Journal of Membrane Science 674 (2023) 121509

Contents lists available at ScienceDirect

Journal of Membrane Science

journal homepage: www.elsevier.com/locate/memsci

Biosynthesis and characterization of bacterial cellulose membranes presenting relevant characteristics for air/gas filtration

Arooj Fatima [a], Paloma Ortiz-Albo [a], Luísa A. Neves [a], Francisco X. Nascimento [b,**], João G. Crespo [a,*]

[a] LAQV/Requimte, Department of Chemistry, NOVA School of Science and Technology, FCT NOVA, Universidade NOVA de Lisboa, Campus da Caparica, 2829-516, Caparica, Portugal

[b] IBET, Instituto de Biologia Experimental e Tecnológica, Apartado 12, 2781-901, Oeiras, Portugal

ARTICLE INFO

Keywords:
Bacterial cellulose
Komagataeibacter
Porous membranes
3D porous morphology
Air/gas filtration

ABSTRACT

The production of bacterial cellulose has gained prominence in recent years as an alternative for the sustainable production of materials that might be used in diverse processes and applications. The present study discusses the possibility of producing tailored bacterial cellulose membranes *in situ*, that present relevant characteristics for potential use in air/gas filtration. Various cultivation processes and characterization studies were performed to ascertain the suitability of *Komagataeibacter* sp. FXV3, *Komagataeibacter* sp. NFXK3, and *K. intermedius* LMG 18909 bacterial strains to produce cellulose membranes with diverse properties. Subsequently, the bacterial cellulose films produced were freeze-dried to obtain stable membranes, and extensively characterized for their physicochemical properties. The results obtained showed that different strains enabled the synthesis of membranes with distinctive morphological properties. Moreover, the different carbon sources and ethanol concentrations employed in the cultivation media led to modifications in the cellulose membranes produced by the different *Komagataeibacter* strains, which further impacted membrane morphology and, ultimately, gas filtration behavior. All the synthesized membranes were fully characterized, showing adequate mechanical properties, and tested for permeance of N_2, CO_2 and O_2, opening perspectives for their use in air/gas filtration.

1. Introduction

The demand for renewable and sustainable bio-based materials has considerably increased in recent years due to environmental concerns. Bio-based materials with high purity, mechanical stability, and barrier properties have become a widespread choice for research community to develop environmentally friendly systems [1]. Among a wide range of biomaterials, cellulose is a highly abundant and widely used biopolymer, produced mainly by plants [2,3]. Still, the use of plant cellulose presents some challenges such as its low purity and crystallinity. In 1886, Brown marked the discovery of bacterial cellulose (BC), produced by *Acetobacter xylinum* (now reclassified as *Komagataeibacter xylinus*) that was chemically identical to plant based cellulose [4]. Off late, bacterial cellulose has attracted great attention due to its remarkable properties in terms of purity, large specific surface area, crystallinity, mechanical stability, water uptake capacity, three-dimensional fiber structure, biocompatibility and biodegradability [5–8].

Members of the *Komagataeibacter* (e.g., *K. xylinus*) and *Novacetimonas (N. hansenii)*, are amongst the best producers of crystalline bacterial cellulose [9]. Under static conditions at the air-liquid interface, the bacterial strains form cellulose membranes exhibiting a three dimensional open network fibrous structure [3] containing nano and microfibrils of cellulose with 10–100 nm diameter. Nevertheless, the cellulose membranes produced by the different bacterial strains possess distinctive physical and chemical properties [7,10], which are greatly influenced by the bacterial cultivation processes. In this way, the overall bacterial cellulose biosynthesis rate can be varied using carbon substrates with different molecular weight and chemical structure (e.g., glucose, fructose, glycerol). Other than carbon sources, components such as ethanol, might be added to the culture media to boost the bacterial cellulose production [11–13]. In addition, bacterial cellulose membrane physical properties (porosity, fiber diameter, permeability, surface wettability, surface area, crystallinity, water absorption and retention, mechanical strength) can also be significantly tuned by

* Corresponding author.
** Corresponding author.
E-mail addresses: Francisco.nascimento@ibet.pt (F.X. Nascimento), jgc@fct.unl.pt (J.G. Crespo).

https://doi.org/10.1016/j.memsci.2023.121509
Received 4 January 2023; Received in revised form 19 February 2023; Accepted 21 February 2023
Available online 22 February 2023
0376-7388/© 2023 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

modulating the medium composition and cultivation conditions, including carbon sources, ethanol addition, pH and temperature [7,14–17].

