Original Article

Filtration and Antibacterial Properties of Bacterial Cellulose Membranes for Textile Wastewater Treatment

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Abstract

Bacterial cellulose (BC) which is characterized by numerous original properties including high purity, crystallinity and mechanical strength has the same molecular formula as cellulose from plant source. The aim of this study was to investigate the possibilities of using environmentally friendly BC membranes for membrane separation. BC membranes produced by *Acetobacter xylinum* were grown in a kombucha tea medium. The effect of cultivation time on filtration properties of BC membranes was tested (from 10 to 30 days). Dead-end filtration was used to determine pure water and permeate fluxes. The hydraulic permeability coefficients (L_p) of BC membranes were 20.5, 16.1, 4.5 L/m^2 h bar for 10, 20, 30 days, respectively. The effects of applied pressure (2.5, 7.5, 12.5 bar) and solution pH (5.0, 7.0, 8.8) were examined on color and chemical oxygen demand (COD) removal. The highest color and COD removals were 85.9 and 73.8% for BC-10 and 90.9 and 75.1% for BC-20 membranes at 12.5 bar pressure and pH 5.0. Moreover, silver nanoparticles with different concentrations (2, 4, and 8 mM) were grown on BC-20 membrane to investigate antibacterial properties of the composite membrane. The results showed that BC membrane could be used to develop eco-friendly antibacterial membranes with potential applications in color and COD removal from textile wastewater.
Keywords: Bacterial cellulose, Membrane, Textile wastewater

1. Introduction

Cellulose is the most plentiful, inexpensive, and easily accessible carbohydrate polymer, which has been used as a bio-based material for all sorts of purposes such as the manufacture of functional foods, cosmetics, nutraceuticals, cosmeceuticals, drug delivery systems, membranes, and fibers (1-3). Lignocellulosic materials consist of three types of polymers – cellulose, hemicellulose, and lignin can be obtained from plants or their wastes. Nevertheless, risky and hazardous chemical processes including harsh alkali and acid treatment are required to extract the pure product (4).

Although plant cellulose is the most important source, various bacteria can produce cellulose as an alternative source. Recently, there has been a growing interest in bacterial cellulose (BC) as a new type of natural polymer (5-7). BC displays different composition in terms of the mechanical properties and microstructure from those of plant cellulose such as higher mechanical strength, higher polymerization degree, higher water-absorbing capacity, higher crystallinity as well as finer web-like network (8). BC has a great potential for wide application in several fields such as food, electronics, biomedical, paper, cosmetics, textile industry, and drug delivery (9-12). BC is also possibly an excellent candidate for membrane applications due to its porous structure and small pore size, good biocompatibility, and mechanical properties (13).

It is well known that wastewater produced from textile industry requires stricter regulation to protect the receiving environment as well as water resources. Dyes are the major pollutants of wastewater from textile industry. Therefore, dyes must be removed from the wastewater due to their toxic properties (14,15). So far, several methods and mechanisms such as physico-chemical sorption, coagulation-flocculation, evaporation, biological degradation, and photocatalytic system have been developed to remove dyes from the waste stream (16-18). Another popular method in textile wastewater treatment is the membrane-based separation processes and their advantages are well known (flexibility, mild operating condition, insensitive to toxic pollutant) (19-21).

The use of membrane technologies are continuously increasing for water purification and wastewater treatment to obtain clean water (22,23). However, most

commercially-available membranes are produced from synthetic (petroleum-based) polymers (24). Nowadays, there is special interest in producing produce membranes based on natural polymers, especially those based on cellulose, such as cellulose nanofibers and BC (25). BC can be used as an ultrafiltration (UF) membrane due to its tight porous structure. In UF, a pressure-driven separation process, small molecules having low molecular weight are able to permeate through a microporous membrane, whereas solute molecules of high molecular weight are rejected in the feed stream. UF membrane is applied in a wide variety of fields to treat water and wastewater. Today, UF technology is being used worldwide for treating wastewater from food and beverage industries to textile industries (26-29).

