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Production and Characterization of bacterial cellulose from *Komagataeibacter xylinus* S4 strain

Komagataeibacter xylinus S4 suşundan bakteriyel selüloz üretimi ve karakterizasyonu

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Abstract

In this study, bacterial cellulose (BC) was obtained from Komagataeibacter xylinus S4 and characterized in detail. The effects of a various of carbon sources and media, different pH conditions, incubation temperatures, Surface area/Volume ratios, and incubation durations were determined for BC production. Considering the carbon types, the amount of BC production from high to low was realized as sucrose, fructose, mannitol, xylose, arabinose, and lactose. The highest BC amount (1.303 g/L) was achieved by combining M1A05P5 broth, 30 °C, 1.06 cm^{-1} Surface area/Volume ratio, pH 3.5 and 21 days. According to scanning electron microscope (SEM) analysis, the cellulose fibril diameters were 34.87-45.97 nm at pH 3.5 and 29.71-102.3 nm at pH 6.5 in M1A05P5. Also, TGA analysis exhibited that the weight loss of BC in the removal of water step initialized between 50 °C and 150 °C and the degradation step initialized between 215 °C and 228 °C. Finally, the electrical conductivity values of the BC samples were determined on the 27-137 °C temperature scale. It was observed that the conductivity was temperature dependent, and the conductivity increased exponentially as the temperature increased. In conclusion, the cellulose from K. xylinus S4 typically showed a semiconducting behavior.

Keywords: Bacterial cellulose, Local isolate, Carbon sources, Electrical conductivity, Thermal analysis, SEM.

Öz

Bu çalışmada, Komagataeibacter xylinus S4'ten elde edilen bakteriyel selüloz (BS) detaylı şekilde karakterize edilmiştir. Çeşitli karbon kaynakları ve ortamlarının, farklı pH şartları, sıcaklıklar, yüzey alanı/hacim oranları ve inkübasyon zamanlarının BS üretimine etkisi tespit edilmiştir. Karbon tipleri gözönüne alındığında, yüksekten düşüğe doğru BS üretim miktarı, sükroz, fruktoz, mannitol, ksiloz, arabinoz ve laktoz şeklinde gerçekleşmiştir. En yüksek BS miktarına (1.303 g/L), M1A05P5 sıvı besiyeri, 30 °C sıcaklık, 1.06 cm^{-1} yüzey alanı/hacim oranı, pH 3.5 ve 21 gün kombinasyonunda ulaşılmıştır. Taramalı elektron mikroskobu (SEM) analizine göre M1A05P5 ortamında üretilen bakteriyel selüloz liflerinin çapları pH 3.5'te 34.87-45.97 nm değerindeyken pH 6.5 değerine yükseldiğinde lif çapları 29.71-102.3 nm olarak ölçülmüştür. Ayrıca, TGA analizi, BS numunelerinde dehidrasyon adımındaki ağırlık kaybının 50 °C ile 150 °C arasında, bozunma adımının ise 215 °C ile 228 °C arasında başladığını göstermiştir. Son olarak, BS örneklerinin elektriksel iletkenlik değerleri 27-137 °C sıcaklık skalasında tespit edildi. İletkenliğin sıcaklığa bağlı olduğu ve sıcaklık arttıkça iletkenliğin üstel olarak arttığı gözlendi. Sonuç olarak, K. xylinus S4 selülozu tipik olarak yarı iletken bir davranış göstermiştir.

Anahtar kelimeler: Bakteriyel selüloz, Yerel izolat, Karbon kaynakları, Elektriksel iletkenlik, Termal analiz, SEM.

1 Introduction

Cellulose is a biodegradable and abundant polysaccharide found in nature. Also, it is a renewable and biocompatible polymer [1]-[5]. Although cellulose is obtained from plant sources, it is also secreted by bacteria, fungi, and algae. Bacterial cellulose (BC) is known possess notable characteristics compared to cellulose from plants in terms of various properties such as purity, macromolecular properties, water holding capacity, and total surface area [4],[6]-[9]. Moreover, there are also some disadvantages to obtaining cellulose from plants, such as cutting trees and removing lignin, pectin, and hemicellulose [10]-[13]. *Acetobacter xylinum* is the first bacterium reported to produce cellulose. Studies over the years have demonstrated the cellulose production capabilities of Gram-negative and Gram-positive bacteria [5],[14]-[15]. *Komagataeibacter xylinus*, the most famous bacterium, has a high bacterial cellulose production capacity from a various carbon and nitrogen sources [13]-[16]. Various researchers have investigated the effects of varied factors (pH, temperature, bacterial species, carbon sources, media, etc.) on the BC production [9],[17]-[18]. In order to discover a new strain with

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increasing cellulose-producing capacity, researchers isolate bacteria from various sources such as fruits (grapes and rotten grapes, apple juice, rotten apples, melons, lemons, and papayas), flowers (passiflora), fermented foods (waste from vinegar fermentation, malt), and kombucha tea [19]-[26].

Bacterial polymers are natural products with commercial value. In particular, the capacity of BC to transform into products with high economic value has caused it to attract great interest in green and sustainable chemistry aspects, and it has become a hope for the development of new technologies. Because bacterial technology has many advantages including the ability to produce polymers in a short time, the purity of cellulose, its high-water holding capacity, and the ability to easily make new arrangements in the production process. On the other hand, although bacterial cellulose is considered an alternative to vegetable cellulose for environmental protection, its production cost is high. For this reason, recent research on the manufacture of BC is directed towards the production of cellulose in different microorganisms, the isolation of new microorganisms that produce cellulose from different environments, new regulations for improving the properties of microorganisms known to produce cellulose and increasing their production. In other words, selecting new species or isolates for efficient cellulose production by bacteria forms the basis of such studies. For this reason, many researchers isolate cellulose-producing bacteria from various sources and investigate their BC production capacities and properties.

The current research aimed to explore the production of BC using local isolate of *Komagataeibacter xylinus* S4, which was isolated from home-made grape vinegar [9]. For this purpose, the effects of growth media, carbon sources, pH, surface area, incubation temperature and period were investigated on manufacture. Obtained BC was characterized by FT-IR, scanning electron microscope (SEM), thermal analysis, and electrical conductivity measurements. In addition, cellulose acetate synthesis was also performed.

