ISSN 2074-9414 (Print)
ISSN 2313-1748 (Online)

https://doi.org/10.21603/2074-9414-2025-3-2587
https://elibrary.ru/JBSFIU

Original article
Available online at https://fptt.ru/en

Effect of Nutrient Medium Composition on Bacterial Cellulose: Yield and Physicochemical Profile

Abdoul-Galil Ahmed Ali[1,*], Olga V. Kriger[1,2,]**

[1] *ITMO University, St. Petersburg, Russia*

[2] *Immanuel Kant Baltic Federal University, Kaliningrad, Russia*

Received: 14.04.2025
Revised: 27.05.2025
Accepted: 01.07.2025

**Abdoul-Galil Ahmed Ali: abdoulgalil49@gmail.com, https://orcid.org/0009-0007-3982-2131*
***Olga V. Kriger: ovkriger@itmo.ru, https://orcid.org/0000-0002-1489-0716*

Abstract.
Bacterial cellulose differs from plant cellulose: its unique properties include a strong crystalline nanostructure and a high degree of polymerization. In addition, it is more pure than traditional cellulose as it contains neither lignin nor hemicellulose. These qualities make it a promising alternative to plant cellulose in several industries. Bacterial cellulose with the specific physicochemical profile can be obtained only if the metabolizing properties of its producer have been considered. This article describes the effect of nutrient medium compositions with different carbon sources, vitamins, mineral salts, and acids on the yield and properties of bacterial cellulose.

Acetic acid bacteria *Acetobacterium xylinum* B-12429 were cultivated statically at 28°C for 72 h on the Hestrin-Schramm medium with varying carbon sources and growth factors.

The highest biomass yield (4.4 g/L) was obtained on cultivation day 10 in the sample with 20.0 g/L fructose. Glucose provided a lower productivity of 3.6 g/L. The bacterial cellulose yield also proved to depend on the concentration of the main carbon source: it was at its maximum at 10%. Adding ascorbic acid and $MgSO_4$ also catalyzed the biosynthesis. The structural profile was studied using infrared spectroscopy and scanning electron microscopy. It included such physicochemical properties as water-holding capacity and crystallinity indices I_α and I_β. The biofilms produced from the media fortified with xylose and sorbitol demonstrated excellent water-holding capacity; all the samples demonstrated a stable crystalline structure regardless of the carbon source.

The composition of the nutrient media had a significant effect on the yield and quality of biosynthesis. An optimized nutrient composition was able to boost biosynthesis, making the method applicable to industrial scales of high-quality bacterial cellulose production.

Keywords. Bacterial cellulose, biosynthesis, carbon sources, physicochemical properties, *Acetobacterium xylinum*

Funding. The research was supported by the Russian Science Foundation, agreement No. 24-24-00169.

For citation: Ali A-GA, Kriger OV. Effect of Nutrient Medium Composition on Bacterial Cellulose: Yield and Physicochemical Profile. Food Processing: Techniques and Technology. 2025;55(3):558–566. https://doi.org/10.21603/2074-9414-2025-3-2587

https://doi.org/10.21603/2074-9414-2025-3-2587
https://elibrary.ru/JBSFIU

Оригинальная статья
https://fptt.ru

Влияние состава питательной среды на продуктивность и физико-химические свойства бактериальной целлюлозы

А.-Г. А. Али[1,*], О. В. Кригер[1,2,]**

[1] *Национальный исследовательский университет ИТМО, Санкт-Петербург, Россия*

[2] *Балтийский федеральный университет имени Иммануила Канта, Калининград, Россия*

Поступила в редакцию: 14.04.2025
Принята после рецензирования: 27.05.2025
Принята к публикации: 01.07.2025

**А.-Г. А. Али: abdoulgalil49@gmail.com, https://orcid.org/0009-0007-3982-2131*
***О. В. Кригер: ovkriger@itmo.ru, https://orcid.org/0000-0002-1489-0716*

Аннотация.
Бактериальная целлюлоза обладает специфическими и уникальными свойствами, которые отличают ее от растительной. Она имеет высококристаллическую наноструктуру, высокую чистоту (отсутствие лигнина и гемицеллюлозы) и более высокую степень полимеризации, что делает ее перспективной альтернативой растительной целлюлозе для специфических применений. Для того, чтобы получить бактериальную целлюлозу с заданными физико-химическими характеристиками, необходимо тщательно исследовать способность ее продуцента метаболизировать различные источники углерода и их влияние на качество синтезированной целлюлозы. Целью данного исследования являлось изучение влияния состава питательной среды, включая источники углерода, витамины, минеральные соли и кислоты, на продуктивность и свойства бактериальной целлюлозы.
Объекты исследования – уксуснокислые бактерии *Acetobacterium xylinum* B-12429 (национальный биоресурсный центр ВКПМ). Культуру выращивали при температуре 28 °C в течение 72 ч на питательной среде Хестрина-Шрамма в присутствии различных источников углерода, а также дополнительных факторов роста в статических условиях при периодическом культивировании. Структурные характеристики полученных пленок бактериальной целлюлозы были изучены с помощью инфракрасной спектроскопии и сканирующей электронной микроскопии (СЭМ). Оценивали физико-химические характеристики бактериальной целлюлозы, включая водоудерживающую способность и индексы кристалличности (I_α и I_β).
Максимальное накопление биомассы бактериальной целлюлозы на среде Хестрина-Шрамма происходило на 10 сутки культивирования в статических условиях. Наибольший выход бактериальной целлюлозы (4,4 г/л) получен с использованием фруктозы в качестве источника углерода с концентрацией 20,0 г/л. При использовании в качестве источника углерода глюкозы продуктивность бактериальной целлюлозы ниже (3,6 г/л). На продуктивность бактериальной целлюлозы оказывала влияние концентрация основного источника углерода: 10 % концентрация способствовала ее максимальному выходу. Внесение дополнительных компонентов в состав питательной среды, таких как аскорбиновая кислота и $MgSO_4$, эффективно влияет на продуктивность синтеза бактериальной целлюлозы. Биопленки бактериальной целлюлозы, полученные на среде HS с ксилозой и сорбитом, обладали наибольшей водоудерживающей способностью. Индексы кристалличности для всех образцов бактериальной целлюлозы были приблизительно равны 1, что свидетельствует о стабильной кристаллической структуре целлюлозы независимо от источника углерода в питательной среде.
Исследование показало, что состав питательных сред оказывает значительное влияние на биосинтез бактериальной целлюлозы. Эти результаты подчеркивают важность оптимизации состава питательных сред для повышения продуктивности ее биосинтеза, что может быть использовано в промышленности для получения высококачественной бактериальной целлюлозы.