In this study, we used a UF membrane from BC produced by *Acetobacter xylinum* for textile wastewater treatment. Moreover, the antibacterial properties of the BC membrane using nanosilver (Ag°) were also investigated. The selected conditions during the filtration procedure to achieve optimal pollutant removal (color and chemical oxygen demand [COD]) from textile wastewater and antibacterial characteristics were discussed. The produced BC membrane exhibited high pollutant removal (90.9% and 71.0% for color and COD, respectively) during real textile wastewater filtration.

2. Materials and Methods

2.1. Materials

Textile wastewater used for filtration experiments was kindly supplied by Kıvanç Tekstil, Adana, south-central Turkey. The specifications of the textile industry were specified in details elsewhere (30). The characterization of the wastewater is given in Table 1.

Analytical grade silver nitrate (ACS reagent, ≥99.0%) was purchased from Merck to obtain silver nanoparticles. Sodium borohydride was provided by Sigma Aldrich to reduce silver ions. *Escherichia coli* were supplied by the Environmental Biotechnology Laboratory, Mersin University (Mersin, Turkey) to investigate antibacterial properties of the membranes. Ultrapure water system (Milli-Q, Academic A-10, resistivity: 18.2 MΩ·cm @ 25°C; TOC ≤ 5 ppb) was used to produce deionized water. All the other chemicals and reagents used in this study were of analytical grade.

The pH and conductivity were measured by pH/Cond 340i Handheld Multimeters, WTW. The COD (closed

reflux titrimetric method) and color (platinum-cobalt method) analyses were followed according to the Standard Methods No. 5220 and No. 2120, respectively (31).

2.2. Preparation of BC Membranes

The bacterial strain used in this study was *Acetobacter xylinum* (ATCC 700178) which was obtained from the American Type Culture Collection (Rockville, MD) and was grown in kombucha medium. Sterilized tap water (4 L) and black tea (30 g) were brewed to prepare kombucha medium. Acetic acid (15 mL) and sucrose (1.5 kg) were added to kombucha medium and stirred until dissolved. Cell suspension (50 mL) was added to kombucha medium (500 mL) in a flask (1000 mL) and statically cultivated at 25 ± 1°C for 5, 10, 20, and 30 days after sterilization at 121°C for 15 minutes. The BC membranes cultivated for 5, 10, 20, and 30 days were named BC-5, BC-10, BC-20, and BC-30, respectively. The BC membranes were treated with 0.1 N NaOH solution at 80°C for 30 minutes to remove the free bacterial cells and medium components and they were dried at 25 ± 1°C for 1 day (32,33). The images of wet BC membranes with different cultivation time are shown in Fig. 1.

2.3. Preparation of BC-Silver Nanoparticles Composite Membranes

BC membranes were washed with distilled water several times until the pH value reached 6.0-7.0 and were treated with ultraviolet radiation. BC membranes cut into 1.5 cm × 1.0 cm pieces were soaked in AgNO$_3$ solutions (30 mL) with Ag^{+2} concentrations of 2, 4, and 8 mM for 12 hours, then, they were rinsed with distilled water several times. Sodium citrate solution (30 mL of 0.1 M) was added after the rinse. Finally, NaBH$_4$ solution (20 mL of 0.2 mM) was added to start the reaction. The solution turned into black color after the reaction was started. The membranes were stirred in the solution for 6 hours. The BC composite membranes containing nanosilver were named BC-Ag2, BC-Ag4, and BC-Ag8, corresponding to the Ag^{+2} concentrations (2, 4, 8 mM, respectively).
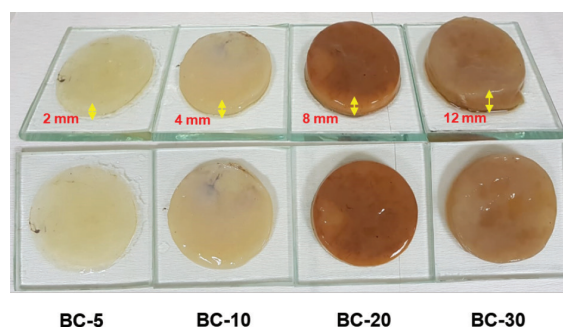
2.4. Filtration Experiments of Textile Wastewater