From the application perspective, the unique properties of bacterial cellulose places it as a promising material for a wide range of applications. These include pharmaceutical (drug delivery systems, tissue regeneration, vascular grafts, antimicrobials and wound healing), food applications (dietary supplements, platform for prebiotics delivery, component to prepare vegetarian meat), electrical and sensor applications, immobilization platforms and energy production (bacterial cellulose hydrogel membrane incorporated with acrylic acid (AAc)-co-acrylamide (AAm)-co-methyl methacrylate (MMA) are being used in osmotic energy conversion), to mention a few [16,18–28]. Importantly, recent studies have demonstrated that bacterial cellulose films, produced biologically and *in situ*, could be used in a wide range of membrane applications [16,17,21,24–26]. For example, bacterial cellulose films containing living microorganisms have been successfully used as self-healing membranes with tunable properties such as permeability and selectivity [26]. Moreover, when compared to other commercial polymeric membranes, bacterial cellulose membranes present lower production costs and higher environmental sustainability as these membranes are biodegradable and their fabrication does not require harmful solvents such as N-methyl-2-pyrrolidone (NMP), tetrahydrofuran (THF), N,*N*-dimethylformamide (DMF) and other chemical pollutants [29]. While bacterial cellulose membranes have been applied in some areas, their use as membranes for air/gas filtration is in an early stage [18,30–33]. The filter like fibrous bacterial cellulose membrane structure allows us to explore the possibility for environmentally benign applications like air filters. Additionally, developing bacterial cellulose in a dry form and assessing its barrier properties is a step toward establishing bacterial cellulose as a competitor among other commercial filters, which has not yet been adequately explored [32].

This work aims to identify the simplest, inexpensive, and suitable bacterial cellulose membrane fabrication conditions. Moreover, present study also defines the best conditions to dry the hydrogel like membranes to obtain stable membranes with reproducible properties. For this purpose, three different *Komagataeibacter* strains were tested for its ability to synthesize cellulose membranes presenting relevant characteristics for application in air/gas filtration. Several carbon sources and ethanol concentrations were tested in the attempt to modulate the properties of the bacterial cellulose membranes produced *in situ*. As a result, differentiated dry bacterial cellulose membranes, with stable functional structures and properties were obtained, opening the possibility to use bacterial cellulose membranes in actual applications.

2. Experimental

2.1. Bacterial strains

The bacterial strains *Komagataeibacter* sp. FXV3, *Komagataeibacter* sp. NFXK3, and *K. intermedius* LMG 18909 were used in this study due to their ability to produce a high yield of cellulose [34]. These strains were routinely cultured in Hestrin and Schramm medium (HS) (5 g/L yeast extract, 5 g/L peptone, 1.15 g/L citric acid, 2.7 g/L Na_2HPO_4, pH 6) supplemented with 20 g/L glucose (original HS formula) or 20 g/L glycerol.

2.2. Preparation of inoculum

Before testing the effect of ethanol and carbon sources in cellulose biosynthesis assays, the bacterial strains were firstly grown in HS (glucose) agar plates for six days at 28 °C. Following this, each grown colony was isolated from the Petri dish containing HS agar and tested for bacterial cellulose production. Three loops (~10 μL) of fresh grown bacterial strains were inoculated in a sterile 50 mL falcon tube containing 10 mL of liquid HS (glucose) medium and vigorously agitated at a maximum speed for about ≥30 s until the growth media became cloudy and cellulose aggregates broke apart. 5 mL of each supernatant was withdrawn and used for the determination of OD_{600}. Solution normalization was performed to adjust the bacterial inoculum standard concentration to $OD_{600} = 0.5$ (~2×10^7 CFU/mL) and this was used in further experiments [34].

2.3. Effect of carbon sources and ethanol on bacterial cellulose production

To assess and compare the bacteria's efficiency to produce cellulose membranes, two different pure carbon sources, glucose and glycerol were used. Glucose is a known main precursor for cellulose biosynthesis [5]. Glycerol was selected due to its capability to be easily metabolized and is also a low cost carbon substrate [35]. Different ethanol concentrations (1.5%, and 3.0% (v/v)) were added in the medium to understand its effects in the overall bacterial cellulose production.

2.4. Bacterial cellulose membrane biosynthesis under different culture conditions and purification

Bacterial cellulose membranes were produced in 60 mm plates under static conditions. Briefly, 7.5 mL of the selected HS medium and 75 μL of working bacterial solution ($OD_{600} = 0.5$) were added in each plate to develop membrane formation. The plates were sealed and incubated without agitation at 28 °C for seven days. Bacterial cellulose membranes formed on the surface of HS medium were collected after the incubation period, rinsed with deionized water, immersed in 0.1 M NaOH solution and incubated at 42 °C overnight. Following this, the pre-treated gel-like membranes were soaked overnight in deionized water at room temperature to remove the remaining growth media embedded inside. The overall procedure for membrane preparation is illustrated in Fig. 1.