2 Material and methods

2.1 Production and purification of bacterial cellulose

Komagataeibacter xylinus S4 was used during the study [9]. BC was synthesized in standard HS [27], Yamanaka [28], and M1A05P5 media [29]. For determining the effects of carbon sources, xylose, arabinose, lactose, mannitol, fructose, and sucrose were added instead of glucose in HS broth. Also, temperature (20, 25, 30 and 35 °C), pH (3.5, 4.0, 4.5, 5.0, 5.5, 6.0, 6.5), incubation time (4, 7, 14, 21 days) and surface area to volume ratio (0.39 cm^{-1}, 0.66 cm^{-1}, 1.06 cm^{-1}) were tested. Harvested BC membrane contains protein, medium ingredients, and bacterial cells. To remove the unwanted contents of the native BC, it was kept at 0.1 M NaOH at 80 °C for 1 h. Then, BC was washed several times with deionized water. After processing, BC was lyophilized (Thermo Fisher ModulyoD Freeze Dryer and Telstar LyoQuest) and stored at -20 °C for analysis [30].

2.2 Water holding capacity (WHC)

The water holding capacity (WHC) is considered one of the most important physical characteristics of bacterial cellulose regarding the biomedical application of BC as wound dressing material. The WHC relates to the BC matrix's porosity and surface area. The high WHC of BC is due to its very porous and hydrophilic nature. In current study, the water holding capacity of the bacterial cellulose samples were the mass of water removed during drying divided by the dry weight of BC.

2.3 Acetylation of bacterial cellulose and preparation of cellulose triacetate

Cellulose triacetate (CTA) is a cellulose derivative. CTA can be used in optical films such as protective films for LCD polarizing panels used in LCD TVs, notebook computers, and mobile phones. CTA was produced with some modifications with a described method by Braun et al. [31]. Uniform wetting of the cellulose was achieved by mixing 10 g of lyophilized BC in 50 mL of acetic acid solution containing 0.5 g of concentrated sulfuric acid in a 250 mL wide-necked flask. The flask was then allowed to stand for 1 hour at room temperature (RT). After the pretreatment, 50 mL of acetic anhydride ($C_4H_6O_3$) and 20 mL of acetic acid (CH_3COOH) solution were added into the flask and replaced in a water bath at 50 °C for 30 min. The solution after this time was called the primary solution. At the end of the period, 25 mL of CH_3COOH (80%) solution was added to the primary solution at 60 °C to break down the remaining acetic anhydride after the reaction, paying attention to avoid any precipitation at this stage. The obtained mixture was kept at 60 °C for 15 min and then poured into the beaker. After carefully adding 25 mL of distilled water, an additional 200 mL of distilled water was added to precipitate cellulose triacetate as a white powder. Obtained precipitate formed by the addition of distilled water was dried in an oven after filtering and washing. The obtained dry precipitate was dissolved in chloroform ($CHCl_3$) and poured into the petri dish. Chloroform was allowed to air-dry, and the obtained CTA was stored in a refrigerator for analysis.

Cellulose triacetate was characterized by determining the degree of substitution by saponification reaction [32]. The degree of substitution is the mean value of acetyl groups (CH_3CO) replacing hydroxyl groups (OH) in glycosidic units. For the saponification reaction, 0.1 g of cellulose triacetate was weighed, 5 mL of 0.25 M sodium hydroxide (NaOH) and 5 mL of ethanol (C_2H_5OH) were added to it, and it was kept at RT for 24 hours. After that, 10 mL of 0.25 M hydrochloric acid (HCl) was added to the mixture. After 30 min, titration with standard 0.25 M NaOH using phenolphthalein indicator was performed. Titration continued until the solution turned completely pink colour. As a result of titration, the percentage of acetyl groups in cellulose triacetate was calculated according to the equality given below.

$$\mathbf{AG}\% = \frac{\left[\{\mathbf{V_{(NaOH)s}} + \mathbf{V_{(NaOH)t}}\}\boldsymbol{X}\mathbf{C_{NaOH}} - \mathbf{V_{(HCl)s}}\mathbf{C_{HCl}}\right]\mathbf{43}\boldsymbol{X}\mathbf{100}}{\mathbf{m_s}} \quad (1)$$

In equation 1, the percentage of acetyl groups (**AG**%), NaOH volume added to the system ($\mathbf{V_{(NaOH)s}}$), consumed NaOH volume ($\mathbf{V_{(NaOH)t}}$), NaOH concentration ($\mathbf{C_{NaOH}}$), the concentration of HCl ($\mathbf{C_{HCl}}$), the molecular weight of the acetyl group (43), the weight of cellulose triacetate ($\mathbf{m_s}$).

2.4 FT-IR analysis

The Fourier Transformed Infrared Spectroscopy data of BC was recorded on a Bruker Vertex 70 V FT-IR spectrometer, with an attenuated total reflection unit between 400-4000 cm^{-1} at RT.

2.5 Thermal analysis

The chemical decomposition of BC was analyzed with Perkin Elmer Diamond branded instrument. Approximately 5 mg of BC was replaced in a ceramic pan. Temperature was increased at

the speed of 20 °C/min from 50 °C to 600 °C under Nitrogen (N_2) gas with 200 mL/min flow rate. Differential scanning calorimetry (DSC) analysis of BC were carried out using a Perkin Elmer Pyris 6 device. The sample was replaced in an aluminum pan and heated from 0 °C to 400 °C under nitrogen atmosphere (10 mL/min).

2.6 Scanning electron microscope (SEM)

For SEM analysis, BC-lyophilized was put on aluminum stamps and coated with gold-palladium approximately 2 min (PAU-ILTAM, Denizli).

2.7 Determination of electrical conductivity of bacterial cellulose

The electrical conductivity measurements of BC were performed between 27-137 °C. In order to provide electrical conduction of samples, conductive copper wires were tied to the samples with silver paste. BC with ohmic contacts was placed in a Janis cryostat, and the temperature was controlled with a LakeShore 331 temperature controller. KEITHLEY 2400 source measure unit was used as a current source in the system.

2.8 Statistical analysis

The results of wet and dry weights, water contents and final pH analysis of BC were analyzed by using the SPSS package statistics program (TEAM EQX SPSS Statistics Version 16.0, 2007). In cases where the difference between changing environmental conditions and incubation times was significant, Duncan's test was applied to show the difference between different environmental conditions and incubation times and between each other. The standard deviations of the values were given in tables created. Statistically significant differences were determined at $p<0.05$, while insignificant differences were determined at $p>0.05$ level.

