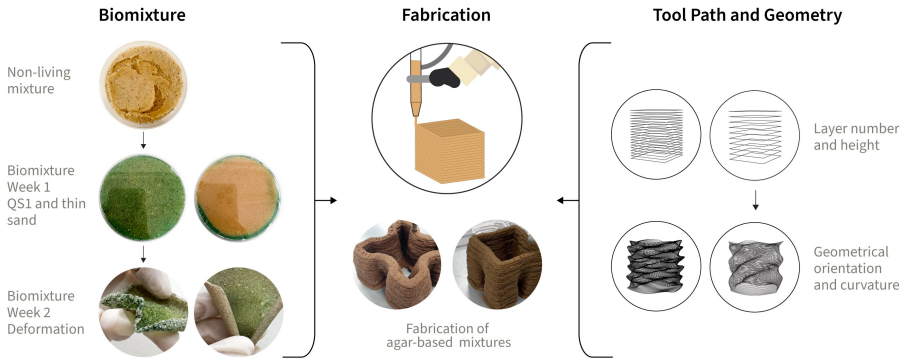

Fig. 3. Diagram, Implementing the pre-fabrication phase towards the second phase of the co-fabrication workflow. (Right) Developing the biomixture and examination of biological activity. Comparison of spatial cell distribution within QS1 and thin sand. (Left) Developing a potential printing tool path and geometrical properties. (Middle) Initial 3D printing experiments with agar-based mixtures for examining agar adhesion and structural integrity.

6. A Next Step Towards Additive Prototyping with Cyanobacteria

In this paper we demonstrate a new methodological approach to prolong the life span of the bacteria within sand-based mixtures towards induced bacterial deposition and enhancing solidification of printed architectural geometrical forms. Constructing protocols for a viable biomixture allows us to continue towards the development of additive prototyping strategies for architectural components.

Future experiments will aim to examine the printing feasibility of sand-based mixtures in relation to cell viability, printing scale and the mechanical performance of the material. Furthermore, fabrication factors will be developed in relation to the geometrical properties of the architectural habitat geometry. For example, the design of the printing tool path could enhance structural stability and strength. Printing patterns and lattice structures could become advantageous not only in minimizing material usage but also in the manipulation of light penetration and therefore, in the effect on bacterial growth and biomass.

Integrating living organisms in design processes holds a great potential in meeting the rising need to design sustainably. Though the newly suggested workflow still faces

many challenges that are yet to be fully resolved, it is a preliminary setup towards an integrated additive biofabrication shift in the architectural discipline.

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