Chapter

Bacterial Cellulose: Multipurpose Biodegradable Robust Nanomaterial

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Abstract

One of actual global problem is clothes and packing materials biodegradability leading to tremendous water contamination. In order to develop ecologically friendly, game-changing in global industry fabric production, we propose a concept to implement kombucha. Kombucha is a symbiotic bacteria and yeast multispecies consortium producing the most abundant polymer on Earth - bacterial cellulose. There are many advantages of bacterial cellulose that are widely used in medicine, material science, food industry and waste management. Unfortunately: long time of bacterial cellulose polymerisation process, lack of its control, diversity in biological composition, finally, acidic smell and disturbances of kombucha growth - all this issues limit the interest of kombucha use to replace easy-accessible and widely applied synthetic materials. In this chapter will be described a revolutionary concept to develop practical and sustainable use of bacterial cellulose as natural alternative for synthetic materials, particularly for a synthetic fabrics and plastics replacement. The optimal cultivation conditions and examples of bacterial cellulose in applications for daily life will be explained.

Keywords: nanocellulose, hydrogel, bio-fabric, design

1. Introduction

One of the most abundant polymers on Earth is cellulose, which is the dominant constituent of plants. It is widely used in daily life in the form of wood and cotton [1–3]. Unfortunately, cellulose obtained from plants has low crystallinity and is contaminated by other polymers like lignins, pectins or hemicelluloses [4]. Such cellulose requires purification processes, which are complex and need use of toxic chemicals, energy and water. Taking the above into account, a more simple and environmentally friendly solution may be use of bacteria-derived cellulose (BC) [5]. BC is a biopolymer synthesised in the fermentation process by various bacteria of genera *Gluconobacter* or *Agrobacterium* [6–9]. The bacteria-derived cellulose is possible the strongest naturally synthesised biological material, which characterises exceptional physicochemical properties, such as high purity [10], crystallinity, water holding capacity, thermal and radiation resistance, mechanical properties, specific surface area, elasticity, relatively high mechanical strength in the wet state, hydrophilicity and excellent biocompatibility [11–15] BC is a very convenient material when it comes to modifications of its applicability since cultivation method determines different shapes, properties and transformations [7]. Among several methods of producing bacterial cellulose, the most simple and ecological one is the use of a symbiotic consortium of bacteria and yeast popularly known as kombucha or SCOBY. Life kombucha cultures are easy to access on the global market and easy to cultivate. Kombucha does not require laboratory conditions, complex growing media or sophisticated cultivation and processing equipment. The optimal growth is obtained on the commonly used sweetened black tea infusion. Each person in the world can grow bacterial cellulose at home without special training. Obtained material is ready to use as an ecological substitute of plastics, storage bags, bandages, and even clothes.

2. Kombucha biological structure

Kombucha microbial consortium (KMC), is a fermented tea and non-alcoholic beverage prepared with water, tea, sugar and kombucha culture (Symbiotic Consortium Of Bacteria and Yeasts known as SCOBY or tea fungus). The Kombucha community is not found in nature. It has been originated by mankind and known already around 220 B.C in Manchuria, northeast China, when it was appreciated for its detoxifying and energising properties [16]. Kombucha microbial consortium is an example of the advanced mutualistic interactions between representatives of two kingdoms of living organisms: bacteria and yeasts [17] (**Figure 1**) . The core bacterial community within kombucha brewing is dominated by bacteria *Komagataeibacter, Acetobacter,* and *Gluconobacter* [19]. It may contain lactic acid bacteria [20, 21], but the most remarkable genera characterised in kombucha microbial consortium are *Bacteroides* and *Prevotella* known as dominant human gut microbiota species [22]. Recent metagenomics screening predicted the presence of opportunistic bacteria like *Bacillus, Pseudomonas* etc. [23, 24], bacteriophages and even yeast viruses [25]. Yeast fraction changes in genera composition depending on geographical origin, and it consists of representatives of *Zygosaccharomyces, Brettanomyces, Schizosaccharomyces, Saccharomyces*, and *Pichia* [19]. The biofilm, as a three-dimensional microbial hydrogel settlement, supports an evolutionary stable social cooperation between its inhabitants, in a way that is analogous to tissues in multicellular organisms. The biofilm may also optimise oxygen concentrations for the microbial microcolonies, which are stratified in the cellulosic matrix, as well as