3 Results and discussion

3.1 Production of bacterial cellulose

BC obtained from standard HS medium was characterized in our previous paper [9]. Our previous finding has shown that the wet and dry weights of bacterial cellulose were between 51.8-52.8 g and 0.43-0.735 g, respectively and, the morphology of cellulose layer was observed as a dense nanofiber network. The weight of BC also decreased with increasing temperature between 50 °C and 150 °C, and the degradation step observed between 215 °C and 228 °C. Moreover, the antibiofilm and antibacterial effects of unmodified BC have also been reported for the first time [9]. The potential values of local isolates and their products have a considerable industrial concern. Therefore, the production of bacterial cellulose from *K. xylinus* S4-local isolate was shown in detail in this study. Different carbon sources (xylose, arabinose, lactose, mannitol, fructose, sucrose), temperature, pH, and Surface area/Volume ratio, which affect the production of BC, were investigated.

Wet weight results of BC were consistent with the dry weight results. In addition, when the results in Table 1 were examined, considering the carbon sources, the amount of BC from carbon sources was achieved in the order of sucrose>fructose> mannitol>xylose>arabinose>lactose. Mannitol (0.19 g/L), fructose (0.233 g/L), and sucrose (0.273 g/L) showed significant increases in BC production among all sources. Especially, the yields of BC in fructose and sucrose medium seem to be relatively higher. The lowest BC yield was achieved in lactose (0.018 g/L), arabinose (0.026 g/L), and xylose (0.095 g/L) medium. In other words, while sucrose, fructose, and mannitol positively affected cellulose production, xylose, arabinose, and lactose did not contribute the manufacture of BC (Table 1).

Table 1. The wet and dry weights of BC from *Komagataeibacter xylinus* S4 (T: 30 °C; pH_i: 6.0; incubation period: 4 days, HS medium containing different carbon sources).

Carbon sources	Wet Weight (g/L)	Dry Weight (g/L)	pH_f	WHC (g·water/ g·dry BC)
Xylose	17.25±0.17	0.095±0.00	4.89±0.0	181±0.58^{c}
Arabinos	8.88±0.08^{a}	0.026±0.00	5.22±0.0	341±1.53^{d}
Lactose	8.78±0.02^{a}	0.018±0.00	5.57±0.0	487±1.00^{e}
Mannitol	22.60±0.12	0.190±0.00	5.54±0.0	118±1.00^{a}
Fructose	33.10±1.02	0.233±0.00	5.35±0.0	141±2.51^{b}
Sucrose	32.41±0.41	0.273±0.00	5.45±0.0	118±4.51^{a}

The differences between the wet and dry weights, pH final and water content of the ones (a, b, c, d, e) shown with different lowercase letters at each carbon source are important.

Nguyen and co-authors reported that *G. xylinus* isolated from Kombucha produced the highest yield with mannitol as the carbon source [26]. Tonouchi et al. [33] claimed that fructose as a carbon source increased the manufacture of BC. A similar study showed that the optimum production of BC with pH resistant *Komagataeibacter medellinensis* was achieved in the medium of glucose>sucrose>fructose [34]. Hungund and Gupta [22] also examined the effects of carbon sources including glucose, fructose, lactose, sucrose, mannitol, maltose, and inositol for cellulose production and obtained the highest BC yield in fructose medium. According to Mikkelsen et al. [35], *G. xylinus* ATCC 53524 produced the highest BC production under HS broth with sucrose and glucose after 96 hours. Sucrose was the favoured carbon source for *Acetobacter* sp. 4B-2 [36]. According to the literature review, it can be understood that the types of bacteria, nutrients, or carbon sources are the main reasons for the differences in cellulose production. Therefore, optimization studies should be carried out in detail according to each bacterial isolate. When the obtained data from our study and the literature data were compared, it was determined that *K. xylinus* S4 strain preferred the environment of glucose, sucrose, and fructose for BC synthesis.

This study also showed the influences of Yamanaka and M1A05P5 media on BC production. Our findings show that while *K. xylinus* S4 did not produce cellulose in Yamanaka medium, the M1A05P5 medium had BC production clearly different from HS medium. In other words, M1A05P5 medium gave higher BC production than HS medium containing different carbon sources, indicating that M1A05P5 on BC production was more effective than *K. xylinus* S4. As can be seen from Table 2-3, three different combinations of surface area/volume ratio (A1: 0.39 cm^{-1}, A2: 0.66 cm^{-1}, A3: 1.06 cm^{-1}) and temperature (25, 30 and 35 °C) were examined to increase BC production in M1A05P5 medium.

Combined with A3 (1.06 cm^{-1}) and 30 °C temperature, the production of BC was higher than the other combinations (Table 2 and 3). The A3 and 30 °C temperature condition (Table 4) was tested in 7, 14, and 21 incubation days at pH 3.5 and 6.5 values, and it was clearly verified that all combinations had high BC production in incubation periods.

Table 2. BC production by *Komagataeibacter xylinus* S4 in M1A05P5 medium (incubation period: 4 days, pH: 3.5).

Surface area/Volume ratio	T (°C)	Wet Weight (g/L)	Dry Weight (g/L)	WHC (g·water/ g·dry BC)
0.39 cm^{-1}	20	23.27 ± 0.84^{Aa}	0.20 ± 0.01^{Aa}	115 ± 4.00^{Ab}
	25	66.02 ± 0.12^{Ab}	0.31 ± 0.05^{Ab}	212 ± 8.00^{Cb}
	30	136.88 ± 0.86^{Ac}	0.63 ± 0.03^{Ac}	216 ± 7.00^{Cb}
	35	154.86 ± 0.54^{Ad}	0.63 ± 0.05^{Ad}	245 ± 4.00^{Bb}
0.66 cm^{-1}	20	60.31 ± 0.46^{Ba}	0.32 ± 0.02^{Ba}	187 ± 3.00^{Ab}
	25	117.91 ± 2.09^{Bb}	0.54 ± 0.07^{Bb}	217 ± 9.00^{Cb}
	30	210.78 ± 3.40^{Bc}	1.04 ± 0.03^{Bc}	202 ± 8.00^{Cb}
	35	193.65 ± 0.78^{Bd}	1.01 ± 0.02^{Bd}	191 ± 5.00^{Bb}
1.06 cm^{-1}	20	93.05 ± 0.26^{Ca}	0.67 ± 0.01^{Ca}	138 ± 2.00^{Aa}
	25	154.08 ± 0.20^{Cb}	0.81 ± 0.04^{Cb}	189 ± 5.00^{Ca}
	30	228.58 ± 0.90^{Cc}	1.19 ± 0.09^{Cc}	191 ± 9.00^{Ca}
	35	96.70 ± 0.20^{Cd}	0.72 ± 0.02^{Cd}	133 ± 1.00^{Ba}

The differences between the wet and dry weights and the water content of those shown with different capital letters (^{A,B,C}) in each Surface area/Volume ratio were important. The differences between the wet and dry weights and the water content of the ones (^{a,b,c,d}) shown with different lowercase letters at each temperature were important.

