The efficiency of bacterial cellulose biosynthesis from the amount of nitrogenous compounds in the nutrient medium

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Abstract. Bacterial cellulose (BC) is a promising material for technological and medical applications. The isolation of effective BC producers from natural symbiotic consortia is a promising direction in relation to industrial biotechnology of BC. The study consists in selecting the composition of the nutrient medium to increase the efficiency of the biosynthesis process of BC by cellulose-synthesizing bacteria isolated from the symbiotic consortium *Medusomyces gisevii*. Morphological features of isolated acetic acid bacteria have been determined - small rods, 0.8-1.2 microns in size with a rounded end, forming cream-colored colonies on agarized nutrient media, 2-7 mm in size with a characteristic luster. The composition of the nutrient medium RAE (AE) was determined, characterized by the maximum efficiency of BC biosynthesis (production of 4.63 g DM/l for 8 days; the degree of glucose conversion - 41.04%).

1 Introduction

An important function of acetic acid bacteria is the ability to oxidize ethanol to acetic acid [1-2], which distinguishes them from other types of bacteria and is of great importance for the food industry (production of acetic acid, ascorbic acid, tartaric acid, cocoa, etc.). The well-known and actively studied potential of acetic acid bacteria is associated with the ability to synthesize cellulose (BC) [3 - 5].

Worldwide research conducted over the past two decades has been related to the study of the structural organization of the cellulose synthase complex, and its operons and genes of acetic acid bacteria [6 – 9] for the efficient and controlled production of BC [5, 10]. Currently, the classification of acetic acid bacteria of the Acetobacteraceae family includes 19 genera (*Acetobacter, Acidomonas, Ameyamaea, Asaia, Bombella, Commensalibacter, Endobacter, Gluconacetobacter, Gluconobacter, Granulibacter, Komagataeibacter, Kozakia, Neoasaia, Neokomagataea, Nguyenibacter, Saccharibacter, Swaminathania, Swingsia and Tanticharoenia*) [11].

Bacterial cellulose synthesized by acetic acid bacteria is a universal material for biotechnology and medicine, representing a pure, highly porous, biodegradable nanomaterial [6]. In a favorable nutrient medium, acetic acid bacteria form gluconic and

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other acids as a result of metabolism, thereby lowering the pH of the medium and subsequently reducing their activity in BC production [12].

Cellulose of plant origin is similar in chemical structure to bacterial cellulose, nevertheless, the ordered ultrathin network of nanofibers of BC gel films has a high moisture retention capacity, mechanical strength, as well as low immunogenicity and environmental stress during production [13-14].

Traditionally, glucose is used as a carbon source in the bio-production of this exopolysaccharide, a number of studies have shown the effectiveness of using cheap carbon sources: combined (galactose with sucrose, lactose, maltose, fructose; fructose with sucrose, lactose, maltose, etc.), hydrolysates (corn cobs, starch, sugar cane pulp, etc.), industrial waste (fruit peel, rice husk, molasses, wheat straw, etc.) [15-17]. Our previous studies [18] showed that the use of sucrose as a substrate of the nutrient medium increases the density, crystallinity and interlacing of the fibrils of the resulting hydrocolloids, in addition, the accumulation of acid metabolites decreases during the biosynthesis of BC by the symbiote *Medusomyces gisevii*.

Despite existing studies, the specifics of BC synthesis using carbon sources other than glucose have conflicting data. The fact that different carbon sources have different molecular weights, chemical structure, and bioavailability leads to changes in the rate of biosynthesis and structural characteristics of BC [19-23]. In our studies, we reported on the molecular assembly, structure and strength parameters of native and modified BC synthesized on various substrates by the symbiote *Medusomyces gisevii* [26].

Thus, optimization of the composition of the nutrient medium, pH, ionic strength and carbon source content remains an urgent task, since many BC production technologies are critically hampered by the low production rate and the degree of conversion in mass production [24-25]. According to the literature data, the symbiote is based on four genera of acetic acid bacteria, namely Komagataeibacter, Acetobacter, Gluconobacter and Pseudomonas, and three genera of yeast fungi, including Brettanomyces, Pichia and Zygosaccharomyces [27].

