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An assessment of the susceptibility of bacterial cellulose films to fouling by mold fungi

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Abstract: *An assessment of susceptibility of bacterial cellulose films to fouling by mold fungi.* The article presents the results of research on the degree of fouling of films made of bacterial cellulose by selected mold fungi. The degree of fouling of the cellulose film was compared with the degree of fouling of pine wood samples. On the basis of the obtained results, it was found that the cellulose film is covered by mold fungi. At the same time, it was found that *T. viride* grows on wood much faster than bacterial cellulose.

Keywords: bacterial cellulose, degree of fouling, kombucha, mold fungi

INTRODUCTION

Bacterial cellulose is an exopolymer composed of β-1,4 D glucopyranose units. In terms of chemistry, it is the same polymer as plant cellulose, but unlike it, it is devoid of encrusting substances that affect its features and properties. Bacterial cellulose is produced by aerobic microorganisms carrying out the acetic fermentation process. The first research on the synthesis of cellulose by microorganisms was published in 1886 by Brown. This polymer can be produced by a variety of microorganisms, both bacteria and fungi, including yeast-like fungi. Wang et al. (2019) indicated that the structure of the polymer, the size of the fibres and its properties depend on the type of microorganisms that synthesize it. Among the cellulose-producing microorganisms, the most common are the species of *Acetobacter xylinum*, *A. hansenii* and *A. pasteurianus*, and those belonging to the genera *Aerobacter, Achromobacter, Agrobacterium, Psedomonas, Sarcina Zooglea and Rhizobium* (Jung et al. 2007; Yamada et al. 2012; Lee et al. al. 2014, Skocaj 2019). The best studied species of biopolymer synthesizing bacterium is *A. xylinum.* Sushil (2018) reports that this bacterium is capable of converting 108 glucose molecules into cellulose in one hour. Unlike its plant counterpart Bacterial cellulose is characterized by a high degree of crystallinity, reaching even 85%, and a higher degree of polymerization than in plant cellulose. The Iα/Iβ ratio of cellulose in the biopolymer can be modified by selecting the appropriate culture parameters and polymer drying methods, which was proved by Stanisławska et al. (2020). Using freeze-drying as a method of drying cellulose, the authors of the studies obtained a value of the degree of crystallinity at the level of almost 92%. The properties of bacterial cellulose depend on many factors, including the method and conditions of cultivation, nutrients available to the microorganisms, the methods and conditions of polymer processing. These factors have a huge impact on the strength of the polymer and its hygroscopicity (Kiziltas et al. 2015, Yim et al. 2017) and the morphological structure (Betlej 2019). Comparing the tensile strength of a polymer dried at 25˚C and 105˚C, Stanisławska et al. (2020) found that the polymer dried at 25˚C is characterized by tensile strength 15 times greater than that of the polymer dried at 105˚C. Similar results were obtained by Domskiene et al. (2019). On the other hand, Betlej et al. (2020) obtained a very low cellulose strength, which was associated with the cultivation of microorganisms that synthesize the polymer on a very poor substrate. The influence of the components of the culture medium on the strength properties and Young's modulus was described by Amorim et al. (2019), Illa et al. (2019) and Skvortsova et al. (2019). Very good physical and mechanical properties of bacterial cellulose make it a potentially promising biomaterial for applications in various sectors of the economy. So far, most studies have been

devoted to the possibilities of using bacterial cellulose in medicine (Cavicchioli et al. 2015, Juncu et al. 2016), in paper and packaging (Lim et al. 2016, Skocaj 2019). Cellulose films can successfully replace paper and plastics used as food packaging. Bacterial cellulose is a completely biodegradable, ecological and non-toxic material. It can be prepared in a relatively short time on media containing various types of saccharides. In the form in which it is synthesized or in a processed form, it can be used for the production of special papers (Skocaj 2019), membranes useful in wastewater treatment technology (Urbina et al. 2018), air filtering materials (Liu et al. 2017), and even modified chipboard (Wacikowski and Michałowski 2020). As a natural material, it is completely biodegradable without threatening the environment, which is a very important aspect in the context of the idea of sustainable product development.

The aim of the presented research was to produce a cellulose film in laboratory culture conditions with the use of microorganisms forming the kombucha ecosystem and to assess the film susceptibility to fouling by mold fungi. The degree of fouling of cellulose films was compared with the degree of fouling of a material containing plant cellulose - wood samples of Scots pine.

MATERIALS AND METHODS

The assessment of the susceptibility of the bacterial cellulose film to fouling by mold fungi was carried out based on the guidelines of the method described by Borysiuk et al. (2020). As model mold fungi, three species of fungi from the collection of the Department of Wood Science and Wood Protection were used. Starter cultures of fungi *Trichoderma viride* Pers., Strain A-102, *Aspergillus niger* strain 287 and *Chaetomium globosum* Kunze 1817 were grown on maltose-agar broth.