2.5. Bacterial cellulose membrane storage, drying, and yield calculation

Bacterial cellulose gel membranes were stored in distilled water at 4 °C (1–2 weeks maximum). Gel bacterial cellulose membranes were dried exploring two different procedures. The first approach was to dry the gel membranes in a desiccator in the presence of a NaCl salt solution with a controlled water activity of 0.75 (75% RH) at 30 °C [36]. It was observed that membrane's water activity did not equilibrate even after two weeks of storage. The second method was to freeze dried the gel membranes at - 55 °C for 24 h and then, weighted in an analytical scale. This approach was relatively fast and preserved the membrane fibrous structure. Moreover, membranes produced under the same conditions and dried according to this procedure show a high degree of reproducibility, with identical membrane appearance, texture and mechanical properties. Therefore, it was decided to process all gel membranes by freeze drying. The bacterial cellulose production was recorded as the dry weight of cellulose obtained per volume of medium (g/L). The following equation was used to calculate the yield of production.

$$Yield\ (\%) = \frac{m_{BC\ dry}}{m_{carbon\ source}} \times 100 \qquad \text{Eq.(1)}$$

Here, $m_{BC\ dry}$ is a dry weight of the bacterial cellulose membrane (g) and $m_{carbon\ source}$ is the initial weight of carbon source (e.g., glucose or glycerol) added in the media (g).

2.6. Characterization of bacterial cellulose membranes

2.6.1. Structural and morphological properties

2.6.1.1. X-ray diffraction (XRD). After drying, the crystallinity of bacterial cellulose material was determined using XRD. The characterization was performed by using a multipurpose diffractometer (Panalytical X'PERT PRO). X-ray diffraction patterns were recorded at CuKα radiation wavelength (λ = 0.154 nm) generated at a voltage of 40 kV and a

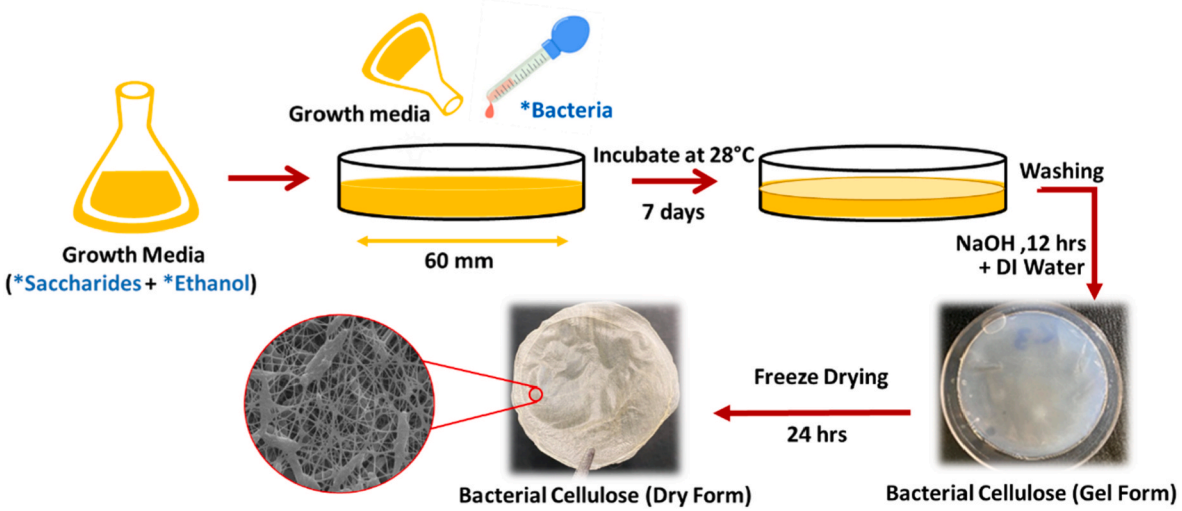

Fig. 1. Procedure for Bacterial Cellulose membrane fabrication. (*) represent the studied variables.

filament emission of 35 mA, with 0.033° step, from 5° to 40° (2θ,angle). The sample crystallinity was calculated based on the X-ray diffraction measurements using the following Equation:

$$Crystallinity\ (\%) = \frac{A_{cr}}{A_T} \times 100 \qquad \text{Eq.(2)}$$

Where, A_{cr} is the area of crystalline peaks and A_T is the total area including the crystalline and amorphous peaks (an example of the calculation process is presented in the supporting information, section S2).

