An Open-Source Bioreactor Enhancing Microbial Cellulose Production and Novel Sustainable substances

Nitzan Cohen(✉), Emma Sicher, Ignacio Merino, and Seçil Uğur Yavuz

Faculty of Design and Art, Free Universtiy of Bolzano - Libera Università di Bolzano, Piazza Università 1, 39100 Bozen - Bolzano, BZ, Italy
nitzan.cohen@unibz.it
http://www.unibz.it/design-art

Abstract. Biodesign is a flourishing field which exemplifies a radical shift in designer's role and a changing approach towards materials, processes and systematic thinking. Instead of choosing among conventional materials and manufacturing processes, designers actively engage in developing new alternative materials by exploring the potential of living organisms as integral part of the process. This emerging field of design brings the need of new tools and enhanced knowledge transfer crossing diverse disciplines to investigate novel sustainable and alternative materials, production systems and scenarios. InnoCell is a design-led interdisciplinary research project exploring Microbial Cellulose (MC) that can be obtained through the fermentation of local apple-related byproducts with a culture of bacteria and yeasts (SCOBY). By turning 'waste' into novel sustainable substances, the project envisions a production system facilitating and optimizing MC production through open-source cultivation protocols and tools. This would optimize its knowledge transfer, support local small-scale iterations as well as possible industrial scale-up. This paper addresses the iterative development of a bioreactor module (InnoCell Bioreactor) for MC cultivation process based on residues of industrial apple processing. As such, it emphasizes the role of open-source design in raising new sustainable practices to 'grow' materials within more circular and distributed systems while fostering local resilience. Furthermore, the potential of locally recovered agri-food waste gives rise to novel applications of MC as edible and non-edible substances.

Keywords: Microbial cellulose · Growing design · Fermentation · Rotating disk bioreactor · Material production · Open source

1 Introduction - Microbial Cellulose and Its Sustainability Aspects

Cellulose is currently used in building, fashion, food, fuel, hygiene, medicine, packaging, paper, wood industries defining one of the biggest business and material flow. It is generally extracted from plants through diverse mechanical and/or chemical processes and its overall life cycle has considerable environmental repercussions [1] like high carbon emissions, eutrophication or soil impoverishment. In view of these aspects, alternative

