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Synthesis of new bacterial cellulose-oriented micropore membrane filter

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This study investigates a novel synthesis method of bacterial cellulose (BC)-oriented micropore membrane filter and its characteristics. BC-oriented micropore membrane filter has been synthesized by preparation of BC-benzol suspension and evaporation of benzol to render dried BC membrane micro-sized porosity. The structures of BC fibrils before and after hydrolysis are studied by X-ray diffraction (XRD). XRD intensity at 23° (2θ) in HCl-treated BC is higher (73%) than the control (60%), however, the diffraction intensity at 23° (2θ) in BC treated with mixture of H_2SO_4 and HNO_3 is lower(53%) than the control. The SEM analysis reveals that the synthesized BC membrane has many micropores in comparison to the native BC film. The FTIR spectra of cotton cellulose, native BC film and the synthesized BC microfilter showed a band at 3345 and 1643 cm^{-1} which is assigned to OH stretching vibration and OH deformation vibration and a band attributed to CO asymmetric and symmetric stretching vibration in a range from 1032 to 1166 cm^{-1}, respectively. Maximum pore size, pure water flux, the breakage pressure, ζ-potential and bacteria-retentive efficiency of the filter are characterized. The synthesized microfilter has maximum pore size of 0.2 µm and high water flux of 220 L/m^2 h under 0.1 MPa. It demonstrates 99.9% filterability against *Escherichia coli* and *Brevundimonas diminuta.* The biodegradability test shows that the newly developed BC microfilter is totally decomposed in a month. This suggests that the novel BC microfilter can be a promising candidate for membrane filer used in pharmaceutical, food, cosmetic and water industry.

Keywords*:* Acid hydrolysis, Bacterial cellulose, Emulsion, Microfilter, Porosity

Introduction

Bacterial cellulose (BC) has been the focus of current research on biomaterials due to its unique structure and characteristics. The chemical compositions of BC and other natural cellulose are very similar. All are unbranched polysaccharide, type β-1,4-glucan, where glucose is linked by β-1,4-glucoside bonds. However, BC has unique physical, chemical and mechanical characteristics other than plant cellulose[1, 2]. BC fibrils are about 100 times thinner than plant cellulose and they are held together by inter- and intra- hydrogen bonds to form a unique ultrafine network structure[3]. BC is very pure in composition without any lignin or other cell wall components and has high water absorbability, crystallinity and mechanical strength[4]. Furthermore, unique characteristics such as heat resistance, pH resistance, non-corrosiveness and biodegradability promote the use of BC as a membrane filter[5].

Membrane filter can be categorized into porous or non-porous, symmetric or asymmetric depending on their structure, filtration behaviour or mechanism. Filtration by porous membranes is divided into 4 categories: microfiltration (MF), ultrafiltration (UF), nanofiltration (NF) and reverse osmosis (RO)[6]. BC pellicle is mainly used as an ultra- or nano- filter because it has a uniform and 3-D nanofibrous network structure with tiny nano-sized pores[1, 7]. Nanofilter is for separation of nanoparticles, so its pore size is about several nanometers. Ultrafilter is the one that separates macromolecules from small molecules under working pressure of 0.2-1.0 Pa. The pore diameter of ultrafiltergenerallyranges from 1 to 50 nm, so BC is typically used as nanofiltersdue toitstiny pores inside the 3D network[5, 8]. However, the pore size of BC pellicles is too small to be used as microfilter.

There is a high demand for microfilters in many fields including food, medicine, pharmaceutical, bioengineering and electronic industry[9]. Especially, bacteria-retentive membrane filter is definitely required to remove bacteria from food, drinking water and injection, or to collect bacterial bodies from culture media, or to provide GMP-standardized surroundings. The most deadly water pollutants are from biological origin: infectious diseases caused by pathogenic bacteria (e.g. *Escherichia coli, Salmonella typhi, Vibrio cholerae*), protozoa (e.g. giardia) or parasites are the most common and widespread health risk associated with drinking water[10]. Furthermore, all materials or ingredients involved in production of food and cosmetics are filtered and purified by bacteria-retentive filters. In consequence, microfilters are a widely-used and essential element in people's everyday lives, as well as in national economy[11]. High demands for microfilters encourages the development of BC-oriented microfilters.

Another reason of study on the BC-oriented microfilter is biodegradability of BC. Everyday a large amount of microfilter is produced and thrown away. The chemically synthesized polymers such as polyvinylidene difluoride, polyether-sulfone and regenerated cellulose which are used for bacteria-retentive filter are non-biodegradable, so they cause secondary environmental pollution. The production of synthetic filter materials itself can also be a cause of environmental pollution. These circumstances inspired many researchers to synthesize BC-oriented mirofilters.

The most important point in synthesis of BC microfilters is to expand the nano-sized pores inside the BC membrane. Some studies on regulating the pore size of BC membrane demonstrated that pores of BC can be changed by varying growth condition such as composition, pH, temperature, and oxygen tension of the culture media[12-14]. The porosity of BC can also be modified by post-production modifications[15]. For example, a pore modifier of BC membrane, polyvinyl alcohol (PVA), was treated to the newly produced BC pellicles to expand the pores[7]. However, these methods could not make a fundamental difference to the pore size of the BC, because they did not destroy the fine 3-D networks constructed by bacteria. Another study showed that BC-soy bean extract complex membrane has a lot of micro-sized pores. In this work, BC was pulverized to get nanofibrils, mixed with soybean extract and dried in the room temperature[16]. However, the membrane synthesized in this way has very low porosity, because hydrogen bonds between nanofibrils which were formed during drying duration eliminate the pores.