The paper presents the results of productivity studies with respect to exopolysaccharide BC of isolated acetic acid bacteria of the symbiote *Medusomyces gisevii* on a liquid nutrient medium of RAE (AE) of different composition.

2 Materials and Methods

Isolation of cellulose-synthesizing bacteria from the symbiotic consortium *Medusomyces gisevii* was performed on a differential diagnostic dense nutrient medium (DNM) RAE enriched with acetate and ethanol (AE): glucose — 20 g/l, peptone — 5 g/l, yeast extract — 5 g/l, citric acid — 0.5 g/l, Na2HPO4 × 12 H2O -6.8 g/l, glacial acetic acid 99,5% — 1.0 ml/l and ethyl alcohol 98% — 10 ml/l, agar for DNM 15 g/l [28].

At the second stage of the research, isolated colonies of acetic acid bacteria were transferred from DNM to a liquid nutrient medium (LNM) RAE (AE), cultured under static conditions, in 250 ml Erlenmeyer flasks, for 5 days. Then the resulting seed material was introduced into the LNM RAE (AE) (ratio 1:3 v/v). Acetic acid bacteria were cultured in a thermostat at a temperature of 32±0.5°C for 2-8 days. Biosynthesis parameters were determined for every second day of the process for the studied compositions of LNM (Table 1). Titrated acidity and glucose concentration in the nutrient medium were monitored during biosynthesis. The amount of synthesized BC was determined after washing the gel films from the remains of the nutrient medium and cells with distilled water, 0.1 M sodium hydroxide, then with newly distilled water to neutral pH values. BC gel films were dried at a temperature of 50°C, and the synthesized dry matter mass was calculated. The storage and study of bacteria was carried out in sterile laboratory conditions

of the Department of Food and Food Biotechnology of the FSBEI HE Omsk State Agrarian University named after P.A. Stolypin. The concentration of the substrate-glucose of the culture liquid was determined by the glucose oxidase method, the test system "Glucose-GOD" produced by Vector-Best. The degree of conversion was determined by the difference between the initial and final amounts of glucose substrate.

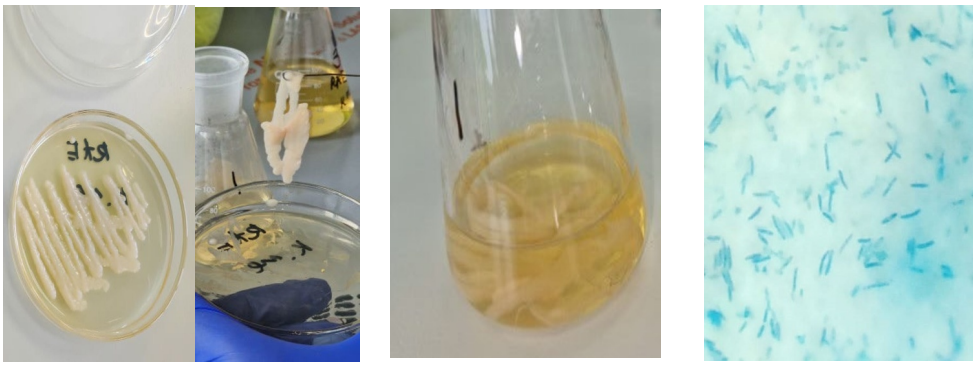
Table 1. The composition of the nutrient medium RAE (AE) and its varieties.