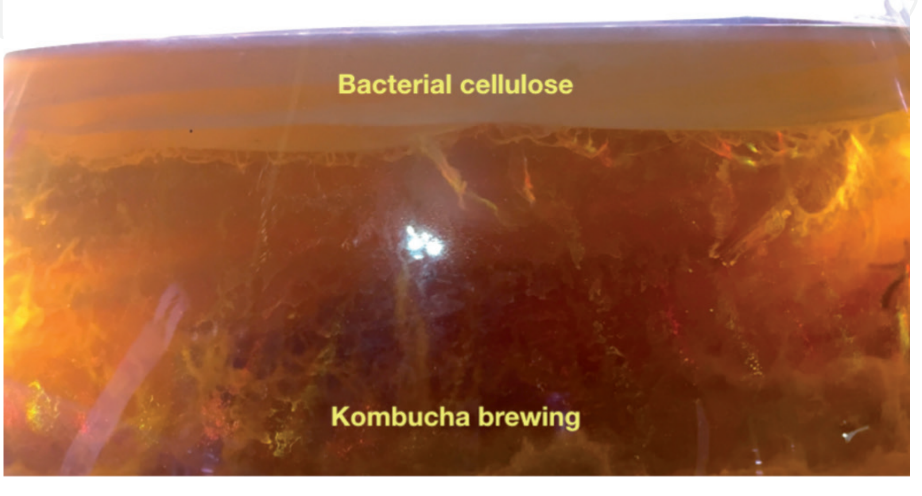

Figure 1.
Kombucha microbial consortium consists of multiple symbiotic species of bacteria and yeasts. On the surface acetic bacteria synthesise the most abundant polymer on our planet - cellulose (modified from [18]).

protect the community from environmental stressors, such as UV radiation [26]. The capability of kombucha microbial consortium to generate and tolerate acidic conditions, metabolise ethanol, and produce organic acids, protects the system from invasion by competitor microbes. The bacterially-produced cellulose-based pellicle biofilm may also provide protection from cheaters by inhibiting the diffusion of their extracellular metabolites.

3. Kombucha chemical composition

Kombucha is diverse not only considering the number of living species inside this micro-ecosystem. Mutualistic interactions between all game players implicate synthesis and transformation of multiple chemical components. Similarly as inside the living cell, many parallel processes can be distinguished. Chemical composition of kombucha beverage depends on cultivation substrate, time and temperature of fermentation process, oxygen tension, microorganisms present in the culture, and the applied method of analysis [27].

Chemical analysis of kombucha revealed *organic acids*: acetic, gluconic, glucuronic, citric, L-lactic, malic, tartaric, malonic, oxalic, succinic, pyruvic, usnic; *sugars:* sucrose, glucose, fructose, and cellulose; *vitamins* B1, B2, B6, B12,K_2 and C; 14 amino acids; biogenic amines; purines; pigments; lipids; proteins; hydrolytic enzymes; ethanol; polyols; antibiotics; carbon dioxide; tea polyphenols; minerals Cu, Fe, Mn, Ni, and Zn; anions; insufficiently known products of yeast and bacterial metabolites; rutin and kombucha-specific theobromine [28–30].