Table 1. The Analysis of the Real Textile Wastewater

Parameter	Value
pH	8.85 ± 0.02
Conductivity, mS/cm	2.58 ± 0.21
Chemical Oxygen Demand (COD), mg/L	1.372 ± 16.6
Color, Pt-Co	240 ± 8.9
Total *E. coli*	$1×10^6$

Fig. 1. The Images of Wet BC Membranes With Different Cultivation Time

A dead-end stirred cell (300-mL, Sterlitech HP 4750, USA) was used to obtain permeate flux. The BC membranes were cut into 5-cm diameter discs (14.6 cm²) and saturated with distilled water for 1 hour. Waterlogged BC membranes were placed in the dead end filtration system and deionized water was passed through the membranes using N_2 gas (30). For wastewater filtration, textile wastewater (200 mL) was filled into the system and it was filtrated for 120 minutes at stirring speed of 250 rpm at 25 ± 1°C. The hydraulic permeability of the BC membranes was calculated by filtration of deionized water.

The hydraulic permeability of the membranes (J_v) was calculated from the initial water permeation according to equation (1) (34).

$$J_v = L_p \times \Delta P \qquad (1)$$

Where ΔP is the gradient of hydrostatic pressures across the membrane and L_p is the hydraulic permeability coefficient.

The permeate flux values (L/m² h) were calculated according to Equation (2).

$$J_v = \frac{V}{A \times t} \qquad (2)$$

Where V is volume flux (L), A is the active filtration area of the BC membrane (m²) and t is the time (h).

Textile wastewater was filtrated through BC membranes using dead-end system. The permeate water quality was determined by measuring color and COD at the end of filtration experiments. Rejection values (%) of color and COD were calculated by equation (3).

$$R\,(\%) = \left(1 - \frac{C_p}{C_i}\right) \times 100 \qquad (3)$$

Where C_p (mg/L) is the concentration in the permeate and C_i (mg/L) is the concentration in the initial feed solution.

2.5. Antibacterial Properties of Nanosilver Loaded BC Composite Membrane

The antibacterial activity of the nanosilver loaded BC composite membranes against *E. coli* was investigated using agar diffusion method based on a previous study (35). *E. coli* solution (100 mL) was filtered using dead-end filtration system at 2.5 bar. After the filtration, bare BC (BC-Ag0) and silver nanoparticles loaded composite membranes (BC-Ag2, BC-Ag4, and BC-Ag8) were cut into 10 mm diameter discs and placed on the plates in order to test the antibacterial activity of composite membranes. The bare BC membrane was considered as the control sample. The plates were incubated at 37°C for 24 hours and the zone of growth inhibition around each membrane after the incubation period was measured by a digital caliper.

2.6. Characterization of BC Membranes

Scanning electron microscopy (SEM, Zeiss Supra 55, Germany) with an accelerating voltage of 5.00 kV and Fourier transform infrared spectroscopy (FTIR, PerkinElmer) with a spectral range of 4000–400 cm⁻¹ were used to demonstrate morphological characterization of BC membranes.

3. Results and Discussion

3.1. Pure Water Permeability of BC Membranes

Fig. 2 shows pure water permeability (PWP) of the BC membranes. The PWP results showed that permeability of BC membrane decreased with increasing cultivation time from 10 to 30 days. The PWP declined from 20.5 to 4.5 L/m² h bar when cultivation time increased from 10 to 30 days. The formation of more cellulose fibers in BC membrane network increased over 30 days. Therefore, the thickness of the BC membrane increased and the pore structure narrowed and the PWP reduced. However, BC-5 membrane ruptured at 2 bar because it was very thin and PWP could not be measured. The obtained results showed that the hydraulic permeability coefficients (L_p) of BC-10 and BC-20 membranes were approximately four and five times higher than those of BC-30 membrane. L_p varies linearly with the porosity and inversely with the thickness (36). In our study, the thickness of BC membranes increased from 4 to 12 mm when cultivation time was increased from 10 to 30 days. This caused a decrease in L_p of the membranes. For textile wastewater filtration experiments, BC-10 and BC-20 membranes were selected for further membrane filtration due to having higher PWP compared to BC-30 membrane.