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and more eco-efficient ways to generate and manufacture cellulose become worldwide relevant for future production and consumption perspectives. Microbial cellulose (MC) is a typology of cellulose which is secreted by several species of single microorganisms and appears in the state of translucent jelly biofilms or ribbons. However, cultures of cooperating bacteria (like Komagataeibacter xylinus) and yeasts (Saccharomyces Cerevisiae) called SCOBY (Symbiotic Colony Of Bacteria and Yeasts) have proven to provide better MC properties [2]. SCOBY, being an acetic 'mother' is renowned for being used for edible purposes namely, to brew the Kombucha beverage, which is a fermented green/black tea, and to produce Nata de Coco from coconut wastewater, a low calories and fiber rich dessert which is popular in the Philippines [3], Vietnam, Thailand and Indonesia [4]. The jelly-like biofilms can be processed into diverse substances that could be either edible, or not, ranging from powder to flakes and sheets, from gel to foam and composites. Being characterized by a three-dimensional fiber-configuration, a hydrophilic capacity and nutritional properties, MC is suitable for diverse applications such as food-ingredients (prebiotic, integrator, fat replacer, texturizer) [5, 6], (non)edible materials (filler, emulsifier, compostable substances), products like single-use packaging, cosmetics, components for electronics, medical and pharmaceutical applications [7, 8]. MC is made almost entirely of nanofibers [9] and its cellulose content is purer in comparison to the one extracted from vegetative sources. Moreover, MC production appears to be more sustainable and resource-efficient when comparing the two typologies [10]. MC is cultivated or 'grown' as microorganisms nourish on the nutrients present in a liquid medium and secrete fibers of which volume is multiplied up to centimeters in thickness. Tea and sugar are commonly used to feed SCOBY however, they are very production-expensive so, other water-based nourishments can be prepared with diverse carbon (sugar containing) sources [11, 12]. It was investigated by diverse scholars that liquid medium prepared substituting virgin sugar with food-production byproducts like molasses and pomace, were suitable and effective for SCOBY fermentation [7, 13]. This practice of further utilizing 'waste' would not only enhance the inherent value of primary resources but would also lower production costs, introduce profitable upcycling processes fostering local production possibilities. Circular production systems revaluing waste can improve current linear production cycles where valuable substances are systematically not used for their full potential and are down-cycled. Indeed, the implementation of MC production could foster a more conscious use of resources generating a positive ecological and even social impact by reducing the total dependence on import, while supporting local economic resilience [14]. As an alternative to the current dichotomy of 'products' and 'byproducts' a new production axiom could be introduced by considering all substances involved in the process as 'co-products' with generic equal value, enhancing the recognition of the primary value of resources [15]. Additionally, different agricultural crops provide diverse properties to MC biofilms and fermented liquid which can be used in various specific applications. Supporting this, data regarding structural determination of MC fibers and their impact on microbiota under simulated gastrointestinal conditions were provided by Micro4DFood team at the Faculty of Science and Technology of the Free University of Bozen-Bolzano. Their unpublished results generated by iterated comparative tests revealed that samples of MC grown from green tea appears to inhibit bacteria strains present in human microbiota and therefore its effects on human digestion range from neutral to negative. This finding suggests that tea-obtained MC is more suitable for non-food applications opening valuable upcycling perspectives for current disposed SCOBY mass generated from Kombucha production. However, MC grown from apple pomace-based broth showed presence of arabinoxylans -which are valuable antioxidants- suggesting promising perspectives in food applications as a prebiotic source. Antioxidants' qualities of Kombucha are already known in literature [16] and further noteworthy data could be discovered revealing inherent qualities of by-products-based broths aimed for MC culture. According to these studies, the use of diverse agricultural resources for MC production could emphasize its local origin and belonging through specialized applications.

By recognizing MC's potential and aiming to push forward its investigation and potential application, this paper presents the development of a bioreactor module called the 'InnoCell Bioreactor' (IB) as a self-produced unit enabling enhanced MC production and yield. The IB is an outcome of the interdisciplinary project InnoCell exploring the (g)local potential of Microbial Cellulose, its production and applications. The project supports knowledge democratization as it openly and transparently shares all the accumulated knowledge through an online platform. This article contextualizes the project by framing Biodesign as an emergent field with a focus on the design of new, sustainable materials that are 'grown' with the aid of existing organisms. It demonstrates how cross fertilization among disciplines supports the development of open-source tools providing optimal conditions for microbial processes making use of agri-food waste. A comparison is made among MC production methods, identifying the 'rotating disk bioreactor' as the most efficient one, and the main principles behind the IB function. Furthermore, the IB is designed as a 'do it yourself' module that could be scaled up or down according to need and technological availability. A following section highlights the role of the open-source platform in disseminating the instructions and the fermentation protocols, ensuring the IB's proper construction, use and maintenance. 'Future perspectives' discusses how improvements in the possible automatization of the IB could further optimize the monitoring and efficiency of the process. Eventually, emphasis is given to further speculation and system thinking towards production models strongly related to (g)local production systems and areas. In the conclusion the importance of the democratization of knowledge through open-source practices is stressed, expanding the act of growing from materials to networks of knowledge.