Table 3. BC production from *Komagataeibacter xylinus* S4 in M1A05P5 medium (incubation period: 4 days, pH: 6.5).

Surface area/Volume ratio	T (°C)	Wet Weight (g/L)	Dry Weight (g/L)	WHC (g·water/g·dry BC)
0.39 cm^{-1}	20	33.04 ± 0.08^{Aa}	0.124 ± 0.01^{Aa}	265 ± 2.25^{Bc}
	25	42.39 ± 0.18^{Ab}	0.154 ± 0.01^{Ab}	274 ± 2.24^{Dc}
	30	86.35 ± 0.34^{Ac}	0.273 ± 0.01^{Ac}	315 ± 1.74^{Cc}
	35	146.92 ± 0.11^{Ad}	0.637 ± 0.01^{Ad}	230 ± 0.60^{Ac}
0.66 cm^{-1}	20	51.27 ± 0.08^{Ba}	0.217 ± 0.01^{Ba}	235 ± 1.21^{Bb}
	25	128.01 ± 0.20^{Bb}	0.566 ± 0.01^{Bb}	225 ± 3.79^{Db}
	30	209.60 ± 0.40^{Bc}	0.970 ± 0.01^{Bc}	215 ± 4.71^{Cb}
	35	209.51 ± 0.31^{Bd}	0.905 ± 0.01^{Bd}	231 ± 1.84^{Ab}
1.06 cm^{-1}	20	102.90 ± 0.07^{Ca}	0.501 ± 0.02^{Ca}	204 ± 3.07^{Ba}
	25	175.18 ± 0.38^{Cb}	0.771 ± 0.02^{Cb}	226 ± 2.43^{Da}
	30	253.34 ± 0.43^{Cc}	1.358 ± 0.02^{Cc}	186 ± 2.88^{Ca}
	35	263.82 ± 0.12^{Cd}	1.280 ± 0.05^{Cd}	205 ± 1.49^{Aa}

The differences between the wet and dry weights and the water content of those shown with different capital letters (^{A,B,C}) in each Surface area/Volume ratio were important. The differences between the wet and dry weights and the water content of the ones (^{a,b,c,d}) shown with different lowercase letters at each temperature were important.

Table 4. BC production by *Komagataeibacter xylinus* S4 in the combination of Surface area/Volume ratio and temperature (1.06 cm^{-1}, 30 °C).

	Wet Weight (g/L)	Dry Weight (g/L)	pH_f	WHC (g·water/g·dry BC)
pH_i: 3.5				
7th day	117.09 ± 0.22^{a}	1.145 ± 0.01^{a}	3.11 ± 0.02^{a}	101 ± 4.00^{a}
14th day	207.85 ± 0.32^{c}	1.161 ± 0.05^{a}	3.20 ± 0.03^{b}	178 ± 1.55^{c}
21st day	204.10 ± 0.23^{b}	1.303 ± 0.01^{b}	3.12 ± 0.03^{a}	156 ± 2.61^{b}
pH_i: 6.5				
7th day	274.61 ± 0.41^{a}	1.535 ± 0.03^{a}	5.80 ± 0.08^{a}	178 ± 2.42^{a}
14th day	463.80 ± 0.43^{c}	2.364 ± 0.05^{c}	8.23 ± 0.03^{b}	195 ± 5.08^{c}
21st day	335.60 ± 0.64^{b}	1.895 ± 0.01^{b}	8.23 ± 0.04^{b}	176 ± 4.81^{b}

The differences between the wet and dry weights and the water content of the ones (a,b,c) shown with different lowercase letters at each day are important.

In particular, the incubation in 14 days showed a significant increase in BC production at pH 6.5. In summary, the environmental conditions for the best bacterial cellulose production were determined as M1A05P5 condition at 30 °C under pH 6.5 in 14 days, with 1.06 cm^{-1} (Surface area/Volume ratio). The wet weight was 463.80 g/L, and the dry weight was 2.36 g/L. Coban and Biyik found that BC production by *Acetobacter lovaniensis* HBB5 was 0.04 g/L after 7 days in Hestrin-Schramm (HS) medium that contained 2% (w/v) glucose and 0.5% yeast extract [37].

Additionally, Cakar et al. [29] stated that the yield of BC decreased on the first day of incubation at pH 6.0≥ values in M1A05P5 medium. Unlike the findings of Cakar and co-authors, *K. xylinus* S4 strain continued producing cellulose at pH 6.5 and it was observed that it produced the highest cellulose yield (2.36 g/L) in 14 days of incubation time. According to Ishihara et al. [38], *Acetobacter xylinus* IFO 15606 produced cellulose at a yield exceeding 0.3 g per 100 mL with pH control. Similarly, our isolate S4 was a strain that can compete with the producers in the literature. In other words, *K. xylinus* S4 strain was at least as good a producer.

Glucose dehydrogenases cause an increase in the acidity of the media and reduce bacterial cellulose production in glucose-containing media [39]-[41]. Seto et al. [42] and Masaoka et al. [43] also support this idea. In the previous studies, high bacterial cellulose production was observed in bacterial media with low glucose dehydrogenase activity and low bacterial cellulose production was observed in bacterial media with high glucose dehydrogenase activity. Nevertheless, *K. xylinus* S4 used in our study showed high cellulose production yields even at near or neutral pH conditions. Therefore, it was considered that the *K. xylinus* S4 had low glucose dehydrogenase activity.