Ключевые слова. Бактериальная целлюлоза, биосинтез, источники углерода, физико-химические свойства, *Acetobacterium xylinum*

Финансирование. Работа выполнена при поддержке Российского научного фонда, соглашение № 24-24-00169.

Для цитирования: Али А.-Г. А., Кригер О. В. Влияние состава питательной среды на продуктивность и физико-химические свойства бактериальной целлюлозы. Техника и технология пищевых производств. 2025. Т. 55. № 3. С. 558–566. (На англ.) https://doi.org/10.21603/2074-9414-2025-3-2587

Introduction

Cellulose is a biopolymer with a high degree of polymerization. It is composed of glucose monomers that are linked by β(1–4) glycosidic bonds to form a long chain. This polysaccharide is present in all living things, from bacteria and algae to plants and animals [1]. Cellulose is a highly abundant renewable biopolymer used in biocompatible and environmentally sustainable solutions.

However, the growing demand for plant-based cellulose has boosted global wood consumption, which has made deforestation a major environmental issue [2].

Although plant cellulose is the most popular natural polymer, its complex chemical composition limits its use in cosmetics and pharmacy since it contains such impurities such as lignin, hemicellulose, and pectin.

Bacterial cellulose is synthesized by various bacteria, including *Komagataeibacter*, *Agrobacterium*, and *Pseudomonas* [3]. The Gram-negative acetic-acid *Komagataeibacter xylinus* often serve as a model organism due to its high cellulose productivity [4]. Bacterial cellulose is different from plant cellulose in some specific properties that depend on the culture conditions. In particular, it has a highly crystalline nanostructure (20–100 nm). It is pure in that it is free of lignin or hemicellulose. Its water-holding capacity is 200 times its dry weight. Finally, it boasts a high degree of polymerization. These properties make bacterial cellulose a good alternative to plant cellulose in biomedicine, cosmetics, high-quality acoustic diaphragms, papermaking, food industry, etc. [5, 6].

Bacterial cellulose is produced in the standard Hestrin-Schramm medium that consists of sources of carbon and nitrogen and growth factors, i.e., yeast extract and peptone, which makes the process economically unfeasible. Moreover, most strains are low-yielding, which means the method cannot be applied on industrial scale [6, 7]. The cultivation conditions are either static or with stirring, each yielding a particular morphological type of bacterial cellulose. The first one is a biofilm formed on the interface between air and liquid. The second is represented by granules that form stable suspensions [8].

Numerous studies have attempted to increase the yield of bacterial cellulose. Some focused on the nitrogen and carbon sources or minerals while others tested different cultivation variables, e.g., temperature, pH, dissolved oxygen, etc. [9]. As different carbon sources have different molecular weight, chemical structure, and bioavailability, their biosynthesis rates also differ significantly, not to mention structural flaws. As a result, bacterial cellulose production remains a costly business. The domestic bacterial cellulose industry needs to optimize its culture medium parameters, including composition, pH, and carbon source. The existing technological procedures face two fundamental limitations. First, the biosynthesis rate remains low. Second, the yield varies from batch to batch [10].

Bacterial cellulose with a specific physicochemical profile is a result of a comprehensive study into the metabolizing properties of each new cellulose producer in relation to various carbon sources and their effect on the quality of cellulose [10, 11].

Recent studies concentrate on alternative culture media obtained from agricultural and industrial wastes. Such economically viable solutions may help to achieve enough bacterial cellulose for industrial production [12]. For example, Saavedra-Sanabria *et al*. [10] used cocoa exudates to obtain 13.13 g/L bacterial cellulose. Sutthiphatkul *et al*. used rice noodles and *Komagataeibacter* sp. PAP1 strain to obtain 11.76 g/L bacterial cellulose [13].

Media obtained from food and agricultural wastes can reduce the cost and time of fermentation, thus producing more high-quality bacterial cellulose for large-scale multipurpose commercial production.

This research describes the effect of nutrient media with different compositions, carbon sources, vitamins, mineral salts, and acids, on the yield and properties of bacterial cellulose.

Study objects and methods

The bacterial strain of *Acetobacterium xylinum* B-12429 was purchased from the National Bioresource Center of the All-Russian Society of Microorganisms (Moscow, Russia) to serve as a model microorganism.

The culture was grown at 28°C for 72 h on a nutrient medium that contained 10.0 g/L yeast extract, 100.0 g/L glucose, and 20.0 g/L $CaCO_3$. Its pH was adjusted to 6.85. The autoclave sterilization lasted for 15 min at 121°C.

To prepare the inoculum, we incubated some of the culture in 50 cm^3 of the sterile Hestrin-Schramm medium with 5.0 g/L yeast extract, 20.0 g/L glucose, 5.0 g/L peptone, 2.7 g/L Na_2HPO_4, and 1.15 g/L citric acid. Its pH was adjusted to 6.85 by adding acetic acid or NaOH.

