BACTERIAL CELLULOSE AS A BASE MATERIAL IN BIODIGITAL ARCHITECTURE (BETWEEN BIO-MATERIAL DEVELOPMENT AND STRUCTURAL CUSTOMIZATION).

Nadine T. El Gazzar, Alberto T. Estévez, Yomna K. Abdallah[1]

1. ABSTRACT

Recently, developing sustainable architectural materials from renewable resources is gaining great interest. This interest is intended to alleviate the drawbacks of petroleum-based materials and their contribution in the escalation of CO_2 emissions causing the current environmental deterioration. Achieving sustainability through developing efficient architectural materials have been always conditioned by technological advancements and economic potential. This has affected the architectural design and construction sectors, especially in times of disasters or economic crisis, resulting in paralysis in the architectural construction and material development. These effects were caused by the capitalization and centralization of architectural construction industries.

The recent trend of self-sufficiency that had first emerged in environmental activities supporting recycling, environmental purification and conservation, oxygen, food, and electricity production, has extended to cover more sophisticated products, such as wearables, gadgets and architecture. Achieving self-sufficiency in architecture is of interest to multidisciplinary researchers who focus on developing both self-sufficient systems and materials as the two main components of the built environment.

Developing architectural materials aims to provide cheap, recycled, renewable, environmentally friendly, durable and sustainable building material regardless of the possibility of the autonomous production of these materials on a popular democratic basis. Architectural building materials production was always and still is considered a massive industry that is centralized in major firms and LTDs, limiting the architectural construction process to the availability of major economic capacity. This centralization had its merits in forcing forward large-scale economies and vitalizing the architectural design and construction market, but only on the large scale; however, this centralization shows its drawbacks every time in disasters or economic crisis, causing almost total paralysis in the construction industry due to economic impotence caused by different reasons. Moreover, the centralization of the building and construction industry have affected developing communities, causing economic drawbacks and creating a ripple-like crisis in housing.

1. Universitat Internacional de Catalunya, iBAG—UIC Barcelona (Institute for Biodigital Architecture & Genetics). nadine.tarekelgazzar@uic.es; estevez@uic.es; yomnaabdallah@uic.es

In this paper, the authors propose the self-sufficiency approach in the development and production of sustainable architectural material from abundant and renewable microbial agents, in order to democratize and popularize material production on a domestic and personalized basis.

The current work presents Bacterial Cellulose (BC) as a structural and membrane material in different architectural elements and applications, developed through simple and domestically applied procedures in order to create distributed and self-sufficient productive units for architectural materials production.

The current study aims specifically at the easiness and simplification of the production practices and procedures of the biopolymers, and specifically bacterial cellulose for encouraging and establishing the popularization of self-sufficient production units of these renewable and abundant biopolymers. In this regard, the current study is part of the ongoing research on enhancing the mechanical properties of bacterial cellulose in order to use it for structural applications, that will be further developed in terms of medium optimization, bacterial cellulose production efficiency analysis, and material mechanical and physical properties testing.

The following sections will contain a literature review on the chemical base and physical/mechanical properties of biopolymers including bacterial cellulose, followed by the experimental work conducted in this paper to develop bacterial cellulose as an architectural material. The results were further analyzed through formal and structural customization proposing possible applications in architectural design.

KEYWORDS

bio films, bio polymers, bacterial cellulose, structural customization

2. BIOFILMS

A biofilm is an assemblage of microbial cells that are enclosed within a surface and an extracellular matrix of primarily polysaccharide, noncellular materials such as mineral crystals, corrosion particles, and clay or silt particles, depending on the environment in which the biofilm has developed. Biofilms may form on a wide variety of surfaces, including living tissues, ecosystems and nonliving surfaces and substrates. (Donlan, R. M., 2002).

The solid-liquid interface between a surface and a moist medium provides an ideal environment for the attachment and growth of microorganisms. The biofilm attachment type and properties cannot be understood without studying the effects of the substratum, conditioning films forming on the substratum, hydrodynamics of the aqueous or moist medium, characteristics of the medium, and various properties of the cell surface.

The substratum or the solid surface has several characteristics that affect the attachment process: the extent of microbial colonization increases relatively with the increase in the surface roughness, as the shear forces are diminished, and surface area is wider on rougher surfaces. The physicochemical properties of the surface also strongly influence the rate and degree of attachment. Most investigators have found that microorganisms attach more rapidly to hydrophobic, nonpolar surfaces than to hydrophilic materials (Fletcher, M., et al., 1979; Bendinger, B., et al.,

1993). A material surface exposed in an aqueous medium will inevitably and almost immediately become conditioned or coated by polymers from that medium, and the resulting chemical modification will affect the rate and extent of microbial attachment. (Donlan, R. M., 2002).

The bacterial cells will behave as particles in a liquid nearly as in particles move in a Brownian motion, and the rate of settling and association with a submerged surface will depend largely on the velocity characteristics of the liquid, under very low linear velocities, the cells must crisscross the sizeable hydrodynamic boundary layer, and association with the surface will depend on cell size and cell motility. As the velocity increases, the boundary layer decreases, and cells will be subjected to increasingly greater turbulence and mixing. (Characklis W. G.,1990).

Other characteristics of the host aqueous medium, such as pH, nutrient concentrations, ionic strength, and temperature, also play a role in the rate of microbial attachment to a substratum. For instance, Fletcher, M., 1988 had found that an increase in the concentration of several cations (sodium, calcium, lanthanum, ferric iron) affected the attachment of *Pseudomonas fluorescens* to glass surfaces, presumably by reducing the repulsive forces between the negatively charged bacterial cells and the glass surfaces; furthermore, Cowan, M., et al., 1991 showed that the increase in nutrient concentration is correlated with the increase in the number of attached bacterial cells.

Cell surface hydrophobicity, presence of fimbriae and flagella, and production of EPS all influence the rate and extent of attachment of microbial cells. The hydrophobicity of the cell surface is important in adhesion because hydrophobic interactions tend to increase with the increasing in the nonpolar nature of one or both surfaces involved (the microbial cell surface and the substratum surface). Most bacteria are negatively charged but still contain hydrophobic surface components, (Rosenberg, M., and Kjelleberg, S., 1986). Fimbriae play a role in cell surface hydrophobicity and attachment, probably by overcoming the initial electrostatic repulsion barrier that exists between the cell and substratum (Corpe, W. A., 1980). Furthermore, Korber, D., et al., 1989 used motile and nonmotile strains of *P. fluorescens* to show that motile cells attach in greater numbers and attach against the flow more rapidly than nonmotile strains. Nonmotile strains also do not recolonize vacant areas on a substratum as evenly as motile strains, resulting in slower biofilm formation by the nonmotile organisms.

Consequently, cell surface structures such as fimbriae, other proteins, LPS, EPS, and flagella all clearly play an important role in the attachment process. Cell surface polymers with nonpolar sites such as fimbriae, other proteins, and components of certain gram-positive bacteria (mycolic acids) appear to dominate attachment to hydrophobic substrata, while EPS and lipopolysaccharides are more important in attachment to hydrophilic materials.

2.1 Biofilm Structure

Biofilms are composed primarily of microbial cells and EPS. Usually, the EPS accounts for 50% to 90% of the total organic carbon of the biofilm (Flemming, H. C., et al., 2000) and is considered the primary matrix material of the biofilm. EPS can vary in their chemical and physical properties, but it is primarily composed of polysaccharides, either neutral or polyanionic, as is the case for the EPS of gram-negative bacteria. The presence of uronic acids confers the anionic property of the EPS (Sutherland, I. W., 2001) which is important to allow association of divalent cations such as calcium and magnesium, that cross-link with the polymer strands and provide greater binding force in the developed biofilm (Flemming, H. C., et al., 2000).

EPS is highly hydrated as it includes large amounts of water into its structure by hydrogen bonding. EPS may be hydrophobic, although most types of EPS are both hydrophilic

and hydrophobic (Sutherland, I. W., 2001). EPS may also vary in its solubility. As noted by Sutherland, I. W., 200, there are two main properties of EPS that have an effect on the biofilm's structure: the first, is the composition and structure of the polysaccharides determining their primary conformation. For example, many bacterial EPS possess backbone structures that contain 1,3- or 1,4-β-linked hexose residues and tend to be more rigid, less deformable, and in certain cases poorly soluble or insoluble (Sutherland, I. W., 2001), the Second feature, is the variety in the EPS of biofilms as they may vary spatially and temporally. (Flemming, H. C., et al., 2000). EPS production is known to be affected by nutrient status of the growth medium; excess available carbon and limitation of nitrogen, potassium, or phosphate promote EPS synthesis. *Slow bacterial growth will also enhance EPS production.* (Donlan, R. M., 2000).