It is most likely that disintegration of BC and remoalding into a membrane under treatment of reasonable pore modifiers can result in a porous BC membrane. Pore modifiers could be evaporable oils which can prevent the hydrogen bonds between BC fibrils and make pores after evaporation.

It has been reported that o/w pickering emulsions of peanut oil were stabilized by 0.05% (w/v) BC nanofibrils[17]. BC nanofibrils surrounded the oil droplets and disturbed the aggregation of small droplets into a large droplet. In the oil/water (o/w) emulsion, however, we focused on stabilization of BC fibrils more than oil droplets. The phenomenon described above can be observed indifferent aspect: small oil droplets are placed between BC fibrils and disturbed aggregation of fibrils during dehydration. Because BC fibrils have many hydroxyl groups, hydrogen bond between them easily occurs. So, BC nanofibrils aggregate with each other during drying duration to create non-porous film. To prevent the aggregation, oil (or benzol) is dipersed in BC suspension before drying. Then BC fibrils are not able to aggregate until the oil droplets evaporate. Benzol which has a lower boiling point than water can evaporate earlier than water. The drying process causes the place where there used to be a benzol droplet becomes a pore. From this idea, we studied the synthesis and characteristics of BC membrane microfilters.

Experimental Section

Materials and Chemicals

Bacterial cellulose was statically produced by culturing *Acetobacterxylinum 10* for 5-7 days in alternative media such as WSCBs (Water Strained off Coagulating Bean curd) or food by product supplemented by 2.0% (w/v) sucrose and 0.1% (w/v) acetic acid (pH 5.0) or alcohol lees[18]. After cultivation, BC was removed from media and rinsed several times to remove the residual media. Next, it was washed by treating with 2 M NaOH at 60°C for 2 h and neutralized by repeated washing. The purified BC pellicles were cut into small pieces, equilibrated with deionized water. The other reagents used in this study are Nitric acid (ANEDRA, ρ=1.4 g/cm^3), Hydrochloric acid (ANEDRA, 30%), Sulfuric acid (ANEDRA, ρ=1.8 g/cm^3), Surfactant OP-10 (SIGMA-ALDRICH), Benzol (ANEDRA).

Disintegration of BC by mechanical pulverization and acid hydrolysis

BC was disintegrated with a high shear blender (HERPUSI) at 7000 rpm for 20 min, drained off by a tamis so that moisture could be removed. Then 20 g of pulverized BC was added to 100 mL mixture of nitric acid (ρ=1.4g/cm^3) and sulfuric acid (ρ=1.8 g/cm^3) (1:1 ratio, v/v) for hydrolysis. HCl (30%, w/w) was also used for acid hydrolysis[19]. After 1 h, the reaction solution was carefully poured into 5L aqueous solution. This dilutes the reactants and stops the hydrolytic reaction. The hydrolyzed BC was thoroughly neutralized by repeated washing and centrifugation (KR20000S, 3000 rpm, 15 min).

XRD (X-ray diffraction) analysis of BC before and after acid hydrolysis

The XRD analysis was employed to determine the crystallinity of BC before and after acid hydrolysis. The XRD pattern of the samples was obtained using X-ray diffractometer (Smartlab). The samples were scanned from 2θ=10° to 2θ=60° at a scanning rate of 1°(2θ)/min.

Fabrication of BC microfilter

10 g of the disintegrated BC (1%,.wt), 1 mL of surfactant OP-10 and 0.13 mL of benzol were mixed in 50 mL of distilled water, which was treated by ultrasonic irradiator (KD-1000 PZT, 20 kHz, 300 W) for 20 min. The BC emulsion was poured into a Petri dish, shaken well, coated on the smooth plate, and dried in a thermostatic dryer at 80°C. At the end of drying, the formed membrane was carefully taken out and was immersed in distilled water for more than 1 h to ensure that all remnants were fully drained out. Then, it was dried again in a refrigerator-vacuum drier (3011D, Czech).

Optical microscopy of the o/w emulsion and SEM analysis of BC microfilter

Benzol/BC emulsion were placed on a microscope slide, covered with a cover slip and observed under 100× and 200× magnification using an optical microscope (Kruss Optronic, Germany). Attention was paid to the size and distribution of benzol droplets in BC suspension. After the benzol volatilized, the dried membrane was observed under scanning electron microscope (SEM) (JSM 6610A) to identify real pores where benzol droplets used to be. Samples were freeze-dried and sputter-coated with platinum.