Yeasts and bacteria are involved in metabolic activities that utilise substrates in different complementary ways. Yeasts hydrolyse sucrose via enzyme invertase into monosaccharides and produce ethanol mainly from fructose via glycolysis [31–33]. Bacteria use these fermentation products to synthesise organic acids. Gluconic acid is synthesised from glucose while acetic acid derives from ethanol. Bacterial cellulose fibrils are synthesised by acetic acid bacteria and secreted out of cells as linear polysaccharide polymer, where D-glucose units are linked by β-1,4-glycosidic linkages, similar to plant-derived cellulose. BC generates structural hydrogel with interconnected ribbons of around 100 μm in length and 100 nm diameter composed of a three-dimensional nanofibrous network, which is free of both lignin and pectin. Such a peculiar supramolecular structure engineered by nature makes BC stable and robust. In static conditions, the surface of the kombucha brewing is covered by multiple interconnected reticular pellicles of bacterial cellulose. This structure is a natural barrier to protect consortiums from external environments [24]. In dynamic conditions cellulose forms irregular sphere-like particles [25, 26]. Several species of acidic bacteria are responsible for bacterial cellulose formation. Some of them, for example *Komagataeibacter xylinus,* synthesise cellulose polymers, while others have potential in both producing cellulose and fixating nitrogen [32].

Natural and biodegradable kombucha-derived bacterial cellulose has two main drawbacks: brown colour and unpleasant acidic smell [34, 35]. The brown colour derives from melanoidins, which appear in the Maillard reaction between amino acids and reducing sugars. Acidic smell of the material is due to the presence of hard to remove fermentation products, mainly carboxylic acids [36]. Mentioned problems can be reduced by application of purification methods to remove microorganisms with their metabolites from BC, and to bleach the unwanted colour. The most popular method is the use of alkaline treatment. During such a procedure, increasing concentrations from 0.5M to 5M of NaOH solutions are used [1, 3, 4, 37, 38]. The alkaline purification method requires, however, the use of significant amounts of water and neutralizers to obtain materials with neutral pH. Akkus et al. [39] examined the effect of BC pretreatment in a mixture of polyethylene oxide and NaOH on degradation of the material that could be used as a soft tissue replacement. Another substance that may be applied for this purpose is sodium dodecyl sulfate [40]. Ecological method to reduce the brown colour of BC is replacing tea breweries with algae-based cultivation medium [18, 33]. KMC cultures set on naturally basic *Chlorella* extract must be reduced to pH = 6. Bacterial nanocellulose produced in green solutions is white, delicate and glossy (**Figure 2**). Other types of algae tested in order to optimise KMC growth in space are extremophilic microorganisms such as endolythic *Galdieria sulphuraria* and *Cyanidioschyzon merolae*. It is important to note that adding a new organism to the consortium takes time and sometimes it is not possible for KMC to accept a new partner. Alternatively, yeasts can be modified with genetic engineering tools. Described examples indicate that experimenting with application of various pre- and post-treatments brings new diversified physicochemical properties of the cellulose material. At the end of these considerations, the author proposes a very simple method to reduce the brown colour and acidic smell of KBC, which can be applied at home. The method is based on boiling bacterial cellulose for 15 minutes in the solution of any household cleaning, which contains a bleach.

Another disadvantage of KBC is the fact that in dry state it resembles paper. It is brittle and its mechanical properties are poor. Therefore the final product should be coated supplementing the cellulose's network with a glycerol to provide water retention. In this simple way a polymer HydroGel Bacterial Cellulose is obtained, in which both water and glycerol provide lubrication at the molecular level [18, 33]. This treatment significantly improves mechanical properties of the material. Furthermore, such material surfaces could be additionally sealed with stearic acid to improve properties of natural fabrics. Such a wax-like additive which is spread on and adsorbed by the surface reduces the unwanted effects associated with a short-time contact of the material with water (**Figure 3**). The BC reveals ultrastructural differences in the surface of kombucha cellulose material in the three stages of its processing: raw, lyophilised and coated. The largest difference in scanning electron microscopy imaging was revealed in morphology of bacteria and polymerization patterns. After

