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Studies on the diversity of substrate composition in the culture medium of Kombucha microorganisms and its influence on the quality of synthesized cellulose

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Abstract: *Studies on the diversity of substrate composition in the culture medium of Kombucha microorganisms and its influence on the quality of synthesized cellulose.* The paper presents the results of the assessment of the effect of nutrients, specifically different nitrogen concentrations in the growth medium of Kombucha microorganisms, on the morphology of cellulose produced and its sorption capacity. Analyzing the obtained research results, we found that polymers formed in different growth environments differ in morphological structure and swelling index. The polymers synthesized on a nitrogen-rich substrate were characterized by a multilayer structure and a lower swelling index than the polymers obtained on a nutrient-poor substrate.

Keywords: bacterial cellulose, Kombucha, carbon and nitrogen source

INTRODUCTION

Bacterial cellulose is a polymer synthesized mainly by acetic fermentation microorganisms belonging to *Gluconacetobacter*, although other groups of bacteria such as *Escherichia* or *Pseudomonas*, and even fungi, also have the ability to synthesize this polymer [1, 2, 3]. Despite the fact that bacterial cellulose is chemically identical to plant cellulose, it has better physico-mechanical properties than plant cellulose. Better properties result from higher purity, uniform and continuous fiber network, higher degree of polymerization and crystallization, and a different ability to absorb and retain water [4, 5]. These features mean that bacterial cellulose has potentially wide application significance. Currently, outside the medical field, interest in using cellulose is centered around the paper industry [6, 7]. Recycled materials have significantly worse properties than those made of primary wood fiber. Thus, the use of bacterial cellulose, whose production method is relatively simple and short in time, may prove to be a highly important solution for the paper industry. A very important quality feature of bacterial cellulose is its high tensile strength. As Stanisławska [8] reports, films made on the basis of bacterial cellulose are more than three times more tear resistant than cellophane films and more than seven times larger than polypropylene films. The quality of bacterial cellulose depends on the type of culture of microorganisms. Numerous studies have found that the type of substrate ingredients, culture temperature, substrate pH, and drying method affect the efficiency of synthesized cellulose and its quality [9, 10]. Zhao et al. [11] compared the efficiency of cellulose synthesis on synthetic and undefined media derived from wastewater from fermentation of polysaccharides. The authors of the study determined the differences and similarities between samples of cellulose synthesized on different types of medium substrates. The influence of carbon and nitrogen sources on the thickness, shape, smoothness and tensile strength of polymers synthesized by Kombucha microorganisms was the subject of research by Yim et al. [12]. The authors of the study have also proved that cellulose tensile strength is twice as high as grain leather of the same thickness.

This paper presents the results of research determining how the composition of Kombucha microbial growth medium affects the basic morphological characteristics and the swelling index of cellulose synthesized by these microorganisms.

MATERIALS AND METHODS

The object of the research was bacterial cellulose obtained in the process of cultivating Kombucha microorganisms on the basis of various nutrient content. Each type of medium contained sucrose (2.5%) as a carbon source and various concentrations of vegetable peptone (0%, 0.1%, 0.25% and 0.5%), a nitrogen-rich component. Cellulose synthesis by Kombucha microorganisms was carried out in stationary culture conditions, in a heat incubator, at temperature-humidity conditions of 24˚C and 68 ± 2%, respectively. After 14 days of culturing, the obtained cellulose was weighed, purified, and then dried at 60˚C for 24h. The cellulose purification process consisted of washing the polymer with distilled water, followed by soaking for 30 min. in 0.1M NaOH, and another three washes in distilled water to stabilize the pH.

For the synthesized polymers, the swelling index was determined based on the methodology presented in patent 227860 B1 [13].

The morphological properties of bacterial cellulose were investigated by means of emission scanning electron microscopy (SEM), FEI QUANTA 200 model, which is available at the SGGW analytical center. Observations were carried out in a low vacuum, with a magnification of 500-4000x. Dried cellulose samples were placed on a carbon belt without dusting.