We used the following reagents (20.0 g/L) as carbon sources: D-glucose (CAS No. 50-99-7, analytical grade, OOO LenReaktiv); D-fructose (CAS No. 57-48-7, analytical grade, OOO LenReaktiv); lactose monohydrate (CAS No. 63-42-3, analytical grade, OOO Reakhim); D-xylose (CAS No. 58-86-6, ≥ 99%, OOO Reakhim); maltose monohydrate (CAS No. 6363-53-7, analytical grade, OOO LenReaktiv); glycerin (CAS No. 56-81-5, analytical grade, OOO LenReaktiv); sucrose (CAS No. 57-50-1, analytical grade, OOO LenReaktiv); D-sorbitol (CAS No. 50-70-4, medical, OOO LenReaktiv); ethanol (CAS No. 64-17-5, 96%, special purity, OOO Kristopharm).

The incubation was carried out in stationary conditions at 28°C for 10 days.

After washing the resulting biofilm three times in an alkaline solution of 1 M (NaOH) at 80°C for 30 min, we washed the bacterial cellulose with hydrochloric acid (1 M) and distilled water until pH 7.

We used the gravimetric method to measure the yield of bacterial cellulose by drying it to a constant mass at 40°C on a second-class scale [14]. The amount was calculated as follows:

$$X = \frac{m_2 - m_1}{V} \quad (1)$$

where m_1 is the filter weight; m_2 is the filter weight with the bacterial cellulose film after drying; V is the volume of the nutrient medium.

The pH in the fermentation medium was controlled by direct potentiometry (ionometry) in an OHAUS

Starter ST300 multiparameter meter (OHAUS, China) with a ST320 pH electrode.

To record the infrared Fourier spectra, we used an infrared FT-IR Nicolet iS5 Spectrometer (Thermo Scientific, USA) in the mid-infrared region between 4,000 and 500 cm^{-1}. Purified and dried cellulose served as samples on an ID7 Diamond ATR unit.

The crystallinity index was calculated as in [15]:

$$IR = \frac{A_{1430}}{A_{893}} \quad (2)$$

The values of I_α and I_β were calculated as in [16]:

$$I_\beta = \frac{A_{710}}{A_{710} + A_{750}} \quad (3)$$

$$I_\alpha = 100 - I_\beta \quad (4)$$

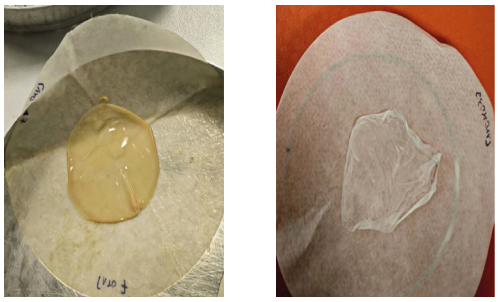
A Tescan Vega 3 scanning electron microscope (Brno, Czech Republic) made it possible to establish and analyze the structure of the purified and dried cellulose. The dimensions of the studied samples for electron microscopy were 20 × 20 × 10 mm.

The water-holding capacity of the bacterial cellulose biofilm was determined by sieving. We soaked the biofilms in distilled water for 1 h. After removing them from the storage containers with tweezers, we put the biofilms in a sieve and shook it twice vigorously to remove any water remaining on the surface. The samples were weighed prior and after being dried to constant weight in a drying cabinet at 50 ± 1°C. The water-holding capacity (*WHC*) was calculated as in [17]:

$$WHC = \frac{m_1 - m_0}{m_0} \quad (5)$$

where m_0 is the initial mass, g; m_1 is the post-drying mass, g.

All experiments were carried out in five independent experiments with three parallel measurements in each. The results were presented as the sum of the mean value and the standard deviation.



a b

Figure 1. Bacterial cellulose biofilms obtained under static conditions: a – before purification, b – after purification and drying

Рисунок 1. Биопленки бактериальной целлюлозы, полученные в статических условиях: a – до очистки; b – после очистки и высушивания

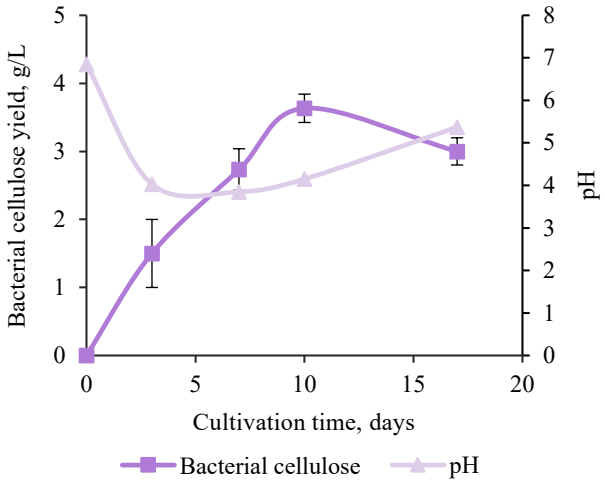


Figure 2. Effect of cultivation time on bacterial cellulose yield and pH of the medium

Рисунок 2. Изменение выхода бактериальной целлюлозы и pH среды в зависимости от продолжительности культивирования

Results and discussion

Optimal cultivation conditions are the key to successful bacterial cellulose production. They depend on the components of the nutrient medium that affect the properties of bacterial cellulose and thus define its further application. The traditional Hestrin-Schramm medium is expensive and, in some cases, ineffective in terms of yield and quality.

Bacterial cellulose absorbs more water than its alternatives, which indicates good prospects for new hydrogels and other polymers used in the food industry.

Figure 1 shows two types of bacterial cellulose obtained under static cultivation conditions before and after purification.