2 Bio-, Material-Design and InnoCell

Biodesign [17] is an emerging field in design also fostering sustainable materials development through the aid of -often integrated- living organisms (like bacteria, algae, fungi and hybrid cultures) in their processing and production. This approach sees microorganisms as co-workers of humans [18] that cultivate and 'grow' materials. Besides, material design is a practice in which designers identify underused resources within artificial production or natural environments [19]. With those, they make new circular materials while envisioning and creating sustainable material scenarios with beneficial social and environmental impacts. These two disciplines have many contact points and are often integrated. However, in order to obtain substances with specific characteristics, designers create new tools and optimize existing methods bridging scientific findings, industrial capabilities and environmental needs. The need for building efficient systems aimed at 'growing materials' is facilitated by today's digital fabrication and numerical control technologies which are distributed and enable efficient open-source knowledge transfer and applications. By highlighting the importance of open-source tools and self-made equipment in Biodesign, the InnoCell project explores the (g)local potential of Microbial Cellulose, its production and applications. Originating from a bachelor thesis project envisioning MC as a sustainable alternative for packaging [20] it furthermore explores a more local and distributed production of food products and (edible) materials. 'What if we made trash that feeds the environment rather than contaminates it?' [21] This scenario is supported by MC's valuable properties, local crafting potential and its inherent compostability. InnoCell, is a project led by a design team and a food-technology team, and partners with international stakeholders. The research takes place in the South Tyrol region which is renowned for its apple monocultures (12% of European production) [22] and develops scenarios proposing circular cycles by using apple-related waste to feed SCOBY cultures. This practice enhances the value of food-production byproducts fostering the development of more circular food and material production [23]. Locally manufactured MC novel food and non-food products could have considerable potential in future business perspectives, regional identity, its value creation and retention, management of resources and overall positive environmental impact. This production principle could be eventually applied to diverse areas around the world encouraging (g)local resilience practices. InnoCell shows how interdisciplinary grounds can become fruitful soils generating innovative scenarios. Through tangible and intangible tools and methods, scientific findings see potential applications and translation into industrial production dynamics proposing sustainable alternatives. Two important aspects of the project are the development of an open-source system for enhanced MC production: the InnoCell Bioreactor (IB) and an optimized recipe for apple-scraps-based broth for SCOBY fermentation.

3 MC Production Methods

Commonly used current systems in Kombucha drink and Nata de Coco industrial production are based on a MC fermentation method called static culture that uses tanks or trays. This procedure starts with the preparation of the liquid medium, which is poured in the meant container. An amount of SCOBY is immersed inside, and a breathable cloth or cover is put on the container to protect the culture from contamination. The culture is kept in static conditions (possibly supported by a system to control the temperature) for a period of 7 to 21 days - for the fermented beverage. At the end of the process a generated MC biofilm floats on top of the liquid, usually covering the complete surface. While being a very simple method to be reproduced, static culture used for MC production has its shortcomings: it is highly laborious to ensure a proper production rate, it provides a low efficiency concerning time and generated yield [24] and it does not enable easy control and adjustment of the culture during fermentation [25] as the MC membranes tend to cover the complete liquid/container surface. As it seems, MC is currently not existing as a raw material in the market, but SCOBY fermentation starters are highly available. However, startups, emerging companies and research institutes [26–29] are investigating and optimizing current methods and enhanced processes to produce MC-based products. With the same purposes, designers have been exploring MC production within the 'growing-design' [30] and digital bio-fabrication fields [31]. Current technologies with their accessibility and availability support designers in creating close relationships with machines and systems, promoting and spreading self-production [32].

Diverse techniques and machines to obtain MC biofilms and three-dimensional shapes [33–37] were developed but stayed eventually on a conceptual/proof-of-concept stage. However, a critical gap is present between home-brewing, experimental procedures and scaled-up production which brings anyone wishing to venture into MC experimentation to rely on DIY methods and often limited resources. Other existing processes which provide more efficient MC production are stirred and agitated cultures, rotating disk bioreactor, rotary biofilm contactor, spin filter-equipped bioreactor, reactor with a silicone biofilm [8, 11, 38]. According to Bungay et al. [11], the technique that appears to have the most effective time-yield ratio is rotating disk bioreactor (RDB). This system is characterized by a rotating shaft with perforated disks which serve as gripping elements for MC. The shaft is positioned partially immersed into a tank aimed to contain the liquid medium and the SCOBY. A motor performs a constant and smooth orbital movement which promotes an enhanced oxygenation into the culture. Over a period of 14–21 days MC thickens on both sides of the disks providing a remarkably higher yield in comparison to static culture thanks to surface optimization [39]. Indeed, a comparative study showed that an RDB system yielded 95% more mass than static fermentation and 31% more than shaken culture [38]. An aspect that needs to be considered is that RDB-produced MC has a higher water retention, and the studies refer to wet mass rather than dry mass.