RESULTS

The composition of the culture medium had a clear effect on the appearance and morphology of cellulose synthesized by Kombucha microorganisms [Figure 1]. Cellulose obtained from cultivation on a nitrogen-rich medium was clearly whiter and coarser, despite having a significantly lower swelling rate. The microscopic image shows numerous layers of polymer superimposed. A 1000x magnification depicts cellulose layers in the form of webs that overlap [Figure 2]. There are also differences in the appearance of cellulose surfaces formed on different nutrient media. The structure of bacterial cellulose, formed on a nutrient-rich medium, is more folded [Figure, 2, 3]. This is probably the result of the cellulose fiber structure and their packing density.

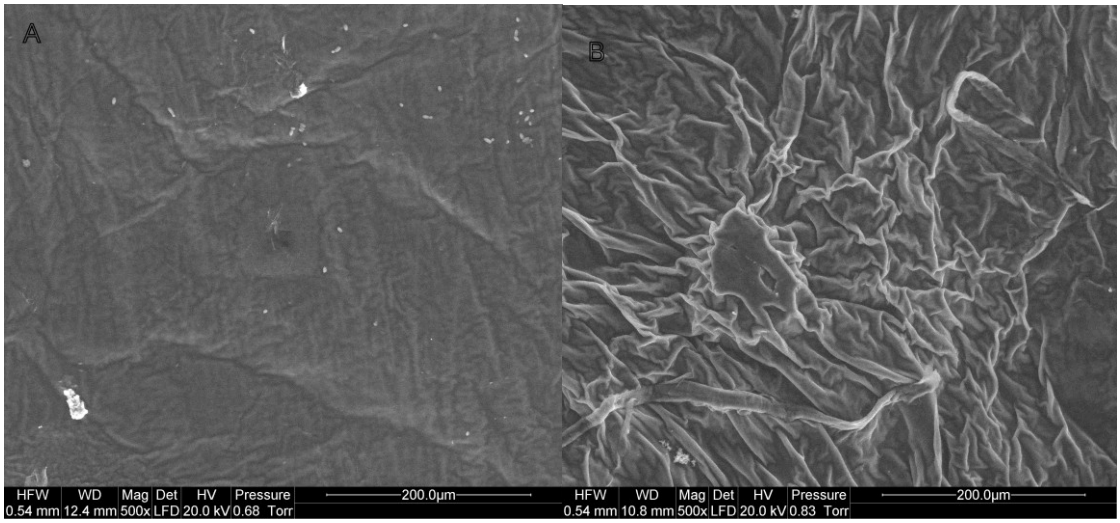

Figure 1. Surface morphology of bacterial cellulose synthesized by Kombucha microorganisms on a medium containing A - 2.5% sucrose B - 2.5% sucrose and 0.5% peptone

