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## Morphological and Physicochemical Properties of Nanostructured Cellulose Obtained through Chemical and Biological Methods

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**Summary.** The authors obtained samples of chemically pure, crystalline, micro- and nanostructured cellulose of various modifications using two approaches – biological and chemical. They studied these cellulose samples via scanning electron microscopy (SEM), thermogravimetric analysis, and infrared (IR) spectroscopy. To prepare cellulose microcrystals, they used the mild acid treatment method based on glycerol-acid mixtures for treating cotton fibers. They showed that the chemical processing of cotton fiber ensured its dispersion with generation of microcrystals surrounded by a partially preserved amorphous shell. The authors produced bacterial cellulose (BC) films using the *Komagataeibacter xylinus* C3 strain in surface cultivation conditions. With a view of obtaining higher-quality SEM images, they applied chemical fixation of lipids and proteins with critical drying to fix the process of nanofiber synthesis by bacterial cells. The two-step fixation method helped find the fibrillar structure of a cellulose film, while the morphology of bacterial cells was not deformed. The authors made a comparative analysis of the IR spectroscopy results between chemically synthesized cellulose microcrystals and BC. The obtained cellulose samples do not contain lignin and hemicellulose, both samples are highly crystalline. The BC has an ordered structure, higher crystallinity and gets carbonized when exposed to air pyrolysis. A thermogravimetric analysis of the samples shows the absence of thermally stable impurities. Both cellulose samples of biological and chemical origin are thermally stable, and the initial decomposition temperature is high enough for cellulose materials. These results show that the authors have managed to create nanocellulose materials that might be potentially applied in various industries, such as pharmaceuticals, functional composites, engineering, etc.

*The paper contains 6 Figures, 2 Tables, 29 References.*

**Keywords:** Bacterial cellulose, cellulose microcrystals, morphology, IR spectroscopy, thermogravimetry

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Научная статья

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## Морфологические и физико-химические свойства наноструктурированной целлюлозы, полученной химическим и биологическим способами

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**Аннотация.** В работе были получены образцы химически чистых, кристаллических, микро- и наноструктурированных целлюлозных материалов различных модификаций с использованием двух подходов – биологического и химического. Полученные образцы целлюлозы исследовали с помощью сканирующей электронной микроскопии (СЭМ), термогравиметрического анализа и инфракрасной (ИК) спектроскопии. Для получения микрокристаллов целлюлозы использовали метод слабокислотной обработки на основе глицерин-кислотных смесей для обработки хлопковых волокон. Установлено, что химическая обработка хлопкового волокна способствовала его диспергированию с образованием микрокристаллов, вокруг которых частично сохраняется аморфная оболочка. Пленки бактериальной целлюлозы (БЦ) были получены с использованием штамма *Komagataeibacter xylinus* СЗ в условиях поверхностного культивирования. Для получения более качественных СЭМ-изображений была проведена химическая фиксация протеинов и липидов с использованием критической сушки для фиксации процесса синтеза нановолокон бактериальными клетками. В результате метода двуступенчатой фиксации была обнаружена фибриллярная структура целлюлозной пленки, а морфология бактериальных клеток не подвергалась деформации. Проведен сравнительный анализ результатов ИК-спектроскопии между химически синтезированными микрокристаллами целлюлозы и БЦ. Полученные образцы целлюлозы не содержат лигнина и гемицеллюлозы, оба образца являются

высококристаллическими. БЦ имеет упорядоченную структуру, более высокую степень кристалличности и подвергается карбонизации при пиролизе на воздухе. Термогравиметрический анализ образцов показал отсутствие термически устойчивых примесей. Оба образца целлюлозы биологического и химического происхождения термически стабильны, а начальная температура разложения достаточно высока для целлюлозных материалов. Результаты продемонстрировали, что наноцеллюлозные материалы были успешно получены и потенциально могут быть применены в различных областях, таких как фармацевтика, функциональные композиты, инженерия и т.д.

**Ключевые слова:** бактериальная целлюлоза, микрокристаллы целлюлозы, морфология, ИК-спектроскопия, термогравиметрия

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## Introduction

Cellulose is the most abundant polymer in nature. It is an important structural component of the plant cell walls. Cellulose could also be produced in living organisms such as bacteria and even some marine animals [1]. It consists of  $\beta$ -1,4-linked glucopyranose groups, which form a linear homopolymer, where the monomers are rotated  $180^\circ$  relative to each other [2].