2.2 Biofilm Architecture

Biofilms are not a continuous monolayer surface deposit, rather, they are very heterogeneous, containing microcolonies of bacterial cells encased in an EPS matrix and separated from other microcolonies by interstitial voids (water channels) (Lewandowski, Z., 2000). This concept of heterogeneity is vivid not only for mixed culture biofilms (such as might be found in environmental biofilms) but also for pure culture biofilms. Stoodley, P., et al., 1997, had defined certain criteria or characteristics that could be considered descriptive of the architecture of biofilms in general, including a thin base film, ranging from a patchy monolayer of cells to a several layers thick film containing water channels. The organisms composing the biofilm may also have a marked effect on the biofilm structure. For example, James, G. A., et al., 1995, showed that biofilm thickness could be affected by the number of organisms. Consequently, biofilm architecture is heterogeneous both in space and time, constantly changing because of external and internal processes. Tolker-Nielsen, T., et al., 2000, had investigated the role of cell motility in forming the biofilm architecture by the flow of cells, through examining the interactions of *P. aeruginosa* and *P. putida* by confocal laser scanning microscopy. When these two organisms were added to the flow cell system, each organism initially formed small microcolonies. With time, the colonies intermixed, showing the migration of cells from one microcolony to the other. The microcolony structure changed from a compact structure to a looser structure over time, resulting in the motility of the cells inside the microcolonies. Motile cells ultimately dispersed from the biofilm, resulting in dissolution of the microcolony.

Structure may also be influenced by the interaction of particles of nonmicrobial components from the host or environment. Soil particles may often collect in biofilms of potable and industrial water systems, providing yet another example of particle interaction with biofilms (Donlan, R. M., 2000).

3. BIOPOLYMERS

Biopolymers are polymers synthesized by living organisms. Thus, they are polymeric biomolecules, as the long chain biomolecules are comprised of covalently linked repeating monomeric units (Pattanashetti, N. A., et al., 2017). Living organisms such as plants, animals, bacteria, fungi and yeast synthesize a wide range of biopolymers such as deoxyribonucleic acid (DNA), ribonucleic acid (RNA), proteins, cellulose, chitin, starch, etc (Dassanayake, R. S., 2018). Biopolymers perform countless number of vital functions, such as storage of energy, preservation and transmittance of genetic information, and cellular construction, in vivo. Proteins not only catalyse reactions (enzymes) and take part in cell signalling but also provide structural support, like collagen (Dassanayake, R. S., 2018).

There are three main classes of biopolymers owing to their universal occurrence and abundance: (i) polynucleotides, (ii) polypeptides/poly amino acids, and (iii) polysaccharides. Polynucleotides (DNA and RNA) are long polymers composed of 13 or more nucleotide monomers (Pattanashetti, N. A., et al., 2017). Polypeptides are the short polymers comprised of amino acids as monomeric units and amide bonds link the monomeric units together (Poly, N. K., 2015). Polysaccharides are composed of monomeric sugars linked together by O-glycosidic linkages. Among hundreds identified polysaccharides, cellulose, starch, chitin, and chitosan are important examples (Rinaudo, M., 2006).

Biopolymers are suitable for various industrial and medical applications, due to their material properties; as they are renewable, biodegradable and, biocompatible. Thus, biopolymers derived from renewable resources possess competitive advantage over synthetic non-renewable polymers. However, the cost of production of biopolymers and biopolymer-based products is of prime importance (Dassanayake, R. S., 2018), if they are going to be produced by mass production for utilizilization as building materials in architectural construction.

Reducing the production costs of biopolymers can be addressed by exploiting the abundant source of biopolymers, at lower cost. Therefore, polysaccharides, which are comprised of monosaccharides (sugars) linked together by O-glycosidic linkages are an obvious choice, including the two most abundant natural resources: cellulose and chitin (Klemm, D., et al., 2005). Additionally, polysaccharides are widely distributed in nature as they can be derived from plants, animals and microorganisms. Furthermore, variation in physicochemical properties, such as mechanical properties, solubility, viscosity, gelling potential, surface and interfacial properties, ruled by monosaccharide composition, degree of polymerization, linkage types and patterns, provide polysaccharides versatility in preparation of materials with diverse applications. In fact, polysaccharides-based materials have different forms including fibres, films, food casing, membranes, hydrogels, aerogels and sponges, with diverse areas of applications (Wang, S., et al., 2016). Thus, polysaccharide-based biopolymers are promising candidates in the preparation of materials that are environmentally friendly and economically sustainable.

3.1 Cellulose

Cellulose is the most abundant renewable resource in plants as it is the major structural component in plant cell walls. Besides plants, some species of bacteria and algae biosynthesize cellulose. Because of its abundance, cellulose can serve as an inexhaustible source of raw material in production of sustainable bioproducts, the so called "green products" (Klemm, D., et al., 2005). Cellulose is a long chain molecule and the degree of polymerization (DP) differs by the source and the treatments employed during its extraction. The DP of cellulose is reported to be as high as 10,000 and 15,000 in wood and cotton fibres (George, J., and Sabapathi, S. N., 2015).

Cellulose in nature is found in multi-level assembly, popularly known as hierarchical structure. During biosynthesis of cellulose, approximately 36 individual cellulose molecules are assembled together to form elementary fibrils, which have dimensions in nano-scale. Elementary fibrils further undergo packing into a larger entity called microfibrils with a cross section of ~20 nm × 8 nm. These microfibrils further assemble into macro sized cellulose fibres (e.g. wood fibre, cotton fibre) (Moon, R. J., et al., 2011). However, specific packing of cellulose may be different based on the source.

3.1.1 Bacterial Cellulose (BC)

As mentioned previously, Cellulose is one of the most abundant biopolymers on Earth and is mainly of plant, wood and bacterial origin. The cellulose of bacterial origin exhibits the highest

purity and has thus attracted the interest of many researchers and industrial sectors. Generally, BC is a hydrogel formed of randomly assembled 3D network of <100 nm wide ribbon-shaped fibrils, composed of 7–8 nm-wide elementary nanofibrils aggregated in bundles. As such, it carries a combination of exclusive properties, such as flexibility, high water holding capacity, hydrophilicity, crystallinity, higher tensile strength (200–300 MPa) and Young's modulus (up to 78 GPa) which make bacterial cellulose 8 times stronger than stainless steel. (Mater, J., and Chem, C., 2015). Because of these features, this type of cellulose attracts interest for different fields of biotechnology applications in nanotechnology, pharmaceuticals, food industry, cosmetics, textiles, paper industry, and medical applications such as artificial skin bioengineering, artificial blood vessels, topical covering for severe wounds, coverings in nerve surgery, wound dressings, electronic platforms, implants for cartilage and bone repair etc.

For efficient bacterial cellulose (BC) production, an efficient and stabile bacterial strain is needed, this strain shouldn't be expensive and should be easily scaled up to industrial settings. The produced cellulose is generally easily separated from its growth medium.

As exhibited, BC is a nanofibrillar, extracellular polysaccharide produced by diverse bacteria when they are growing statically, but also when bacteria are submerged in liquid and cultured by shaking. Bacteria produce BC in media with different carbon sources, although the efficiency of BC production differs substantially among various growth substrates. The substrate supplies energy to bacterial metabolism during the exhaustive energy-consuming pathway of cellulose synthesis. Theoretically, every carbon block which the bacterial cell metabolizes into glucose, can be used for cellulose production. (Wang, S. S., et al., 2018; Krasteva, P.V., 2017).

The capacity of BC production is widespread among bacteria, but the most prominent and well-known BC-producer is *Komagataeibacter xylinus*, which belongs to the group of acetic acid bacteria (AAB). AAB are strictly aerobic Gram-negative bacteria classified into *α-Proteobacteria* (Trček, J., Barja, F., 2015). The species has been for many years known as *Acetobacter xylinum*, but has been later classified into *Gluconacetobacter xylinus* and due to further taxonomic changes finally reclassified into *Komagataeibacter xylinus*. *K. xylinus* is not the only species among AAB with an immense potential for BC production, since other species, such as *Komagataeibacter hansenii*, *Komagataeibacter medellinensis*, *Komagataeibacter nataicola*, *Komagataeibacter oboediens*, *Komagataeibacter rhaeticus*, *Komagataeibacter saccharivorans* and *Komagataeibacter pomaceti* have been characterized as strong cellulose producers (Škraban, J., 2018). An important aspect of using AAB for cellulose production is their characteristic of being food-grade or GRAS bacteria (generally recognized as safe).

BC is synthesized in the bacterial membrane from nucleotide-activated glucose (Morgan, J. L.W., et al., 2012). Bacteria then channel BC through pores of cell membrane as fibrils composed of D-glucose units which are linked with β-1,4-glycosidic bonds. The chain is linear and extruded from the cell, then the lateral and unidirectional aligned chains form intra- and interchain hydrogen bonding through all available hydroxyl groups. In this way the chains merge into insoluble nanofibrils of up to 25 nm in width and 1 to 9 μm in length which represents 2000 to 18,000 glucose residues (Ross, P., et al., 1991). These nanofibrils further aggregate into <100 nm wide ribbon-shaped fibrils (McNamara, J.T., et al., 2015).

