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Preparation and Properties of Bacterial Cellulose Gel Films

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Abstract

Results are presented concerning the studies on the physicochemical properties of a bacterial cellulose film, obtained by culturing the symbiotic acetic-acid bacteria *Acetobacter xylinum* and the yeast of genus *Brettanomyces, Zygosaccharomyces, Saccharomyces*. A block diagram is presented for the production and purification of the cellulose from impurities. High consumer properties of bacterial cellulose are demonstrated. Sorption properties were determined with respect to ε-aminocaproic acid and sea buckthorn oil. Prospects were estimated concerning the application of bacterial cellulose gel films in order to create transdermal therapeutic systems.

Key words: bacterial cellulose, gel film, transdermal therapeutic systems, sea buckthorn oil, ε-aminocaproic acid, *Medusomyces gisevii* J. Lindau

INTRODUCTION

In the early 60s of last century, the biotechnology advances have provided a broad application of microbial polysaccharides, often called "biopolymers". At present, microbial polysaccharides are widely used in various spheres of human activity: in medicine, pharmaceutical, food, chemical industry, agriculture and even in such "heavy" industries such as hydrometallurgy, oil production, refining non-ferrous and rare metals [1–5].

Such products as Xanthan (E415), Kurdlan (E424), Skleroglyukan, Pullulan (E1204), produced by microbiological industry in the form of fine powders, are successfully used as thickeners and gelling agents [6] .

In order to obtain bacterial polysaccharides as a film, researchers as a rule, use bacteria such as *Acetobacter xylinum*. These polysaccharides are synthesized by individual strains of bacteria those are sensitive to the incubation conditions [7] and the composition of a medium [8–10]. These disadvantages are almost completely absent in the system involving acetic acid bacteria *Acetobacter xylinum* symbiosis with yeast of genera *Brettanomyces*, *Zygosaccharomyces*, *Saccharomyces*, also known as *Medusomyces gisevii* J. Lindau. The waste products of the symbiosis are presented by a culture fluid, better known as brewing Kombucha tea and an extracellular polymer β-1,4-glucan formed as a film on the surface of the culture fluid. Such a film consisting of bacterial cellulose (BCF) can be used in medicine as a highly efficient wound dressings and for the creation of transdermal therapeutic systems (TTS) due to its high elasticity, strength and low BCF adhesion with respect to wound surface [11, 12]. This substance is identical to the structure of plant cellulose and has other names such as acetan [13], xylinan [14]. The symbiosis is cultivated in liquid medium consisting of 10 % sucrose solution and diluted tea extract with no introducing trace elements to the medium [15].

The present work was aimed at studying the physicochemical properties of purified BCF, obtained *via* cultivating symbiotic *Medusomy-*

ces gisevii J. Lindau and at the evaluation of the possibility for using BCF as a matrix for a transdermal therapeutic system containing water and fat-soluble pharmaceutical preparations.

EXPERIMENTAL

In order to prepare the culture medium, to a glass container were placed 100 g of sucrose and 500 mL of black tea extract obtained *via* maceration of 2 g of tea leaves (GOST 1938–90) at the temperature of 70–90 °C during 15 min; after complete sucrose dissolution the solution volume was adjusted up to 1 L. The medium was prepared by means of boiling tap water with total hardness amounting to 4.2 mmol/dm^3. The culture medium prepared was cooled down to 25 °C and transferred to a fermentation tank; after that there was introduced 30 mL of a 7-day culture of *Medusomyces gisevii* J. Lindau. The biosynthesis was carried out during 7 days at a temperature of 25–29 °C under static conditions. The resultant BCF was then taken off and cleaned.

In order to remove soluble ash, tea polyphenols and other lignin-like substances, the film was placed to a 0.5 % NaOH solution during 24 h at a temperature of 25–27 °C, under periodical stirring. After washing with distilled water, the film is placed to a 0.5 % solution of acetic acid or hydrochloric acid for 24 h and then it was washed with distilled water until obtaining the pH value of washing water to be equal to pH 6–7. After that the film was dried in air at a room temperature until obtaining constant mass. The yield of dry purified cellulose was equal to 6.12 % (as calculated for 100 g of sucrose).

Figure 1 demonstrates a block diagram of obtaining and purifying the BCF.

IR spectra were registered by means of an Infralum FT-801 FT-IR spectrometer in a pellet of KBr.

The samples of bacterial cellulose were investigated with the use of a Jeol JSM-840 SEM (Japan) with a Link System 860 Series II X-ray microanalyzer.