Despite the common chemical composition, depending on structural features, there are several types of cellulose: cellulose nanofibrils, also known as nanofibrillated cellulose; cellulose nanocrystals, with other designations such as nanocrystalline cellulose, cellulose (nano)whiskers, rod-like cellulose microcrystals; bacterial cellulose (BC), also known as microbial cellulose [3].

By subjecting microfibrils to a certain mechanical, chemical, or enzymatic treatment, it is possible to extract highly crystalline regions of cellulose microfibrils, which in turn leads to the formation of cellulose nanocrystals [4]. Normally they are rigid particles with rod-like structure. In contrast to cellulose with high content of amorphous fractions, nanocrystals have high specific strength and modulus of rigidity, as well as significant surface area. Tensile strength of cellulose nanocrystals is 7.5-7.7 GPa, which is significantly higher than that of steel wire and Kevlar [5].

Another important property of cellulose nanocrystals is their ability to behave like liquid crystals. Under suitable conditions and critical concentrations, asymmetric rod-like and lamellar particles tend to self-assemble into ordered

structures. Size, charge, electrolyte, and other factors affect the liquid crystal properties of cellulose nanocrystals. Combined with the ability to refract rays, it leads to interesting optical phenomena. The acid used for hydrolysis also affects the process. For example, the use of sulfuric acid results in negatively charged nanocrystals, which facilitates the formation of water dispersion. Cellulose crystals obtained with sulfuric and phosphoric acids usually lead to chiral nematic structures, whilst crystals obtained with hydrochloric acid and sulfonation are likely to form a birefringent glassy phase [6].

Along with plant sources, the non-pathogenic bacteria *Komagateibacter*, for example, *K. xylinus*, could be highlighted here [7]. Some strains of these bacteria, amenable to isolation from various sources, are capable of producing highly crystalline cellulose networks and fibers that form biofilms of various thicknesses to maintain high oxygen levels on the surface and create a protective barrier against drying, radiation, and contamination [6].

In addition to such pros as biodegradability, non-toxicity, and biocompatibility, an important advantage of BC in comparison with plant analogs is its unique purity, which in practice allows skipping additional purification stages. The chemical composition of such biological celluloses is equivalent to cellulose of plant origin; however, an important advantage is that BC does not contain such by-products as lignin, pectin, hemicellulose, and other substituents in lignocellulosic materials [8].

BC is a nanostructured polymer with diverse levels of organization (protofibrils - ribbons - pellicle). It is obtained by fermentation in a medium containing only microbial cells, nutrients, and secondary metabolites, which could be easily removed to obtain the purest nanostructured cellulose films. Films formed in this way possess a unique three-dimensional structure that is not inherent for plant-based celluloses. As a result, physical and mechanical properties also differ.

BC aggregates form long fibrils (about 1.5 nm wide), providing high specific surface area, elasticity, resistance, and flexibility [9]. It is important that the physical properties of BCs strongly depend on their method of production and processing. Hereby, for dried samples, it was stated that, as a rule, tensile strength is about 240 MPa, Young's modulus is approximately 10 GPa and maximum deformation is about 3%, although it is believed that the modulus for a single fiber can reach 114 GPa [10].

Regarding hydrated samples (water content 98%), results showed the following properties: tensile strength 380 kPa, maximum deformation 21%, and water vapor transmission rate  $2,900 \text{ g}\cdot\text{m}^{-2}\cdot\text{d}^{-1}$  [11]. Determined compression modulus for such samples was 0.06 MPa.

Concerning morphological properties of hydrated samples, it was found that BC had a specific surface area of about  $60 \text{ m}^2/\text{g}$ , a specific pore volume of  $0.2 \text{ cm}^3/\text{g}$ , and an average pore diameter of about 13 nm [12].

Another important parameter in the analysis of BC applicability is its water-retaining properties. To assess it, there are quantitative parameters such as water capacity and water release rate. The water capacity of cellulose is 60 to 700 times its dry weight, depending on synthesis conditions. In typical films

cultured under static conditions, cellulose weight itself consists of about 1% of total weight [13].