Bacterial cellulose samples were produced by the microorganisms that make up the kombucha ecosystem. The cultivation of cellulose-synthesizing microorganisms was carried out for a period of 14 days in a calf incubator at a temperature of 26 ± 2˚C and a relative air humidity of 66 ± 2%. After the end of the cultivation period, the produced cellulose was removed from the surface of the liquid, which was then cleaned of the remnants of microorganism cells. The polymer purification procedure consisted of washing with detergent, and then washing it twice in water. Subsequently, the cellulose purification consisted of soaking for 30 minutes in 0.1% NaOH, rinsing again in water, and then rinsing in 0.1% citric acid and rinsing again several times in water. After the purification process was completed, the polymer was ground in a laboratory blender to obtain a pulp. The cellulose pulp was deaerated in a vacuum oven with a vacuum of 100 mbar for a period of 2 hours. The right amount of cellulose pulp was placed and spread in a silicone form. The cellulose prepared in this way was dried at a temperature of 26±2˚C until the weight of the polymer stopped changing. The cellulose film obtained in this way was cut into 25 x 25 mm samples, which were stored in the dark until the beginning of the tests. The thickness of the film samples was measured with a thickness gauge and ranged from 0.14 to 0.17 mm.

An assessment of the degree of fouling of cellulose films by mold fungi was carried out on a maltose-agar medium on Petri dishes with a diameter of 90 mm. The samples were placed on glass plates of the same size as the cellulose samples. Glass plates were placed centrally on empty plates, to which the nutrient solution was then added in such a way that the surface of the glass plate remained free of the nutrient. After placing the foil on the plates, the inoculum of the tested fungi was placed on the surface of the medium. The degree of fouling of the cellulose films was assessed at 24-hour intervals. Each sample was photographed and the photo image analyzed with Image J2-Fiji v1.52i image analysis software (Schindelin et al., 2012; Tinevez et al., 2017). The degree of mycelium development on the samples was expressed on a point scale in which point equal to 1 meant 100% of the area occupied in

relation to the total top surface of the samples. Each study was performed in five replications. Control samples were subjected to the same test procedure but without the exposure to the fungi. The assessment of the degree of fouling of bacterial cellulose films by mold fungi was compared to the degree of fouling of the whitish pine wood samples *P. silvestris* by the tested test fungi. Wood samples with dimensions of 50x50x3 mm were tested in the same way and under the same conditions as the samples of bacterial cellulose.

RESULTS

Graphical evaluation of the degree of the growth of bacterial cellulose film samples by selected mold fungi is shown in Figures 1 and 2. The tested cellulose samples showed similar susceptibility to mold growth, especially in the initial period, i.e. from the second to the fifth day. At the same time, lower dynamics of film fouling by *Ch. globosum*. *A. niger* fungus completely covered the upper surface of the cellulose film already in the sixth day of incubation. In the case of *T. viride* fungus, complete overgrowing of the film took place in the last day of observation. In the case of samples infected with *Ch. globosum*, the complete covering of the film was not obtained in the assumed experimental period.