Synthesis of nucleotide-activated glucose takes place in bacterial cytoplasm. If the starting substrate is glucose, the uridine diphosphate (UDP)-glucose is produced in three steps: phosphorylation of glucose by glucokinase, isomerization of glucose-6-phosphate into glucose-1-phosphate by phosphoglucomutase and synthesis of UDP-glucose by uridylyltransferase (UTP)-glucose-1-phosphate. Finally, cellulose synthase transfers glucosyl residues from UDP-glucose to the nascent β-D-1,4-glucan chain. Cellulose synthase is a membrane-embedded glycosyltransferase

composed of two or three subunits (Römling, U., 2015). The catalytic subunit of cellulose synthase is a major determinant of chemical and physical properties of BC, meaning that different bacterial species are able to generate cellulose with different lengths (Yang, H., 2019).

3.1.1.1 Different Carbon Sources for Bacterial Cellulose (BC) Production

The production of BC is extremely expensive, due to high costs of the synthetic media used for its production. The most well-known complex synthetic medium for growing cellulose producing AAB is Hestrin–Schramm medium (HS), composed of 2% (w/v) glucose, 0.5% (w/v) peptone, 0.5% (w/v) yeast extract, 0.27% (w/v) Na_2HPO_4 and 1.15 g/L citric acid (Schramm, B. M., and Hestrin, S., 1954). During BC production, other by-products, such as gluconic and other acids are formed that can decrease the BC yield (Zhang, H., et al., 2016). The composition of HS medium can be further optimized for the highest cellulose yield by replacing glucose with other carbon sources, such as maltose, fructose, cellobiose, mannitol, xylose, sucrose, galactose etc.; however, in most cases glucose turned out to be the best energy source for bacteria, besides, glucose can be directly used as a precursor for the assembly of glucose units into cellulose. The process for BC production can be further optimized by adding buffers into the medium for keeping pH at an optimal value for growing bacterial strains (Castro, C., et al., 2012).

To reduce the costs for BC production, alternative natural carbon sources are utilized, such as waste substrate from different sectors of the food industry, sugar cane molasses etc. (Gorgieva, S., and Trček, J., 2019). The BC yield can be improved also by the addition of additives into the growth medium such as glycerol, agar, xanthan, sodium alginate, ethanol, carboxymethyl cellulose (CMC), etc. Lu, Z., et al., (2011) reported enhanced BC production with *K. xylinus* in chemically defined medium under static cultivation by the addition of pyruvic acid, malic acid, lactic acid, acetic acid, citric acid, succinic acid, and ethanol in concentrations 0.15%, 0.1%, 0.3%, 0.4%, 0.1%, 0.2%, 4%, respectively. Li, Y., et al., (2012) improved cellulose production with the strain *K. hansenii* M2010332 by the addition of ethanol and sodium citrate. Lu, Z., et al., (2011) reported that the addition of 1% of methanol, 0.5% ethylene glycol, 0.5% of n-propanol, 3% of glycerol, 0.5% of n-butanol and 4% of mannitol produced 21.8%, 24.1%, 13.4%, 27.4%, 56% and 47.3% higher yield of cellulose by culturing strain *K. xylinus* 186 statically in glucose medium. There is also a report on improved BC production with *K. xylinus* ATCC 10,245 by adding vitamin C in growth medium (Cheng, Z., et al., 2017).

As the production of BC in synthetic media with different carbon sources and growth factors is expensive, researchers have been searching for inexpensive raw material containing high levels of sugars as substrates for BC production. Several raw materials have been examined for BC production, such as tobacco waste extract (Toda, K., et al., 1997), sugar beet molasses, cheese whey media (Lu, H., et al., 2016), distillery effluent (Li, Y., et al., 2012), corn steep liquor (Lu, Z., et al., 2011), fruit juice (Matsuoka, M., et al., 2011), corn stalks (Yang, X.Y., et al., 2016), litchi extract (Fan, X., et al., 2016), beverage industrial waste (Huang, C., et al., 2015), and waste beer yeast (García-Lomillo, J., and González-SanJosé, M.L., 2017). Another possible natural growth medium would be waste material from wine production. According to recent reports (Bayrak, E., Büyükkileci, A.O., 2018), 1.17 kg of grapes are used to produce 750 mL wine, and after the grapes are squeezed, about 20% of that weight remains in the form of grape skins, seeds and stems, counting for ~12 million tons each year. This substrate contains soluble carbohydrates (white grapes), fibers, acids, salts, and phenolic compounds (red grapes) (Molina-Ramírez, C., et al., 2018) and as such it is often considered as a convenient source of carbon for microbial processes. Moreover, grape waste as a carbon source in BC production may contribute to reduce winery residuals and reduce BC production costs.

The carbon source used for growing BC affects BC properties such as water holding capacity, surface area, porosity, polymerization degree, molecular weight, crystallinity index, mean crystallite size, intrinsic viscosity, oxygen and water vapor transmission rates, mechanical properties, etc. However, a recent study of Wang, S., et al., (2018) reported similar morphology and microfibrils of BCs from different carbon sources, meaning that these characteristics have to be checked for each bacterial strain before starting BC production at large scale.

The production of BC can be simply performed in bowls with large surface area which support a direct supply of oxygen and assembly of large cellulose sheets. To improve the efficiency of BC production and to produce cellulose of desired characteristics, different technological approaches can be used. For instance, Static production (Rani, M.U., et al., 2011) is the most commonly used method at lab scale, the processes last for up to 2 weeks, and it produces a hydrogel sheet Cellulose. Another method is production in shaking culture (Wang, J., et al., 2019), this method depends on the increased delivery of oxygen to bacteria; however, it might cause reduced genetic stability of bacteria and lower BC production yields. It also results in production of cellulose of different particle sizes and various shapes (spherical shape) suitable for economic scale production only. Production in an airlift bioreactor (Wang, J., et al., 2019) employs efficient oxygen supply with low power supply to produce Cellulose in pellet. Production in rotating disc bioreactors (Serafica, G., et al., 2002) produces homogenous cellulose. Production in trickling bed reactor (Lu, H., and Jiang, X., 2014), provides high oxygen concentration and low shear force to produce BC in the form of irregular sheets.

3.1.1.2 In Situ Modifications

Several studies had identified various in situ modifications as a straightforward approach for the introduction of particular functionality to BC by the addition of reinforcement material (chitosan, gelatin, poly-3-hydroxybutirate, nanomaterials, clays, silica) to the bacterial culture medium, mostly at the beginning of BC production. The great advantage of such a process is encaging materials that become part of the fibrils, thus enhancing BC by altering mainly the physical–mechanical properties of BC fibrils. Moreover, new functionalities also can be introduced (Dassanayake, R. S., 2018).

The in-situ modification approach also presents integration of reinforcement materials that also have antibacterial activity against special BC strains, the insolubility of various materials in culture media, high surface tension towards hydrophobic materials, the lack of structure control of BC nanofibers, and introduction of particles with low suspension stability within BC growing media, etc. (Dassanayake, R. S., 2018).

In situ modification of BC porosity is not affected by the aforementioned limitations and several studies demonstrate facile procedure for pore size manipulation. As shown by Lu, X., et al., 2016, the addition of potato starch to culture medium increases BC viscosity by interrupting BC assembly during static culture and thus creating more free spaces within the fibrous network. The procedure for the processing of macro-porous and foam-like BC was recently reported by Rühs, P. A., et al., 2018; they cultured *K. xylinus* in mannitol-based media by foaming and then stabilized the product with surfactant Cremodan and viscosified with Xanthan to prevent water drainage.

3.1.1.3 Ex Situ Modifications

Ex situ modifications are either chemical such as oxidation and grafting (Oliveira Barud, H.G., 2015), crosslinking reactions or physical absorption from solutions or particle suspensions (Cai, Z., and Kim, J., 2010).

4. MATERIALS AND METHODS

The experimental work conducted in this study was categorized into four groups, the first group aimed to culture the bacterial strain *Acetobacter xylinum* to produce bacterial cellulose film, analysing the growth medium constituents and the growth factors, as temperature, and incubation period, as well as manipulating the growth medium constituents by utilizing organic waste as a cheap and sustainable carbon source for the growth of the bioagent *A. xylinum*.

The second group of experiments aimed to test different fibrous reinforcement that would customize the mechanical properties of the produced bacterial cellulose to be suitable for structural applications as an architectural building material.

The third group aimed to test the moulding potentials of the bacterial cellulose, utilizing 3D printed scaffolds, in order to formally customize the resultant biopolymer BC for the further processing of structural customization.

The fourth group tested the BC self-healing potentials to introduce a regenerative aspect into the architectural built environment.

4.1 Bacterial cellulose production

In this experiment, the main criteria were the availability of medium constituents on a domestic scale and the simplicity of experimental procedures.

