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Bacterial cellulose synthesis by Kombucha microorganisms on a medium with a variable composition of nutrients

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Abstract: *Bacterial cellulose synthesis by Kombucha microorganisms on a medium with a variable composition of nutrients.* The paper presents the results of the assessment of the impact of various sucrose contents and the presence of various sources of nitrogen compounds in the growth medium of Kombucha microorganisms on the synthesis efficiency and the obtained bacterial cellulose mass. The analysis of obtained research results revealed that the efficiency of cellulose synthesis by Kombucha microorganisms depends on the quantity and quality of nutrients available in the growth medium.

Keywords: bacterial cellulose, Kombucha, carbon and nitrogen sources

INTRODUCTION

From the chemical point of view, bacterial cellulose is the same polymer as plant cellulose, but it has additional, definitely better features than cellulose derived from plant tissues. First of all, it is characterized by high purity, which is guaranteed by the lack of lignin and hemicelluloses, high crystallinity, susceptibility to forming to any shape, high hygroscopicity and very high mechanical strength, as well as high biological compatibility [5, 8, 10]. These features guarantee great opportunities for the use of bacterial cellulose in various industries. Bacterial cellulose is already successfully used in medicine, as a dressing material or surgical implants, in optics as biosensors, and also in the food, pharmacy and paper industries [7]. In the paper industry, bacterial cellulose is mainly used for bleaching waste paper and as a filler for defects in printing [6]. The use of cellulose in the woodworking and packaging industries also seems to be prospective. Bacterial cellulose is synthesized by a large group of microorganisms, both bacteria and yeast. Among cellulogenic micro-organisms, those belonging to the genera: *Acetobacter, Aerobacter, Achromobacter, Agrobacterium, Psedomonas, and Sarcina* have been identified [1]. These microorganisms often appear as a conglomerate, biofilm, often described as a “scoby”. Despite many unique physicochemical features and a very promising application perspective, the use of bacterial celluloses on a large scale poses some difficulties. This is mainly due to still high production costs and low productivity. A high yield of synthesis depends not only on the culture method, associated with the availability of nutrients for microorganisms, but also on the dynamic interaction of microorganisms with each other. The nutritional requirements of individual strains vary widely. Ramana and Singh [9] found that the best carbon source for the development of Acetobacter xylinum, the NUST4.1 strain, is glucose, and the growth of microorganisms and cellulose synthesis further increased in the presence of sodium alginate, while the growth of Acetobacter xylinum, the BRCS strain, is more dynamic with the additional presence of ethanol. An assessment of cellulose synthesis efficiency was conducted by Coban and Biyik [2] using variable sources of carbon and nitrogen. Fan et al. [3] evaluated the rate of synthesis and quality of bacterial cellulose on a substrate with the addition of waste from the food industry.

In this work, attempts were made to assess the efficiency of bacterial cellulose synthesis by strains of microorganisms included in the Kombucha biofilm, using three types of media, differing in the amount of carbon source and the availability of nitrogen source. The

consumption rate of sucrose used as a carbon source was assessed in relation to cellulose synthesis efficiency.

MATERIALS AND METHODS

Studies on the effect of nutrients on the intensity of cellulose synthesis by Kombucha microorganisms were carried out on three different media, the basic component of which was sucrose as a carbon source. Three types of media were prepared based on the literature data provided by Goh et al. [4] and Sharma and Behardwaj [3]. The sucrose content in the media ranged from 1.5% to 10%. The first type of medium contained only different sucrose concentrations. The second medium variant, apart from sucrose, contained a constant addition of vegetable peptone (0.25%), while the last medium variant contained a constant addition of tea extract (0.1%). The microorganisms were cultured under the temperature and humidity conditions of 24˚C and 68 ± 2%, respectively. The cultivation time of the microorganisms was 14 days. After a specified cultivation time, the synthesized cellulose was purified, weighed, then dried at 60˚C for 24h and weighed again. The dry mass and the percentage yield of polymer synthesis were determined based on the assumptions presented by Sharma and Behardwaj [10]:

dry mass of polymer formed = mass of dried polymer [g]/volume of medium [l]
percentage of synthesis [%] = (dry mass of polymer formed [g]/sum of the initial concentration of sucrose and additional component [g]) x 100

At the time of liquidation of the microorganism culture, the sucrose content was measured. The sucrose concentration in the post-culture fluid was determined polarimetrically, using a polarimeter model P3000 Kruss. After each measurement, the post-culture fluid was cleaned by syringe filters. The specific rotation of the sugar solution was measured in a 1 dm long glass tube. Due to the fact that the result of determinations obtained by the polarimetric method is the value of specific curvature, the concentration of sugar contained in the medium was determined from the following relationship:

test concentration [%] = concentration of sugar solution at the beginning of the culture [%] x (specific torsion capacity of the test solution/specific torsion capacity of the solution of known concentration)

In order to determine the rate of consumption of the primary carbon source during cultivation, the sucrose consumption rate (g L^{-1} d^{-1}), was determined, according to the assumption presented by Sharma and Behardwaj [10]:

sucrose consumption rate (g L^{-1} d^{-1}) = (sugar content in time 0 [g/l] - sugar content in final time K [g/l]) / cultivation time [d]