2.6.1.2. Scanning electron microscopy (SEM). Scanning electron microscopy (SEM) images were obtained with a Jeol (JSM70011F) SEM, Japan at 15 kV to observe the morphology of the bacterial cellulose membranes. The samples were freeze dried and gently placed on Aluminum stub using a conductive tape. Before doing the SEM, the samples were coated with a thin layer of Au/Pd using a sputter coater (Q150T ES) to improve conductivity. The top and bottom surfaces of each sample were examined at magnifications of×1000 and × 10,000. Moreover, the fiber size, pore size and surface porosity were quantitatively determined from the SEM images acquired, using the ImageJ software [37].

2.6.1.3. Nitrogen adsorption/desorption. N_2 adsorption/desorption was used to measure the specific surface area of bacterial cellulose. Isotherms were obtained at 77 K using a Micrometrics ASAP 2010 equipment, USA. Prior to the measurement, the freeze-dried bacterial cellulose samples were degassed at 80 °C for about 12 h in continuous dry N_2 flow to remove any adsorbed water molecules. A freeze-dried bacterial cellulose sample mass of 15 mg was used for each measurement.

2.6.2. Chemical-physical properties analysis

2.6.2.1. Fourier transform infrared (FTIR). FTIR was used to analyze the chemical composition of freeze-dried bacterial cellulose membranes. FTIR spectra were recorded with a PerkinElmer Spectrometer (PerkinElmer Inc., Waltham, MA, USA). The spectra were recorded in the 4000 cm^{-1} to 600 cm^{-1} region, with a 1 cm^{-1} resolution. Each data point was the result of the accumulation of 10 scans.

2.6.2.2. Bacterial cellulose surface wettability. To evaluate the membrane surface wettability, the bacterial cellulose membranes were sliced into a square with a dimension of 2 × 2 cm and attached to a drop shape analyzer (DSA 25B, Kruss GmbH, Germany). Water and glycerol were used as the probe liquid for measuring surface wettability. The droplet was manually placed on the membrane surface using a small syringe. Immediately after the droplet landed on the membrane surface, a 10 s frame measurement was collected. The surface wettability measurements of each sample were repeated three times.

2.6.3. Mechanical properties analysis

The Young's Modulus and tensile strength of bacterial cellulose membranes were determined with a texture analyzer equipment (TAXT plus, Stable Micro Systems, England) using the load cell of 5 kg. Freeze dried membranes were cut into rectangular membrane strips of 45 mm × 15 mm dimensions. Subsequently, the membrane test samples were clamped in between the grip of the texture analyzer. The tensile force was tracked by moving the upper clamp upward at a cross head speed of 0.5 mm/s for each sample until the sample broke. The experiments' command was assured by a Texture analysis software and results in the form of Stress Strain curve were recorded in a computer connected to the equipment. Stress was calculated in Pascal as the tensile force divided by the cross-sectional area measured as the product of the length and the width of sample. Strain was calculated using **Eq. (3)**:

$$\varepsilon = \frac{\Delta L}{L_0} = \frac{(L - L_0)}{L_0} \qquad \text{Eq.(3)}$$

where L (mm) is the elongation from initial length L_0 (mm). The Young's Modulus and tensile strength of bacterial cellulose membranes were expressed in mega Pascal. Three strips of each membrane were analyzed, and the average value was calculated. Calibration of force was done for each sample using a load cell of 5 Kg.

2.6.4. Membrane gas permeation

Gas permeation studies for the membranes produced were performed by using two different set-ups, according to the permeability of the membranes tested.

Membranes which were dense or having low permeability were characterized by using the set-up present in Fig. 2a, reported previously [38]. The membranes permeability was tested for three different gases: CO_2, N_2 and O_2, using an absolute pressure difference of 0.5 bar as the driving force. Prior to their characterization, the freeze-dried bacterial cellulose membranes were stored in a desiccator at controlled temperature and relative humidity (30 °C, 75% RH).

Highly permeable membranes could not be characterized in the same set-up and therefore were characterized using a continuous gas feed flow set-up (Fig. 2b). This set-up is constituted by a pure gas feed stream