Components	RAE 1	RAE 2	RAE 3	RAE 4
Glucose	20,0 g/l	20,0 g/l	20,0 g/l	20,0 g/l
Peptone	5,0 g/l	-	5,0 g/l	2,5 g/l
Yeast extract	5,0 g/l	5,0 g/l	-	2,5 g/l
Citric acid	0,5 g/l	0,5 g/l	0,5 g/l	0,5 g/l
$Na_2HPO_4 \times 12H_2O$	6,8 g/l	6,8 g/l	6,8 g/l	6,8 g/l
Glacial acetic acid 99,5%	1.0 ml/l	1.0 ml/l	1.0 ml/l	1.0 ml/l
Ethyl alcohol 98%	10.0 ml/l	10.0 ml/l	10.0 ml/l	10.0 ml/l

Source: "Compiled by the authors"

3 Results and Discussion

Isolated colonies on the DNM RAE (AE) were obtained from the culture fluid of *Medusomyces gisevii* using the "depletion stroke" method. Microscopy of the cells of these colonies made it possible to differentiate the acetic acid bacteria shown in Figure 1.
The study of morphological and cultural signs of microorganisms grown on DNM RAE (AE) proves the presence of acetic acid bacteria – small sticks, 0.8-1.2 microns in size with a rounded end, forming cream-colored colonies on agarized nutrient media, 2-7 mm in size with a characteristic luster.



a b c

Fig. 1. Visualization of colonies and cells of cellulose-synthesizing bacteria of the symbiotic consortium: (a) colonies of acetic acid bacteria on the DNM RAE (AE); (b) transplanting colonies of acetic acid bacteria on the LNM RAE (AE); (c) acetic acid bacteria under a light microscope magnification by 630 times. *Source:* "Compiled by the authors".

Further, isolated acetic acid bacteria were used in the study of their productivity in relation to bacterial cellulose on LNM RAE (AE) of different compositions. On the 1st day of the consortium cultivation process, gel films of bacterial cellulose synthesized by acetic acid bacteria were formed on the surface of the culture medium. Figure 2 shows

synthesized BC samples on LNM RAE (AE) by colonies of acetic acid bacteria isolated from the symbiotic consortium of *Medusomyces gisevii* (Fig. 2).

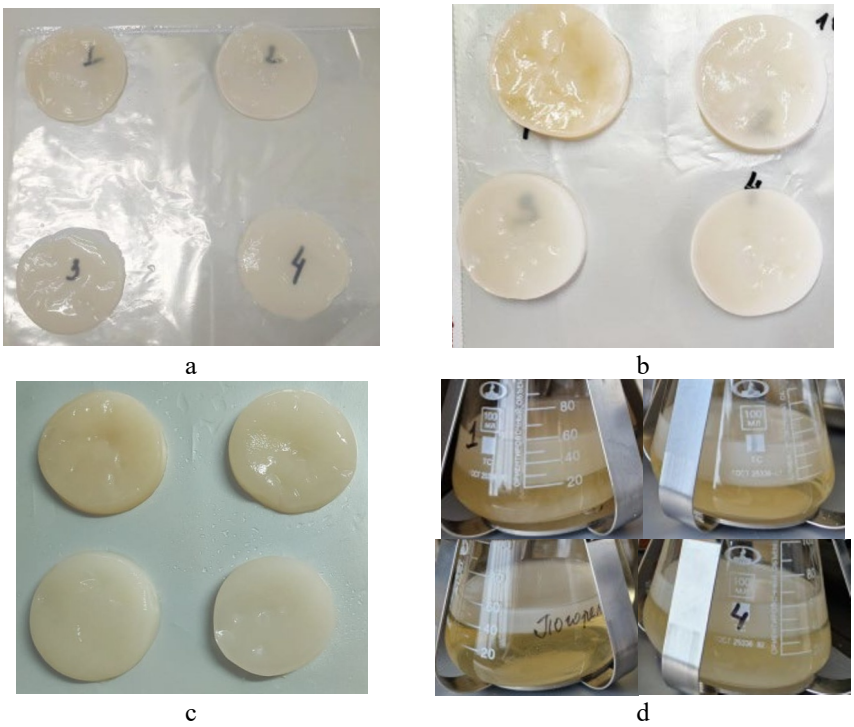


Fig. 2. Biosynthesis of bacterial cellulose on LNM RAE (AE) of different composition lasting 2 days (a); 4 days (b); 6 days (c); 8 days (d). *Source:* "Compiled by the authors".