The use of various strategies during the synthesis of nanostructured cellulose films can increase their water retention capacity [14]. There is a direct relationship between pore size and the ability to regain moisture [15]. It was found that a decrease in pore size and surface area resulted in a decrease in water retention and an increase in the rate of water release. The ability of BC fibers to retain and release water is especially important for biomedical applications.

Due to the flexibility and strength of such cellulose films, it is possible to create flexible electronics for flexible displays, portable electronic devices, and electronic skin. Some of the main applications are reflected below in Fig. 1.



**Fig. 1.** Nanocellulose materials applications

From an ecological point of view, it is relevant to search for methods that will cause minimal harm to the environment, for example, obtaining cellulose using bacteria, which gives a chance to perform waste-free production of chemically pure nanostructured cellulose. However, like any product of biosynthesis, the production cost of BC is quite expensive, since the development of the producer requires a nutrient medium and operating costs [16]. It is easier to obtain cellulose from cotton fiber since nearly 90% of the cotton fibers are cellulose [17].

Table 1 shows the comparison between nanocrystalline cellulose and BC (source of isolation, production method, and structural features).

Table 1

**The main characteristics of nanocrystalline cellulose and bacterial cellulose**

Type of nanocellulose	Cellulosic source	Preparation methods	Features	References
Nanocrystalline cellulose (cellulose (nano)whiskers, rodlike cellulose microcrystals, cellulose nanocrystals)	Cotton, wood, wheat straw, corn-cob residue, cellulose from algae, cornhusk, sugar-cane bagasse, blue agave bagasse, jute, spruce bark, lax fibers, pineapple leaf and coir, banana	Acid hydrolysis, oxidation method, enzymatic hydrolysis, ionic liquid treatment, subcritical water hydrolysis	Rod or needle-like particles, high crystallinity and specific surface area, biocompatibility, biodegradability, optical transparency, low cost, high tensile strength, elasticity, low density, and purity; crystallinity index: 54-88%; degree of polymerization: 500-15,000; width: 4-70 nm; length: 100-6,000 nm	[3, 18-27]
Bacterial cellulose (microbial cellulose)	<i>Aerobacter</i> , <i>Agrobacterium</i> , <i>Rhizobium</i> , <i>Glucanacetobacter</i> (presently <i>Komagataeibacter</i> ), <i>Acetobacter</i> , <i>Sarcina</i> , and <i>Pseudomonas</i>	Microbial fermentation	Mechanical strength, biocompatibility, polyfunctionality, purity, non-toxicity, high stability of the single cellulose fibers and thermal stability; water holding capacity: from 60 to 700 times its dry weight; crystallinity index: 84-89%; degree of polymerization: 800-10,000; diameter: 20-100 nm	[1, 3, 18, 28, 29]

Thus, the development of mild modifications of acid hydrolysis, making it possible to obtain micro- and nanostructured cellulose crystals in accordance with the principles of green chemistry, becomes a promising task. Since, as previously known, BC obtained by microbial synthesis can be attributed to nanocellulose, nanocrystalline cellulose obtained by mild acid hydrolysis was compared with BC in several key parameters. This was the purpose of this research work.

### Materials and methods

*Obtaining BC in static cultivation condition.* Synthesis of cellulose by *Komagataeibacter xylinus* C-3 strain was conducted in Hestrin-Schramm (HS) nutrient medium (pH 6), which consists of following components (g/L): glucose - 20, peptone - 5, yeast extract - 5, Na<sub>2</sub>HPO<sub>4</sub> - 2.7 and citric acid - 1.15. The inoculum was a 48-hour culture of acetic acid bacteria grown in a medium containing yeast extract and beer wort (6° Balling) in a 1:1 ratio with 2 wt. % of glucose, 1 vol. % of ethanol.

To obtain BC films of regular shape and equal diameter (0.5 cm), cellulose-producing microorganisms were cultured for 7 days under stationary conditions in a 24-well plate at 28°C.