4.1.1 Standard growth media

In the current study, the bacterial strain used is *Acetobacter xylinum*, cultured in Symbiotic Culture of Bacteria and Yeast medium (scoby) 10g, 1500 ml black tea brew, 500 ml Kombucha 100 ml Sucrose, and 100 ml Apple cider vinegar. The mixture was prepared in a sterile glass jar as follows: the black tea bags were dissolved and stirred in 1000 ml boiling water and left to cool down until it reached 25 °C, the 100 g Sucrose was added along with the 100 ml apple cider vinegar to the mixture and stirred to dissolve and homogenize; the Scoby was then added to the medium, the prepared and cultured medium was plugged and covered by light blocking and air permeable cloth and incubated at 25°C, pH 4 for 10–18 days. (Figure 1).

4.1.2 Optimizing BC growth medium by employing organic waste from domestic use

The medium constituents were modified to partially replace the used carbon source in media from Sucrose to simple organic waste from domestic use. The optimized media was composed of Scoby 10g, Black tea brew 1500 ml, Apple cider vinegar 150 ml, Sucrose 70 g, ground and dried Raspberry waste 100 g. the medium constituents were stirred in a glass sterile jar, covered with light blocking, air preamble cloth and incubated for 14 days in pH 4, and 25°C. (Figure 4).

4.2 Material development for mechanical properties reinforcement

This experiment aimed to simplify the procedures of material processing on a domestic scale in order to enhance the solidification of the BC to make it more suitable for structural applications.

4.2.1 Natural fibres reinforcement of BC

This group of experiments aimed to adjust the mechanical properties of the BC film to be suitable for structural application in architecture and interior design. The reinforcement was tested by adding different natural fibres: including palm tree leaves, sponge and cotton fibres, separately, and respectively. The standard growth medium was prepared as exhibited previously in

(4.1.1), the natural fibers of palm leaves and sponge were added into the medium, the medium with the bioagent and natural fibers were covered with light blocking, air permeable cloth and incubated at 25°C for 10 days. After the growth of bacterial cellulose film encompassing the natural fibers, the BC film was removed from the cultivation medium and washed with water + ethanol 70% to remove any bacterial cells possibly attached to the BC pellicles. The resultant BC film was dried at room temperature for 3 days. (Figure 6).

4.2.2 Cotton disk Reinforcement of BC

A system was made in order to ensure the stability and floating of the cotton mat on the surface of the medium during the fermentation process. The system is composed of one piece of cotton mat 12cm in diameter, vessel, vertical support made with two tubes, two cubes made of ceramic clay to fix the vertical support, threads to attach the cotton into the vertical support and movable needle attached with the threads in order to adjust the height level of the cotton. After adjusting the supporting scaffold system that holds the cotton mat floating on the top of the vessel, the standard growth medium and the bioagent as in (4.1.1) were added in the vessel. The vessel was covered with the light blocking, air permeable cloth and incubated for 10 days in 25°C. (Figure 11).

4.3 Material formal customization

This experiment tests the ability of BC to be formed and to maintain specific formal and textural features.

4.3.1 Molding

In this experiment, the material formal properties were manipulated by using 3D printed scaffold to grow the BC film on it in order to take its shape. The standard medium with the bioagent was prepared as in (4.1.1), then the 3D printed scaffold of a Voronoi shape was added to the liquid inoculated medium, the mixture was covered with light blocking air permeable cloth and incubated for 10 days at 25°C, after 10 days the BC film was removed from the vessel and separated from the scaffold and left to dry in room temperature for 3 days. (Figure 14).

4.4 Material self-healing

In this experiment self-healing was tested as it is one of the most unique characteristics of BC films. A young cultured BC film (5 days) was cute to create two integrated parts, a hollow loop of 30 cm diameter and a circle of 20 cm diameter. The two parts were then placed in standard growth medium that is not inoculated with the bioagent *A. xylinum*, to test the ability of the material to bond and self-heal with its integrated part. The mixture was covered with light blocking, air permeable cloth and incubated for 7 days at room temperature. (Figure 20).

5. RESULTS AND DISCUSSION

The presented results are observed by simple tools that are available on domestic scale in correspondence with the main objective of the paper of domesticating and democratizing architectural material production, however, the research is still under development to apply material mechanical standard experimentation to be generalized and applied in construction materials' industry.

5.1 Bacterial cellulose production

The BC film was produced after 10 days of fermentation in standard growth medium at 25°C, the BC was composed of strong and lightweight tiny fibres, (Figure 2), those fibres vowel together to a gelatinous mat floating at the air-liquid interface. Scoby was used in the standard growth medium as it is the most commonly studied source of bacterial cellulose because of its ability to produce relatively high levels of polymer from a wide range of carbon and nitrogen sources. Normally, the Scoby found floating at the air-liquid interface as in (Figure 3). These results are congruent with Aswini, K., et al., (2020). That produced BC film after 14 days of incubation in aerobic conditions as the pellicle of BC is formed on the air-liquid interface of the media for providing sufficient oxygen to the bacterial cells.

Carbon source is a pivotal factor in the production of cellulose and cell growth. The maximum production of BC was achieved by using sucrose as the main carbon source, this is supported by Rangaswamy, B. E., et al., (2015). The pH plays a significant role for cellulose production. The utilized medium pH in the current study was tuned to pH4. This is congruent with Rangaswamy, B. E., et al., (2015) that reported the highest BC production was achieved at pH range 3–7. As temperature plays an important role in the fermentation process, in the current work the culture was incubated at 25°C, this is supported by Aswini, K., et al., (2020), that reported that *A. xylinum* culture needs a warm and static condition with the temperature between 20–30°C.

5.1.2 Optimized growth medium using organic waste as the carbon source

Using organic wastes from domestic use resulted in facilitating and fastening the growth and formation of BC after 8 days of fermentation, the used waste of raspberry have further added

FIGURE 1. Bacterial cellulose simple production steps, that enables the autonomous mass production of BC on a personal scale, that will be utilized as an architectural material in different applications. Step 1: black tea bags were dissolved and stirred in 1000 ml boiling water and left to cool to 25°C. Step 2: 100 g of sucrose was added to the brew along with 100 ml apple cider vinegar. Step 3: the Scoby culture was added into the medium. Step 4: Finally, the container was covered with a light-blocking, air permeable cloth. **By the authors.**

FIGURE 2. Different produced BC thicknesses, showing the relative growth in thickness of the Biopolymer film in relation to the incubation period. Left, BC formation after 10 days of incubation, Right, BC layers after 24 days of incubation. **By the authors.**

FIGURE 3. The resultant BC film after 10 days of fermentation using *Acetobacter xylinum*. **By the authors.**

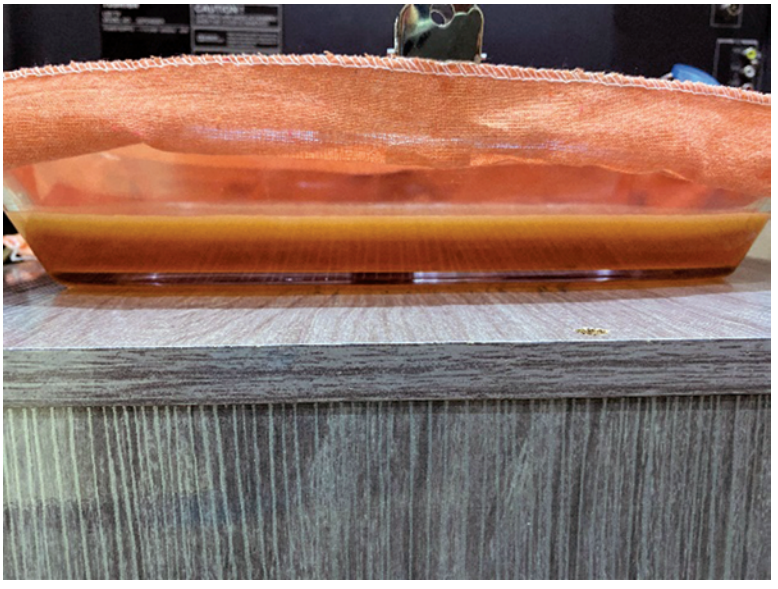
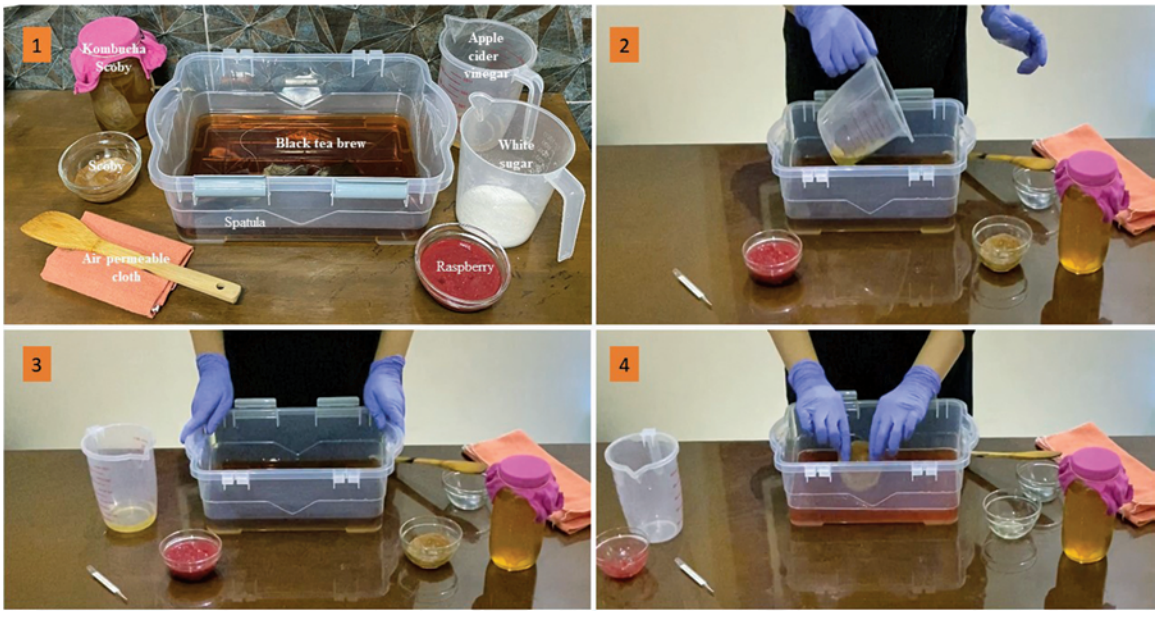