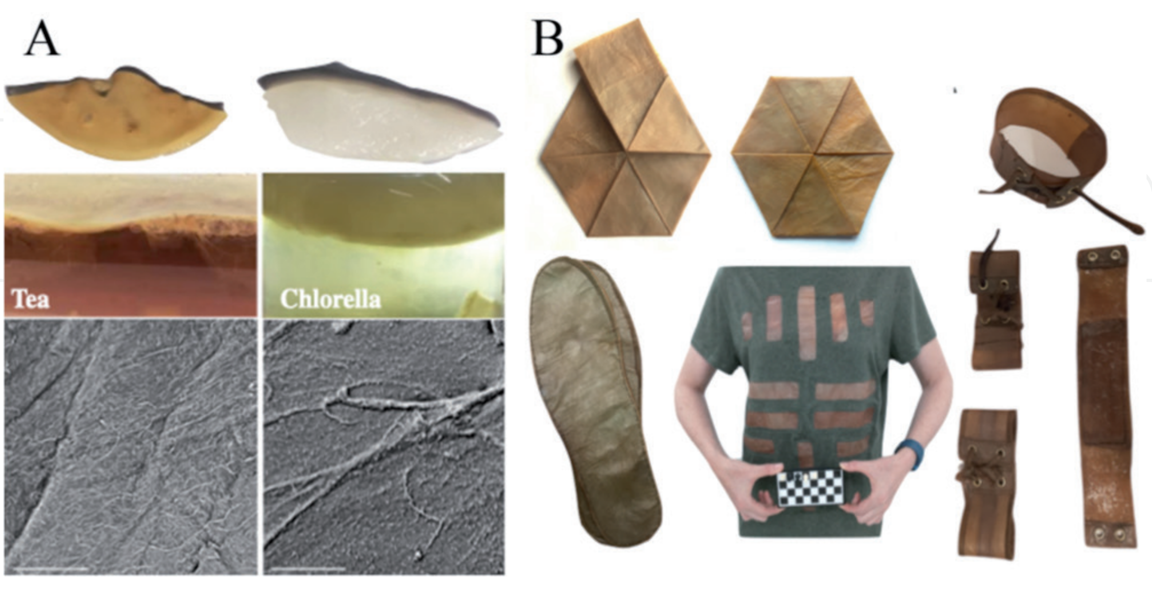

Figure 2.
(A) Two types of KMC cultures: (left) grown on tea, and (right) grown on algae. Hydrogel bacterial cellulose is similar in chemical composition but physical properties are slightly different. Nanocellulose grown on algae infusion is much softer and more prone to damage. Scanning electron microscope images reveal the ultrastructure of nanocellulose filaments. Scale bar 50 microns. (B) KMC's material absorbs odours. It can be easily cleaned and sterilised. It folds gently to very compact volumes. Folding does not implicate changes in structure of the material. Kombucha's cellulose does not generate allergic responses in direct contact with human skin. It makes skin softer and visibly healthier [18, 33].