Cellulose was separated and periodically washed with 0.5-1% aqueous NaOH solution while boiling until removal of cells. Then, the cellulose film was washed from NaOH solution with distilled water, 0.5% acetic acid solution, and again with distilled water until neutral pH. The resulting cellulose was stored as a gel film in distilled water at 5°C. Membranes were subsequently dried to determine their morphological and physicochemical properties. Biomass of BC samples was determined after preliminary drying in a dry heat thermostat at 80°C up to constant sample weight.

*Obtaining microcrystals using mild acid hydrolysis in glycerol.* To obtain cellulose with a high degree of crystallinity, dried cotton fibers were subjected to acid hydrolysis with an excess of 9% sulfuric acid solution in glycerol. Then, the reaction mixture was heated in a water bath at 90°C for 3 hours.

After cooling, the mixture was centrifuged to remove the hydrolysis solution. The resulting precipitate was repeatedly redispersed in water and centrifuged to remove residual acid and glycerol. Finally, cellulose crystals were characterized.

Obtained samples of cellulose were investigated by scanning electron microscopy (SEM), thermogravimetric analysis (TGA), and infrared (IR) spectroscopy.

*Characterization of cellulose samples by SEM.* Samples of cellulose were precoated with a thin layer of a platinum-palladium alloy (Pt/Pd 80/20) and examined using a scanning electron microscope SUPRA 55VP-31-04 (Zeiss, Oberkochen, Germany). Image analysis of SEM micrographs was performed using Image J software (version 1.8.0, National Institutes of Health, Bethesda, MD, USA).

*Determination of the IR spectrum.* The Fourier transform IR spectra of the polymers were measured using an FT/IR6200 spectrometer (Jasco, Easton, MD, USA). The spectra were obtained with a resolution of no higher than 4 cm<sup>-1</sup> (4-point apodization function) after more than 50 scans in the range of 4000-400 cm<sup>-1</sup>.

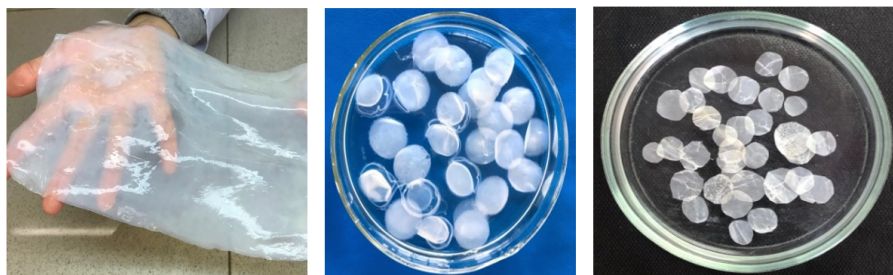
*TGA.* Cellulose materials of various origins were analyzed by the thermogravimetric method by measuring the rate of weight change. In addition, the samples were analyzed by TGA to determine the amount of water. Setaram Set-sys 92-12 (Setaram, Lyon, France) was used in the range of 50-9,000°C at a heating rate of 100°C min<sup>-1</sup>.

*Statistical analysis.* Statistical comparison was performed using an unpaired test, followed by a one-way analysis of variance (ANOVA) using Dunnett's multiple comparison test. All statistical data of the analysis were carried out using SPSS software package (version 16.0, SPSS Inc., Chicago, USA).

## **Results and discussion**

BC synthesized by acetic acid bacteria can be obtained by superficial cultivation of producer strains. At the first stage, it was necessary to obtain stable and

durable BC films. For this, producer strain *Komagataeibacter xylinus* C-3 was grown on HS medium for 7 days. Obtained films of cellulose synthesis are shown in Fig. 2.



**Fig. 2.** BC gel-film obtained after 7-day static cultivation of producer strain:  
A - BC gel-film; B - wet discs of BC; C - dried cellulose films

Samples of BC films obtained using *K. xylinus* strain were examined by SEM. This method is one of the most important methods in the study of bacterial samples. Of special interest is the possibility to record the process of nano-fiber synthesis by bacterial cells.

To obtain better images, a two-stage fixation of proteins and lipids was performed to preserve the structural appearance of fibers and cells. Lipid fixation was carried out using Karnovsky's fixative and osmium tetroxide [30]. During the interaction, osmium dioxide is placed at polar groups in lipid micelles, allowing them to be fixed and further investigated by various microscopic and chromatographic techniques.