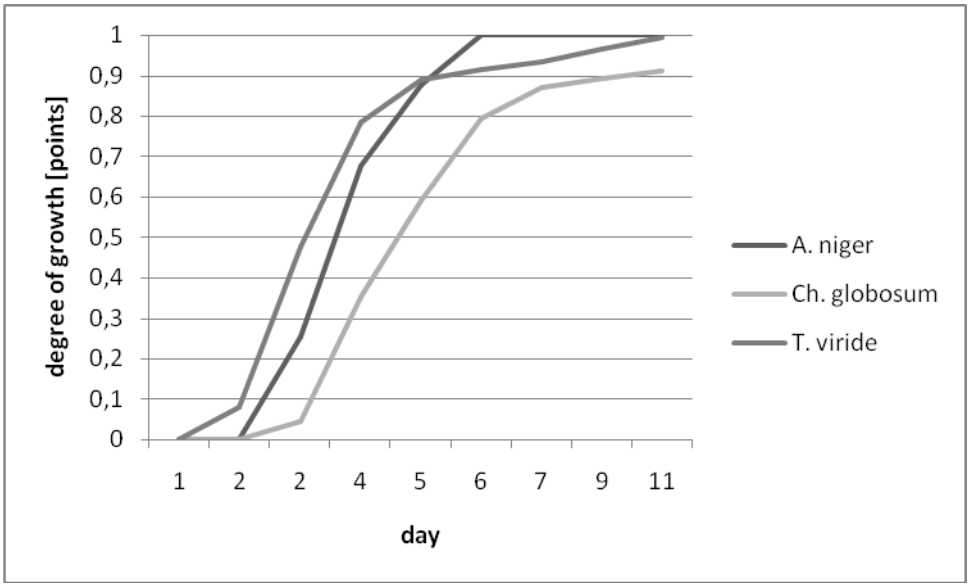

Figure 1. Degrees of bacterial cellulose foil growth by selected mold fungi

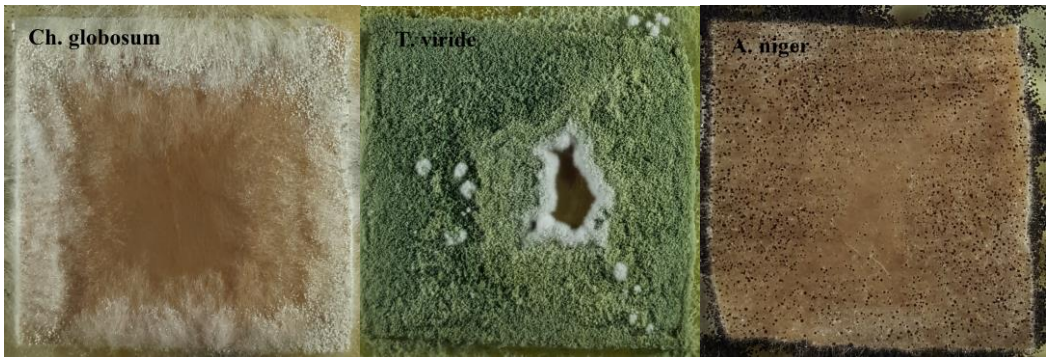

Figure 2. A visual image of bacterial cellulose foil growth by the tested mold fungi on the last day of the measurement

By comparing the degree of fouling of the cellulose film and pine wood samples by the tested test fungi, interesting observations were obtained (Figures 3, 4 and 5). *T. viride* fungus grew completely faster on the upper surface of pine wood than on cellulose film.

Already on the fourth day of cultivation, the pine sample was completely overgrown by the tested fungus. Observing the dynamics and the degree of *A. niger* fouling of the tested samples, it was found that this fungus grows much better on pure cellulose. Also interesting results were obtained for samples of cellulose film and pine wood infected with *Ch. globosum*. At the assumed cultivation time, the degree of wood fouling was lower than that of the cellulose film. However, in the final period of the experiment, the wood samples, unlike the film, were completely overgrown by the tested fungus.

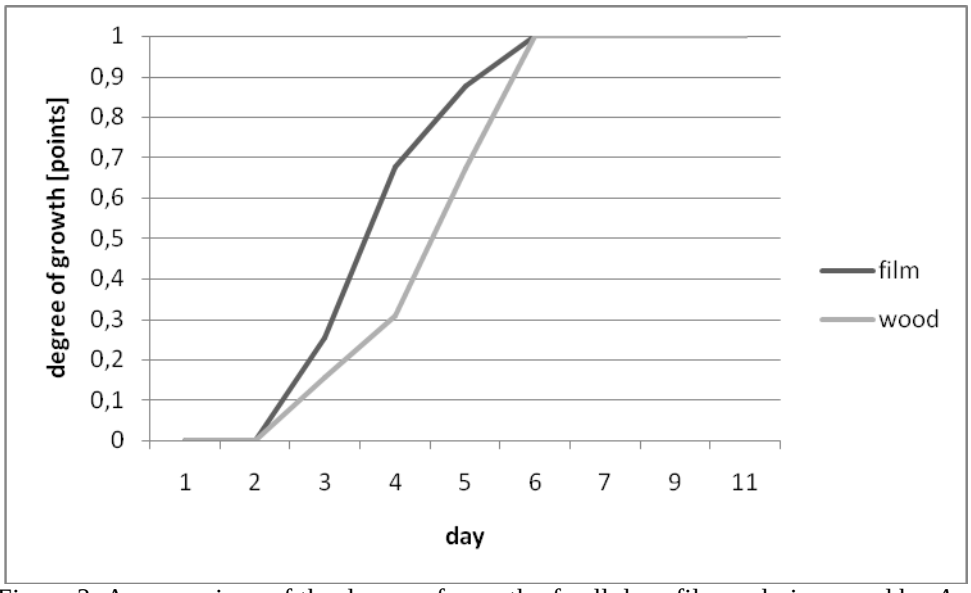

Figure 3. A comparison of the degree of growth of cellulose film and pine wood by *A. niger*