For the biochemical process of BC synthesis by acetic acid bacteria, glucose is the main substrate for the assembly of exopolysaccharide, which is explained by a decrease in glucose in the nutrient medium RAE (1) and its utilization to 0.08% within 8 days. According to the results of the study (Fig. 3), the final glucose concentration of the nutrient medium after 8 days of biosynthesis is 9 times less for the standard composition of the LNM RAE (1) in comparison with the RAE nutrient medium that does not contain yeast extract (3). This may indicate less favorable conditions for the cultivation of BC producers, in the absence of nitrogenous compounds – yeast extract.

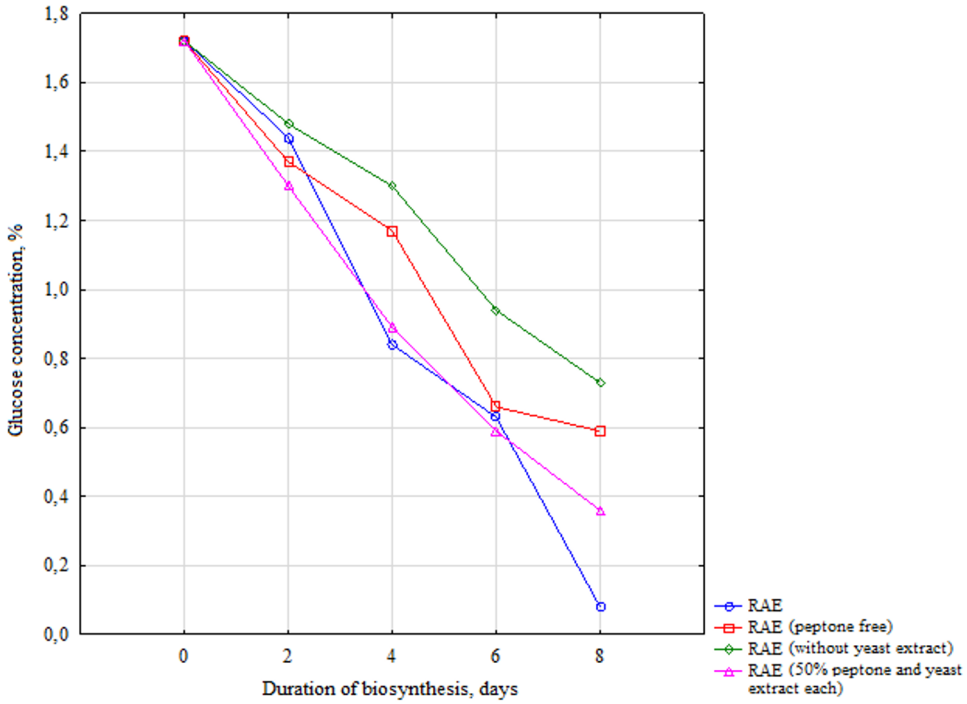


Fig. 3. The change in glucose concentration depends on the duration of biosynthesis and the composition of LNM RAE (AE). *Source:* "Compiled by the authors".

It was determined that during 4 days the mass of BC with LNM RAE (AE) of different composition does not change significantly (Fig. 4). Nevertheless, on the 6th day, studies of BC biosynthesis on LNM RAE (AE) of different composition showed an increase in BC mass by 1.2 times and on the 8th day without the addition of peptone - by 1.5 times compared with standard RAE (1) (Fig. 4).