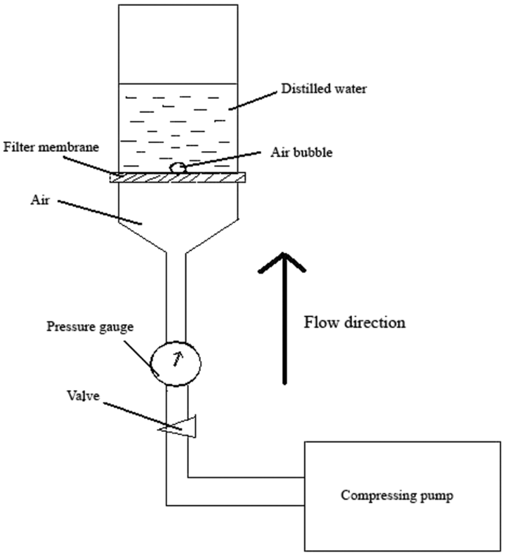
Maximum pore size of BC microfilter

The BC membrane was equipped in the apparatus as shown in Scheme 1 and the maximum pore size was determined by the bubble-point test[20]. The pore size of an ideal capillary filter which has the same bubble point as the actual filter is called "equivalent pore diameter". The maximum pore diameter can be calculated from the following equation[21].

$$D = \frac{4\gamma\cos\theta}{P} \times 10^6$$

Where, D is maximum pore diameter (μm), P is bubble-point air pressure (Pa), γ is surface tension of the liquid (N/m) and θ is contact angle between the liquid and the filter material.

Fig. 1 shows the relationship between pressure of the first air bubble formation and pore size. In order to measure the equivalent pore size by the bubble-point test, it is necessary to know the contact angle θ, the viscosity of the liquid, the pressure difference between both sides of the membrane, and the surface tension of the filtrate. The contact angle θ was set to 0 as the filter was completely wetted. The viscosity of the liquid was set to 1×10^{-3} (Pa s) as distilled water was used, and the surface tension of the water was considered to be 72.8 N/m at 25°C(Ref.21). Pressure difference between upper side and lower side of the membrane (△P) was measured just when the first air bubble formed. Here △P is considered as P.



Scheme 1 — Schematic diagram for measurement of maximum pore size of the filter

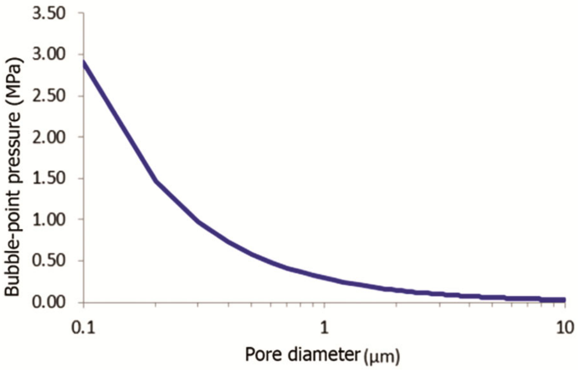


Fig. 1 — Pore diameter vs. air pressure for ideal filter in bubble-point test[21]

Pure water flux of the membrane

To evaluate the pure water flux and hydraulic permeability coefficient of the membrane, the permeate volume of water under various pressures was measured. Apparatus shown in Scheme 2 was employed to measure the pure water flux. The apparatus (Scheme 2) was set up and a circular membrane filter of 7×10^{-4} $m^2$was placed on a polypropylene support to measure water flux (J).Each membrane was immersed in distilled water for at least 1 h to make sure that water flux was not affected by swelling property of the membrane.

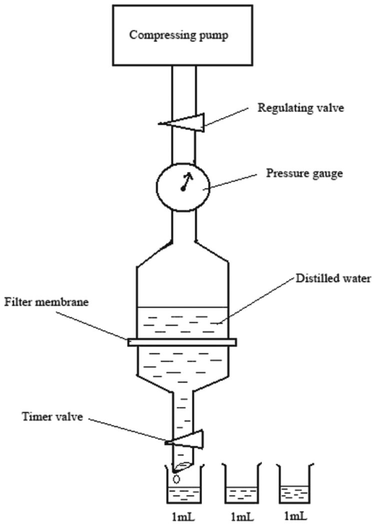
According to the Hagen-Poissuille equation, water permeability coefficient (L_P) and water permeation velocity through a pore (J) can be obtained from equation given below. The L_P is related to membrane pore number (N), mean pore size (r), viscosity of feed solution (η) and membrane thickness (△x)$^{(\text{Ref.14})}$. △P was 1×10^5 Pa due to the usage of vacuum pump, and the viscosity of liquid was $\eta = 1\times10^{-3}$ Pa sas the feed solution was distilled water.

$$J = \frac{N\pi r^4}{8\eta \triangle X} \times \triangle P = L_P \times \triangle P$$

N is calculated by weight difference method[22] and mean pore size is determined from the equation. Porosity (ρ) of the filter can be obtained by Eq 1.

$$\rho = \frac{N\pi r^2}{S} \qquad \text{... (1)}$$

where, S is the area of the membrane filter. Then water permeation velocity (v) is calculated from the Eq 2



Scheme 2 — Schematic diagram for measurement of water flux of the filter

$$v = \frac{J}{\rho} \qquad \text{... (2)}$$

FTIR analysis of BC microfilter

Chemical functional groups of BC membrane filter were analyzed by Fourier transform infrared spectroscopy (FTIR) (NICOLET 6700) with a range of frequency from 4000 cm^{-1} to 400 cm^{-1} and a resolution of 8 cm^{-1}. Any changes in functional groups occurred during the synthesis of microfilter can be observed in the FTIR spectra.