It takes bacterial cellulose three days to start to develop in the Hestrin-Schramm medium (Fig. 2). Its yield is very low (1.5 g/L) because the pH of the medium is low due to the accumulation of secondary metabolites, e.g., acetic acid. As a result, the maximal yield in the Hestrin-Schramm medium occurred on day 10, when the pH of the medium fell down from 6.85 to 4.15.

We observed the yield of bacterial cellulose under static condition in the Hestrin-Schramm medium with different carbon sources (20.0 g/L) as the main substrate for 10 days (Fig. 3). The carbon sources included glucose, fructose, lactose, xylose, maltose, glycerin, sucrose, sorbitol, and ethanol.

Acetobacterium xylinum B-12429 proved able to use various carbon sources (2%) for growth and cellulose synthesis. The highest yield of 4.4 g/L bacterial cellulose was observed in the fructose medium. This monosaccharide triggered such an intense fermentation

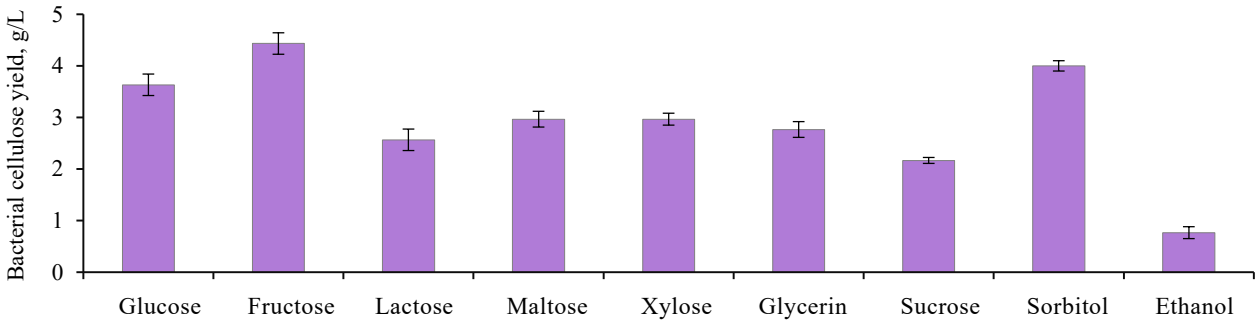


Figure 3. Effect of different carbon sources on bacterial cellulose yield over 10 days

Рисунок 3. Производство бактериальной целлюлозы при разных источниках углерода за 10 дней

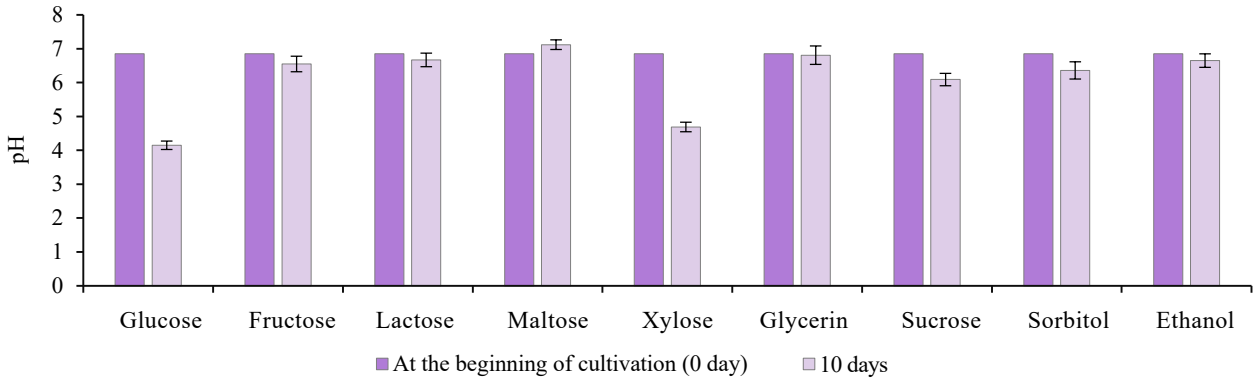


Figure 4. pH of the cultivation medium of *Acetobacterium xylinum* B-12429 over 10 days

Рисунок 4. Измерение pH среды культивирования штамма *Acetobacterium xylinum* B-12429 в течение 10 суток

due to the activity of phosphokinase, which inhibited the conversion of fructose-1-phosphate to fructose-1,6-bisphosphate [18].

The sorbitol sample also demonstrated a high yield of 4.0 g/L. Glucose yielded much less bacterial cellulose (3.60 g/L) due to the activity of gluconic acid, a by-product of glucose oxidation. The maltose and xylose substrates yielded a much lower amount (3.0 g/L), with lactose showing an even worse performance (2.6 g/L). Yet, these substances did have a certain substrate potential, although with a much lower efficiency.

In our study, glycerol and sucrose showed a rather low fermentation efficiency with yields of 2.8 g/L and 2.2 g/L, respectively. Mohammadkazemi *et al*. [19], however, obtained the highest bacterial cellulose yield from sucrose and mannitol.

Ethanol was responsible for the negligible yield of 0.8 g/L, which indicates its unsuitability as a carbon source for cellulose production under these particular conditions. Figure 4 illustrates the change in pH in the culture medium for different carbon sources over 10 days.

Glucose reduced the pH value from the initial 6.85 to 3.21, probably, due to the formation of organic acids. Maltose, glycerol, and fructose led to a small decrease in the pH value from the initial 6.85 to 6.75, 6.65, and 6.28, respectively. Perhaps, these carbon sources formed no gluconic acid. Lactose, xylose, and sorbitol could act as a substrate for glucose dehydrogenase, with a significant drop in pH from 6.85 to 3.85.