3.2. The Effect pH on Permeate Flux and Pollutant Removal

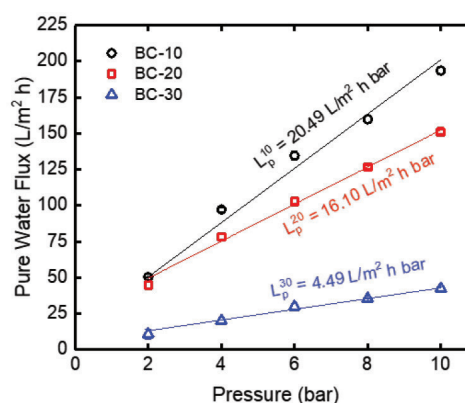It is well known that solution pH is very important in membrane filtration and pH affects directly the permeate

Fig. 2. PWP of BC Membranes for 10, 20, and 30 days

flux. Fig. 3 shows the flux values of BC membranes as a function of time versus solution pH for BC-10 (Fig. 3A) and BC-20 (Fig. 3B) membranes. The flux values of initial and pseudo steady state are presented in Table 2. The highest initial (J_o) and steady state (J_{ss}) flux were observed in BC-10 membrane at pH 5.0 (Fig. 3A). The lowest flux was obtained at the original pH (8.8) of the textile wastewater for both membranes (Fig. 3A and 3B). Therefore, to obtain the highest permeate flux and color and COD removal, the optimum pH was determined to be 5.0.

Color and COD were analyzed in permeates versus solution pH and the results are shown in Figs. 3C and 3D. Color and COD rejection efficiencies in the permeate increased at acidic pH. Color and COD removal efficiencies increased from 51.6% to 85.9% and from 49.2% to 73.8%, respectively, when pH decreased from 8.8 to 5.0 for BC-10 (Fig. 3C). Color and COD removal efficiencies increased from 66.4% to 82.7% and from 55.0% to 75.1%, respectively, when pH decreased from 8.8 to 5.0 for BC-20 (Fig. 3D). Accordingly, the operating pH of 5.0 was chosen to obtain the highest permeate flux and color and COD removal. It could be concluded that the decrease in flux and the increase in the removal of color and COD at low pH could be caused by a change in the shape of color molecules and/or the easier formation of a gel layer, which might result in plugging of the membrane pores. It seemed that the decrease in removal of color and COD at low pH was ascribed to the change in properties of coloring matters (37).

## 3.3. The Effect of Operating Pressure on Permeate Flux and Pollutant Removal

The flux values of BC-10 and BC-20 membrane versus pressure as a function of time are shown in Fig. 4. Moreover, Table 3 presents the initial (J_o) and pseudo steady state (J_{ss}) flux values. The initial robust drop happened in the flux within the first 10 minutes for BC-10 membrane (Fig. 4A). However, BC-20 membrane showed smoother flux decline compared to BC-10 (Fig. 4B). Interestingly, steady state flux was the same for BC-10 and BC-20 membranes. The initial permeate flux increased from 82.2 to 163.9 L/m^2 h (LMH) for BC-10 membrane and from 57.9 to 94.1 LMH for BC-20 membrane when pressure was increased from 2.5 to 12.5 bar. The reason for obtaining a lower initial flux for BC-20 can be attributed to the decrease of membrane pore size when cultivation time increased from 10 to 20 days. The increase in cellulose fibrils and membrane thickness (from 4 to 8 mm) decreased initial flux but it did not affect the steady state flux.