ζ-potential of BC microfilter

ζ-potential of BC filter was measured by the electroosmosis method. The electroosmotic velocity, the current intensity, and the specific conductivity of the dispersion solvent are measured to determine the ζ-potential. The electroosmotic velocity (*v*) was determined by measuring the separation distance of the dispersion solvent (1 mol/L KCl) through capillary. The specific conductivity was set to $2.06\times10^{-2}\ \Omega^{-1}\cdot m^{-1}$, and the current intensity was measured at the interface using the ammeter installed within the apparatus. The equation for determining the ζ-potential by electroosmosis method is as follows.

$$\zeta = \frac{\eta X v}{\varepsilon_0 \varepsilon I} \qquad \text{... (3)}$$

Where, η= Viscosity of dispersion solvent (Pa·s), x = Specific conductivity of dispersion solvent ($\Omega^{-1}\cdot m^{-1}$), v= Electroosmotic velocity ($m^3\cdot s^{-1}$), ε_0= Absolute permittivity of vacuum ($8.85\cdot10^{-12}$ $F\cdot m^{-1}$) and ε= Relative permittivity of dispersion solvent

Bacterial retention efficiency

Escherichia coli (E. coli) and *Brevundimonas diminuta (B. diminuta*) are commonly used to evaluate the performance of the BC membrane filter, so we selected these two bacteria. *E. coli*, a rod-shaped bacterium with a thickness of about 1µm is a major group of pathogen in polluted water. *Bs. Diminuta* which has a diameter of 0.5 to 0.8 mm is widely used to test bacteria-retention ability in liquid filtration[23]. *E. coli* was cultured in LB medium (Sigma) at 30°C for 48 h to be activated and *B. diminuta* was cultured in a rotary shaker at 30°C for 48 h. About 300 mL of each culture was centrifuged, and the pellet was washed and re-suspended in deionized water 3 times.

Prior to the fiterability test, the pellet was suspended into 400 mL sterile deionized water to prepare the feed solution. 100 µL of feed solution (positive control), post-filtration solution (sample) and distilled water (negative control) were transplanted into Nutrient Agar plates, which were incubated at 30℃ for 48 h. The amount of colony forming units (cfu) of each plate was determined automatically or manually. Bacteria-retentive efficiency was determined by the following equation.

$$F_e(\%) = \frac{N_b - N_f}{N_b} \times 100 = \left(1 - \frac{N_f}{N_b}\right) \times 100$$

Where, F_e= Filter efficiency, N_b= Colony numbers on the surface of feed solution smear and N_f= Colony numbers on the surface of filtrate smear

Biodegradability

The BC microfilter was buried in humus soil in depth of 10cm, and was dug out to be taken a photo every week. The ratio of decomposed area to the total was evaluated as a degradation rate.

Results and Discussion

XRD analysis on BC before and after hydrolysis

BC was mechanically pulverized and hydrolyzed by acid (mixture of sulfuric acid and nitric acid or hydrochloric acid) to get fine fibrils. X-ray diffraction analysis showed the results of different acid hydrolysis. As shown in Fig. 2, X-ray diffraction intensity at 23° (2θ) in HCl-treated BC was higher (73%) than the control (60%). Here 23° (2θ) is corresponding to the crystallinity of BC. According to the previous studies, hydrolysis of cellulose by acid usually takes place in amorphous regions more than in crystalline regions andas the breaking process in amorphous region continues, the proportion of crystalline region to the total increases, that is, the crystallinity of BC increases[24,25]. In our study, hydrolysis of BC by hydrochloric acid increased the crystallinity of BC, however, hydrolysis by acid mixture showed a contradictory result to the previous study where BC nanocrystals was produced by either HCl or H_2SO_4 treatment[17,19]. The diffraction intensity at 23° (2θ) in BC treated with mixture of H_2SO_4 and $HNO_3$was lower (53%) than the control (60%). This is because the hydrolysis reaction by mixture of sulfuric acid and nitric acid occurs in the crystalline regions as well as amorphous regions. The strong oxidation by H_2SO_4 and HNO_3 breaks the glycosidic bond inside the sugar chain and even oxidizes the circular structure of sugar in the crystalline regions. This intense oxidization by acid mixture makes the crystallinity of cellulose decrease.

The oxidation capacity of HCl is not as strong as the mixture of H_2SO_4 and HNO_3, so HCl-based hydrolysis only destroys the amorphous regions and yields BC nanocrystals[17, 19, 26]. In some studies where they tried to get BC nanocrystals as a stabilizer for o/w emulsion, HCl hydrolysis was employed[24, 25]. However, in this work BC must be broken into nano-fibrils and modified so that H-bond cannot be reformed during the drying process to be fabricated into a microfilter. For this reason