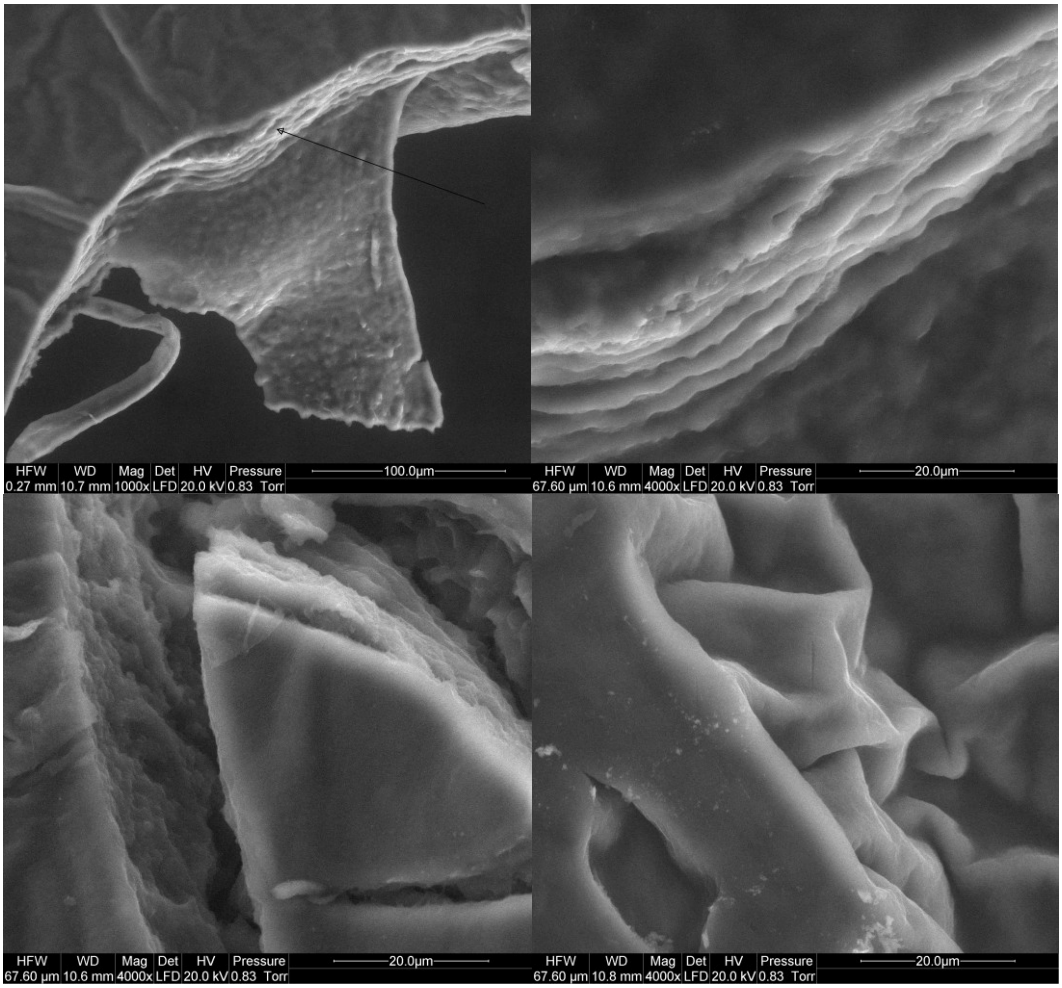

Figure 2. Cellulose morphology synthesized by Kombucha microorganisms on a medium containing 2.5% sucrose and 0.5% peptone

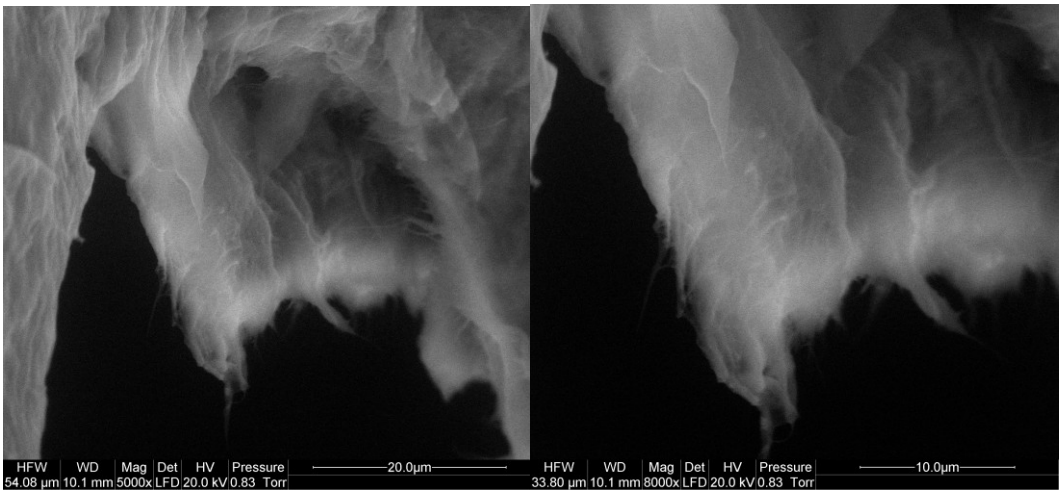

Figure 3. Cellulose morphology synthesized by Kombucha microorganisms on a medium containing 2.5% sucrose and 0.1% peptone