Table 1 summarizes the effect of inorganic and organic components on cellulose biomass yield.

Other studies also reported the effect of various additives on bacterial cellulose production in the Hestrin-Schramm medium [8]. In this research, vitamins, ethanol, $MgSO_4$, tartaric acid, and agar were able to boost the yield, which allowed us to identify them as stimulators of bacterial reproduction. The media with $FeSO_4$, $CaCO_3$, and $ZnSO_4$ demonstrated no bacterial growth and cellulose biosynthesis, which means they inhibited the growth of *A. xylinum* B-12429.

The highest yield belonged to Medium 9 with agar, Medium 4 with $MgSO_4$, and Medium 2 with ascorbic acid, amounting to 5.04, 4.86, and 4.05 g/L, respectively. Rutin, ethanol, and tartaric acid (1%) did not increase the biomass of bacterial cellulose, compared to the control sample (3.60 g/L).

Vitamins, ethyl alcohol, and all mineral salts but $CaCO_3$ reduced pH in the medium from 6.85 to 3.05 during cultivation. Probably, when *A. xylinum* B-12429 consumed these substances, they developed more organic

Table 1. Cultivating *Acetobacterium xylinum* B-12429 on nutrient media with different compositions

Таблица 1. Культивирование штамма бактерий *Acetobacterium xylinum* B-12429 на питательных средах различного состава

Sample	Composition	Cellulose yield, g/L
Control	Hestrin-Schramm	3.60 ± 0.18
Medium 1	Hestrin-Schramm + ethanol	3.34 ± 0.17
Medium 2	Hestrin-Schramm + ascorbic acid	4.05 ± 0.20
Medium 3	Hestrin-Schramm + rutin	0.84 ± 0.04
Medium 4	Hestrin-Schramm + $MgSO_4$	4.86 ± 0.24
Medium 5	Hestrin-Schramm + tartaric acid	3.54 ± 0.18
Medium 6	Hestrin-Schramm + $FeSO_4$	0
Medium 7	Hestrin-Schramm + $CaCO_3$	0
Medium 8	Hestrin-Schramm + $ZnSO_4$	0
Medium 9	Hestrin-Schramm + agar	5.04 ± 0.25

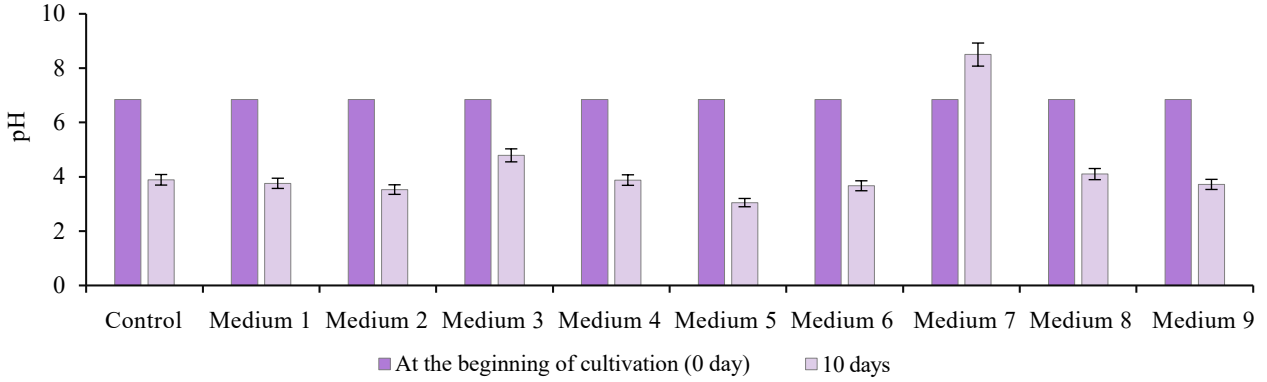


Figure 5. pH of different nutrient media with *Acetobacterium xylinum* B-12429 over 10 days

Рисунок 5. Изменение pH культуральной жидкости при выращивании *Acetobacterium xylinum* B-12429 на различных вариантах питательных сред в течение 10 суток культивирования

acids as by-products, which inhibited their growth and, in turn, the yield of bacterial cellulose. The sample with $CaCO_3$ raised its pH up to 8.5. This effect could be caused by the lack of gluconic acid.

The cellulose fermentation profiles showed that the glucose concentration was important for the effective cultivation of *A. xylinum* B-12429 (Fig. 5). The highest cellulose yield (5.6 g/L) was achieved on day 10 of static cultivation. The lowest cellulose yield was observed both at the lowest glucose concentration (1%) and when it reached 10%. In this respect, our results were consistent with some previous research that observed the highest cellulose yield at 8% glucose [10]. Probably, the cellulose synthesis went down at ≤ 2% glucose because glucose was the only available source of carbon and energy in the medium. The low cellulose yield at ≥ 10.0% glucose might be due to high the osmotic pressure, low free water, or competitive inhibition caused by the excess substrate (Fig. 6).

The pH values went down as the glucose concentration increased, stabilizing at ~ 5.5 when glucose was > 15% (Fig. 7). As reported elsewhere, bacterial cellulose-producing strains can maintain the pH of the medium within 3.0–5.0 during the enzymatic process [8].