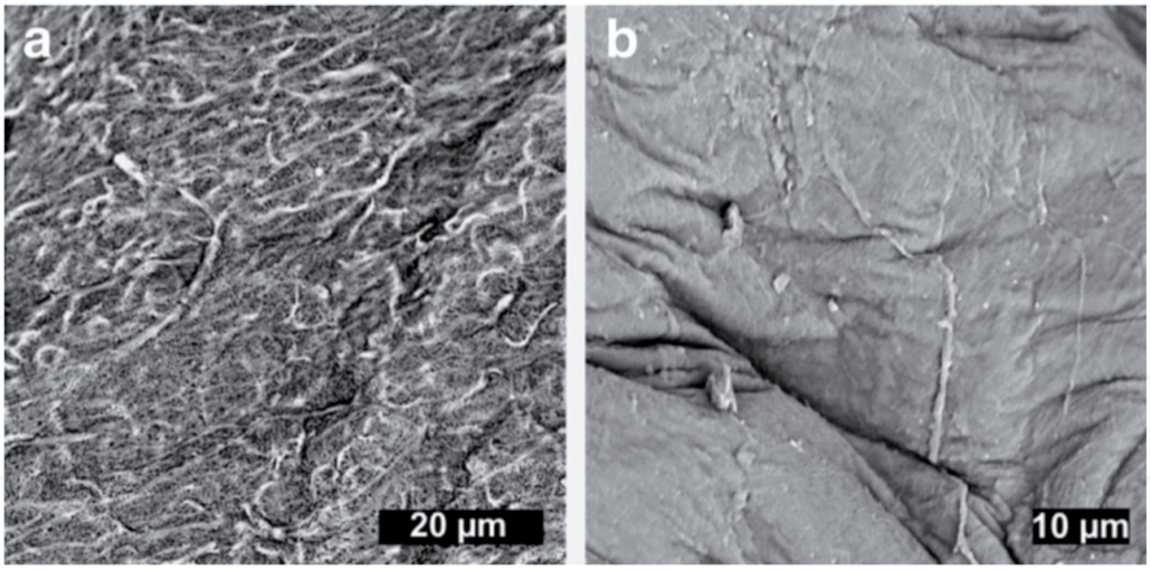

Figure 3.
Scanning electron microscopy images revealing the ultrastructure of kombucha material. (a) A view of a large fragment of the pellicle with yeast (green arrow) and bacteria (red arrow) that are embedded in the cellulose fibres (blue arrows). (b) A close-up of cellulose fibres (blue arrows) and a bacteria cell (red arrow).

sterilization of kombucha samples, no bacteria producing cellulose were visible. Microorganisms were predominantly seen as covered with multiple, distinctive cellulose fibers. After adding the coating solution, the surface of kombucha material has smoothed significantly.

4. Bacterial cellulose

Bacterial cellulose is a very interesting material for the manufacture. First, it may be produced from completely renewable and reusable sources according to the principles of waste-free technologies. Secondly, BC has higher quality for applications because of multiple advantages compared to cellulose produced by plants. It has higher thermal stability, thinner fibrils and higher tensile strength. The highly porous structure of its nanonetwork and controlled shape makes this type of cellulose the source of new ultra-light and stable nanomaterials and cellulose-based nanocomposites. When not needed, BC can easily be degraded with no ecological impact. The material is fully biodegradable, and can be reused for example in soil remediation processes.

There are two types of bacterial cellulose: raw cellulose and processed pure material. Each type of cellulose has several applications in food and package sectors, biomedicine and industry.

The most critical stage of cellulose production is its growth. The BC grows from 3 weeks up to two months depending on cultivation conditions. The procedure of synthesising the almost ready-to-use product from the raw material is very simple. To obtain the most mechanically resistant and optimally thick kombucha material for further processing, 1-2 cm thick raw bacterial cellulose must be grown. The next step is to gently remove the water from the roar hydrogel. It can be done using several methods, such as lyophilisation, drying in the oven, or using mechanical forces. The final thickness of kombucha material is about 0.1mm-1.5mm. Cellulose should be grown in a sterile dish with desired form. For example, to obtain A4 format of a raw pellet of bacterial cellulose, the material should grow in a box of this format. It is important to note, that it should be a minimum 5 cm height of the kombucha brewery level in the dish in order to obtain the best quality of the cellulose. If there is a lower volume of the kombucha solution, the cellulose will not develop properly. The container with kombucha brewing should be separated from the external environment to prevent bio contamination. It should be always covered with a lid allowing air to flow.

Raw cellulose resembles mammal skin. It has high biocompatibility, antimicrobial properties, water holding capacity and valuable metabolites. It is used as wound dressing for skin regeneration and healing, especially after bites of insects. BC exceptional bioaffinity promotes the development of biomedical products for tissue-engineered scaffolds, wound-dressing materials, dental implants, artificial blood vessels and nerves, surgical mesh, bone fillings, heart valve, meniscus, artificial cartilages, etc. [41]. Raw cellulose finds application in the food and package sector in the form of cellulose puree, fat replacer, artificial meat and food package with living biosensors.