FIGURE 4. Optimized medium constituents using organic waste from domestic use as an alternative carbon source for the growth of BC. Step 1: preparing the medium constituents following the description in (4.1.2). Step 2: the container was filled with boiling black tea brew that was then left to cool to 25 °C. Step 3: 70 g sucrose was added and stirred along with 150 ml apple cider vinegar, and 100 g raspberry powder. Step 4: the Scoby culture was added into the medium. **By the authors.**



the variety of different red shaded colours to the BC film as shown in (Figure 5), which have a particular importance in the field of architectural design application, achieving quadratic benefit by: reducing the fermentation time, reducing the production costs, recycling domestic organic wastes and having variety of colours utilizing natural pigments. A coloured layer of cellulose has grown at the surface of the medium and it has adjusted to the shape of the container in which it is located (Figure 5).

5.2 Material development (reinforcement)

The results revealed in this section exhibits the enhanced mechanical properties of the film, lessening its liquidity and hardening its final consolidation, however, this part of the research is still under development, and the provided results were observed from manual stress applied to the reinforced film.

5.2.1 BC reinforcement by Natural fibres.

The dried BC film encompassing the natural fibres, exhibited flexibility and enhanced overall consolidation of the BC resistance to manual and mechanical pressure (Figure 7, 8, 9, 10).

5.2.2 BC reinforcement on cotton disk

The developed scaffold system holded the cotton matt floating on top of the liquid medium, Figure 12.

FIGURE 5. The produced BC after 8 days of fermentation, using organic waste as an alternative carbon source. The figure shows the pigmentation effect achieved by the organic waste of raspberry powder. **By the authors.**

FIGURE 6. Production process of BC in standard medium with natural fibres reinforcements; using palm tree leaves, sponge and cotton disk. **By the authors.**

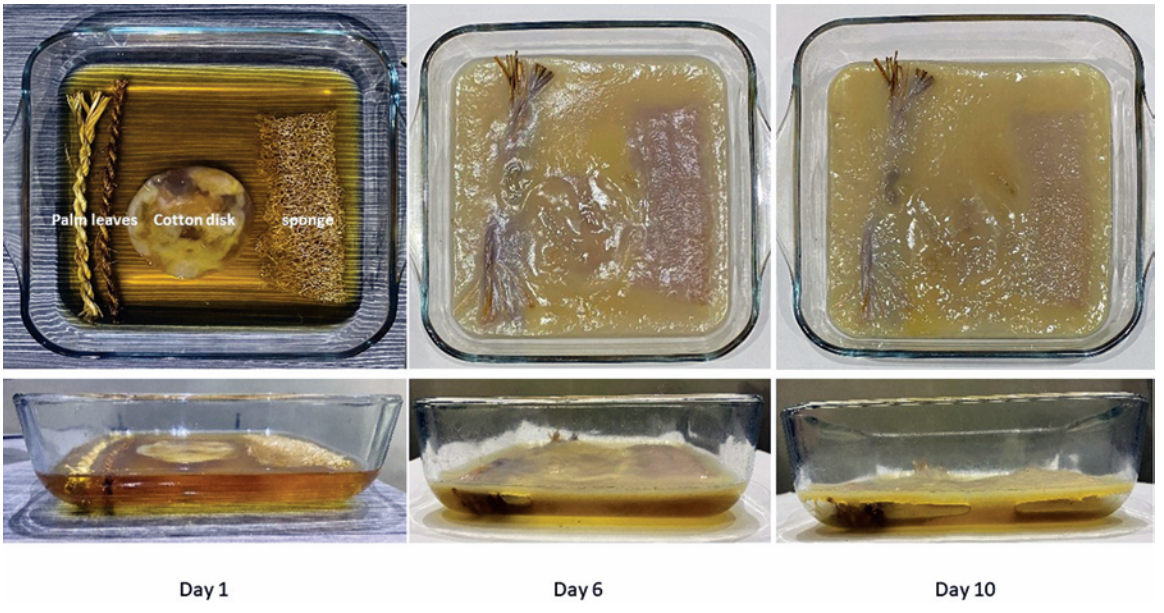


FIGURE 7. The BC + natural fibres film growth. **By the authors.**

FIGURE 8. The BC+ natural fibres film growth after 10 days of fermentation. **By the authors.**

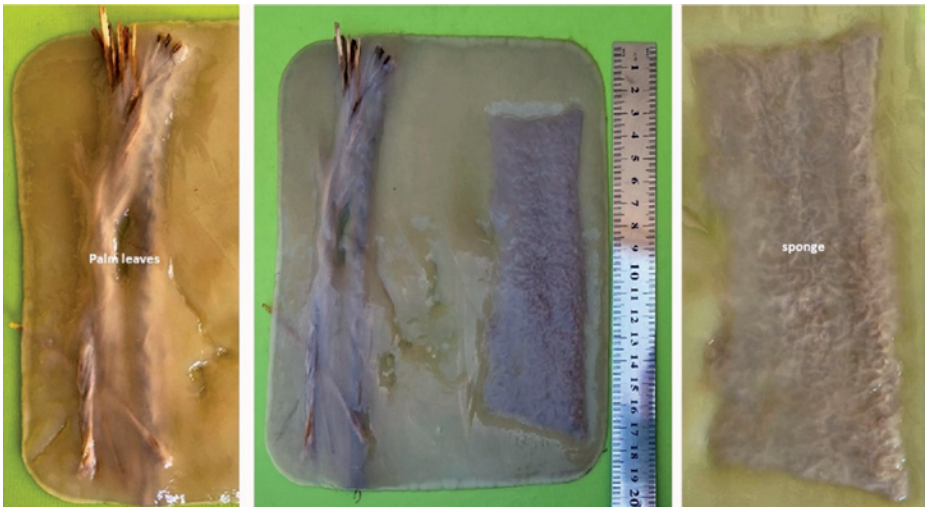


FIGURE 9. The BC+ natural fibres film growth after 10 days of fermentation showing the integration and attachment of the BC film and the encompassed natural fibres. **By the authors.**

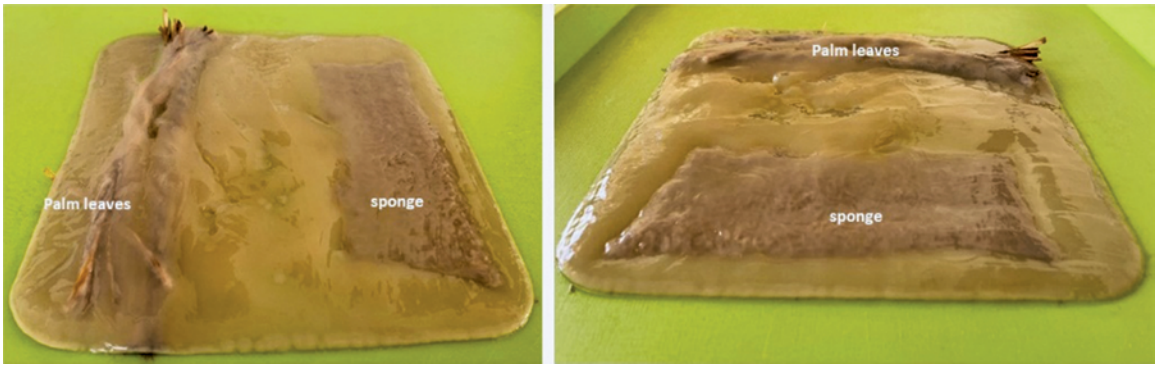


FIGURE 10. The washed and dried BC reinforced film. **By the authors.**

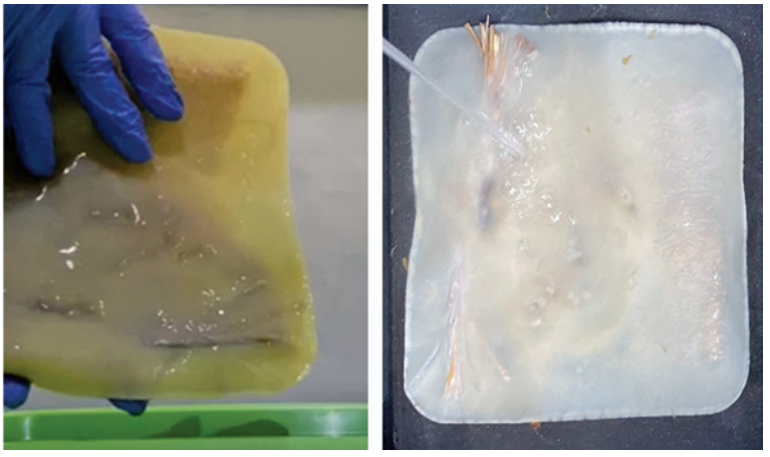


FIGURE 11. The developed scaffold system held the cotton matt floating on top of the medium surface in order to prepare it for the attachment of the bioagent in order to grow the BC on the cotton matt. **By the authors.**