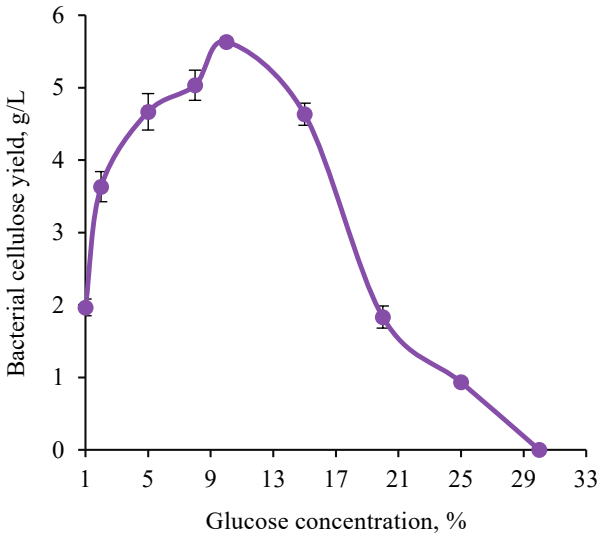


Figure 6. Effect of glucose concentration on bacterial glucose synthesis by *Acetobacter xylinum* B-12429 in Hestrin-Schramm medium during 10 days of cultivation

Рисунок 6. Влияние концентрации глюкозы на синтез бактериальной целлюлозы *Acetobacter xylinum* B-12429 на среде HS в течение 10 суток культивирования

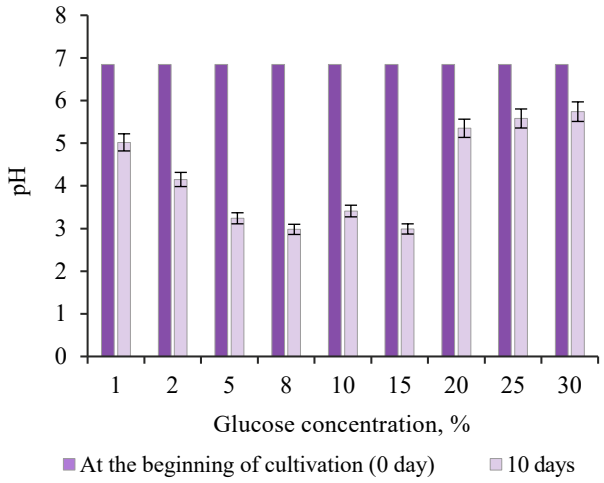


Figure 7. Effect of glucose concentration on pH of the culture medium with *Acetobacterium xylinum* B-12429 during 10 days

Рисунок 7. Изменение pH культуральной жидкости при выращивании *Acetobacterium xylinum* B-12429 на различных концентрациях глюкозы среды HS в течение 10 суток культивирования

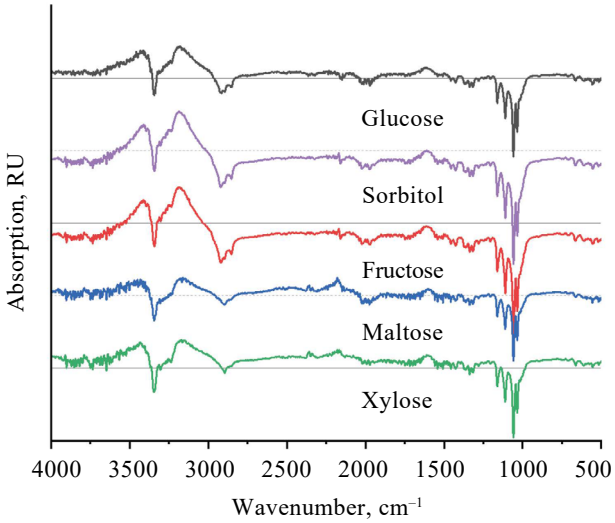


Figure 8. FTIR spectra of bacterial cellulose from different carbon sources

Рисунок 8. ИК спектры бактериальной целлюлозы, получаемой при разных источниках углерода

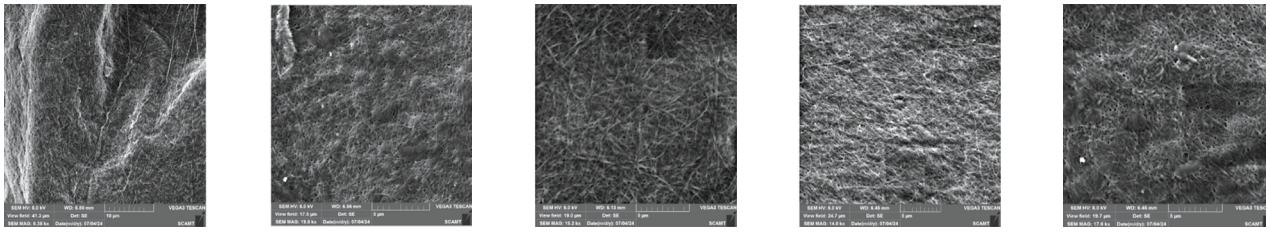


Figure 9. Scanning electron microscopy of bacterial cellulose obtained from different carbon sources: a – glucose; b – fructose; c – maltose; d – xylose; and e – sorbitol

Рисунок 9. Изображения бактериальной целлюлозы (СЭМ), полученной на различных источниках углерода: a – глюкоза; b – фруктоза; c – мальтоза; d – ксилоза; e – сорбит

To define the effects of glucose, fructose, maltose, xylose, and sorbitol on bacterial cellulose, we studied its structure using infrared spectroscopy and scanning electron microscopy (SEM). We also identified such physicochemical properties as water-holding capacity and crystallinity indices I_α and I_β of purified cellulose biofilm.

Figure 8 shows the results of infrared spectroscopy obtained from different carbon sources.

The infrared spectroscopy revealed no significant differences in the structure of the spectra across different carbon sources. Obviously, the use of different carbon substrates did affect the basic structure of the resulting cellulose.

In Figure 8, the strong absorption band at 3,444 cm^{-1} corresponded to the hydroxyl (OH) group. The absorption band that peaked at 2,918 cm^{-1} corresponded to C-H bond vibrations. The band at 1,058 cm^{-1} marked the stretching vibrations in the C-O-C structures. The absorption band at 1,111 cm^{-1} corresponded to the glucopyranose stretch. The spectra were consistent with the data in [20, 21], which reported bands typical of cellulose in the FTIR spectra.