Depending on the processing method, different types of cellulose may be produced. The most simple processing method is using cellulose for fabrics and clothes in the form of vegan leather. After drying the raw cellulose and removing the acidic smell, the surface should be coated with one or two layers of glycerin. After drying, the material is ready to use. In order to make the material resistant to water, many types of heterophobic coatings can be applied. One of the simplest solutions and ecological ways is the use of stearic acid to obtain a "wax canvas". Processed and purified bacterial cellulose is applied in production of hydrophilic films for packaging, eye lenses, artificial scaffolds, bandages, clothes, and even fuel cells. When bacterial cellulose is modified with conductive compounds, such as metal nanoparticles or graphite, it naturally becomes conductive for electricity and, therefore, promising in developing materials for electrical applications [42]. KMC living cellulose-based materials can sense and respond to their environment.

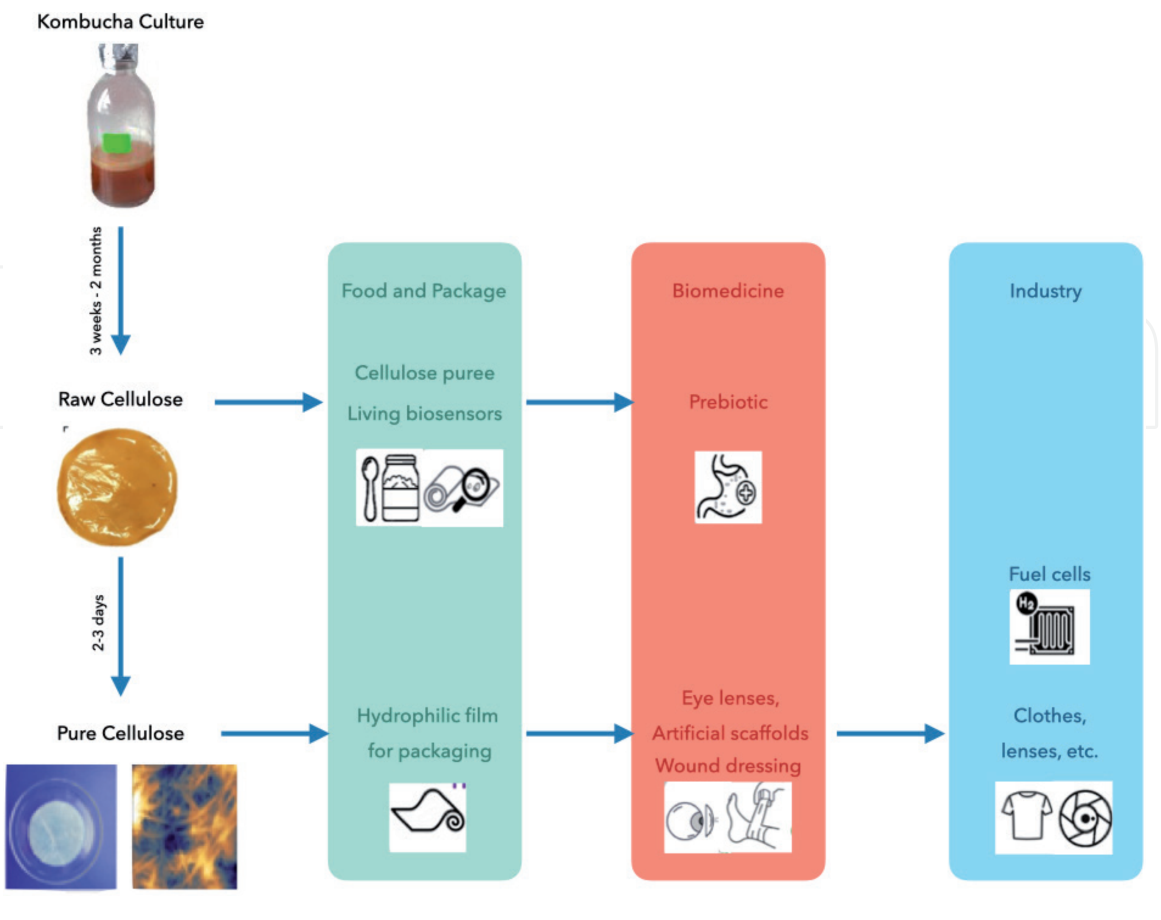

Figure 4.
Different applications of the kombucha microbial community. Modified after Kozyrovska et al. [45–47].

Synthetic biology methods provide tools to create hybrid cellulose materials with predictable novel or enhanced characteristics matching the special purposes of the developing settlements, which cannot be achieved by conventional tools. For example, engineered to secrete enzymes into bacterial cellulose, yeast strains can be taught to sense and respond to chemical and optical stimuli. This means that the modified KMC can be biosensors for many purposes [43]. Moreover, another especially potential use will be intelligent packaging for foods with short shelf-life, as well as biosensors of microbial contamination and mycotoxins.

Growing interest in bacterial cellulose makes it a multipurpose biodegradable robust nanomaterial. The worldwide market for bacterial cellulose is valued at 324.5 million USD in 2020 and is expected to increase put rill 785.1 minion USD in 2026 [44]. Interestingly, since economic and environmental issues will be critical in the early beginning of extraterrestrial settlements, kombucha is considered to be used in space as a sustainable biobased product providing biodegradable and re-usable goods and materials (**Figure 4**).