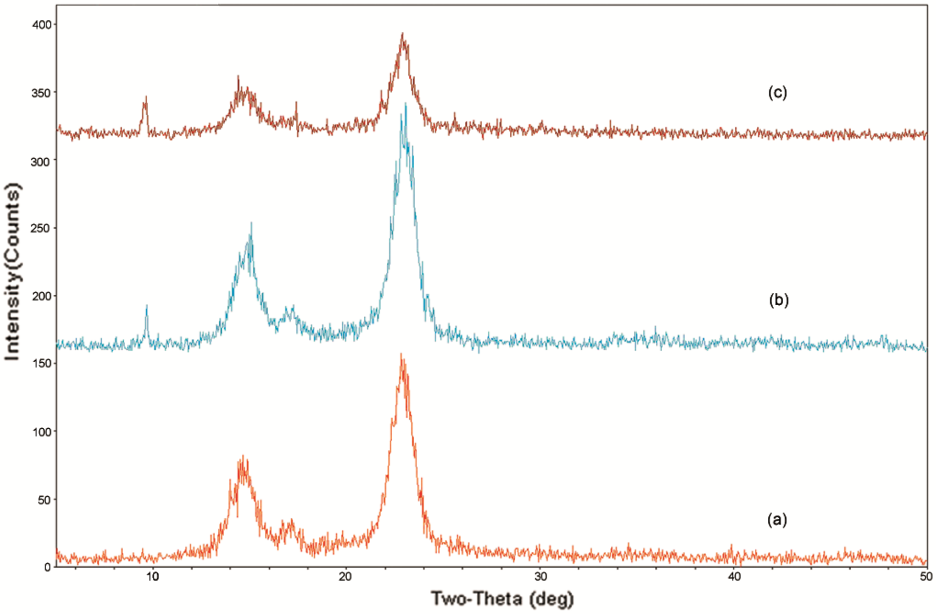


Fig. 2 — X-ray diffractograms of (a) mixed acid-treated BC, (b) HCl-treated BC and (c) untreated BC

hydrolysis by the mixture of H_2SO_4 and HNO_3 appears to be better than HCl.

Optical microscopy of the o/w emulsion and SEM analysis of BC microfilter

After preparation of BC hydrolysate, benzol and surfactant (OP-10) were added to prepare BC/benzol emulsion. 10 g of disintegrated BC (1%, wt), 1 mL of OP-10 and 5 mL of benzol were mixed and distilled water was poured until the total volume reached 50mL. It was ultrasonically irradiated for 20 min to become to the uniform emulsion. After 2 h, this emulsion was observed under light microscope. Microscopy showed tiny benzol spheres scattered over the whole area, whose diameter ranged approximately from 0.1 µm to 2 µm (Fig 3). The diameter of benzol spheres was measured by Adobe Photoshop CS6.

It is supposed that surface tension of benzol droplets becomes lower thanks to the surfactant OP-10, so emulsion is stabilized. While the membrane is being dried, water surrounding the benzol sphere evaporates first. As the water evaporates, the content of benzol in dispersed system increases, thus fusion of benzol droplets takes place. When the ratio of benzol to water reaches 1, stability of the dispersed system is destroyed and benzol evaporates rapidly. This makes the pore left in the place where there used to be a benzol droplet. SEM analysis of the fabricated BC microfilter confirmed this supposition.

Fig. 4 shows SEM figures of the native BC membrane (Fig. 4a) and BC microfilter fabricated by