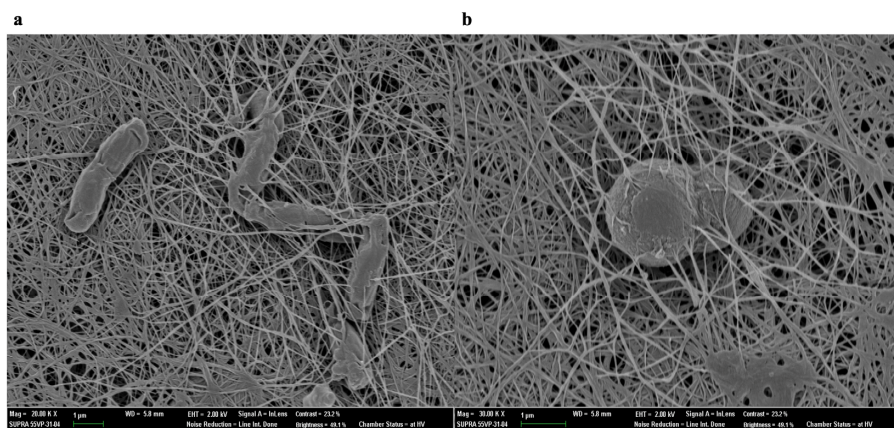
Besides lipids, it is also important to fix proteins in cells. For that, the most versatile critical drying method was used. During this process, water molecules in biological tissues are replaced by an inert liquid, whose critical temperature for achieving appropriate pressure is just slightly higher than ambient temperature. In this work, carbon dioxide was used, the critical point of which is 35°C. Thus, water in the cell structure was replaced by liquid CO<sub>2</sub>, followed by heating above a critical temperature. As a result, the liquid-gas phase transition of CO<sub>2</sub> occurs without changing density. This helps to avoid surface tension effects, responsible for morphology distortion. The method also uses ethanol to achieve complete mixing with carbon dioxide.

From the images of SEM in Fig. 3, it could be seen that as a result of this two-step fixation, not only the fibrillar structure of the cellulose film was detected, but also, the morphology of bacterial cells was perfectly preserved without any deformation. The images show that bacterial strain is viable and participates in synthesis under created conditions. Each bacterium produces many nano-fibers, surrounding itself with a three-dimensional network.

BC synthesis by *K. xylinus* occurs between outer and cytoplasmic membranes assisted by a cellulose-synthesizing complex that binds to pores of the bacterial surface. During the synthesis, glucose chains created inside the cell are



released through tiny pores in the cell membrane. About 30 cellulose molecules are formed into larger units known as elementary fibrils (protofibrils), which are then gathered into microfibrils, later assembling to form cellulose ribbons [31].



**Fig. 3.** SEM images of the process of synthesis of cellulose nanofibers by *K. xylinus* strain at 20,000-fold magnification (a), at 30,000-fold magnification (b)

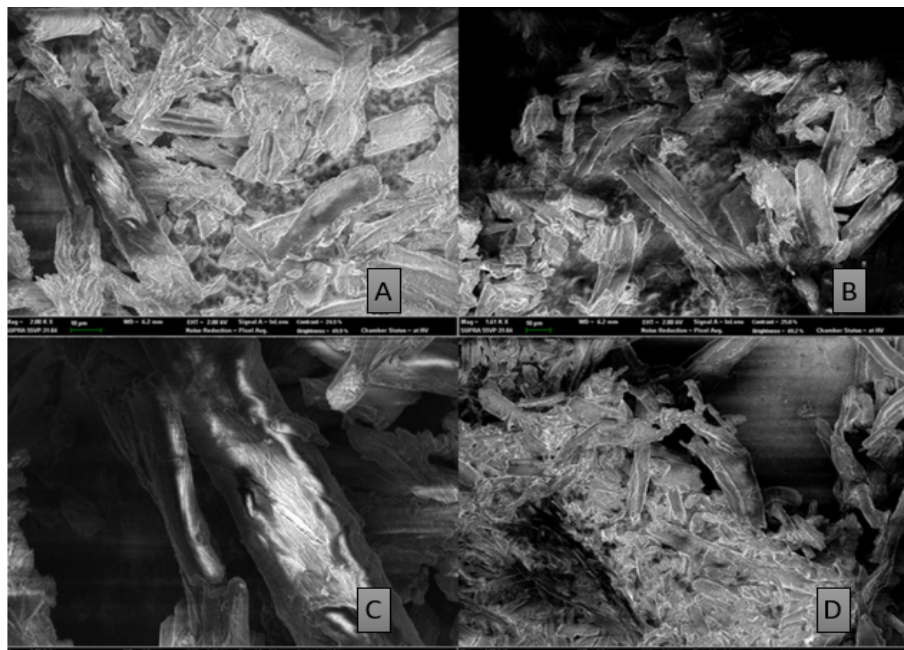
Microfibrils connect by hydrogen bonds, making possible the formation of fibers, flat layers, and film structures. As a result, flat fibers containing cellulose are formed on the surface of the bacterial cell. These fibers, with diameters from 10-20 to 30-40 Å, are built side by side on the horizontal axis.