Figure 9 shows the scanning electron microscopy images of bacterial cellulose synthesized by *A. xylinum* B-12429 with various carbon sources.

The method of scanning electron microscopy made it possible to describe the structure of bacterial cellulose synthesized by *A. xylinum* B-12429 in media with different carbon sources. The images for all samples showed cellulose fibers with a distinctive and highly compact morphology, indicating a fine and uniform matrix structure typical of cellulose I. We detected no large pores, which means that all the carbon sources developed tightly interwoven fibrous structures rather than interstitial spaces. This compactness may have good implications for bacterial cellulose to be used as a biomaterial.

Indeed, the absence of porosity provides a number of advantages, including high resistance to enzymatic degradation and excellent mechanical properties, which indicates good prospects for the packaging industry.

Biopolymers and their physicochemical profiling are a popular research object since their physical and chemical properties define the application options, e.g., in pharmacy or food storage. These qualities often depend on the proper choice of carbon source to be used in the synthesis process. We measured the water-holding capacity and crystallinity indices I_α and I_β for bacterial cellulose obtained from glucose, fructose, xylose, maltose, and sorbitol (Table 2).

Water-holding capacity is an important indicator that determines the ability of a material to retain water. In our research, the water-holding capacity of bacterial cellulose depended on the carbon source. The bacterial cellulose obtained from glucose had a water-holding capacity of 63.57%. The highest water-holding capacity belonged to the sample obtained from xylose (79.02%), with similar rates observed for sorbitol (79.32%) and maltose (75.01%). These substrates formed more stable hydrophilic interactions due to their chemical structure. Such complex carbohydrates as maltose provided a lower water-holding capacity compared to simple monosaccharides. This discovery opens up new prospects for using sorbitol as a carbon source for bacterial cellulose with strong water-holding properties.

Crystallinity indices I_α and I_β characterize the order of cellulose macromolecules and their crystalline structure (Eqs 2–4). In our case, the crystallinity indices were approximately the same across the samples, which means that the carbon source had no effect on the crystallinity of bacterial cellulose. The biofilms obtained from different substrates demonstrated a fifty-fifty equilibrium ratio of forms 1 and 2 of cellulose I. The crystallinity of bacterial cellulose slightly depended on the carbon source, as confirmed by crystallinity indices I_α and I_β, which ranged from 49.980 to 50.020% for all samples, with a high degree of cellulose crystallinity regardless of the carbon source. Cellulose molecules indicated a stable structure and ordered organization, which indicated a better mechanical and thermodynamic quality of the final products.

Table 2. Physicochemical properties of bacterial cellulose from various carbon sources

Таблица 2. Характеристика образцов бактериальной целлюлозы из различных источников углерода

Carbon source	Water-holding capacity, %	Crystallinity index	I_α, %	I_β, %
Glucose	63.57	0.992	50.014	49.986
Fructose	70.69	0.989	50.020	49.980
Xylose	79.02	0.992	50.018	49.982
Maltose	75.01	0.991	50.020	49.980
Sorbitol	79.32	0.989	50.020	49.980

Conclusion

We studied the mechanisms of bacterial cellulose biosynthesis by *Acetobacterium xylinum* B-12429. This strain proved able to use various carbohydrates as carbon sources for cellulose synthesis. The cellulose yield depended on the carbon source. Monosaccharides, such as fructose and sorbitol, provided the highest yield while disaccharides and ethanol performed much worse.

The nutrient media and additives also affected the process of cellulose biosynthesis. Unlike iron and calcium, ascorbic acid and $MgSO_4$ were able to boost the cellulose production. When optimized properly, the nutrient media catalyzed the biosynthesis, thus opening prospects for industrial high-quality bacterial cellulose production. Carbon sources also affected the physicochemical profile of the resulting bacterial cellulose. The samples obtained from xylose and sorbitol exhibited the best water-holding properties, which renders them good prospects as part of hydrogels and artificial tissues used in medicine. The high crystallinity indices emphasized the structural quality and stability of biofilms obtained from all carbon sources considered in this study. Further research may reveal the exact effect of the abovementioned properties on the functionality of bacterial cellulose in practical applications.

Contribution

A-G.A. Ali developed the research concept, designed the research, collected and analyzed the data, and drafted the manuscript. O.V. Kriger wrote the review, processed the illustrations, and proofread the manuscript. Both co-authors have agreed and approved of the final version and are equally responsible for its integrity, reliability, and plagiarism.

Conflict of interest

The authors declared no potential conflict of interest regarding the research, authorship, and / or publication of this article.

Критерии авторства

А-Г. А. Али – разработка концепции и дизайн исследования, сбор, анализ и интерпретация данных и материалов, подготовка и редактирование текста статьи. О. В. Кригер – сбор и анализ литературных данных, обработка иллюстраций, подготовка статьи к публикации. Все соавторы согласовали и утвердили окончательный вариант текста статьи и несут равную ответственность за его целостность, достоверность материалов и плагиат.

Конфликт интересов

Авторы заявляют об отсутствии потенциальных конфликтов интересов в отношении исследования, авторства и / или публикаци данной статьи.