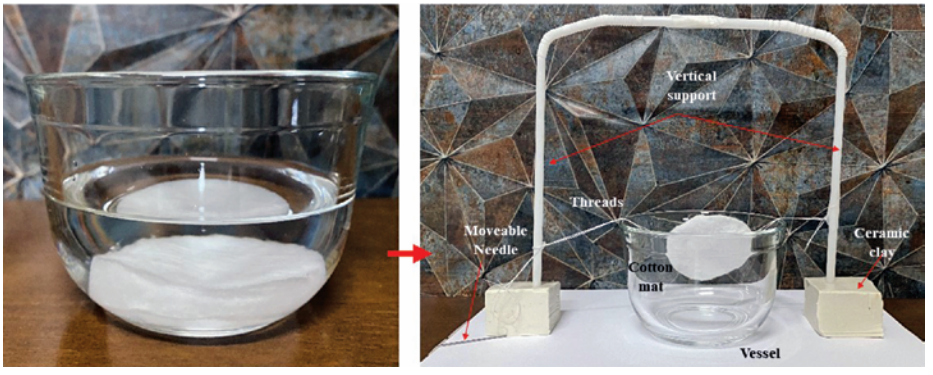


FIGURE 12. Inoculating the system with standard medium +the bioagent *Acetobacter xylinum* to produce the reinforced BC. **By the authors.**

After 10 days the BC film was grown attached to the cotton matt showing the capacity of vertical and horizontal attachment of the bioagent and the growth and formation of the BC as exhibited in Figure 13.

5.3 Material formal customization

The results exhibited the moldability of the BC to be textured and maintain the texture after the fastness process, proposing endless plastic potentials in design applications.

FIGURE 13. The grown reinforced BC film after 10 days of fermentation. **By the authors.**

FIGURE 14. The preparation of the BC standard medium and the 3D printed scaffold, for customizing the formal properties of the BC film. **By the authors.**

FIGURE 15. The growth process of the BC film after 6 days of fermentation showing the attachment and emersion of the printed scaffold within the growing BC film. **By the authors.**

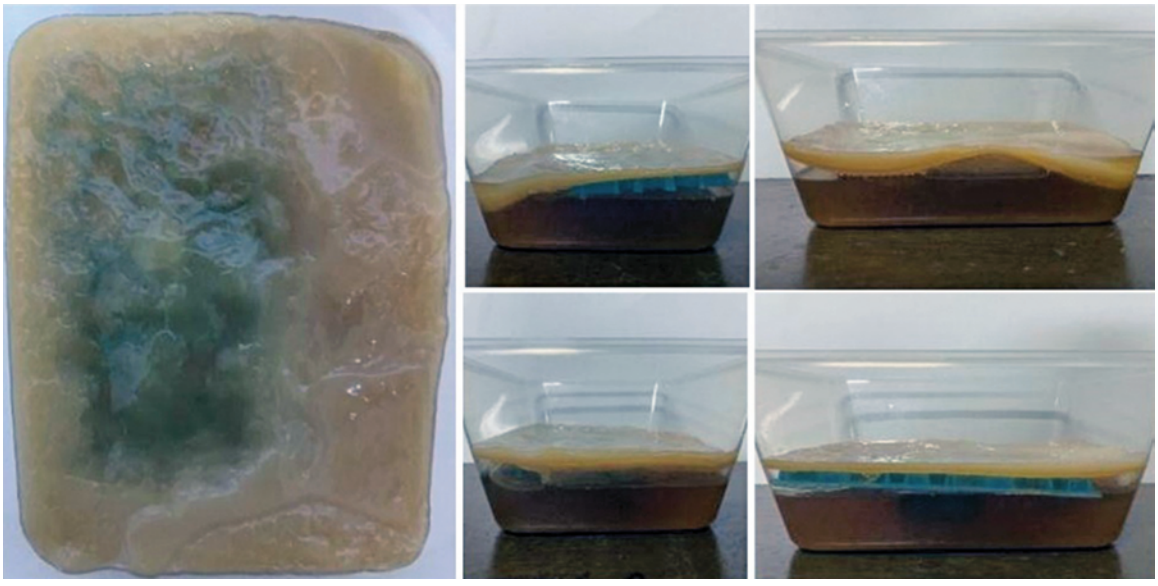
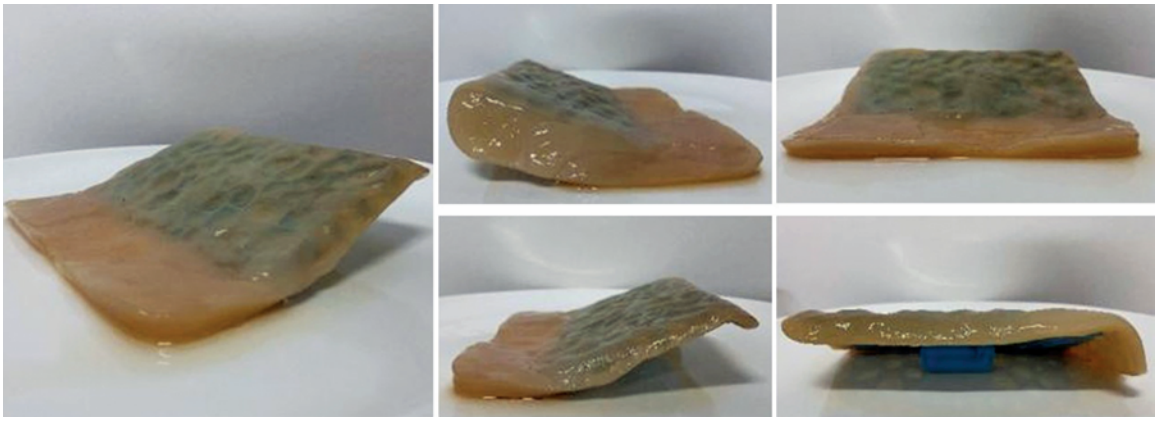


FIGURE 16. The grown BC film after 10 days of fermentation, showing the total attachment on the 3D printed scaffold. **By the authors.**



5.3.1 Molding

After 10 days, the produced BC film was in attachment on the 3D scaffold of 3D printed hollow net of Voronoi cells' shape (Figure 15, 16, 17), however after the separation of the produced BC from the scaffold, it still maintained the shape of the 3D Voronoi cells after drying the BC in 3days at room temperature (Figure 18, 19).