Color and COD were analyzed in permeates against applied pressure and the results are shown in Fig. 4C and 4D. Increasing the applied pressure improved both color and COD removal efficiencies of the permeate. In this study, the color was removed better than COD for all experimental conditions. Color and COD removal efficiencies increased from 75.1% to 85.9% and from 57.7%

Table 3. Permeate Flux Values Versus Pressure for BC-10 and BC-20 Membranes

BC membrane	ΔP (bar)	J_o (LMH)	J_{ss} (LMH)
BC-10	2.5	82.2	8.3
	7.5	132.7	10.7
	12.5	163.9	12.5
BC-20	2.5	57.9	8.9
	7.5	93.7	10.8
	12.5	94.1	12.8
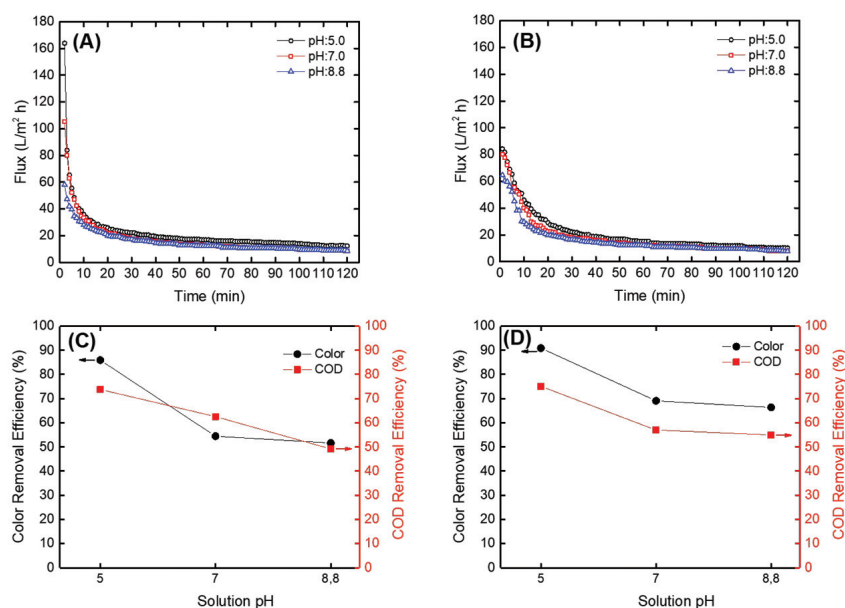
Fig. 3. Change of Fluxes with Time for **(A)** BC-10 Membrane **(B)** BC-20 Membrane and Change of Color and COD with pH for **(C)** BC-10 Membrane **(D)** BC-20 Membrane (experimental conditions: inlet color: 240 Pt-Co; inlet COD: 1,372 mg/L; operating pressure: 12.5 bar).

to 73.8%, respectively, when applied pressure increased from 2.5 to 12.5 bar for BC-10 (Fig. 4C). However, color and COD removal efficiencies increased from 82.7% to 90.9% and from 60.0% to 75.1%, respectively, when applied pressure increased from 2.5 to 12.5 bar for BC-20 (Fig. 4D). Therefore, the operating pressure of 12.5 bar was determined to obtain the highest steady-state permeate flux and color and COD removal. Higher removal efficiency for color and COD was observed for BC-20 membrane because of having narrower pore size than BC-10 membrane. This may be also attributed to mechanical compaction of BC membranes at higher operating pressure. Compaction can be defined as the decrease in membrane volume because of mechanical deformation upon the application of a high mechanical pressure (38). Thicker BC-20 membrane could be compacted better than BC-10 membrane and density of the active layer of BC-20 membrane could be changed. Therefore, the application of a high mechanical pressure can cause a change in free volume available. This will have a significant influence on transport of permeating components such as color and soluble organic molecules (39).

Despite its many advantages, the use of BC membranes for treating wastewater has not been widely investigated; relatively few studies were published on using BC membrane for wastewater treatment. The design of reusable novel membranes based on BC and chitosan was carried out using two different preparation routes, in situ and ex situ, for the filtration and elimination of copper in wastewater. The copper removal capacity of the BC membranes was analyzed and the prepared membrane showed the highest values (about 50%) for

initial concentrations of 50 and 250 mg/L. Moreover, the reusability of the membranes was assessed and after two cycles a decrease of less than 10% of the removal efficiency was observed. They reported that BC membrane was used to provide physical integrity for chitosan to develop eco-friendly membranes with potential applications in heavy metal removal (40).