5. Zero-waste philosophy

Biofabrication of cellulose for industrial purposes based on the kombucha microbial consortium is an elegant example of eco-friendly and zero-waste production. All substrates and side products may be reused and applied in diverse ways. Residues of bacterial cellulose can be used as animal food or in formation of protosoil in various types of agricultural innovative systems including aquaponics, hydroponics and urban gardens. KMC may be stored for many years without need of any type of intervention and can be reused for cultivation any time is needed. Very sour kombucha solution can be used as vinegar, balms, or disinfectants. *Komagataeibacter* cells, as a by-product of the BC production, may be used as antioxidants, to prevent skin dryness.

6. Space application

Space is a challenging environment. Astronauts take all necessary clothes for the time of their mission. They cannot reuse them because there are no washing machines on board spaceships. The price for each kilogram lifted into space ranges from $10 000 to $25 000, which means that clothes in space are very expensive considering long-term missions [48]. There were many discussions about solving the clothing problem in space. One idea is to use bacterial cellulose. KMC-derived nanocellulose may be easily and ecologically processed, even in harsh space conditions [18, 33]. Recent studies revealed that bacterial cellulose retains robustness after 18th months of exposure on the International Space Station. Observed cellulose polymer integrity in exposed samples was not significantly changed. Only after a long-term exposure experiment, the mechanical properties of the newly synthesised cellulose were slightly changed compared to ground control BC pellicles. 2.5 years after the exposure experiment, the kombucha microbial consortium did not return to the initial composition. Among cellulose-producing species, komagataeibacter show the most significant potential of survival in extraterrestrial conditions [49, 50]. This observation indicates the need to modify the bacterial consortium to be more resistant to stressors. Genetic modification of the appropriate candidates for cellulose biofabrication meets increasing attention not only for extraterrestrial conditions but also for earthly use (**Figure 5**).