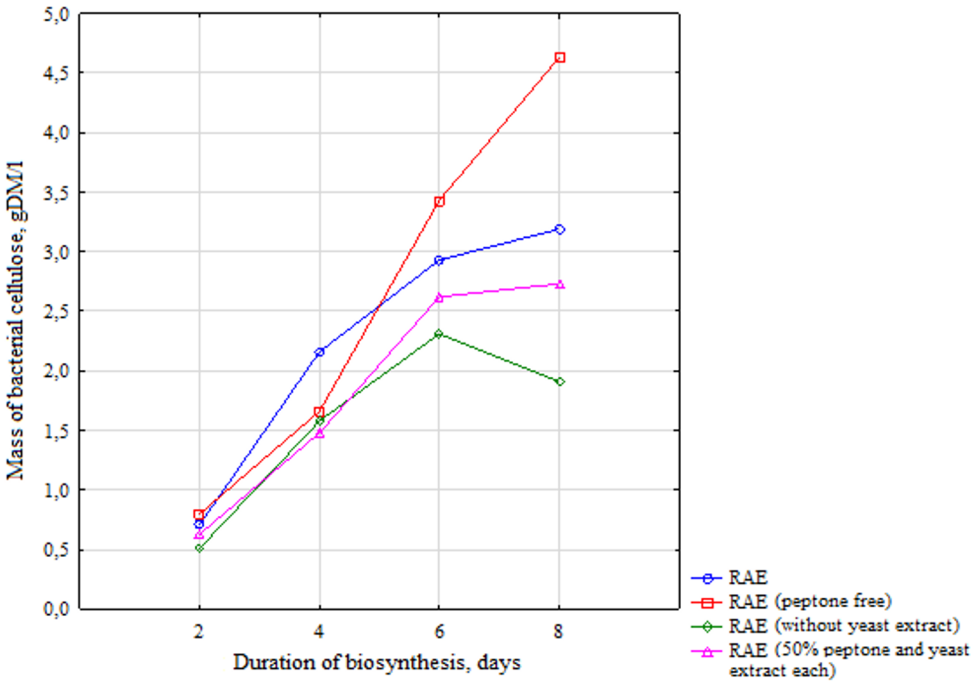


Fig. 4. The change in the mass of bacterial cellulose (in terms of dry matter) from the duration of biosynthesis and the composition of LNM RAE (AE). *Source:* "Compiled by the authors".

Next, the efficiency of the use of glucose substrate by acetic acid bacteria in the biosynthesis of exopolysaccharide was determined depending on the composition of the nutrient medium. The lowest index of the degree of glucose conversion (DC) during 6 days of biosynthesis was determined for the LNM RAE (4), in which the content of peptone and yeast extract is 2 times lower compared with the composition of standard RAE (1) (Fig. 5). Interestingly, on the 8th day, glucose concentration is highest for the nutrient medium RAE (2), which does not contain peptone (41.04%) (Fig. 5).

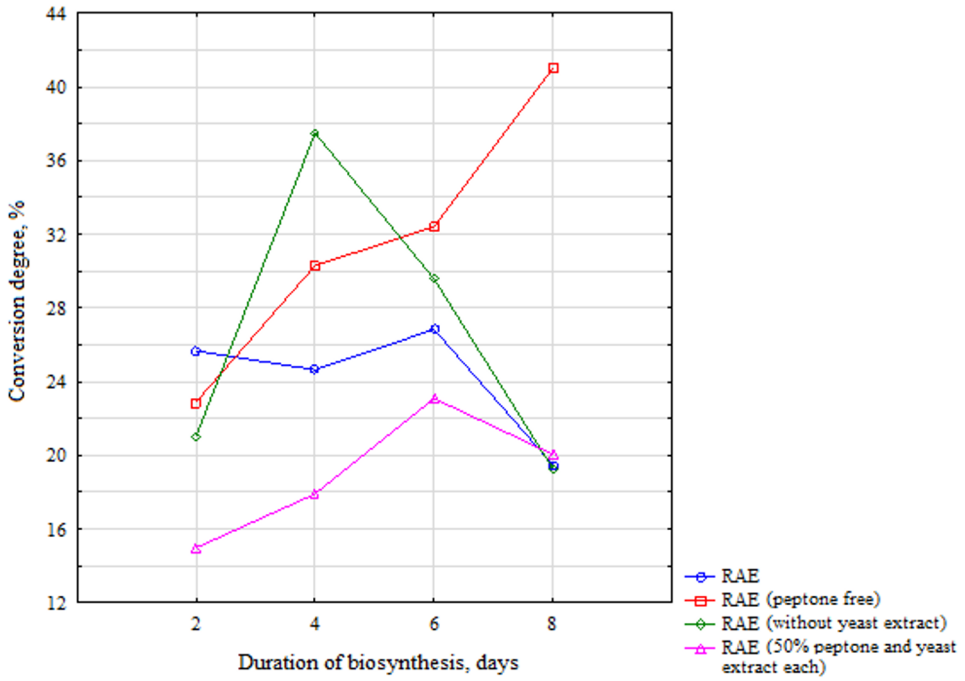


Fig. 5. The change in the degree of glucose conversion (in terms of BC dry matter) from the duration of the process and the composition of the LNM RAE (AE). *Source:* "Compiled by the authors".