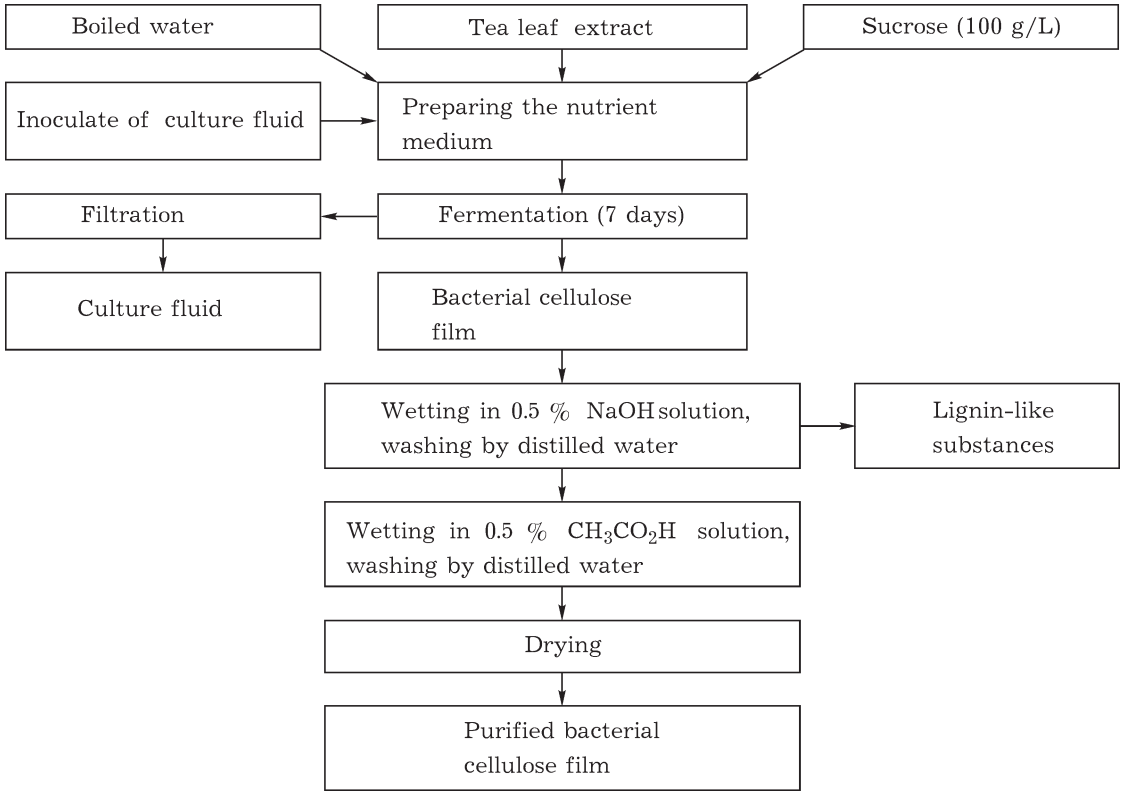

Fig. 1. Block diagram of obtaining the bacterial cellulose film.

RESULTS AND DISCUSSION

The use of the symbiosis *Medusomyces gisevii* J. Lindau for the biosynthesis of the gel films of bacterial cellulose allows one to replace a multi-component nutrient medium used for cultivation of the individual strains of *Acetobacter xylinum*, by a nutrient medium consisting of sucrose solution and the extract of black or green tea. In addition, in the course of studying the symbiosis we established that the symbiotic system excretes amylolytic enzymes to the culture fluid as the result of the vital activity. Because of this, in our opinion, the biosynthesis of cellulose could be carried out using not only hexoses, but also with the use of disaccharides, or short-chained oligosaccharides as a source of carbon those can be obtained by hydrolysis of low-grade plant pulp.

In order to identify the material obtained and purified we registered IR spectrum that exhibited the following characteristic absorption bands of functional groups: 3347 cm^{-1} – stretching vibrations of OH groups, 2896 cm^{-1} – stretching vibrations of CH_2 and CH groups (a wide band indicates the presence of several groups), 1640 cm^{-1} – HOH bending vibrations of crystallization water, 1429 cm^{-1} – deformation vibrations of CH2 and CH groups, 1360 cm^{-1} – deformation vibrations of CH, 1336 cm^{-1} – planar deformation vibrations of OH primary alcohol group, 1281 and 1205 cm^{-1} – deformation vibrations of OH and CH [16], 1163 cm^{-1} – asymmetric stretching vibrations of the C–C bridge [17], 1060 cm^{-1} – stretching vibrations of C–O in the C^3H–OH group, 1029 cm^{-1} – stretching vibration of C–O in the primary alcohol group in different conformations.