5.4 Material self-healing

After 7 days of fermentation, the material was self-healed, forming one BC film as shown in (Figure 21), that maintained its translucency and physical properties of elasticity and shape memory. However, the welding edges were still visible marking the separate integrated parts

FIGURE 17. The dried BC film encompassing the 3D printed scaffold, after 3 days dehydration process. **By the authors.**

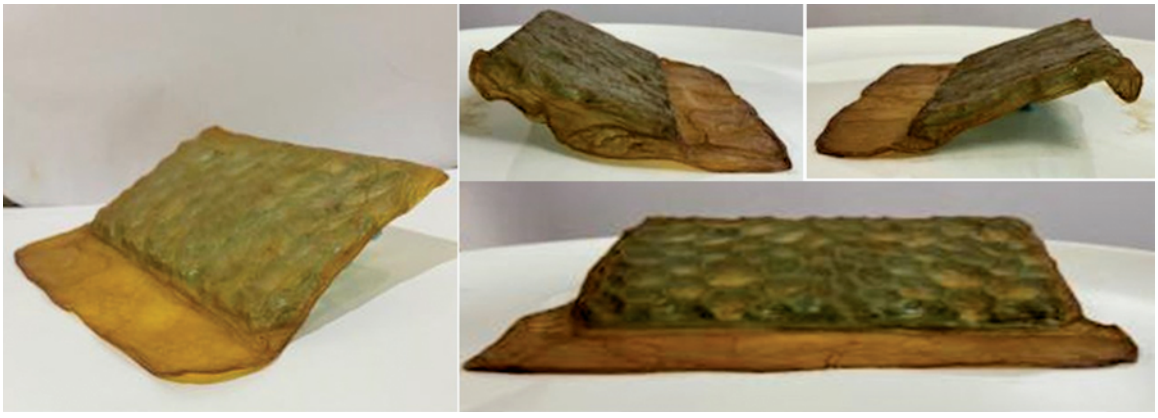


FIGURE 18. The separated dried BC film, showing the patterned texture that was achieved by growing the film on the 3D printed scaffold. **By the authors.**

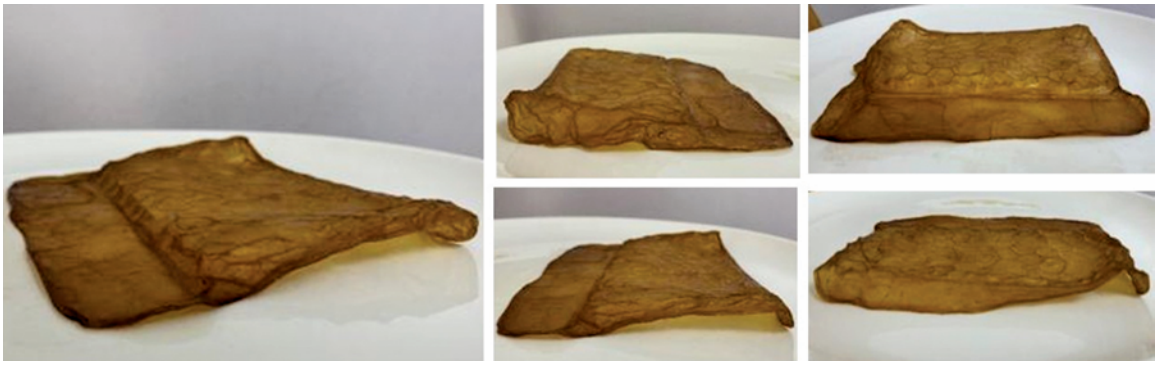


FIGURE 19. The patterned BC films. **By the authors.**

FIGURE 20. The self-healing experimentation procedures, showing the two separate integrated parts, cultivated in the same standard medium. **By the authors.**

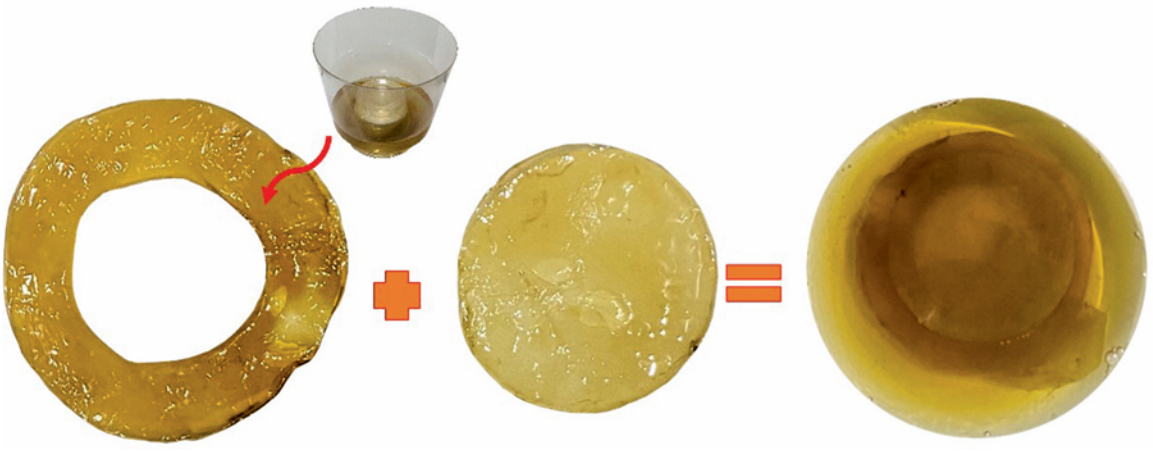


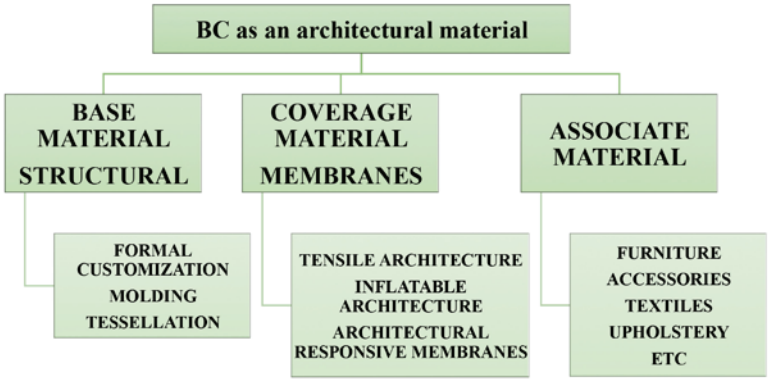
FIGURE 21. The resulting BC self-healed film after 7 days of fermentation, showing back and forth views, exhibiting material translucency and showing the welding edges between the two parts. **By the authors.**

of the BC film, and after drying the film 3 days it exhibited shrinkage in the its dimension by total 2 cm along its diameter. (Figure 21).

6. BC AS AN ARCHITECTURAL MATERIAL

The previous results of different experimentation on culturing BC, shows the unique characteristics of this biopolymer that makes it a competitive sustainable and renewably produced architectural material. The Bacterial Cellulose properties relevance to ecological Architecture can be summarized in the following: biodegradability, high mechanical and tensile strength,

FIGURE 22. Integration of BC film as an architectural material. **By the authors.**



High water holding capacity, composition of integrated and interwoven Layers, Self-healing ability, Plasticity, brittleness, and different levels of translucency. Thus, the application of BC as an architectural material could be categorized in the following:

Utilizing BC film in architectural built environment is an emergent trend recently, derived by exploiting the availability and simplicity of producing BC films and its environmentally friendly and sustainable aspect. Although the unique mechanical properties that this material exhibits, its application was limited in the interior and furniture design aspects. The architectural scale current applications of BC are still in the conceptual and experimental phase, due to the continuous development of the BC material properties and experimenting its hybridization with other natural or synthetic substrates to enhance or add special characteristics to adjust it for architectural applications.

As exhibited in Figure 22. BC film can be more likely utilized as architectural membranes or coverages, to this extent, BC films are competitive tensile material that could be exploited in tensile architecture due its unique tensile strength as inferred from the literature review. There are various recent attempts to employ BC in tensile architecture, as in the Bio-Fabric project by John, N., et al., (2017). Institute for Advanced Architecture of Catalonia (IAAC), that focused on reinforcing BC biofilm by introducing fibers to the material with two different ways: growing with fiber, where they combined the fibers and the membrane through the culturing process, and the fiber reinforcement where they added fibers at the end of the culturing process.

BC film is also a sufficient candidate for inflatable and pneumatic architecture thanks to its flexibility and shape maintaining ability. This inflatable architecture application was recently investigated by numerous researchers, for example, The Inflated Membrane by Gazit, M., (2016) at the Master of Science in Architecture, (MIT), developed a bio-pneumatic cellular envelope that functions—through pneumatic and fluid actuations—as a system for growing, shaping, and enhancing the material properties of 3D bacterial cellulose inflated structures. The developed pneumatic system is computationally controlled in order to measure the flow of air and the liquids in and out of each cell. Consequently, this system facilitates the creation of cellulose-based material with changeable properties in each cell. The most important idea in this research is the use of PDMS vessels in which the growing of microbial cellulose as 3D membrane occurred. As the BC grows only on the air-liquid interface of the media for providing sufficient oxygen to the bacterial cells. PDMS has a unique property of being liquid-proof and at the same time oxygen-permeable. Therefore, culturing BC cells in 3D PDMS vessels filled with

growth medium enables the replication of the shape of the oxygen-medium interface created by the PDMS substrate, hence allowing the ability to shape BC as it grows. Furthermore, the PDMS vessels can be produced with any shape according to the designer need.

Despite the shortage in BC applications as a base or structural material in architecture, limited attempts were conducted utilizing the formal customization, molding and tessellation methods in order to build a structural element, for instance, Li, L., et al., (2016). Urban Morphogenesis Lab, Bartlett School of Architecture, UCL, Have developed a column structure from tessellating molded cups of BC together, they designed a joint which can connect various units together in order to create an assembled structure from BC, after the assembling and connection step, they developed various domes while changing certain parameters for example height, radius and extrusion, and they connected different domes together in order to create more complicated design and self-support system.