The water holding capacities of different BCs produced in different media were shown in the tables. According to the results, it was seen that the WHC values of the produced BC was quite high (101±4.00 g·water/g·dry BC to 487±1.00 g·water/g·dry BC). It was noteworthy that the WHC values of our BCs were generally higher than the values of similar studies in the literature [12],[44]-[45].

3.2 SEM analysis of bacterial cellulose

The scanning electron microscope photos of the synthesized BC with *K. xylinus* S4 under different conditions were also investigated (Figure 1 and 2). Although there were similarities in the morphological structures of bacterial cellulose, the fibril thicknesses were not identical. The thickness of the fibrils was found to be between 29.71nm and 102.3 nm. Moreover, the fibril structure of cellulose obtained from all conditions (except mannitol) was thin.

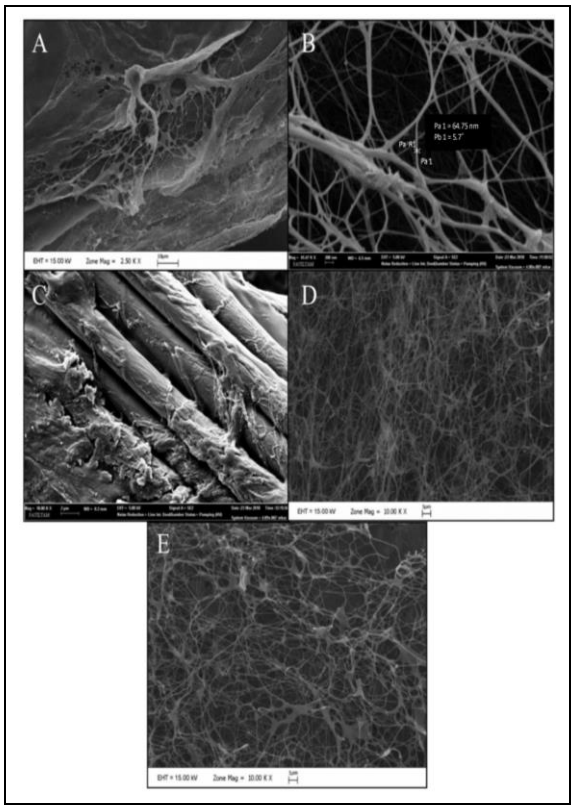

Figure 1. SEM images of BC obtained from *Komagataeibacter xylinus* S4. **A:** Main source, **B:** Lactose **C:** Mannitol **D:** Fructose **E:** Sucrose.

In addition to that, fibril distribution and interfacial adhesion of cellulose in each medium were also different. It was observed that cellulose obtained from all media (except lactose) had a more stable and compact network structure. In other words, the less fibrillary structure was imaged in the medium containing lactose, and the fibril diameter was found to be 64.75 nm. We noticed the uniaxial oriented cellulose bands and strong interfacial adhesion of fibers in mannitol medium (Figure 2). In addition, cellulose from M1A05P5 medium was observed to be in dense fibril bundles and their diameters were between 34.87-45.97 nm under pH:3.5 and 29.71-102.3 nm under pH:6.5. BC is defined as a reticular substance composed of ultra-fine cellulose fibrils and, various factors including nutrients, growth conditions, and bacterial species affect both the production of BC and its fibril structure [17],[46].

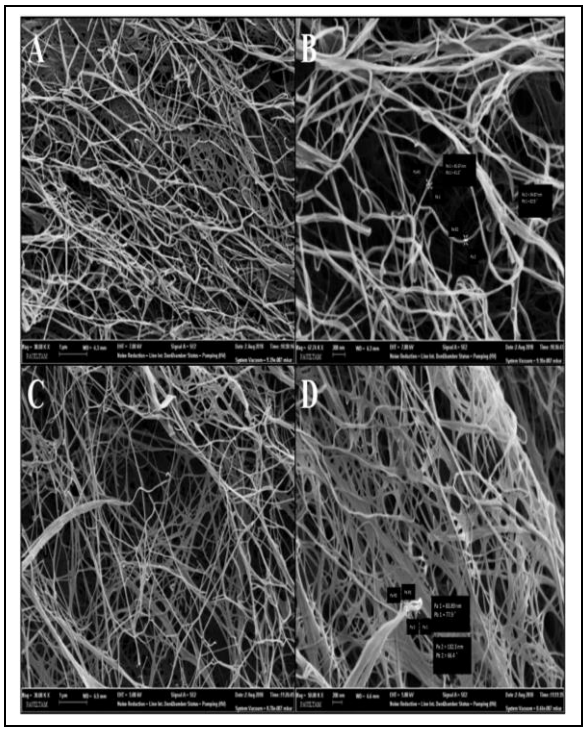

Figure 2. SEM pictures of the produced BC with *Komagataeibacter xylinus* S4 under M1A05P5 medium at various pH values. **A-B:** pH 3.5, **C-D:** pH 6.5.

For example, according to Jung et al. [46], BC from *Acetobacter* sp. was a reticulated structure with ultrafine fibrils and its diameter was approximately 65-80 nm and 75-90 nm in glycerol and glucose medium, respectively. However, Vazquez et al. [47] showed that cellulose obtained from modified HS, glycerol, and molasses had a fibrillary network structure and the fibril diameter varied from 35 to 70 nm. In another study, it was reported that the diameter of *Acetobacter xylinum* cellulosic fibrils ranged from 128-207 nm and BC fibrils were the same as fibrils in pure microcrystalline cellulose [48]. In a study investigating the influence of ascorbic acid on the production of BC with *Komagataeibacter xylinus*, it was determined that the size of BC nano-fibrils was found to be in the range of 45-55 nm by SEM analyzes [13].

3.3 Fourier transform infrared (FT-IR) spectra analysis

Fourier transform infrared spectra results of the obtained BCs were recorded on using an ATR-FT-IR spectrophotometer (Bruker Vertex 70 V, Germany) between 400 and 4000 cm^{-1} wavelengths (Figure 3). These spectra were used to investigate vibrational bands of the functional groups of BC produced under various carbon sources and culture media.