The band of medium intensity within the region of 1429 cm^{-1} with very weak overtone of 900 cm^{-1} indicates that the IR spectrum corresponds to highly crystalline cellulose I, nearly identical to the cotton cellulose (CC) [18].

In order to determine the physicochemical properties of the films obtained we used standard methods. The results obtained concerning the comparative characteristics of bacterial cellulose and cotton cellulose are demonstrated in Table 1. It is seen that the bacterial cellulose even surpasses the cotton cellulose in the basic quality parameters. It should be noted that the use of hydrochloric acid instead of acetic acid at the stage of BCF purification can significantly reduce the ash content therein.

The high values of BCF swelling level and wettability, as well as a satisfactorily low adhesion with respect to wound surface (determined organoleptically) allow us to justify the choice of BCF as a base for the TTS of matrix type.

Particularly noteworthy are the results of the investigations concerning the microstructure of cellulose film, in particular, revealing the fundamental difference between bacterial cellulose and plant species. Figure 2 demonstrates the micrographs of the film surface, the location of the individual polymer microfibrils relative to each other, as well as micrographs of cotton cellulose obtained by means of SEM.

TABLE 1

Comparative characteristics of the bacterial cellulose (BC) and cotton cellulose (CC)

Physicochemical parameters (determination method)	BC	CC, I grade (GOST 595–79)
Moisture content, % (GOST 16932–93)	3.8	10.0
Ash content, % (GOST 595–79)	1.0 (0.2)*	0.2
Swelling level, % [19]	186.7	–
Average polymerization level (GOST 9105–74)	947	716
Wettability level, g (GOST 595–79)	179.7	140.0
Determination of leaf density, g/m^2 (GOST 11720–76)	80.6	–
Linear expansion, % [19]	68.7	–
Mass fraction of α-cellulose, % (GOST 16932–71)	98.5	97.7

Note. GOST – State Standard.

* The use of hydrochloric acid instead of acetic acid at the purification stage.