6.1 Structural Customization

The possibility of utilizing BC as a structural architectural material is dependent on enhancing the mechanical properties and overall consolidation of the BC film, thickening it while maintaining its other tensile and flexibility properties. However, the current state of the BC film could be also utilized as a structural material in architecture applications, mainly through manipulating the geometric formation and composition of the BC. As exhibited previously in the experimental phase, the BC film is mouldable and have the ability to maintain different forms after its fastness, these properties could be exploited to structurally build a structure from BC films that are organized together to deliver the final form. This is called structural customization in favour of exploiting BC film as a structural material. This structural customization is achieved through loop optimization in the design process, that integrates the free formation of structural design with the structural efficiency simulation and the feedback that is informing the structural optimization process. This structural customization is achieved through these two interconnected strategies, that are ruled by formal customization and material development, the following Figure 23. Shows these strategies:

As exhibited previously in the current experimental work, the material reinforcement with natural fibers have enhanced the overall consolidation of the BC and increased its mechanical resistance, however, the BC aqueous base causing the liquidity prevents the potential of using

FIGURE 23. BC application as a structural material in architecture. **By the authors.**

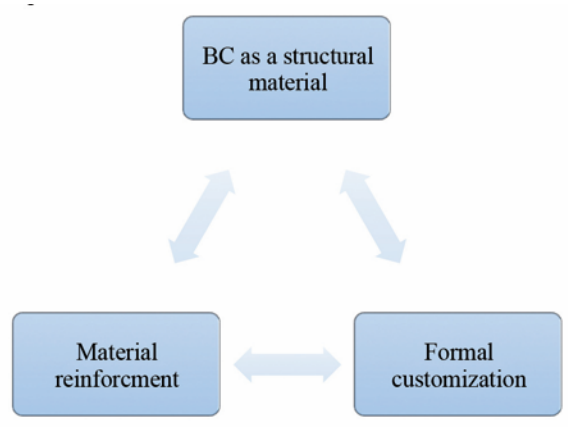


FIGURE 24. A prototype to a structural system based on natural fibers reinforcement, tessellation and molding to utilize BC as a structural material in architecture. The authors developed a mould by utilizing formed cardboard (egg box), this was a base that the wet BC film was shaped on and left to dry on it taking its shape. By the authors.

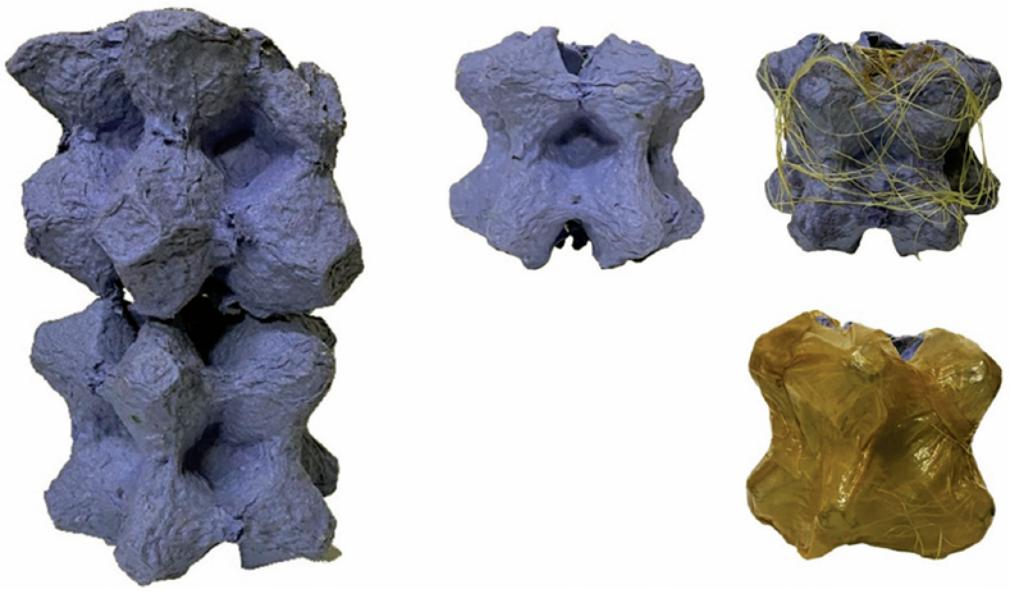
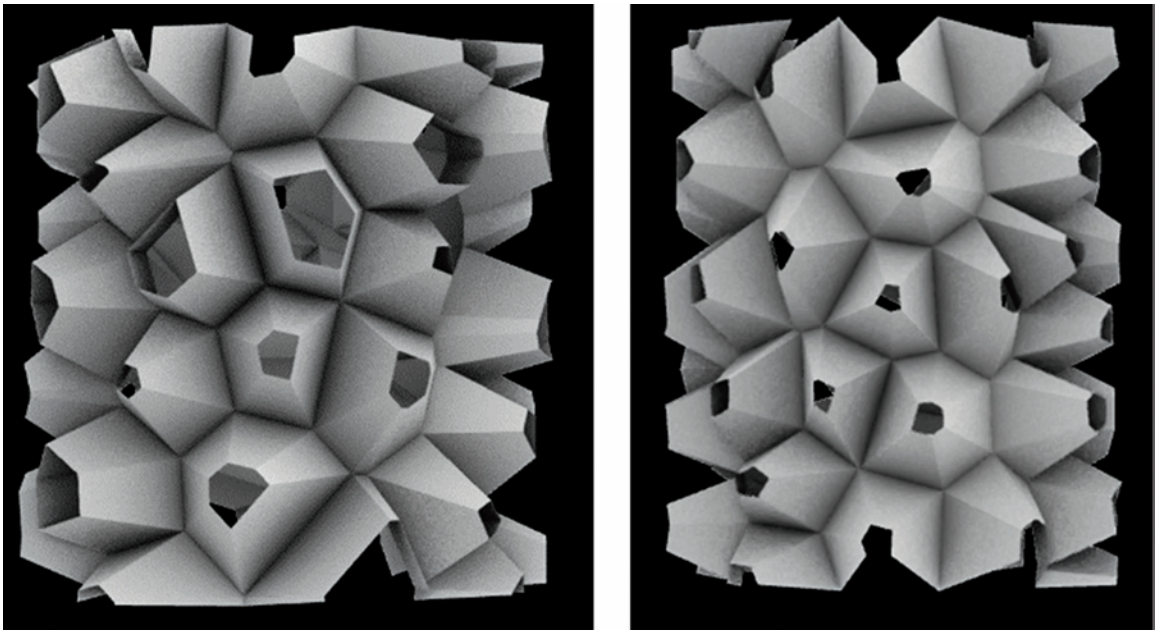


FIGURE 25. Visualization of an architectural structural element that could be developed from BC. This model was developed in Rhino+ Grasshopper 3D modelling, the structural form was optimized in order to maximize the surface area for attachment of the BC, enabling structural reinforcement through the double layer of the BC film that will grow on the outer and inner surface of each protrusion of these cones. This structural unit is 50 cm Hight, 30 cm length, and 20 cm in width, which forms a brick that could be stacked horizontally and vertically to form a bearing structural element (column, wall, partition, etc.). **By the authors.**



BC as a bearing structural material even after its fastness. The solution for exploiting all BC unique characteristics as a structural material depends on either; molding the BC layers into a building brick or block in a similar fashion of production of mycelium bricks, or formally customize the BC film during the fastness and dehydration process through molding process to produce pixels of structural units or tiles to be aggregated in a tessellated method. The tessellation methodology is similar in soul to producing building bricks; however, it enables more formal manipulation and diversity. The formal customization of the whole is dependent on the form of the pixel (the single module of dried and shaped BC film), the joinery system, and the forces direction. In Figure 24, the authors exhibit simple prototype of a BC structural system developed by combining material reinforcement with natural fibers, tessellation and molding.

CONCLUSION

The current study is part of ongoing research proposing Bacterial cellulose BC as an architectural base or structural material. The study aims to popularize and democratize the production of architectural building materials from renewable and sustainable resources on a domestic scale, in order to overcome the drawbacks of centralization and capitalization of the building materials industry, resulting in escalation in CO_2 emissions, monopolism and housing crisis. Thus, the experimental work conducted in this study focused on the simplicity and easiness of the methods and procedures of BC production process as well as the availability of the medium constituents and the bioagent to make it accessible and available for a wide range of communities, especially in developing countries. This objective was achieved through the different experimental phases in the current study, including the optimization and reinforcement of the bacterial cellulose for adjusting it to architectural application. The results have showed that maximum BC production was achieved after 10 days using *Acetobacter xylinum* as the main bioagent, sucrose as the main carbon source, and incubated at 25°C, pH4. The results also exhibited increase in shear forces resistance and overall mechanical strength by using natural fibers from palm tree as reinforcement of the BC film. The tested BC have shown mouldability and plasticity potential besides the unique ability of self-healing. To exploit all these material properties of BC, the authors have proposed a dual strategy for employing BC as a structural unit, based on material reinforcement with natural fibers and formal customization of the structural system using molding and tessellation to create the different structural elements.

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