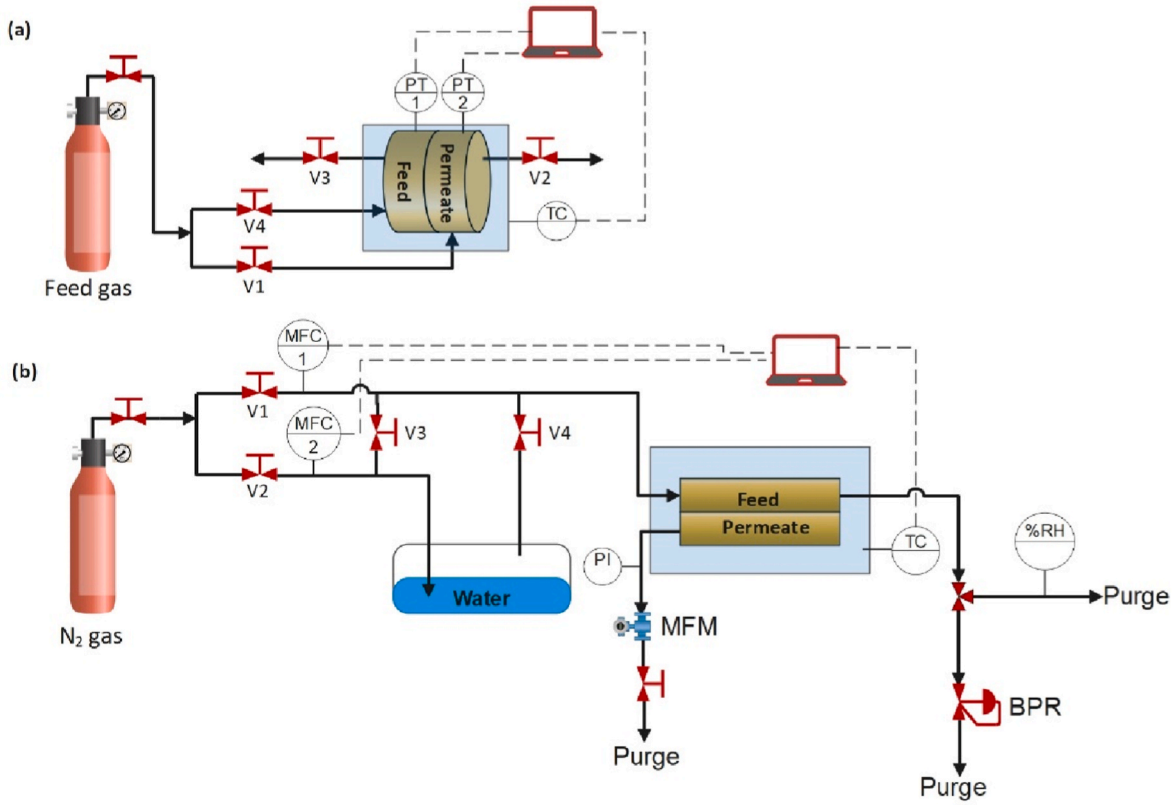

Fig. 2. Gas permeation set-ups: (a) low permeable membranes – closed system (b) high permeable membranes – open system and continuous flow. **NOTE:** V refers to valves; PT, pressure transmitter; PI, pressure indicator; TC, temperature controller; MFC, mass flow controller; MFM, mass flow meter; BPR, back pressure regulator; and % RH, relative humidity indicator.

controlled with two mass flow controllers (MFC1 and MFC2), a back-pressure controller in the retentate side (BPR), and an outlet mass flow meter in the permeate side (MFM). The experiments were carried out at 30 °C, with pure gases with a controlled relative humidity of 75% RH (relative humidity). The desired relative humidity was obtained from the mixture of a non-humidified gas stream (MFC1, as in Fig. 2b) and a humidified gas stream (MFC2, as in Fig. 2b). Gas humidity percent was confirmed using a hygrometer-thermometer probe (% RH in Fig. 2b). The gas flux through the membrane was obtained from the permeate outlet flow (Eq. (4)) at different driving forces of up to 0.5 bar. Membrane gas permeance was calculated using the Fick's law equation **Eq. (5)** which was converted into permeability by dividing by the membrane thickness measured with micrometer. Subsequently, the ideal selectivity was also calculated as the ratio of the gas permeability values, obtained from experiments with each individual gas. Permeability measurements performed with the two different set-ups show a very good adherence, when plotted against the membranes surface porosity or fiber diameter.

$$J = F^{p}_{out}/A \qquad \text{Eq.(4)}$$

$$J = L \cdot \left(p^{f} - p^{p}\right) \qquad \text{Eq.(5)}$$

Where J is the flux through the membrane ($cm^3 . cm^{-2} . s^{-1}$); F^{p}_{out}, permeate flow measured by outlet mass flow meter (MFM) ($cm^3 . s^{-1}$); A, membrane area (cm^2); L membrane permeance (cm^3(STP). $cm^{-2} . s^{-1} . cmHg^{-1}$); and, p, pure gas pressure, given by back pressure regulator (BPR) at the retentate and pressure indicator (PI) for feed and permeate side, respectively.