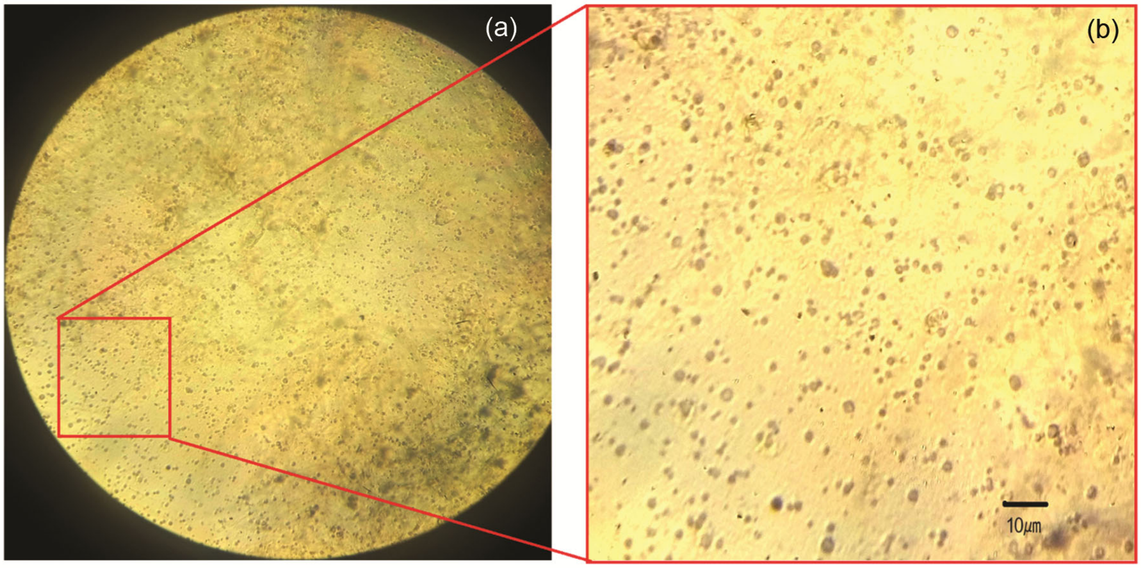


Fig. 3 — Optical microscopy of BC-benzolemulsion; magnification ×200

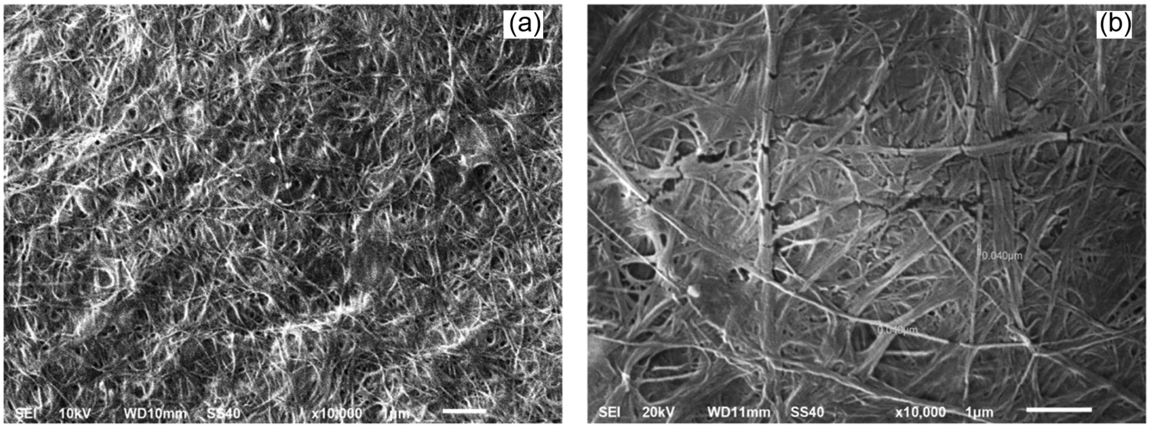


Fig 4 — SEM images of the surface of (a) native BC and (b) BCmicrofilter; magnification ×10 000 acid hydrolysis and o/w emulsionization (Fig. 4b). The SEM analysis illustrated some morphological differences between two samples. Native BC had very dense fibrillar structures and few pores, whereas the surface of the fabricated filter was less organized and had a lot of pores. These pores were formed by evaporation of benzol droplets. The reason why the pore size is much smaller than the real droplets can be explained because contraction of the fibrils around the pores occurs as the evaporation proceeds.

Effect of additive amount of benzol on the maximum pore size

SEM analysis shows the pores on the surface of the membrane, but it cannot indicate the size of actual path transpiercing the membrane. As the filtering performance depends on the actual size of transpiercing passage, a term of "equivalent pore size" is introduced[7, 21]. The equivalent pore size of the BC microfilter was determined by bubble-point test[20]. The equivalent pore size of the membrane filter was adjusted by varying additive amount of benzol to BC hydrolysate. Increasing the additive amount of benzol from 1 to 10 mL, the equivalent pore size of BC membrane filter increased from 0.1 to 0.5 µm (Fig. 5). When more than 10 mL of benzol was added, pore size variation was not observed. The maximum pore size of the microfilter was about 0.2 µm when the additive amount of benzol was 5 mL.

Water flux of BC membrane filter

The liquid permeability is one of the physicochemical characteristics of the membrane filter, indicating the filtration capacity in a definite time. Under the same pressure the higher the liquid permeability, the higher the filtration capacity. The liquid permeability which is related to pore numbers and pore size of the membrane is usually represented as water flux[21]. Water permeation velocity of the BC microfilter is shown in Fig. 6. Water permeation velocity which represents the pore size of the filter increased as the amount of added benzol increased. This indicates that the pore size depends on the amount of benzol. The water permeation velocity was 0.034 m·s^{-1} in the membrane prepared by addition of 5 mL benzol. According to the measurement of the pore size, the membrane with maximum pore size of 0.2 µm had water permeation velocity of 0.034 m·s^{-1} and water flux of 220 L/m^{2}h under the pressure of 1×10^5 Pa. The breakage pressure of this membrane was 1.2×10^6 Pa and water permeation velocity at this pressure was about 0.9 m·s^{-1}. These values are much higher with respect to the other BC membrane filters[5, 7, 15]and are not inferior to some commercial microfilters such as Millipore.

FTIR analysis

The FTIR spectra of cotton cellulose, native BC film and the fabricated BC microfilter were recorded in a range of frequency from 4000 cm^{-1} to 400 cm^{-1} and with a resolution of 8 cm^{-1} to identify the functional groups in the BC microfilter. All samples showed a band at 3345 and 1643 cm^{-1} which is assigned to OH stretching vibration and OH deformation vibration, respectively. They also showed a band attributed to CO asymmetric and symmetric stretching vibration in a range from 1032 to 1166 cm^{-1}, which confirms the presence of cellulose as the backbone of the samples. The BC microfilter showed band due to NO_2^- group at 1512 cm^{-1}, 1247 cm^{-1} and 832 cm^{-1}but hemicellulose and native BC film did not, which means BC fibrils were modified with NO_2^- group when it was hydrolyzed by the acid mixture. However, band for SO_3^{2-} group was not observed. When cellulose is treated with the mixture of undiluted sulfuric acid and nitric acid (ratio 1:1, v/v),