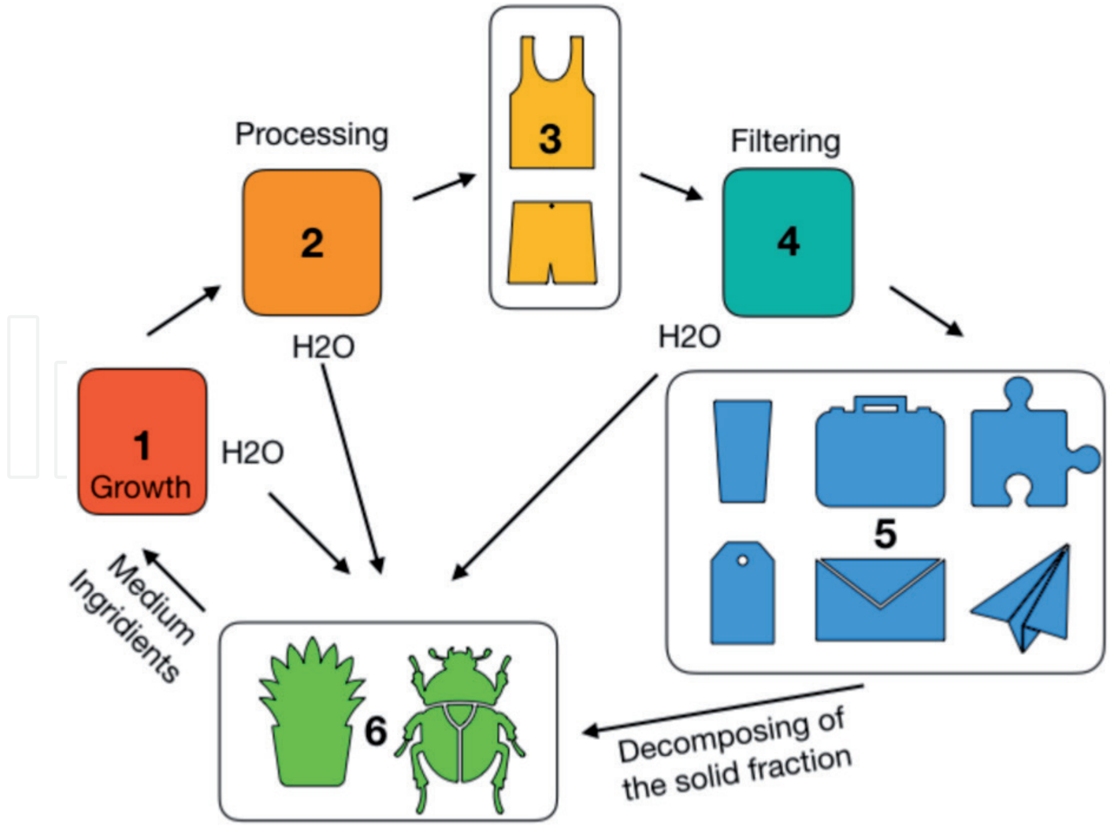

Figure 5.
The summary diagram presents a cycle of multiple processing stages of kombucha cellulose. After bacterial cellulose growth in the kombucha brewery (1), the material is processed by lyophilisation, autoclaving and coating with water-resistant medium (2). Kombucha soft material is used for clothes (3). Used clothes are transformed into filters (4). After drying, the cellulose can be used for various applications as paper, storage boxes, labels, kitchen dishes (5). Finally, bacterial cellulose is digested and decomposed in animal breedings and plant cultivations (6). Converted biomass is then used to prepare a growing medium for the kombucha brewery that encloses the cycle.

7. Summary

Kombucha Microbial Consortium is adapted to live with humans in artificial environments. Several experiments with this microbial community revealed an endless range of plasticity toward desired applications. Despite its health-promoting properties it produces one of the strongest natural biodegradable materials on our planet. A significant advantage of KMC is the zero-waste production in systems ranging all scales of cellulose biofabrication. Its organization as a micro ecosystem provides strong advantages over most microorganisms of biotech value: resistance to contamination, ease of cultivation, and high versatility. Systems and synthetic biology could be used to enhance KMC's biotechnological features but the pure beauty with this multi species community is that everyone can use it at home without sophisticated science. Each person can produce healthy probiotic drinks and eco-fabrics for daily life. Plastic packages and bags can be replaced by home-made bacterial cellulose.

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