3. Results and discussion

3.1. In situ bacterial cellulose production

The cellulose biosynthesis potential of the strains NFXK3, LMG 18909, and FXV3 was tested in HS media using two pure carbon sources (glucose and glycerol) and different ethanol concentrations (0%, 1.5%, and 3.0% v/v). It was found that strain NFXK3 produced denser and homogeneous bacterial cellulose membranes in all the tested conditions, when compared to strains LMG 18909 and FXV3 (Fig. S1). Moreover, strain NFXK3 produced the highest cellulose amount using glucose and glycerol as carbon sources (9.61 and 11.46 g/L), respectively (Fig. 3). Nevertheless, strain LMG 18909 biosynthesized cellulose in higher amount when using glucose as carbon source (5.93 g/L), while no significant differences were observed in strain FXV3 cellulose production abilities in terms of the effect of carbon source (glucose and glycerol) under the same cultivation conditions (5.65 and 5.05 g/L) (Fig. 3).

Previous studies have demonstrated that other than bacterial strains and carbon sources, bacterial cellulose production is highly influenced by the addition of ethanol in the growth medium [5,10,39,40]. The results obtained in this work showed that the bacterial cellulose production in the presence of ethanol is higher than without ethanol (Fig. 3), further corroborating the previously described results. Ethanol is oxidized to acetic acid, which is a feature of acetic-acid bacteria. High levels of acetic acid are extremely toxic and kill other bacterial competitors and may also be used as C source by the producing strain [13]. As different strains present different tolerances to acetic acid this may impact their ability to synthesize cellulose. It should be noted that, for strain NFXK3 with the glycerol source, addition of 1.5% ethanol is the optimum amount if the objective is to favor the maximum production of cellulose. Whereas, for the other strains, 3.0% of ethanol leads to a

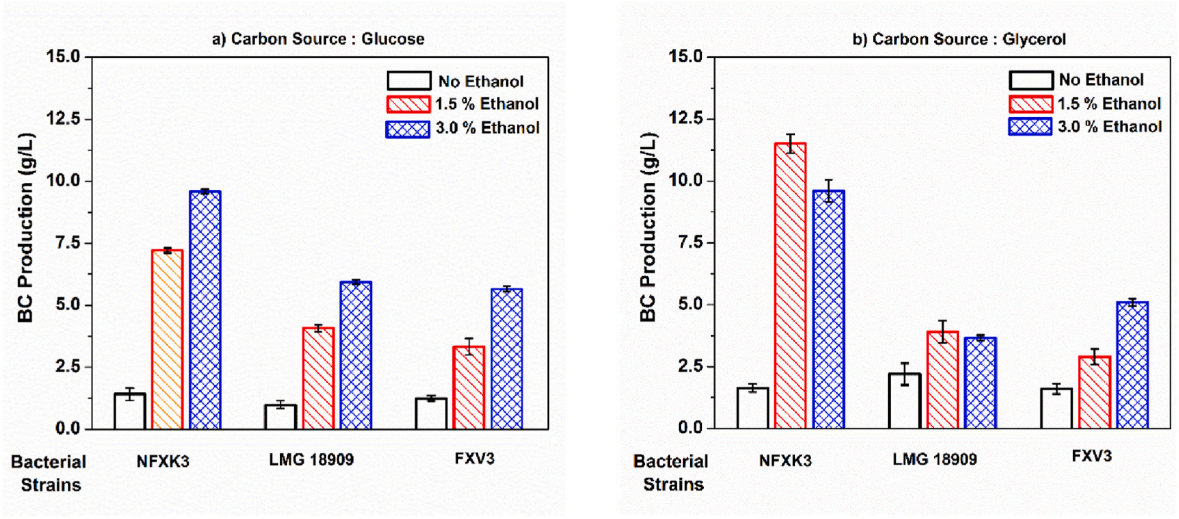

Fig. 3. Bacterial cellulose production (g/L) by NFXK3, LMG 18909, and FXV3 strains under the following conditions: 28 °C, 7 days incubation, pH 6. Cultivated in media with (a) glucose; (b) glycerol, under different ethanol concentration (0%, 1.5% and 3.0%).

higher bacterial cellulose production either using glucose or glycerol. Due to the limited cellulose production under no ethanol addition, the remaining section of this work will only include the study of bacterial cellulose membranes produced with ethanol concentrations of 1.5% and 3.0%. Moreover, the strain and cultivation conditions selected correspond to the membranes with the best properties to act as a filter.