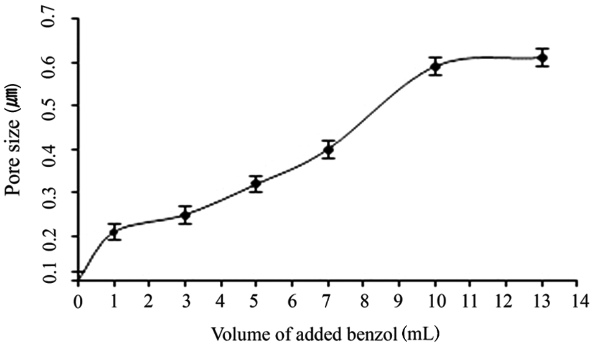


Fig. 5 — Variation of pore size of the membrane with the volume of added benzol

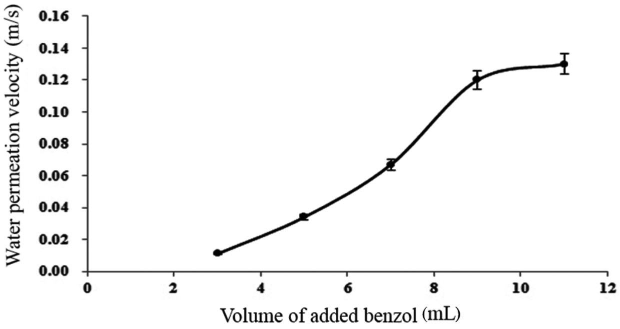


Fig. 6 — Variation of water permeation velocity of the BC microfilter with the volume of added benzol

it is considered that undiluted sulfuric acid acts as a dehydrator and nitric acid adds NO_2^- to cellulose. In consequence, nitrocellulose is obtained and the nitration degree depends on water content of reaction system and concentration of HNO_3. The higher the concentration of HNO_3, the higher was the nitration degree[8]. The experimental result shows that when BC is hydrolyzed, nitration occurs slightly but BC contains a large amount of water and has strong network structure and high crystallinity unlike plant cellulose, thus, acid hydrolysis reaction of cellulose is more predominant than nitration[17]. The analytical results are shown in Fig. 7.

ζ -potential of the BC microfilter

It is necessary to characterize the electrostatic interaction between filter and separating particles to develop a new biofilter and improve its filter efficiency. When the absolute value of zeta potential is high, the electrostatic repulsion force between particles is greater than van der Waals attraction force[21]. Since ζ-potential of most bacteria is about -10 mV to -50 mV, the filter should have a negative electrochemical potential to enhance bacterial filter efficiency.The higher the absolute value of ζ-potential, the higher was the filtering efficiency by electrostatic repulsion. ζ-potential of BC filter membranes was measured by electroosmosis method. 0.1 mol/L KCl (pH 7.0) was used as an electrolyte solution. Temperature (T) was considered as 293 K, the permittivity of water (ε) was 81 and viscosity (η) was 1.0× 10^{-3} Pa s. ζ-potential of the membrane filters fabricated under various additive amount of benzol was measured. The result was given in Table 1. As the table shows, there was no significant potential variation of the membrane filters varying the amount of added benzol. This indicates that benzol affects only pore size and pore numbers, but not ζ-potential. The average value of ζ-potential was about -20 mV, which can further enhance the filterability of the membrane filter by electrostatic repulsion.

Bacteria-retention efficiency of BC microfilter

The filter efficiencies (F_e) of the BC microfilter were shown in Tables 2 and 3. The filter efficiency against *E. coli* and *B. diminuta* with high. CFU (7.6×10^7/mL and 4.3×10^7/mL, respectively) was over 99.9%. F_e was 100% for the 10^5 times diluted solution of bacteria culture. It seems that the newly developed BC microfilters are comparable to the other

Table 1 — ζ-potential of membrane filters varying the additive amount of benzol (n=3, $p<0.05$)

Additive amount of benzol (mL)	$v\cdot10^8$ ($m^3\cdot s^{-1}$)	-ζ (mV)
0	0.35±0.01	5.0±0.2
1	1.39±0.05	19.3±0.6
5	1.49±0.02	21.0±0.3
7	1.40±0.03	19.8±0.4