Dried BC membrane was used for the removal of oil from stabilized and non-stabilized oil-in-water emulsions with droplet sizes less than 1 μm. BC harvested after different incubation time periods (2 to 10 days) did not show a change in the width of the nanofibers, but only the thickness of the membranes increased. BC membranes showed a higher flux and efficiency in removing oil from oil emulsions (25).

A novel anti-biofouling UF membrane was prepared based on reduced graphene oxide (RGO) and bacterial nanocellulose (BNC), which incorporates GO flakes into BNC in situ during its growth. The RGO/BNC composite membrane exhibited excellent chemical stability at solution pH varying from 4 to 9 and stable water flux under 100 psi. The new environmentally friendly anti-biofouling membranes were used for water purification (41).

3.4. The Effect of BC Membrane Cleaning on Flux Recovery

Membrane cleaning is a very significant topic for operational cost and membrane life. Membrane cleaning can be classified into two categories, physical and chemical cleaning (42). Physical cleaning is not a very effective way to reduce membrane fouling; thus, the flux recovery rate does not improve satisfactorily (43).
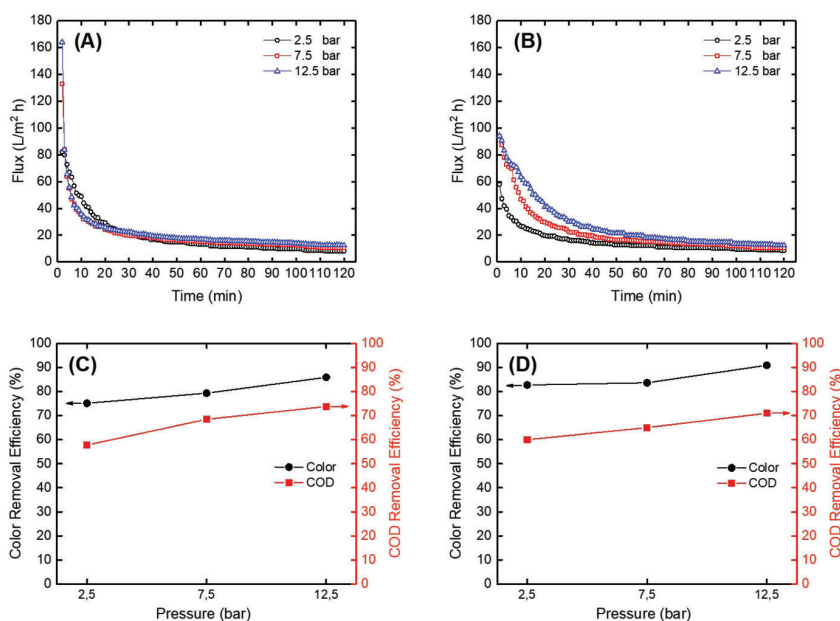
Fig. 4. Change of Permeate Fluxes with Time for (A) BC-10 Membrane (B) BC-20 Membrane and Change of Color and COD Removal with Applied Pressure for (C) BC-10 Membrane (D) BC-20 Membrane (experimental conditions: inlet color: 240 Pt-Co; inlet COD: 1,372 mg/L; solution pH: 5.0).

Therefore, we preferred chemical cleaning using sodium hydroxide (NaOH) to remove organic foulants from BC membrane surface to improve preferred flux recovery (Fig. 5). Physical cleaning using deionized water was also tested for 15 minutes but we obtained only 15% flux recovery. For chemical cleaning, NaOH (0.1 N) was filtrated through fouled BC membrane for 15 minutes and was tested after four cycles. After the first chemical cleaning, the initial flux was lower than the first filtration. After the first and second chemical cleaning, the initial flux and pseudo steady-state flux were the same as 41.9 and 8.6 LMH. After the third chemical cleaning, the initial flux decreased to 32.9 LMH but pseudo steady-state flux was similar to the third filtration (Fig. 5).
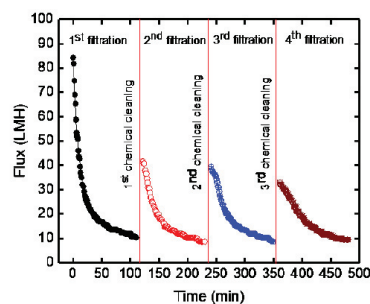

Fig. 5. Chemical Cleaning in Series for Flux Recovery of BC-20 Membrane (experimental conditions: applied pressure: 12.5 bar; pH: 5.0; inlet color: 240 Pt-Co; inlet COD: 1,372 mg/L; cleaning time: 15 min).