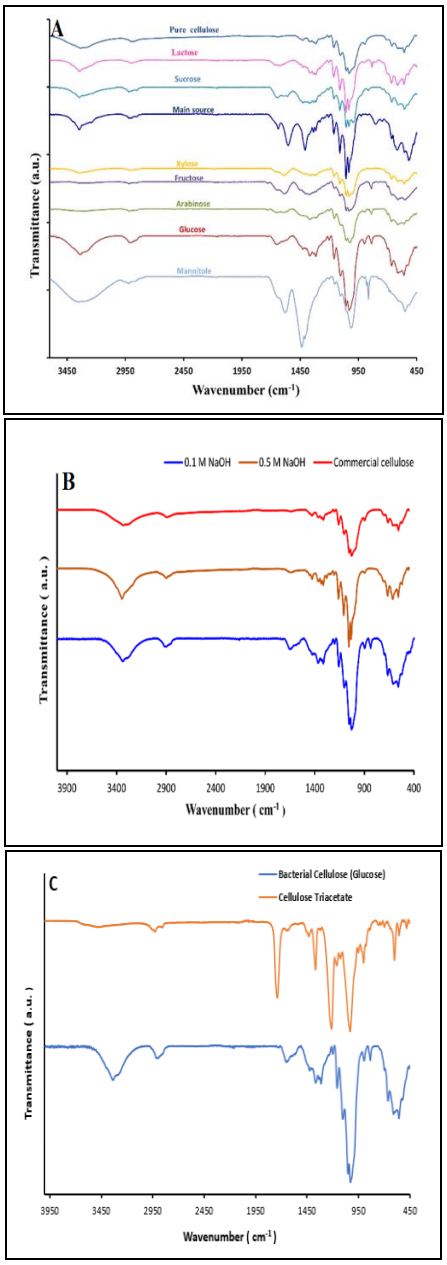

Figure 3. FT-IR spectra of BC samples and Cellulose triacetate synthesized from BC. **A:** Monosaccharides and Disaccharides as carbon source, **B:** The effect of different concentrations of NaOH in the workup procedure, **C:** Cellulose triacetate synthesized from BC.

The following determinations were determined to analyze FTIR spectra of BC. The broad bands that appeared at 3300 cm^{-1} were assigned to -OH stretching vibrations of samples due to the formation of hydrogen bonds. The peak at 2950 cm^{-1} showed C-H stretching. This peak was ascribed to the C–H stretching vibrations of $-CH_2$ and $-CH_3$ groups. The peaks in the wavenumber range of 1750-1400 cm^{-1} were associated with protein, lipid, and nucleic acid. The vibration bands at 1426 and 1315 cm^{-1} were attributed to the bending vibrations of CH_2 and C-H groups of polysaccharides. The peak of C–O stretching vibration was located at 1159 cm^{-1}. The bands in the range of 1000-1200 cm^{-1} could be associated with the vibrations of C–O and C–O–C functional groups in pyranose ring. The further vibration peaks seen for BC (Figure 3A-B) were fixed to -C-H out-of-plane bending (896 cm^{-1}) and –OH out-of-plane bending (666-619 cm^{-1}) vibrations. Obtained vibration bands from FT-IR spectra proved the successful synthesis of BC. Fig. 3C shows the difference between FT-IR spectra of BC from glucose and CTA produced from BC. According to Fig. 3C, the intensity vibration band appeared around 1737 cm^{-1} representing the carbonyl group of CTA. -OH stretching vibration of CTA was disappeared around 3360 cm^{-1}. The disappearance of -OH stretching vibration and the existence of carbonyl group in FT-IR spectra proved the cellulose triacetate formation from BC. In other words, FT-IR spectra evidenced the production of BC and CTA. In addition, the percentage of acetyl groups of cellulose triacetates synthesized from bacterial cellulose was calculated as 44.9% by using equation 1. The cellulose triacetate has a degree of hydroxyl substitution 2.98. The degree of substitution value was calculated using the acetyl group value [49].

3.4 Thermal analysis (TG-DTG)

Thermal degradation of bacterial cellulose was characterized with Perkin-Elmer Diamond brand instrument. All analyzes were performed under N_2 atmosphere with a 200 mL/min flow rate, using a ceramic pan at temperatures between 50 and 600 °C with 20 °C/min heating rate. Approximately, 5 mg of BC was used in the analysis.

Thermogravimetric analysis (TG) of bacterial cellulose was accomplished to better understand the thermal degradation behaviors of BC during the thermochemical transformations. Because the results of TG analysis depend on many parameters, including sample geometry, sample mass, and heating rate. These parameters should be constant during the analysis process. The weight loss of BC obtained from different carbon sources was determined by increasing temperature. Thermogravimetric analysis (TG) and Differential Thermogravimetric (DTG) curves were respectively shown in Figure 4A and 4B.

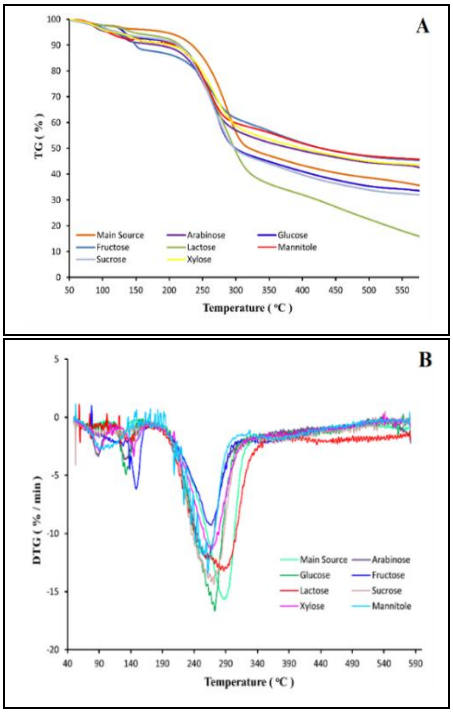

Figure 4. TG and DTG curves of BCs of *Komagataeibacter xylinus* S4 grown in HS medium with the addition of different carbon sources; **A:** TGA curves, **B:** DTG curves.

In addition, some results such as the starting degradation, the final degradation temperature, the maximum degradation temperature, and the pyrolysis residue value after heating to 550 °C obtained from the TG and DTG curves were summarized in Table 5.

Table 5. The thermal decomposition temperatures of BC from *K. xylinus* S4 grown under HS medium with different carbon sources.