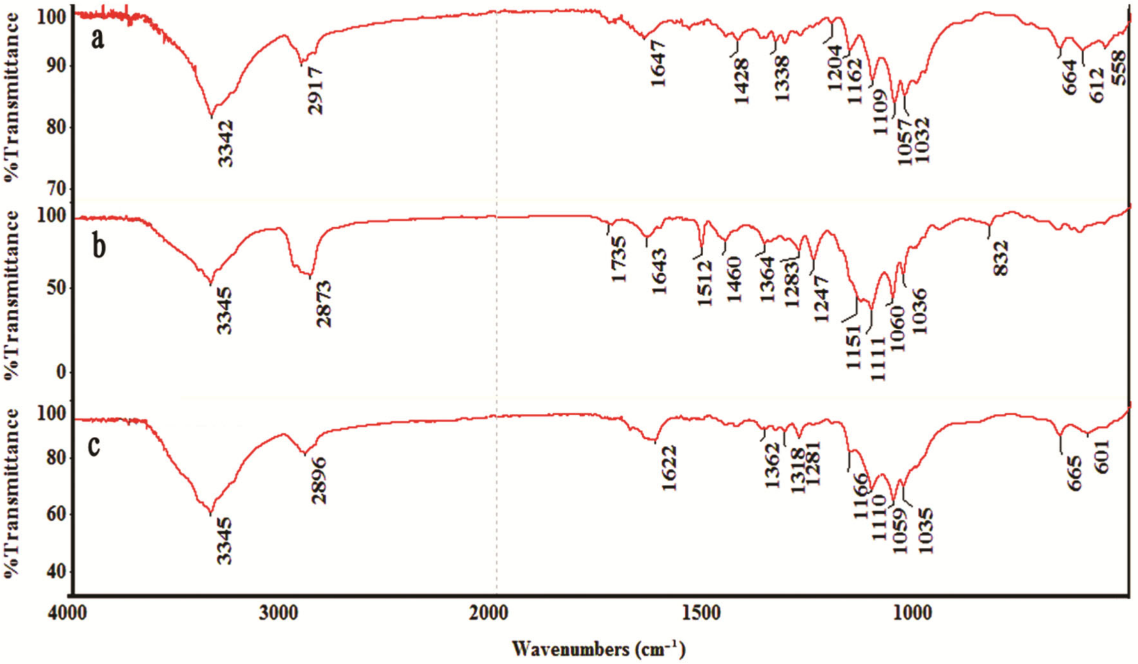


Fig. 7 — FTIR spectra of (a) cotton cellulose, (b) BC filter membrane and (c) native BC

Table 2 — Filter efficiency of various filter membranes against *E. Coli* (n=3, $p<0.05$)

Filter	CFU before filtration (mL)		CFU after filtration (24 h culture)	CFU after filtration (36 h culture)	CFU after filtration (48 h culture)	F_e (%)
BC micro-filter	Undiluted	7.6×10^7	0	1.0±0.3	7.7±1.1	99.9
Millipore			0	4.0±0.7	8±1.3	99.9
BC micro-filter	10^5×diluted	760±10	0	0	0	100
Millipore			0	0	0	100

Table 3 — Filter efficiency of various filter membranes against *B. diminuta* (n=3, $p<0.05$)

Filter	CFU before filtration (mL)		CFU after filtration (24h culture)	CFU after filtration (36 h culture)	CFU after filtration (48 h culture)	F_e (%)
BC micro-filter	Undiluted	4.3×10^7	0	2.0 ±0.7	7.7±1.1	99.9
Millipore			0	3.6±0.9	8.7±1.1	99.9
BC micro-filter	10^5×diluted	430±10	0	0	0	100
Millipore			0	0	0	100

Fig. 8 — Degradation process of the BC microfilter inside humus soil: (a) beginning day, (b) 2 weeks later and (c) 4 weeks later

conventional bacteria-retentive filters such as Millipore (PVDF, US, 0.22 μm)[11].

This result shows the potential of development of BC-oriented microfilter. Microfilters are used for several purposes such as production of ultra-pure water, separation of blood cells, air filtration etc. Bacteria-retentive filter is a kind of microfilter, which is essentially required in food and water processing, cosmetic production, drug industry, GMP standardization and so on, has a great significance in the development of national economy. BC is a natural biofibre produced on a large scale by food wastes and has several advantages of membrane filter. Another benefit of BC is biodegradability and non-toxicity. Disintegration, o/w emulsionization by addition of benzol and drying process provides BC membrane with pores of suitable size. The pore size in BC can be regulated by amount of added benzol. BC-oriented microfilters are cost-effective, eco-friendly, versatile (applicable in wide range of pH, temperature and pressure) and efficient. One considerable problem in synthesis of BC filter is utilization of strong acids, which is not good for environment. This problem would be successfully overcome, if appropriate receipt for physical disorganization of BC was established. Studies on mechanical and ultrasonic pulverization of BC will be carried out.

Biodegradability of the BC microfilter

The fabricated BC filter showed good biodegradability. Its degradation rate was about 50% after 2 weeks, and it was totally decomposed after 4 weeks (Fig.8).

Conclusion

The synthesis method involving disintegration of BC by acid hydrolysis, o/w emulsionization by addition of benzol and drying process enables to fabricate a new BC-oriented membrane microfilter. The membrane filter manufactured by addition of 5 mL benzol to 10 g BC hydrolysate (1%, wt) had maximum pore size of about 0.2 μm and 220 $L/m^2\cdot h$of water permeation flux under 0.1 MPa. The breakage pressure was 1.2×10^6 Pa. The microfilter with maximum pore size of 0.2 μm demonstrated filterability of over 99.9% against *Escherichia coli* and *Brevundimonas diminuta.* These values are comparable to the other commercialized microfilters. The new BC filter was totally decomposed within a month. This strategy gives a new prospect to the development of cost-effective, eco-friendly and highly efficient biofilter. Non-corrosiveness, pH- and temperature- resistance, high mechanical strength and biodegradability of BC provide its versatile application in filtration and separation field. The newly developed BC microfilter can be used for water processing, removal of bacteria from food, cosmetic and drug materials, collection of bacterial bodies from culture media and purification of bioengineering products, etc. In order to guarantee 100% friendliness to environment, more eco-friendly method of BC disintegration than acid hydrolysis should be developed.

Conflicts of Interest

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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