Besides, another advantage is that as the biofilms do not grow directly on the liquid but on the disks so, controlling and adjusting the temperature, acidity (PH), sugar content (Brix) is practical and efficient. Moreover, the RDB is proven to be a space optimization production module, opening further industrialization possibilities and high-volume production.

4 Developing the InnoCell Bioreactor

The InnoCell Bioractor (IB) is based on scientific literature findings [11, 24, 25] and an expired patent [40]. It was developed for achieving optimal production and to be easily reproduced with distributed means available in small workshops, Fablabs, etc. Further on, its direct dissemination would follow through a dedicated online platform and would consist of a complete materials and components overview, their production and the IB maintenance instructions. All is aiming towards an optimized knowledge transfer and its democratization facilitating enhanced research. The development phase consists of iterative cycles of prototyping and testing that led to the final version of IB. In this phase, since the main goal is to create an open-source machine, digital fabrication techniques and easy to access components are used. A first 16l prototype was developed for testing the perforated disk patterns. A geared motor with encoder (12 V/28 RPM/80 kg) and a speed controller (5 V–30 V 6 A 150 W) enabled the identification of the ideal mechanical parameters for MC growth. The liquid medium preparation protocol and fermentation control were further-on iteratively tested by the project partners of the Food Technology Lab. A following 25l version (Fig. 1) was developed in order to produce bigger amounts of SCOBY. The IB, while having the same configuration of a rotating disk bioreactor, embeds further improvements and differences. The final version is assembled with components made through the aid of digital fabrication tools (3D printing, laser cutting) and a geared motor (52 MM, 12 MM, 6–12 VDC, 488:1) which is constantly turning; rpm change according to nourishing liquid ranging from 8,5 (apple) to 11 (tea). The design choices of the IB are strictly functional. Predominant materials are acrylic and polycarbonate because of microbial suitability and to simplify the checking of the MC culture and growth from the outside. Two removable external surfaces have disassemblable bearings and 3D printed holding structures which ensure a smooth rotation of the shaft positioned among them. The shaft has two inox holders at its ends which are inserted through the bearings. An additional central bearing placed on a support contributes to better distribute the weight of the shaft. The half cylinder shape of the tank optimizes the inner volume by following the profile of the twenty-eight disks that have a diameter of 25 cm. They are mounted on a square-profiled polycarbonate shaft, separated with 3D printed spacers and fixed with 3D printed stoppers. A specific pattern of holes, together with a surface treatment optimizes the growth of MC on the disks: a recent cycle generated wet MC mass per disk with an average of 500 g. per disk, reaching a total yield of 13,5 kg. Accessories like a removable cloth cover and a heating system (made of filter, universal aquarium pump, vertical liquid heater, tubes and a metal holder) were also designed to retain a constant temperature optimizing the fermentation conditions. In particular, the heating system creates a circulation of the liquid medium inside the tank which maintains the homogeneity of the culture.