Carbon Source	T_{onset} (°C)	T_{offset} (°C)	T_{max} (°C)	Residue at 550 °C (wt %)
Main Source	214.49	325.84	287.46	36.80
Fructose	227.91	299.92	266.76	45.71
Xylose	227.11	290.72	268.25	43.97
Sucrose	223.97	294.16	271.36	32.50
Lactose	221.32	318.48	286.64	18.02
Arabinose	216.9	279.22	259.72	43.53
Mannitol	221.74	276.33	261.99	46.14
*Glucose**	227.23	288.10	272.72	34.19

*: Standart HS medium containing glucose.

The thermal degradation behavior of BC consists of weight loss steps including dehydration and depolymerization of the polymer network [50]. First weight loss occurred in the dehydration step, which is related to evaporation of water molecules attached to cellulose fibrils, which occurs between the temperatures of 50-150 °C. As can be seen in Figure 4, our results were also compatible with the literature [47],[51]-[53]. Cellulose is a polymer with moderate thermal stability and degrades rapidly at temperatures between 250 °C to 350 °C [54]. In our study, the main degradation step with high percent weight loss appeared between 215 °C and 290 °C for all samples. It was seen that the second degradation step initialized between 215 °C and 228 °C temperatures.

DTG analysis also confirmed that the maximum weight loss rate was between 260-288 °C. The maximum decomposition temperatures of BC from various carbon sources were observed between 277 °C and 326 °C (Table 5). These thermal values were somewhat lower with respect to the similar works [47],[53],[55]-[57]. It is known that the thermal degradation behavior of cellulose can be altered by some structural parameters including the cellulose fibres orientation, crystallinity, and molecular weight [53]. In addition to these structural parameters, thermal degradation behavior could be affected by many factors including the cleaning procedure in the purification step, the preparation of sample for thermal analysis, and the morphology and size of the sample [58]. As stated in the information mentioned above, this difference could be due to the purification step of the BC. After changing the work-up procedure of BC, the observed degradation steps of BC were consistent with the results in the literature. After heating to 550 °C, the high pyrolysis residues of BC also supported the accuracy of this evaluation.

TG and DTG curves of BC samples obtained at various pH values in M1A05P5 medium of *K. xylinus* S4 bacteria and BC-based cellulose acetate obtained from these samples were given in Figure 5. Also, the detailed results obtained by thermogravimetric analysis of bacterial cellulose produced in Hestrin-Schramm medium with different carbon sources and in M1A05P5 medium with different pH values were shown in Table 5. And finally, the detailed results for thermogravimetric analysis of cellulose acetate in M1A05P5 medium were presented in Table 6.

Table 6. Thermal properties of BC obtained at different pH values in M1A05P5 medium of *K. xylinus* S4 bacteria and BC-based cellulose triacetate obtained from those samples.

	T_{onset} (°C)	T_{offset} (°C)	T_{max} (°C)	Residue at 550 °C (wt %)
M1A05P5, pH 3.5	308.85	379.12	356.67	12.42
M1A05P5, pH 6.5	301.27	375.38	349.15	2.89
Cellulose Triacetate	332.52	395.02	367.11	15.36

It was determined from the TG and DTG curves of BC-based cellulose acetate that the initial temperature of degradation was 332.52 °C, the final temperature of degradation was 395.02 °C and the maximum decomposition temperature was 367.11 °C. As seen in Figure 5, the decomposition step took place in a single step, as the BC samples were dried before thermal analysis.

The decomposition phase of BC obtained in M1A05P5 medium started between 301.27 °C and 308.85 °C and was completed between 375.38 °C and 379.12 °C. After the BC and BC-based cellulose acetate were heated to 550 °C, the remaining pyrolysis residue values varied between 2.89 to 15.36 % (Figure 5).

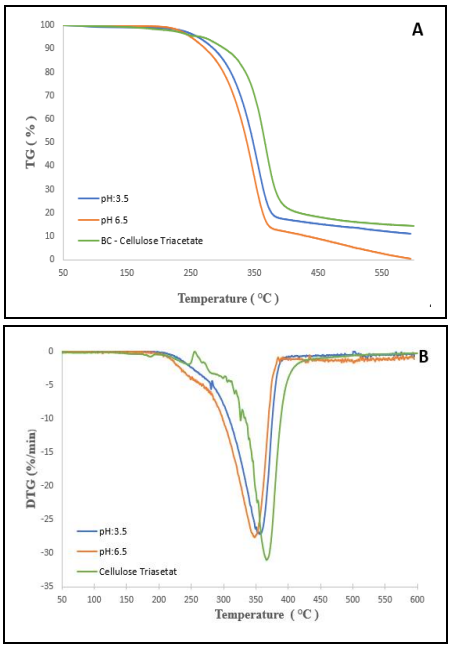

Figure 5. Thermogravimetric analysis curves of BC samples of *Komagataeibacter xylinus* S4 grown in M1A05P5 medium at different pH values and BC-based cellulose acetate obtained from those samples; **A:** TG curves, **B:** DTG curves.

Thermal analysis results also confirmed the synthesis of cellulose triacetate (CTA). Cellulose triacetate showed different thermal behavior during the analysis. The residue of BC was 2.89-12.42% at 550 °C while the residue of cellulose triacetate was 15.36 % at 550 °C. According to thermal analysis data, CTA was more resistant to high temperatures than BC. Moreover, the mass loss of CTA was less than that of BC. In the current study, acetylation of BC was studied for 24 h. According to Barud et al. [53], there is a significant relationship between acetylation time and mass loss in thermal analysis. They studied three different acetylation duration 1 hour, 6 hours, and 24 hours and reported increased crystallinity and thermal stability at the 24 hours of acetylation duration.

A high degree of purity is mainly looked for in the synthesis of cellulose derivatives. For example, CTA is one of the most essential cellulose esters due to its low cost, non-toxic and non-flammable, renewable, and biodegradable properties. Cellulose triacetate can be synthesized through the reaction between cellulose with acetic anhydride and acetic acid in the presence of sulfuric acid [59]. CTA has been synthesized from bacterial cellulose by some scientists, and its acetylation methods, physical and thermal properties have also been studied. It has been determined that bacterial cellulose can be an alternative to plant-derived cellulose in the synthesis of cellulose triacetate [60]-[63]. The cellulose acetate synthesis steps were illustrated in Figure 6 and SEM images of cellulose triacetate were presented in Figure 7.