Cells producing cellulose-like fibers move forward parallelly to microfibrils [32]. Hence, the bacteria will move forward as they secrete cellulose in a jet propulsion manner. As a result, cellulose synthesis forces cells to rotate along their longitudinal axes as they push and twist the cellulose fibers. Since *K. xylinus* is an aerobic bacterium, it tends to move towards the surface of the liquid medium, where the oxygen level is higher [33]. A ligament formed by "zigzag movement" and cell division forms a branch of delamination. As a result, microfibrils crystallize into a bunch of fibers, which form ultrafine reticular structure and thick cellulose mat-film [34]. Cellulose molecules bind to each other through hydrogen bonds near the cell surface.

Thus, during the formation of a gel film, *K. xylinus* cells move in the opposite direction of polymer chain secretion. It is believed that cells of bacteria synthesizing cellulose are immobilizing in a polymer network to maintain the entire population in the space between air and liquid. Therefore, the biosynthesis of cellulose is physiologically expedient and is an important evolutionary mechanism for the survival of cellulose producers.

As a result of mild acidic treatment of cellulosic material, their amorphous shells are destroyed resulting formation of crystalline particles. The treatment was carried out in a glycerol-sulfuric acid system for 3 hours. The resulting product - a mixture of suspended cellulose particles in a liquid phase - could be used independently, for example, to obtain thin films, or can be dried to acquire micronized powder cellulose powder.

The resulting white powder was characterized by SEM to assess the effectiveness of acidizing treatment of cotton fiber. SEM images are shown in Fig. 4.



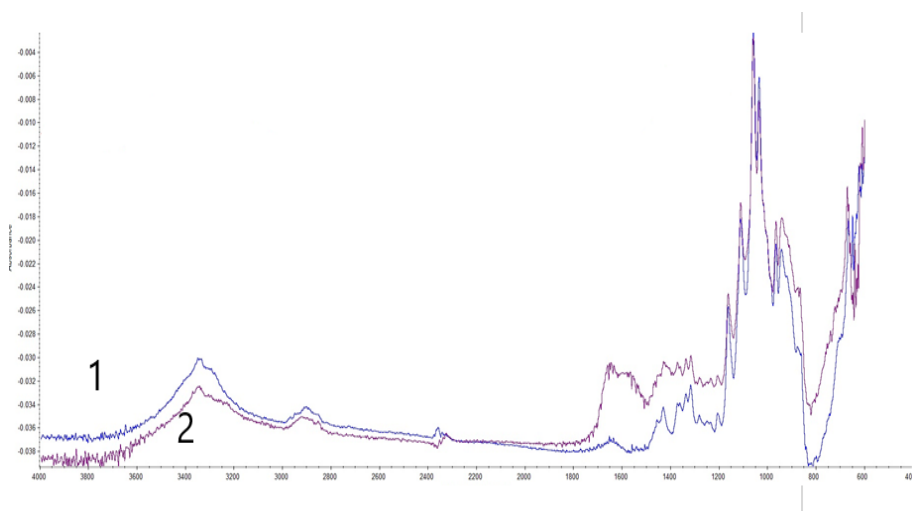
**Fig. 4.** SEM images of samples obtained by mild acid hydrolysis at 2,000-fold magnification (a), crystal structure at 1,610-fold magnification (b), crystalline and amorphous structures of an individual particle at 5,000-fold magnification (c), at 1,000-fold magnification (d)