RESULTS

Based on the results obtained, it can be concluded that the composition of the culture medium clearly affects the increase in dry matter of the biopolymer. In a medium richer in carbon source, the increase in dry matter of synthesized cellulose was clearly greater. On a medium containing 1.5% sucrose, the dry weight of the polymer produced was 14 times lower than the dry weight obtained on a medium containing as much as 10% sucrose (Table 1). Sucrose is the basic carbon source for yeast forming the Kombucha biofilm, used for the production of ethanol, which in turn is the main nutrient for acetic acid bacteria that synthesize cellulose. At the same time, it should be noted that the increase in bacterial cellulose dry matter also depends on the presence of nitrogen-rich components. The efficiency of cellulose synthesis in a nitrogen-rich environment was clearly higher than in a nitrogen-free environment. In the presence of peptone, the yield of cellulose synthesis was from 116% to

218% greater than in a medium without a nitrogen source (Table 2). Tea extract is also a nitrogen-rich component found in compounds such as alkali, peptides, and essential oils, however, the yield of bacterial cellulose synthesis in the presence of tea extract was significantly lower than in the presence of peptone. The reason for this phenomenon may be poorer availability of components present in the extract, but also their concentration or even the formation of by-products of their metabolism, which impede the synthesis of cellulose.

Table 1. The average value of obtained dry matter of cellulose synthesized by Kombucha microorganisms on various types of nutrient media

Sucrose content [%]	Peptone content [%]	Tea extract content [%]	Dry mass of polymer formed [g/l]
1.0	0.25	-	2.03
2.5			3.30
5.0			7.22
7.5			12.89
10.0			22.34
1.0	-	0.10	0.81
2.5			2.77
5.0			2.65
7.5			8.89
10.0			15.74
1.0	-	-	0.2
2.5			1.36
5.0			1.08
7.5			2.24
10.0			2.81

Table 2. The average value of the obtained cellulose synthesis efficiency by Kombucha microorganisms on various types of nutrient media

Sucrose content [%]	peptone content [%]	Tea extract content [%]	Polymer synthesis yield [%]
1.0	0.25	-	116.0
2.5			120.0
5.0			137.0
7.5			166.0
10.0			218.0
1.0	-	0.10	50.6
2.5			106.5
5.0			51.9
7.5			117.0
10.0			155.8
1.0	-	-	13.0
2.5			54.0
5.0			21.6
7.5			29.0
10.0			28.1

The increased efficiency of cellulose synthesis on substrates rich in nitrogen correlates with increased sucrose. On the medium containing peptone or tea extract, the sucrose content in the medium, after 14 days of culturing Kombucha microorganisms was lower than in the medium containing only sugar (Figure 1). Faster sugar consumption resulted in a greater increase in dry matter of synthesized cellulose.

When determining the rate of sucrose consumption, it was found that in the case of microorganisms cultured on peptone-containing medium, it was from 0.050 to 0.380 g / L per day. The yield of cellulose synthesis with such daily sugar metabolism ranged from 116% to

218%. In a medium containingsucrose and tea extract, the sucrose consumption rate per day was lower than in the sucrose and peptone medium. Thus, a lower yield of cellulose synthesis was observed in this medium. The lowest sucrose consumption rate was found in the medium containing only sucrose as a nutrient for Kombucha microorganisms (Figure 2). Based on the above data indicated in Figures 1 and 2, the conversion of sugar to cellulose with the participation of Kombucha microorganisms was presented. The data shows that both the nutrient content with the necessary amount of carbon and the source of nitrogen have an impact on the mass of the synthesized polymer and its synthesis efficiency.