3.2. Structural and morphological properties

3.2.1. X-ray diffraction analysis

The crystallinity of the obtained bacterial cellulose membranes was evaluated under the various cultured conditions as reported in Table 1. All bacterial cellulose membranes showed a diffraction profile of cellulose with prominent peaks at 2θ of 14.5°, 16.6°, and 22.7° that correspond to the Miller indices of diffraction planes of (100), (010), and (110), respectively (Fig. 4) [41,42]. It is clearly identified that the analyzed set of peaks attributed to the highly crystalline structure of cellulose I, tended to have a specific orientation in dried bacterial cellulose films [2,3,5]. Our data indicate that bacterial cellulose membranes produced from both carbon substrates show similar diffraction with high crystallinity ranging from 71 to 85%. The same trend was reported for bacterial cellulose produced by other bacterial strains [5, 43]. Overall, both carbon sources have the potential to produce bacterial cellulose with high crystallinity. In contrast, cellulose produced from plants only show 40–60% crystallinity [7,44].

3.2.2. Bacterial cellulose morphology and SEM image analysis

The detailed morphology of the bacterial cellulose membranes produced by the three bacterial strains (NFXK3, LMG 18909, and FXV3) with two carbon sources using the optimal ethanol conditions selected in this study were examined by SEM (Fig. 5). Both top and the bottom surfaces of the membrane were analyzed. In all cases, the densest surface was the top surface (the surface in contact with air during the cutivation process), which seems almost identical for all the selected strains (Fig. S3). The bottom surfaces were clearly less dense (Fig. 5). The images of the bacterial cellulose bottom surface revealed that each bacterial strain remarkably lead to a different interwoven pattern when producing bacterial cellulose. Therefore, to understand the differences in morphology, the fiber size, pore size and surface porosity were quantitatively determined from the SEM images and summarized in Table 2. The results showed that strain NFXK3 formed comparatively more compact, smooth, and dense bacterial cellulose surfaces, where fibers were tightly aggregated. Whereas the bacterial cellulose membranes formed by the FXV3 strain exhibited the thinnest porous cellulose fibers with high surface porosity, up to 48% and 30% using glucose and glycerol, respectively. It is also worth mentioning that the increase in ethanol concentration from 1.5% to 3.0% altered the bacterial cellulose morphology significantly by lowering the surface porosity and the pore size, resulting in surfaces with more compact densely interweaved fiber structures (Fig. 5b). A similar effect has been reported and explained in a study by Kazemi et al. [45].

The calculated average pore size range for bacterial cellulose membranes are typically below 200 nm (Table 2), which is similar to the results reported in a study by Mautner et al. [46]. This pore size range makes these membranes excellent candidates for air/gas filtration, if the aim is to retain not only particles but also bacteria. Moreover, carbon source comparison showed that glycerol-based membranes were more compact than glucose-based membranes. This morphology variation could be explained in terms of various factors such as microorganism properties, how they consume the carbon source, their metabolic path and kinetics [35,47,48]. Besides the bacterial strain, ethanol content and carbon source, it is also important to take into account the effect of oxygen diffusion that has an impact in order to grow the selected bacteria strains and tune the surface morphology [49].

Table 1
Crystallinity (%) of bacterial cellulose membranes produced by NFXK3, LMG 18909, and FXV3 strains using as carbon source glucose or glycerol, at various ethanol concentrations (1.5%, 3.0%).

Bacterial Strains	Ethanol (%)	Crystallinity (%)	
		Glucose	Glycerol
NFXK3	**1.5**	76	83
	3.0	78	80
LMG 18909	**1.5**	83	80
	3.0	84	71
FXV3	**1.5**	79	78
	3.0	84	85

3.2.3. Nitrogen adsorption/desorption

The surface area of bacterial cellulose membranes was determined using N_2 adsorption/desorption isotherms and reported in Table 2. Both glucose and glycerol supported the bacterial growth that led to the production of membranes with diverse thicknesses, surface porosity and fiber diameter (Table 2). SEM images revealed that pores and fiber channels of varied sizes were present in the structure of all the bacterial cellulose membranes. Among all strains, FXV3 produced membranes