As could be seen from the images, in this case, the chemical processing of cotton fibers led to obtaining particles with heterogeneous size distribution and morphology (Fig. 4a). In images 4b and 4c, we observe that the particles demonstrate predominantly crystalline structure, however, in many particles, their highly crystalline core seems to be surrounded by an amorphous shell preserved even after treatment with sulfuric acid. This indicates that highly crystalline cotton fiber requires more severe processing conditions to remove the amorphous phase. Thus, to obtain nanocrystalline particles, it is necessary to change the parameters of acid hydrolysis (an increase in acid concentration, temperature, or processing time), or improve the availability of crystalline regions by introducing structural defects using mechanical activation. Therefore, mild acid treatment based on glycerol-acid mixtures for treating cotton fibers is more suitable for obtaining microcrystalline cellulose.

For our research, it was interesting to carry out a comparative analysis of IR spectroscopy results between chemically synthesized cellulose (microcrystals obtained by the method of soft glycerol hydrolysis) and BC.

Comparing the spectra of obtained compounds (Fig. 5), several conclusions could be drawn. First of all, the purity and ordering of biological and chemical samples could be estimated using IR spectra. In both cases, the spectrum does

not have vibrations at  $1740\text{ cm}^{-1}$  and  $1600\text{ cm}^{-1}$ , corresponding to hemicellulose and lignin impurities, respectively. The presence of such impurities, as a rule, is especially frequent for celluloses isolated from natural plant raw materials. It happens since, in plant materials, cellulose is traditionally accompanied by lignin and hemicellulose, which are relatively difficult impurities to remove. Cotton is a chemically pure cellulosic raw material. As could be seen from the spectrum, microcrystal samples obtained from cotton do not require additional purification from this type of contamination.



**Fig. 5.** Comparison of spectra of chemical crystalline (1) and bacterial (2) celluloses

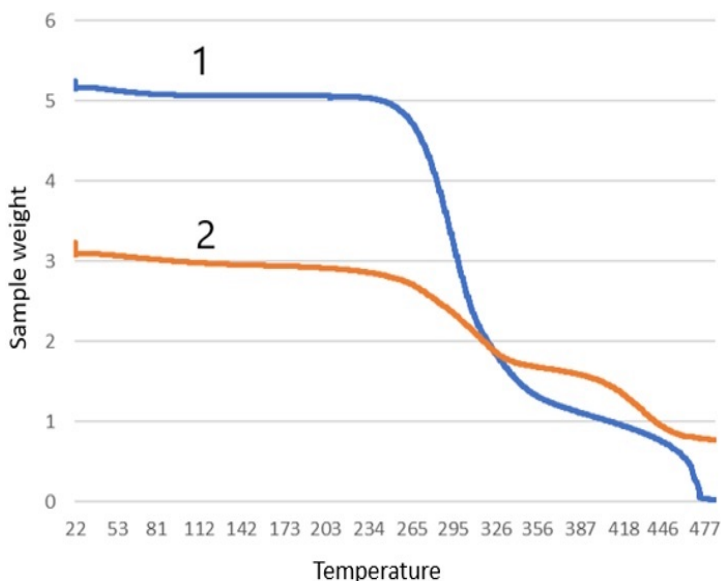
In general, evaluating the spectra, it is obvious that in the case of the biological sample, peaks are generally more pronounced. Exceptions could be found in regions of  $3200\text{--}3600\text{ cm}^{-1}$  and  $2800\text{--}3000\text{ cm}^{-1}$ , which characterize vibrations of methine and methylene groups, as well as stretching vibrations of hydroxyl groups. These peaks are always well-pronounced in cotton-originated celluloses, hereby they are more pronounced for chemical cellulose isolated from cotton fiber. For the rest, chemically synthesized cellulose generally has a more diffuse spectrum, which may correspond to its less ordered structure and the presence of a neglectable amount of impurities.