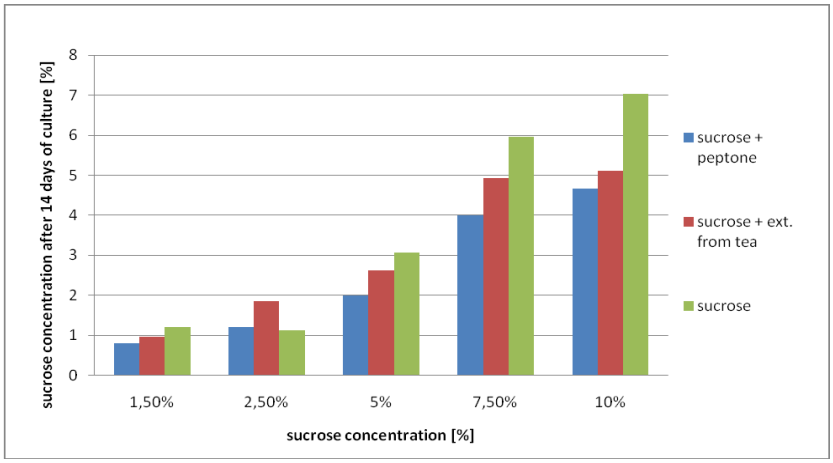

Figure 1. Sucrose content in growth media for Kombucha microorganisms

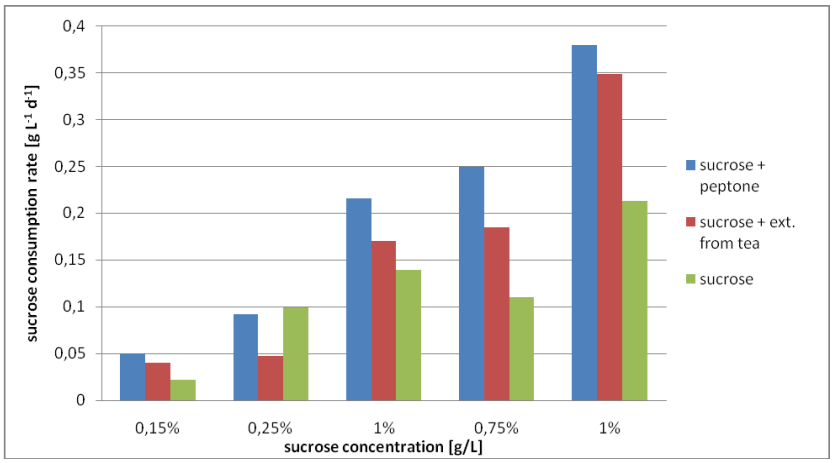

Figure 2. The rate of sucrose consumption by Kombucha microorganisms on various types of media

CONCLUSION

The analysis of the obtained research results indicates that the yield of bacterial cellulose synthesis by Kombucha microorganisms is dependent on the quantity and quality of nutrients available in the growth medium. Both the amount of sucrose as a carbon source and the addition of substances rich in nitrogen affects the mass of cellulose obtained and its synthesis efficiency. In the presented work, it was found that the addition of peptone as a nitrogen source and an increased amount of sucrose in the medium clearly stimulate microorganisms for cellulose synthesis.

REFERENCES:

1. CAZÓN P., VELÁZQUEZ G., VÁZQUEZ M., 2019: Characterization of bacterial cellulose films combined with chitosan and polyvinyl alcohol: Evaluation of mechanical and barrier properties, Carbohydrate polymers nr 216; 72-85
2. COBAN E.P., BIYIK H., 2011: Effect of various carbon and nitrogen sources on cellulose synthesis by Acetobacter lovaniensis HBB5, African Journal Biotechnology nr. 10; 5346–5354
3. FAN X., GAO Y., HE W., HU H., TIAN M., WANG K., PAN S., 2016: Production of nano bacterial cellulose from beverage industrial waste of citrus peel and pomace using Komagataeibacter xylinus, Carbohydrate Polymers nr. 151; 1068-1072
4. GOH W.N., ROSMA A., KAUR B., FAZILAH A., KARIM A.A., BHAT R., 2012: Microstructure and physical properties of microbial cellulose produced during fermentation of black tea broth (Kombucha). II. International Food Research Journal nr. 19; 153–158
5. JEONG S. I., LEE S. E., YANG H., JIN Y.-H., PARK C.-S., & PARK Y. S. 2010: Toxicologic evaluation of bacterial synthesized cellulose in endothelial cells and animals, Molecular & Cellular Toxicology nr. 6(4); 370–377
6. URBINA L., CORCUERA M. Á., ECEIZA A., RETEGI A., 2019: Stiff all-bacterial cellulose nanopaper with enhanced mechanical and barrier properties, Materials Letters nr. 249; 67-70
7. MENENDEZ E., GARCIA-FRAILE P., RIVAS R., 2015: Biotechnological applications of bacterial cellulases, Bioengineering nr. 2(3) 163-182
8. ROUABHIA M., ASSELIN J. R. M., TAZI N., MESSADDEQ Y. S., LEVINSON D., ZHANG Z. 2014: Production of biocompatible and antimicrobial bacterial cellulose polymers functionalized by RGDC grafting groups and gentamicin, ACS Applied Materials & Interfaces nr. 6(3); 1439–1446
9. RAMANA K. V., SINGH L., 2000: Effect of various carbon and nitrogen sources on cellulose synthesis by Acetobacter xylinum, World Journal Microbiol. Biotechnol nr. 16 (3); 245–248
10. SHARMA CH., BHARDWAJ N.K., 2019: Biotransformation of fermented black tea into bacterial nanocellulose via symbiotic interplay of microorganisms, International Journal of Biological Macromolecules nr. 132; 166-177

Streszczenie: *Synteza celulozy bakteryjnej przez mikroorganizmy Kombucha na podłożu przy zmiennym udziale składników pokarmowych*. W pracy przedstawiono wyniki oceny wpływu różnych zawartości sacharozy oraz obecności różnych źródeł związków azotu w podłożu wzrostu mikroorganizmów Kombucha na wydajność syntezy oraz uzyskaną masę celulozy bakteryjnej. Analizując uzyskane wyniki badań, stwierdzono, że wydajność syntezy celulozy przez mikroorganizmy Kombucha jest zależna od ilości i jakości składników pokarmowych dostępnych w podłożu wzrostu.

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