Analyzing the results of biosynthesis studies (Fig. 6), the rate of BC biosynthesis reached a maximum value (0.579 g DM/l × day) on the 8th day of cultivation of LNM RAE (2) without the addition of peptone.

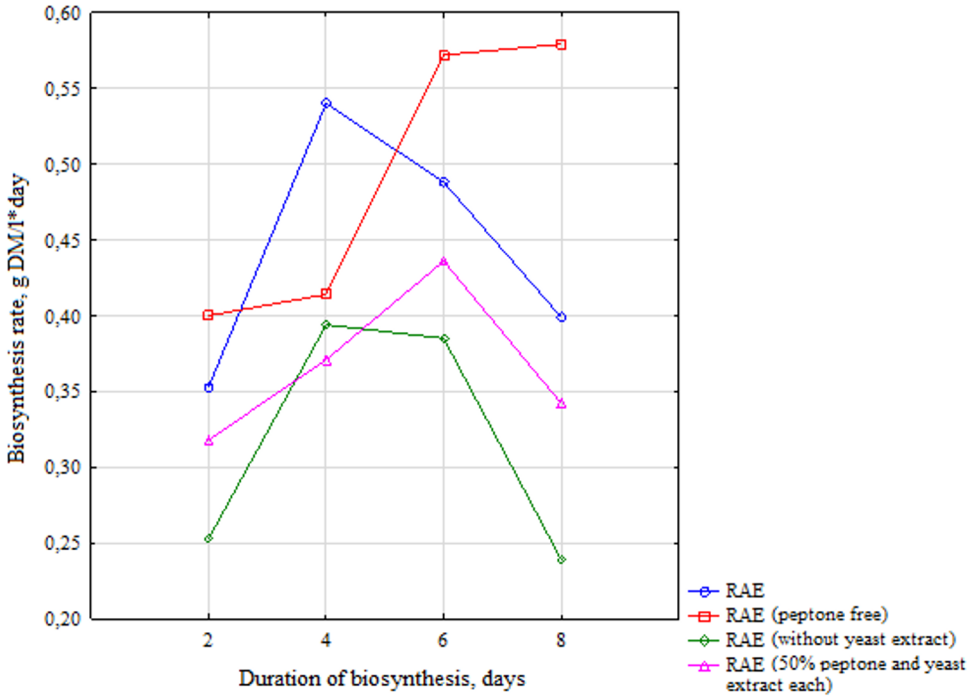


Fig. 6. The change in the rate of BC biosynthesis depends on the duration of the process and the composition of the LNM RAE (AE). *Source:* "Compiled by the authors".

4 Discussion

Thus, as a result of the study of the biosynthesis process of BC on LNM RAE (AE) of different compositions, the maximum efficiency in relation to the production of exopolysaccharide of a nutrient medium containing peptone was determined. The parameters of the biotechnological process were determined within 8 days: the rate of biosynthesis is 0.579 g DM/l × day, glucose concentration is 41.04%, the value of BC production is 4.63 g DM/l.

5 Conclusion

In this study, high indicators of BC production, conversion rate and biosynthesis rate are indicated when using LNM RAE (AE) without peptone addition, which may indicate an excess of nitrogenous compounds in the nutrient medium when peptone is added. Isolated colonies of acetic acid bacteria isolated from the symbiotic consortium of *Medusomyces gisevii* are highly effective producers of bacterial cellulose.

Acknowledgements

Financing. The work was carried out with the financial support of the Russian Science Foundation (project No. 23-24-10052 dated 04/20/2023).

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