Table 4. Diameters of Antibacterial Circle of the Bare BC and BC-Ag Composite Membranes Against *Escherichia coli*

| Membranes | Parallel test | | | |
	1	2	3	Width (mm)
BC-Ag0	0	0	0	0
BC-Ag2	6.52	6.85	6.72	6.69
BC-Ag4	8.15	8.32	8.46	8.31
BC-Ag8	15.02	14.98	15.12	15.04

3.5. Evaluation of Antibacterial Properties of Nanosilver Loaded BC Composite Membrane

Inhibition zone and colony counting are commonly used for evaluating antibacterial activities. In this study, BC membrane was loaded with silver (Ag) nanoparticles and compared to bare BC membrane. The images of inhibition zones of silver loaded composite BC membranes (BC-Ag0, BC-Ag2, BC-Ag4, BC-Ag8) against *E. coli* are given in Fig. 6. The results showed that the inhibition zone increased by increasing silver nanoparticles loaded on the membrane surface. The composite membranes with silver nanoparticles exhibited good antimicrobial activity against *E. coli*. The diameters of the inhibition zone for BC-Ag composite membranes with different silver concentrations against *E. coli* are shown in Table 4. We did not observe an inhibition zone in the bare BC membrane as the control group (Fig. 6 and Table 4). However, clear zones surrounding the membrane discs were observed by containing different concentrations of silver nanoparticles. The widest zone was detected by BC-Ag8 (loaded 8 mM silver nanoparticles). It was concluded that the antimicrobial activity was due to silver nanoparticles loaded into BC membranes and they showed good antimicrobial activity against *E. coli*. A possible mechanism was proposed for the antimicrobial activity of Ag nanoparticles by Morones et al in 2005 (44). The mechanism involves the interaction of monovalent silver ions (Ag^+) with biological macromolecules through proteins thiol groups (-SH). Ag^+ would replace H^+ ions of sulfhydryl or thiol groups, inactivating the protein, decreasing membrane permeability, and eventually causing cellular death (44,45). Silver nanoparticles were loaded on the surface of BC to form BC-Ag composite membrane and antimicrobial activity of the BC-Ag nanocomposites were evaluated. They reported that the antibacterial ratio against *E. coli* reached 99.4% (46). Antimicrobial BC-silver nanoparticles composite membranes had been obtained by "in situ" preparation of Ag nanoparticles (45). The composite membranes exhibited a strong antimicrobial activity against *Staphylococcus aureus* (gram-positive bacteria), *Pseudomonas aeruginosa* and *E. coli* (gram-
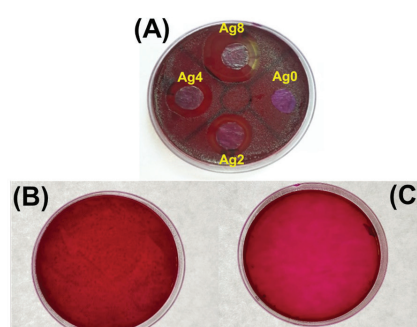

Fig. 6. (A) Inhibition Zone of the Bare BC (BC-Ag0) and Silver Nanoparticles Loaded Composite Membranes (BC-Ag2, BC-Ag4, BC-Ag8) Against *E. coli* (B) E. Coli Before Filtration (C) BC-Ag8 Permeate

negative bacteria). In another study, the antibacterial activity of nanocomposites of silver and bacterial or vegetable cellulosic fibers was studied (47). An inhibition zone of 2 cm was observed confirming the diffusion of silver nanoparticles from BC-Ag-triethanolamine composite to culture medium. No inhibition zone was observed in the bare BC control.