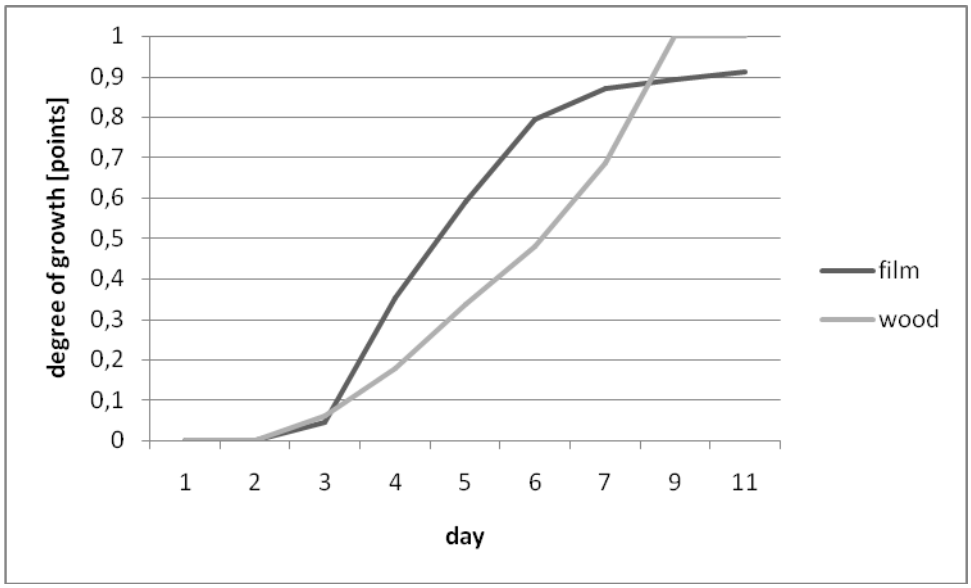

Figure 4. A comparison of the degree of growth of cellulose film and pine wood by the fungus *Ch. globosum*

The obtained results show that cellulose film is susceptible to mold growth, which suggests that it can also be easily biodegradable. On the one hand, different rates of foil fouling by the tested fungi may result from the physiology of the fungi themselves. However, based on the results presented for wood samples, it can be assumed that the type of infected material and its chemical composition determine the speed and the degree of its growth by fungi.

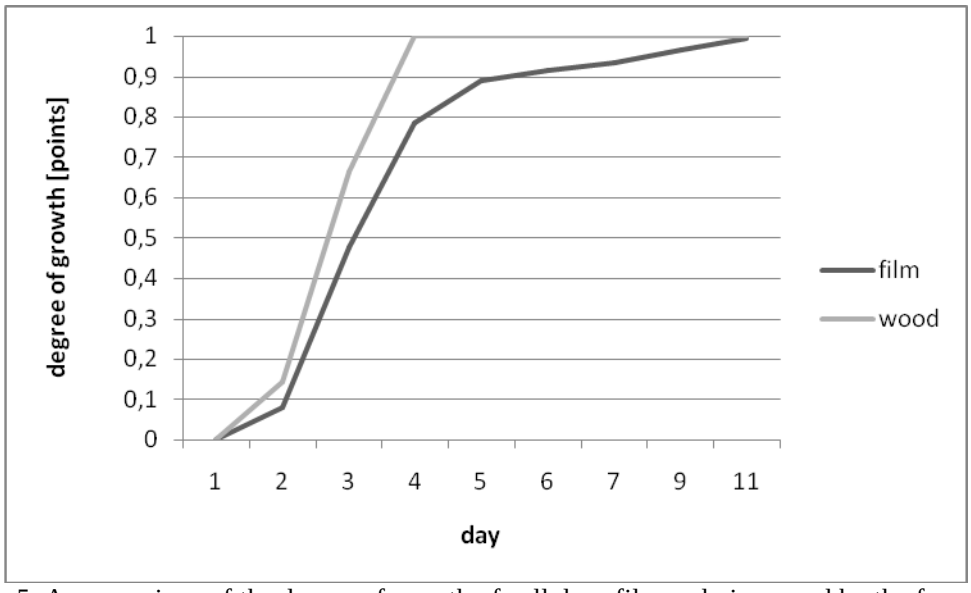

Figure 5. A comparison of the degree of growth of cellulose film and pine wood by the fungus *T. vride*

CONCLUSION

1. The tested test fungi are able to cover the bacterial cellulose film, which suggests that bacterial cellulose is an easily biodegradable material.
2. *Ch. globosum* shows less fouling of the cellulose film than *A. niger* and *T. viride*.
3. The *T. viride* fungus grows much faster on the pine wood surface than on bacterial cellulose.

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Streszczenie: W artykule przedstawiono wyniki badań stopnia porastania folii wytworzonych z celulozy bakteryjnej przez wybrane grzyby pleśniowe. Stopień porastania folii celulozowej porównano ze stopniem porastania próbek drewna sosny. Na podstawie uzyskanych wyników stwierdzono, że folia celulozowa ulega porastaniu przez grzyby pleśniowe. Jednocześnie ustalono, że grzyb *T. viride* znacznie szybciej porasta drewno niż celulozę bakteryjną.

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