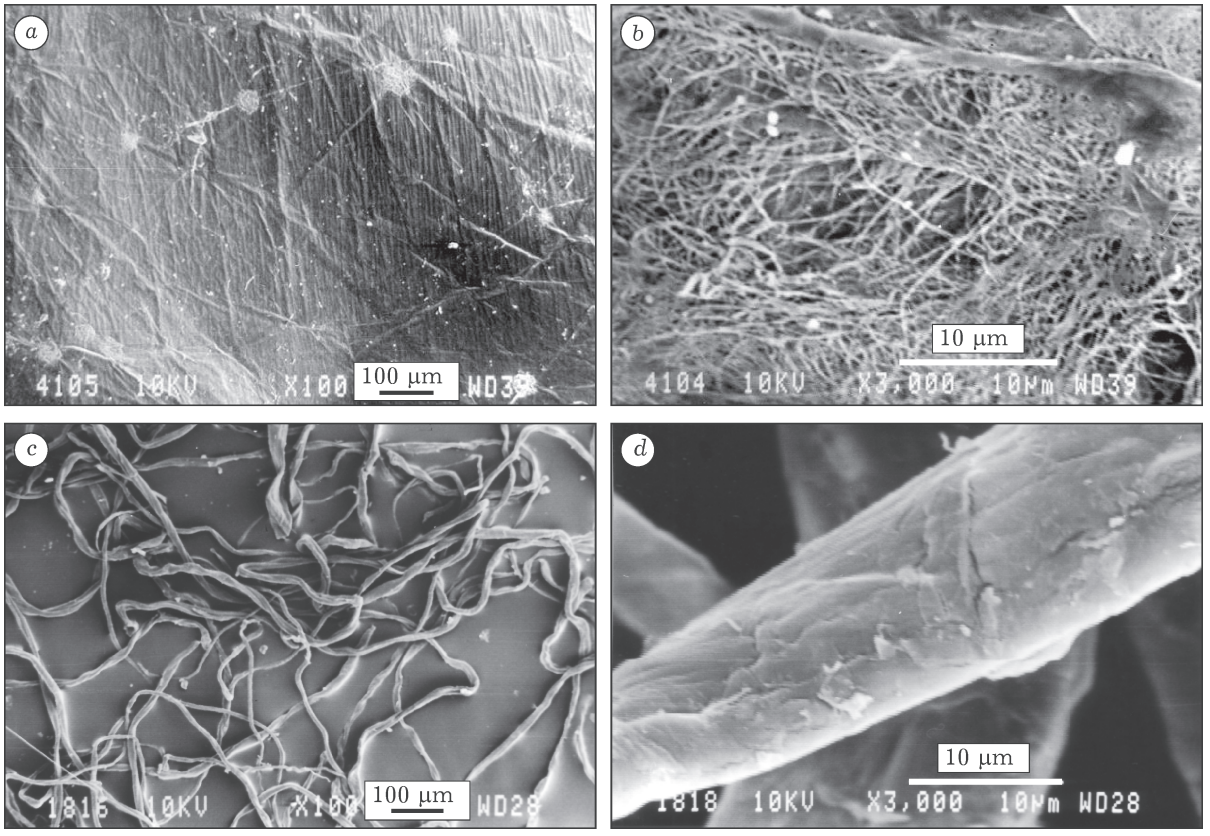

Fig. 2. Photomicrographic images of BCF (*a*, *b*) and cotton cellulose (*c*, *d*).

It is seen that, unlike the textile dressings made of cotton, the film surface is flat and smooth (see Fig. 2, *a*). With a sufficient moistening, such a film should easily be taken off from a wound, with no harming a "fresh" epithelium.

The structure of the film obtained (see Fig. 2, *b*) is formed by microfibrils exhibiting the form of irregular grid structure. Moreover, a strictly parallel arrangement of the bacterial cellulose (BC) molecules in the crystalline microfibrils guaranteed by the conditions of the biosynthesis of the extracellular polymer provides a minimum thickness of microfibrils within a single fibre unit. According to our data obtained by SEM, the thickness of a single fibber unit ranges within 15–150 nm being much thinner than cellulose fibres of plants (see Fig. 2, *d*). In addition, it is obvious that there is a uniform density distribution of skeleton fibres observed, which provides a high strength of the film.

Owing to such a structure, the system is able not only of providing the necessary steam and gas permeability, but also of retaining biologically active compounds within the structure of the film whereas in contact with the surface of a wound it can gradually release them.

In order to prove theoretical assumptions, we performed a number of experiments concerning the adsorption and desorption the solutions of biologically active substances in water and oil. In the first case we used a 5 % solution of ε-aminocaproic acid as a fibrinolysis inhibitor; in the second case we used sea buckthorn oil, a natural multicomponent remedy, the solution of anti-burn substances of sea buckthorn fruits (carotinoids, vitamins F and E) in vegetable oil.

Adsorption properties of the BCF

The ratio between solid phase (BCF) and liquid phase (5 % aqueous solution of ε-aminocaproic acid (ACA) and sea buckthorn oil) was equal to 1 : 25.

BCF samples cut according to a template, with the natural thickness of 5–7 μm were conditioned for 4 h at a relative humidity of 63–

TABLE 2

BCF adsorption capacity

Adsorbates	Adsorption duration, h	Sample mass before adsorption, g	Adsorbate mass, g	Adsorption level, g/g
5 % ACA solution	5	0.1000	0.1091	1.091
Sea-buckthorn oil	5	0.1000	0.0597	0.597

67 % and the temperature of 20–25 °C, were weighed to accuracy within 0.0001 g and were then placed in adsorbate (the solution of sea buckthorn oil, or ACA) for 5 h. After the 5 h exposure, the BCF were taken out and placed for 20 min on a porous plate, then dried in an oven at 40 °C to obtain constant mass, by the analogy with the method for the thermogravimetric determination of moisture content (State Standard GOST 28561–90).

The amount of the adsorbate was calculated as a difference between the mass of impregnated BCF and the mass of the carrier before the adsorption procedure. As the result of the measurement, we took an arithmetic mean of 10 parallel measurements. The results of determining the adsorption capacity of BCF are demonstrated in Table 2.

In order to determine the rate of adsorption we performed a kinetic study (Fig. 3).

Experimental data for both adsorbates demonstrated that the process is almost entirely completed within 3 h, whereas the character of changing the magnitude of adsorption indicates a multilayer adsorption on the surface of bacterial cellulose.

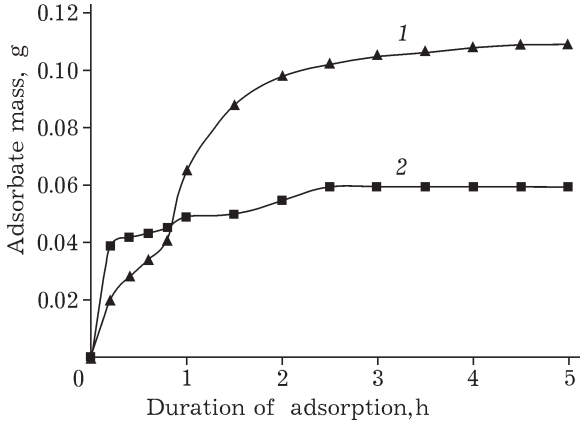

Fig. 3. Kinetics of the adsorption of ACA (*1*) and sea-buckthorn oil (*2*) of BCF.