References

1. Ul-Islam M, Ha JH, Khan T, Park JK. Effects of glucuronic acid oligomers on the production, structure and properties of bacterial cellulose. Carbohydrate Polymers. 2013;92(1):360–366. https://doi.org/10.1016/j.carbpol.2012.09.060

2. Moran-Mirabal JM, Cranston ED. Cellulose nanotechnology on the rise. Industrial Biotechnology. 2015;11(1):14–15. https://doi.org/10.1089/ind.2015.1501

3. Ullah H, Santos HA, Khan T. Applications of bacterial cellulose in food, cosmetics and drug delivery. Cellulose. 2016;23:2291–2314. https://doi.org/10.1007/s10570-016-0986-y

4. Park SU, Lee BK, Kim MS, Park KK, Sung WJ, *et al*. The possibility of microbial cellulose for dressing and scaffold materials. International Wound Journal. 2014;11(1):35–43. https://doi.org/10.1111/j.1742-481X.2012.01035.x

5. Almihyawi RAH, Musazade E, Alhussany N, Zhang S, Chen H. Production and characterization of bacterial cellulose by *Rhizobium* sp. isolated from bean root. Scientific Reports. 2024;14:10848. https://doi.org/10.1038/s41598-024-61619-w

6. Mohammad NH, Hammad AA, Nour SA, Askar AA, Gamal E. Optimization of bacterial cellulose production using Plackett-Burman and response surface methodology. Egyptian Journal of Medical Microbiology. 2021;30(4):93–101. https://doi.org/10.21608/ejmm.2021.197467

7. Lin S-P, Liu C-T, Hsu K-D, Hung Y-T, Shih T-Y, *et al*. Production of bacterial cellulose with various additives in a PCS rotating disk bioreactor and its material property analysis. Cellulose. 2016;23:367–377. https://doi.org/10.1007/S10570-015-0855-0

8. Ali A-GA, Krieger OV. Production and application of bacterial cellulose. Vestnik of Immanuel Kant Baltic Federal University. 2025;(1):82–102. (In Russ.) [Али А-Г. А., Кригер О. В. Получение и применение бактериальной целлюлозы. Вестник Балтийского федерального университета им. И. Канта. 2025. № 1. С. 82–102.] https://doi.org/10.5922/vestniknat-2025-1-6

9. Saavedra-Sanabria OL, Durán D, Cabezas J, Hernández I, Blanco-Tirado C, *et al*. Cellulose biosynthesis using simple sugars available in residual cacao mucilage exudate. Carbohydrate Polymers. 2021;274:118645. https://doi.org/10.1016/j.carbpol.2021.118645

10. Digel I, Akimbekov N, Rogachev E, Pogorelova N. Bacterial cellulose produced by *Medusomyces gisevii* on glucose and sucrose: biosynthesis and structural properties. Cellulose. 2023;30:11439–11453. https://doi.org/10.1007/s10570-023-05592-z

11. Abdelraof M, Hasanin MS, Farag MM, Ahmed HY. Green synthesis of bacterial cellulose/bioactive glass nanocomposites: Effect of glass nanoparticles on cellulose yield, biocompatibility and antimicrobial activity. International Journal of Biological Macromolecules. 2019;138:975–985. https://doi.org/10.1016/j.ijbiomac.2019.07.144

12. Abol-Fotouh D, Hassan MA, Shokry H, Roig A, Azab MS, *et al*. Bacterial nanocellulose from agro-industrial wastes: Low-cost and enhanced production by *Komagataeibacter saccharivorans* MD1. Scientific Reports. 2020;10:3491. https://doi.org/10.1038/s41598-020-60315-9

13. Sutthiphatkul T, Suwanposri A, Ochaikul D. Optimization of bacterial cellulose production from wastewater of noodle processing by *Komagataeibacter* sp. PAP1 and bio-cellulose paper production. Walailak Journal of Science and Technology. 2020;17(11):1241–1251. https://doi.org/10.48048/wjst.2021.6508

14. Khattak WA, Khan T, Ul-Islam M, Ullah MW, Khan S, *et al*. Production, characterization and biological features of bacterial cellulose from scum obtained during preparation of sugarcane jaggery (gur). Journal of Food Science and Technology. 2015;52(12):8343–8349. https://doi.org/10.1007/S13197-015-1936-7

15. Ciolacu D, Ciolacu F, Popa VI. Amorphous cellulose – Structure and characterization. Cellulose Chemistry and Technology. 2011;45(1):13–21.

16. Szymańska-Chargot M, Cybulska J, Zdunek A. Sensing the structural differences in cellulose from apple and bacterial cell wall materials by raman and FT-IR spectroscopy. Sensors. 2011;11(6):5543–5560. https://doi.org/10.3390/s110605543

17. Hanemann T, Szabó DV. Polymer-nanoparticle composites: From synthesis to modern applications. Materials. 2010;3(6):3468–3517. https://doi.org/10.3390/ma3063468

18. Liu M, Liu L, Jia S, Li S, Zou Y, *et al*. Complete genome analysis of *Gluconacetobacter xylinus* CGMCC 2955 for elucidating bacterial cellulose biosynthesis and metabolic regulation. Scientific Reports. 2018;8:6266. https://doi.org/10.1038/s41598-018-24559-w

19. Mohammadkazemi F, Azin M, Ashori A. Production of bacterial cellulose using different carbon sources and culture media. Carbohydrate Polymers. 2015;117:518–523. https://doi.org/10.1016/j.carbpol.2014.10.008

20. Casaburi A, Rojo ÚM, Cerrutti P, Vázquez A, Foresti ML. Carboxymethyl cellulose with tailored degree of substitution obtained from bacterial cellulose. Food Hydrocolloids. 2018;75:147–156. https://doi.org/10.1016/j.foodhyd.2017.09.002

21. Gladysheva EK. Investigation of the structure and chemical structure of bacterial cellulose. Polzunovskiy Vestnik. 2015;(4–2):100–103. (In Russ.) [Гладышева Е. К. Исследование структуры и химического строения бактериальной целлюлозы. Ползуновский вестник. 2015. № 4–2. С. 100–103.] https://elibrary.ru/VMDLLL