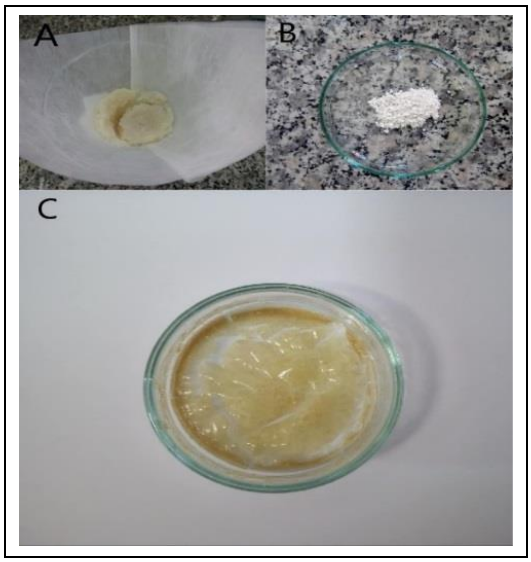

Figure 6. The photography of cellulose acetate. *Komagataeibacter xylinus* S4 was grown in M1A05P5 medium. After filtration **(A)**, Cellulose triacetate in powder **(B)** and dry forms **(C)**.

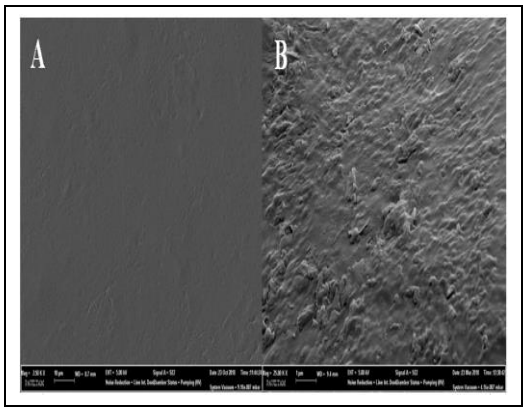

Figure 7. SEM images of CTA, **A:** 250 KX, **B:** 2500 KX.

3.5 Electrical conductivity of BC

Today, biopolymers modified with organic or inorganic components are promising composites for organic electronic material production and medicine. Sensors, flexible electrodes, conductive flexible screen applications with good mechanical properties, polymeric films, electrolyte gels, capacitors, carbon nanotubes, or new potential application areas including tissue engineering due to their cell regeneration properties are also important to study BC-based materials [64]-[71]. For this purpose, the electrical conductivity measurements of three BC samples were accomplished at 27-137 °C. Figure 8 shows the variation of conductivity with inverse temperature. It was observed that the conductivity increased exponentially with increasing temperature. Thus, it was concluded that BC exhibited semiconducting behaviors. The room temperature electrical resistivity values were measured to be 1.23x10^5 *ohm-cm,* 1.03x10^6 *ohm-cm* and 2.80x10^6 *ohm-cm* for BC-1, BC-2 and BC-3, respectively.

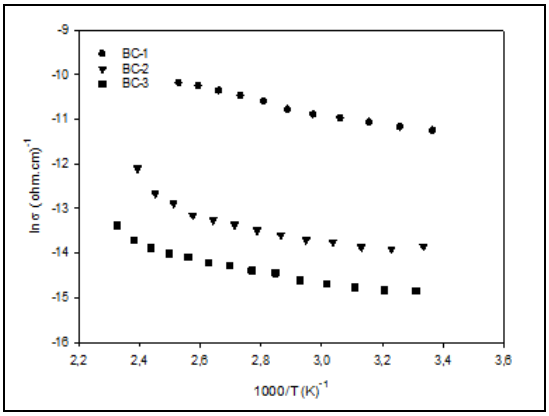

Figure 8. Temperature dependence of electrical conductivity of BCs (BC-1; bacterial celluloses obtained from HS medium, BC-2; pH: 3.5, BC-3; pH: 6.5).

The temperature dependence for the electrical conduction in the entire temperature range was analyzed by using the conductivity expression.

$$\sigma = \sigma_0 e^{-\frac{E_a}{kT}} \quad (2)$$

In equation 2, σ_0 is a constant, E_a is the thermal activation energy for conductivity, and k is the Boltzmann constant [72]. The plot of $\ln\sigma$ versus $1000/T$ in Figure 8 indicates that the slope of the linear region gives the activation energy. According to Figure 8, there was only one activation energy owing to a single slope region. In the temperature range of 27-137 °C, the activation energies of samples were calculated as 123 meV, 115 meV and 103 meV for BC-1, BC-2 and BC-3 respectively.

4 Conclusions

The need for increasing cellulose demand due to the increasing world population shows that alternative cellulose production is necessary. Especially, the decrease in vegetable sources shows that it would be wise to discover alternative sources for cellulose production. Undoubtedly, cellulose produced from bacteria is not yet cheap and economical. However, human beings know that they must use the world's resources rationally. In the current study, bacterial cellulose from *Komagataeibacter xylinus* S4 was characterized by SEM, FT-IR, and thermal analysis and, its electrical conductivity was also investigated. According to SEM analyses, there were unidirectional cellulose fibers and the interfacial adhesion between fibrils was detected in mannitol. The fibrils of bacterial cellulose obtained from other carbon sources and M1A05P5 medium also had a network-like appearance. In addition, BC showed semiconductor property. Improving the conductivity properties of BC can be used in various engineering branches. Last of all, a detailed investigation of bacterial cellulose-based cellulose triacetate will excite for future studies as an essential natural material.

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6 Author Contribution Statements

In the study, Nazime MERCAN DOĞAN has made contribution to the concepts of literature review, research planning, design and direction of experimental studies, evaluation of results, supply of resources and materials, and writing of the article.

In the study, Erdal UĞUZDOĞAN has made contribution to the concepts of literature review, design and direction of experimental studies, evaluation of results, and writing of the article.

In the study, Burak TOP, Naime Nur BOZBEYOĞLU, Duygu TAKANOĞLU BULUT and Orhan KARABULUT have made contributions to the concepts of literature review, experiments and analysis, spelling, creation of graphics and tables from the results of experiments and analysis and writing of the article.

7 Conflict of interest statement and ethics committee approval

"The authors declare no competing interests. All co-authors have seen and agree with the contents of the manuscript".

"There is no need to obtain permission from the ethics committee for the article prepared".

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