To determine the strength of binding the adsorbate molecules with the surface of BCF we investigated desorption of these compounds under the influence of buffer solutions within pH range of 2.0–8.0.

Desorption properties of BCF

The samples obtained were subjected to desorption, which procedure was performed as it follows. Samples were placed into buffer solu-

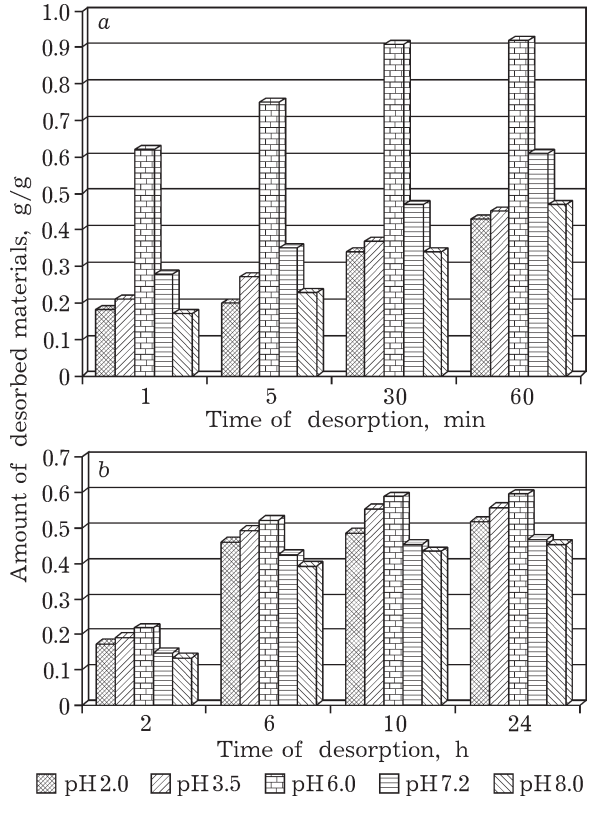

Fig. 4. Kinetics of ACA desorption (*a*) and sea-buckthorn oil (*b*) from BCF. The mass of the ACA in the sample before desorption being equal to 1.091 g/g, the mass of sea buckthorn oil in the sample before desorption being equal to 0.597 g/g.

tions with pH 2.0, 3.5, 6.0, 7.2, 8.0 with the ratio of solid and liquid phases amounting to 1 : 50 at 35–40 °C. The solutions with pH value of 2.0 and 3.5 were prepared using glycine buffer, solutions with pH 7.2 and 8.0 were prepared using phosphate buffer, and the solution with the pH value of 6.0 was presented by distilled water. It should be noted that the interaction between the components of the buffer solution with the adsorbate does not occur because of a high water duty level and too low temperature for chemical reactions to occur. The samples prepared were placed into a container with a buffer solution, after the exposure they were removed and placed for 20 min on a porous plate, then dried in an oven at 40 °C to obtain constant mass. After drying, the amount of desorbed material was calculated as the mass difference between the BCF and the impregnated sample after desorption. As the result of the measurement we took an arithmetic mean of 10 parallel measurements.

The results of studying the desorption properties of BCF (Fig. 4) allow one to conclude that both the adsorbates are faster desorbed to the solution with pH 6.0. The desorption process of ε-aminocaproic acid from the film to the buffer solution is almost entirely completed during 1 h. ε-Aminocaproic acid is used as a styptic agent, thus such a rate of desorption could be considered optimal for an application of hemostatic effect. The process of sea buckthorn oil liberation from the film into the buffer solution is almost entirely completed within 24 h, which indicates the value of an optimal rate of desorption for the applications with prolonged action, namely anti-burn one.

The pH value of human skin varies within the range from slightly acid to neutral; this fact confirms the possibility of producing TTS based on BCF, soaked either aqueous or oil solutions of pharmaceutical preparations.

CONCLUSION

Physicochemical properties have been studied for β-1,4-glucan obtained by culturing *Medusomyces gisevii* J. Lindau. A method is worked through for film obtained to purify from non-cellulose impurities. Possibility is demonstrated for using BCF in obtaining TTS either with water-soluble or liposoluble drugs.

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