A study was also carried out by impregnation of silver nanoparticles on BC surface to prepare antimicrobial wound dressing material (48). Antimicrobial activity of silver nanoparticle-impregnated BC had been investigated against the model Gram-positive bacteria (*S. aureus*) due to its drug-resistant nature. They reported that a high porosity of BC supplied the sustainable release of the antimicrobial compound. The porosity of BC could hold the silver ions and release them gradually. This slow-release property of BC helped to maintain the moisture
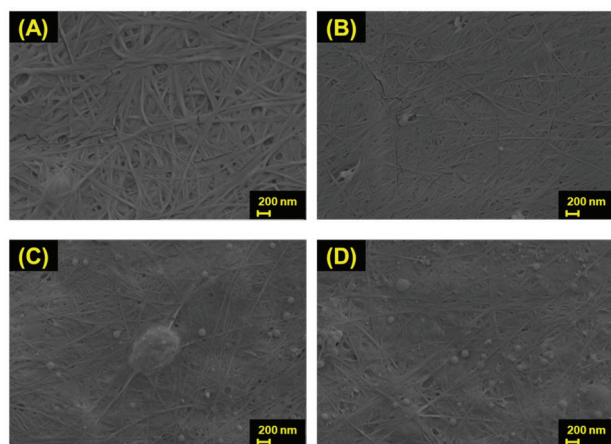
Fig. 7. SEM Images of the Bare Membranes (A) BC-10 (B) BC-20 Membrane and Fouled Membranes after Filtration of Textile Wastewater (C) BC-10 Membrane (D) BC-20 Membrane.

and set aside the microbial dominance at the wound site (48).

BC functionalized with silver nanoparticles was evaluated as an antimicrobial membrane for wound-healing (49). Antibacterial activity of the composite membrane was tested against the Gram-negative bacteria (*E. coli*) by disk diffusion and growth dynamics showed high bacteria-killing performance. The distance from the outer surface of Ag/BC to the bacterial colony-forming zone was measured to be about 6.5 mm. No significant amount of silver release was observed from the Ag/BC pellicles even after a long soaking time.

Morphologic characterization of the BC membranes was screened by SEM analyses (Fig. 7). BC-10 membrane (Fig. 7A) showed more porous structure compared to BC-20 membrane (Fig. 7B). The fibril diameters of BC-10 membrane (ranging from 35 to 50 nm) were thicker than those of BC-20 membrane (ranging from 10 to 25 nm). The width of BC fibrils decreased when the cultivation time of BC was increased, but the thickness of the BC-20 membrane increased due to the greater mass of BC being formed (Fig. 7B). Moreover, the fibrils were tightly packed which were thought to be amorphous regions of BC-20 membrane (50). SEM images of fouled membranes after filtration of textile wastewater showed that BC membranes removed pollutants from wastewater. Furthermore, fouled BC-20 membrane (Fig. 7D) removed more pollutants compared to fouled BC-10 membrane (Fig. 7C) due to narrower pore size, higher fibril density, and higher thickness.

Functional groups of BC membrane identified by FTIR spectrum and their wave numbers were given as follows. The absorption bands of 3400 cm^{-1} and 2897 cm^{-1} were assigned to the -OH group and C-H stretching. Other characteristic bands of cellulose such as 1429 cm^{-1}, 1380 cm^{-1}, and 899 cm^{-1} were also evidenced H-C-H and O-C-H bending inside of plane vibration, C-H deformation vibration, and C-O-C, C-C-O, and C-C-H

deformation modes stretching vibrations, respectively. The wave number of 670 cm^{-1} was proved C–OH bending out of plane.

4. Conclusion

BC was successfully used as a bio-based membrane to filtrate real textile wastewater. Removal of color and COD was affected by the cultivation time of BC as well as pH of wastewater in order to change membrane pore size and thickness. For the highest color and COD removal from wastewater, optimum pH and cultivation time were detected to be 5.0 and 20 days, respectively. Moreover, BC-20 membrane was successfully used four times after cleaning with alkali reagent. The composite membranes including silver nanoparticles demonstrated strong antimicrobial activity against *E. coli*.

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