Based on the results obtained, it can be concluded that the composition of the culture medium also clearly affects changes in the bacterial cellulose's swelling ability. Based on the obtained swelling index, it was found that the presence of nitrogen in the growth medium reduces the sorption potential of the biopolymer. In the nitrogen-rich medium (0.5%), the examined indicator was slightly over 850%. In the case of cellulose obtained from a culture

on a substrate with only sucrose as a nutrient, the swelling ratio was 2814.67% [Figure 4]. The swelling index on sucrose medium was over three times higher than the swelling value obtained for cellulose from the culture with the highest peptone content in the culture medium composition. The differences in the ability to absorb water may closely depend on the structure and packing of individual cellulose fibers in a three-dimensional network. Probably, the fibers in the multilayer structure obtained on a substrate rich in nitrogen create a more packed network than it is in a polymer synthesized on a substrate poor in nutrients.

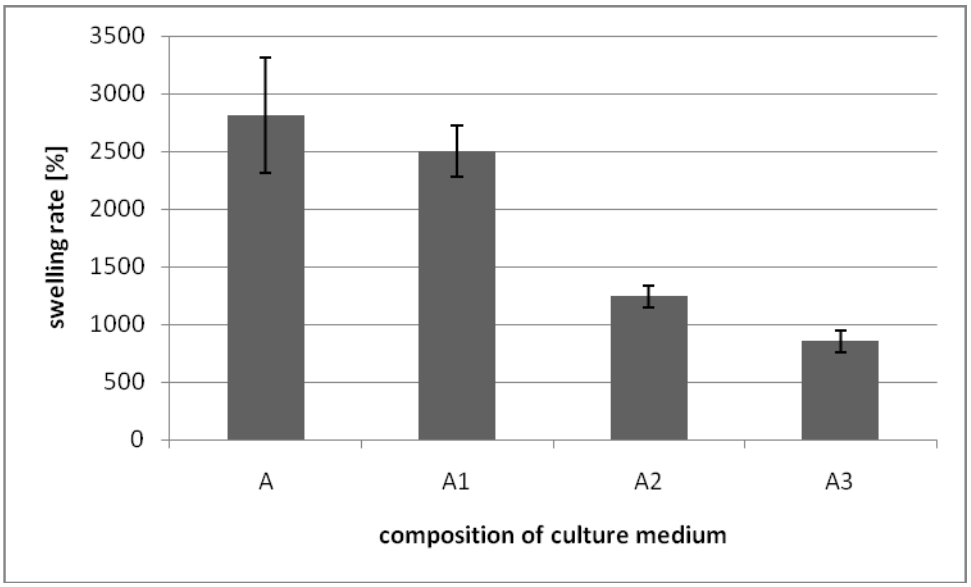

Figure 4. Swelling index of bacterial cellulose obtained from various culture media
A - 2.5% sucrose, A1- 2.5% sucrose + 0.1% peptone, A2 - 2.5% sucrose + 0.25% peptone, A3 - 2.5% sucrose + 0.5% peptone

CONCLUSION

The following conclusions can be made based on the obtained results:

- the composition of the growth medium of cellulose synthesizing microorganisms has an impact on the morphological characteristics of cellulose,
- cellulose synthesized on nitrogen-rich medium has a lower swelling rate.

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Streszczenie: *Ocena zróżnicowania składu podłoża w hodowli mikroorganizmów Kombucha i jego wpływ na jakość syntetyzowanej celulozy*. W pracy przedstawiono wyniki oceny wpływu składników pokarmowych, a dokładnie różnych stężeń azotu w podłożu wzrostu mikroorganizmów Kombucha, na morfologię oraz zdolność do pochłaniania wody celulozy syntetyzowanej przez te mikroorganizmy. Analizując uzyskane wyniki badań, stwierdzono, że polimery powstałe w różnych środowiskach wzrostu różnią się pod względem struktury morfologicznej i zdolności do pochłaniania wody, wyrażonej wskaźnikiem spęcznienia. Polimer syntetyzowany na podłożu zasobnym w azot odznaczał się wyraźną wielowarstwową strukturą i mniejszym wskaźnikiem spęcznienia niż polimer otrzymany na podłożu ubogim w składniki odżywcze.

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