Structure ordering could be 2 types: inter- and intramolecular. The first one is determined by the interaction between molecules and the conformation of polymer chains. The second one is responsible for the interposition of units in the chain and the conformation of hydroxyl and pyranose groups [35]. To characterize intermolecular three-dimensional ordering, as a rule, X-ray diffraction methods are applied. IR spectroscopy, in turn, makes it possible to draw conclusions about intramolecular interactions. According to [35], absorbance in ranges of  $1200\text{--}1400$  and  $800\text{--}900\text{ cm}^{-1}$  indicates rotation of  $\text{CH}_2\text{OH}$  groups in crystal regions and, thus, makes it possible to estimate intramolecular ordering. These peaks are well pronounced in spectra of both samples, meaning high crystallinity

and structure ordering. However, absorbance at these frequencies is more intense for biological cellulose, indicating the most ordered structure here. The structure of chemical microcrystals is also quite crystalline but contains more amorphous regions. This result is in accordance with SEM data, which has confirmed that a partially preserved amorphous shell is still present in the chemically extracted sample.

Additional information about the structure could be obtained by evaluating vibrations between  $1700$  and  $1650\text{ cm}^{-1}$ . They indicate deformation vibrations in H-O-H bond and, therefore, correspond to the presence of bound water in the sample structure. This peak is more visible for BC, which contains a greater amount of water even after drying. This feature is provided by its fibrous, nanostructured morphology.

Integral graphs of TGA from Fig. 6, allowed us to carry out a comparative analysis of different cellulosic materials.



**Fig. 6.** Thermogravimetry spectra for samples of chemical (1) and bacterial cellulose (2)

Thereby, it was found that weight loss associated with dehydration was longer for an extremely hydrophilic fibrillar BC sample than for crystalline cellulose.

The location of the main TGA parameters, given in Table 2, in general, is quite close for both samples. Both samples are thermally stable, and the IDT (initial decomposition temperature) is high enough for cellulosic materials.

In  $235\text{--}330^\circ\text{C}$  zone, we can observe that stage of oxidative pyrolysis begins for both materials. Temperatures of maximum decomposition rate were determined using differential TGA plots. Two pyrolysis regions were observed for BC, which, remembering IR spectroscopy data, could be caused by the presence of three-dimensional ordering - the necessity to destroy intermolecular bonds responsible for the formation of ordered fibrillar structure.

Table 2

**Comparative analysis of TGA spectra for biological and chemical celluloses**

Parameter	Description	Values for chemical cellulose	Values for biological cellulose
IDT (onset)	Initial decomposition temperature, at which the material begins to disintegrate. This parameter is an indicator of the thermal stability of the material	260°C	235°C
MRDT	Maximum rate of decomposition temperature	298°C	312°C, 431°C
D <sub>1/2</sub>	Temperature at which 50% of material is decomposed	307°C	400.5°C
FR	Final residue - the amount of material at the end of heating, indirectly characterizes the composition of test sample	0 g, 0%	0.75 g, 23.5 %

Half-decomposition temperatures and final residue weight describe the final stage of the pyrolysis process. The absence of any residue during pyrolysis may indicate a lack of thermostable impurities, however, 23.5% of BC residual mass, altogether with other data and characteristics of synthesis, permits us to suggest that this material, as in [36], undergoes carbonization with the formation of stable graphitized films.

Higher values inherent in chemical cellulose are suitable for use in functional composites, engineering, and lithography, while BC could be involved in medical applications, such as developing wound healing dressings or capsules for targeted drug delivery.

### Conclusion

As a result of the studies, we obtained samples of chemically pure, crystalline, micro-, and nanostructured cellulose of various modifications using two approaches - biological (bacterial synthesis to obtain nanostructured films) and chemical (glycerol hydrolysis of cotton fiber to obtain crystalline powder). It was demonstrated that mild acidic hydrolysis in glycerol promoted the dispersion of cotton fiber with the formation of microcrystals surrounded by a partially preserved amorphous shell.

Comparative analysis of the samples shows that both are stable, and do not contain lignin and hemicellulose, as well as any heat-resistant impurities, moreover, both samples have a crystalline structure. The BC sample has a higher degree of crystallinity and chemical purity, and during pyrolysis in air, it is getting carbonized.

The study showed that chemical fixation of lipids and proteins with the use of critical drying was required to obtain qualitative information on the structure of bacterial cells and cellulose nanofibers of biological origin.

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