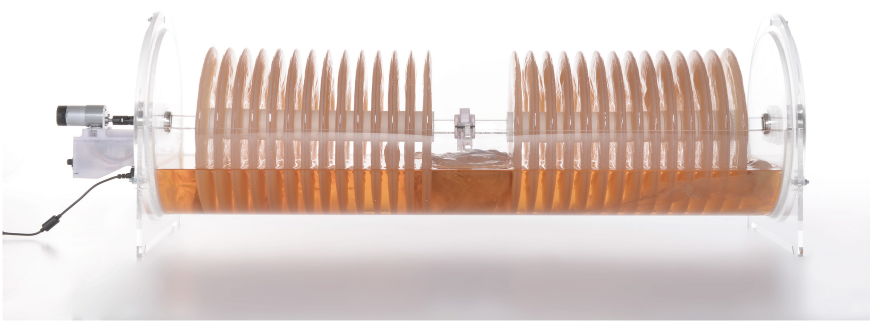

Fig. 1. 25l prototype (*heating and covering systems are not shown*)

5 Open Source

With the current design as an alternative to static culture production, the aim of the IB is to support anyone interested in venturing into MC through a more advanced production system. Instructions to build, use and maintain the IB will be disseminated through an online open-source platform currently under development. This means of knowledge transfer would possibly raise another dimension in the action of 'growing' by providing advanced tools to turn mere material tinkering into a more reliable and controllable food and non-food production possibilities. The concept of growth relates not only to the extension of production practices but also to 'growing' networks of knowledge. This principle is following a broader scope related to social sustainability, which is achieved when open knowledge makes sustainable development accessible by anyone. Therefore, an open online platform would serve as a dissemination tool to reach communities and individuals and foster further related research around the globe.

6 Future Perspectives

The current version of IB is a valuable prototype for further advanced iterations and a base for future scale-up and down. The knowledge and developed protocols are just as much suitable for both, industrial and more experimental scopes. Further evolution of the bioreactor could regard the automatization and monitoring of the process. These enhancements would considerably reduce the manual labor which is currently required throughout the cycle. Another aspect for future follow-up is the development of customized SCOBY cultures and optimized broth recipes related to diverse single resources recycled from agri-food production. Thus, this would optimize further-on the MC production and yield while potentially adapting it to stakeholders' needs and food/non-food applications.

Systemic thinking [41] is fundamental in design practice to envision and propose meaningful products and production alternatives. With the current design, the IB is a highly effective, space-efficient module enabling high volume production. The module could aid anyone who is willing to venture into MC experimentation and production. The current module logic enables human-scale handling and management in 'semi-handcrafting dimension' [42]. Moreover, its integration in diverse systemic models could take place, not only MC production could be in-housed but specialized facilities could foster the creation of distributed systems. Speculative design scenarios developed within the InnoCell project envisioning the possible integration of MC manufacturing in current production cycles, aim at the creation of more resilient and connected communities [43]. Indeed, the IB can contribute to connecting food and non-food productions through MC fermentation. Indeed, benefits of the implementation of IB modules in the facilities or infrastructures of local producers (of apple products or derived from other fruit and vegetable sources) would regard the further utilization of byproducts generating new profitable foods or material-related profitable streams.

7 Conclusion

Microbial cellulose is recognized as a valuable edible and non-edible substance with promising potential for envisioning sustainable product solutions and new local production scenarios. Despite these favourable perspectives, it is observed that there is little accessibility to MC mass amounts and related R&D practices. By reacting on problematic aspects related to the most common MC production method namely static culture, the InnoCell project developed a bioreactor (IB) based on established scientific studies. The principle of rotating disk bioreactor was developed into a reproducible module that is open-source. This bioreactor can play a valuable role in future production and consumption perspectives related to food and non-food production realities. Indeed, the implementation of IB modules in the facilities or infrastructures of local producers could generate new profitable streams while applying a more circular approach towards byproducts.

All the information concerning the physical building and maintenance of the IB would be disseminated on an online platform. Moreover, protocols for optimized cultivation, applications and system implementation of apple-based MC are other key elements in the project's knowledge transfer, supporting further on (g)local MC research and production. In this way, the meaning of 'growing' would shift from alternative production practices to growing networks of knowledge. Through an interdisciplinary approach bringing together the design world with food-technology, the democratization of MC production knowledge and technologies would foster systemic sustainable alternatives. This concept is translated in the practice of fermenting underused resources to produce valuable and functional MC foods and materials with peculiar qualities.

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