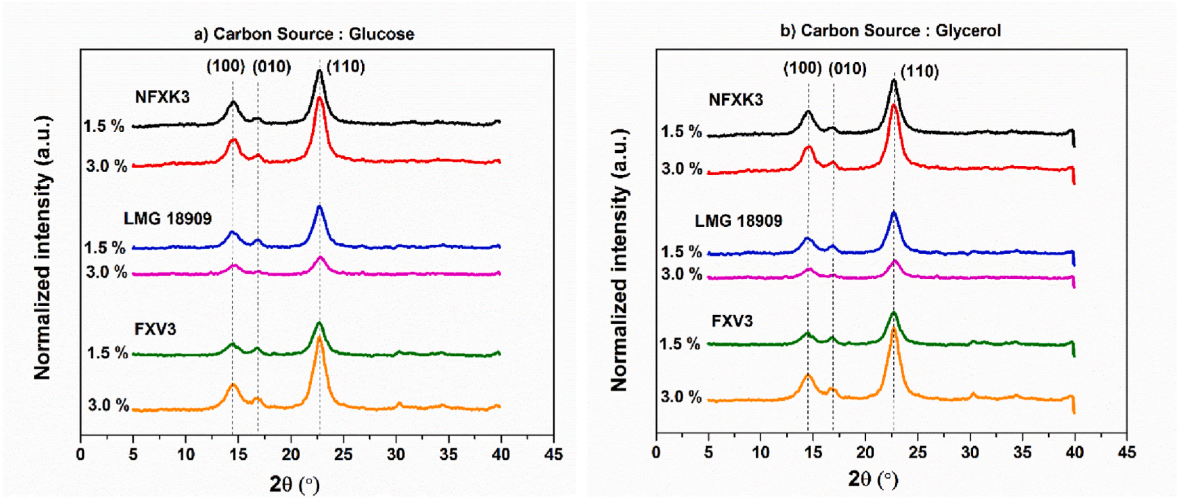

Fig. 4. Comparative X-ray diffraction (XRD) analysis of bacterial cellulose membranes produced by NFXK3, LMG 18909, and FXV3 strains using two carbon sources (a) glucose; (b) glycerol, at various ethanol concentrations (1.5%, 3.0%).

with the highest surface area. Furthermore, and overall, adding ethanol made the bacterial cellulose membrane structure denser, resulting into low surface areas. Interestingly, NFXK3 showed the lowest surface area using both glucose and glycerol.

3.3. Chemical-physical properties analysis

3.3.1. Fourier transform infrared (FTIR) spectroscopy analysis

The FTIR spectra of bacterial cellulose membranes prepared from strains NFXK3, LMG 18909, and FXV3 under different conditions were obtained (Fig. 6). Despite the fact that carbon substrates and ethanol concentration had a considerable impact on bacterial cellulose production (Fig. 3), all FTIR spectra revealed the typical cellulose vibration bands with little variations. The broad absorption band at 3340 cm^{-1} is attributed to the –OH strectching present and C—H streching appears at around 2800-2900 cm^{-1} in the bacterial cellulose network. The strong CH_2 bond stretching is visible at 1427 cm^{-1}. The absorption peaks from 800 to 1200 cm^{-1} are associated with the chemical groups (C — C) present in polysaccharides [5,42,43,50]. The results obtained showed that the bacterial cellulose prepared from both glucose and glycerol presented the same chemical composition, with high purity. Moreover, the purity of cellulose produced from bacteria is higher than that of plant based cellulose [51]. The complete analysis of functional groups corresponding to the vibrational bands in Fig. 6 from the bacterial cellulose membranes can be found in Table S4.

3.3.2. Bacterial cellulose membrane surface wettability

To evaluate the bacterial cellulose surface wettability, the apparent contact angle between probe liquid (water or glycerol) and the bacterial cellulose membrane surface was measured. It was observed that water (used as a probe liquid) immediately penetrated the bacterial cellulose porous fiber structure and caused swelling of the surface. Therefore, to assess the surface wettability was quite challenging with water, which indicates that these membranes are highly hydrophilic. To quantify the apparent contact angle, glycerol was used instead. It should be noted that it was not possible to measure the contact angle for more than 10 s (Fig. 7). The penetration of both probe liquids (water or glycerol) in 10 s of interval is shown in Fig. 7c. It can be hypothesized that both chemistry of bacterial cellulose material and morphological characteristics were responsible for the liquid/surface interaction. The results revealed that in both case (1.5%, and 3.0% ethanol), the contact angles after 10 s of equilibrium were below 90°. However, the bacterial cellulose surface contact with glycerol (probe liquid) was more stable with 3.0% ethanol concentration. It can be speculated that the surface morphology (membrane porosity) is the dominant membrane surface feature that explains the contact angle effect at 1.5% ethanol concentration (Fig. 7a). On the other hand, the contact between the surface and the probe liquid at 3.0% ethanol concentration (Fig. 7b), could be related to the surface chemistry and distribution of the –OH group on the membrane surface rather than just the membrane morphology.

Among all the strains, it was found that the effect of carbon source was not significant for the contact angle of membranes produced by strain LMG 18909. In contrast, the use of glycerol as a carbon source led to higher contact angle values for both NFXK3 and FXV3 strains. It should kept into account that for FXV3, the bacterial cellulose membrane surface was more porous, therefore making the penetration of probe liquid easier. In terms of surface chemistry, it can be hypothesized that the studied experimental conditions could greatly influence on the distribution of – OH groups inside the material, which may impact the surface interaction with the probing liquid.

3.4. Membranes mechanical properties

The mechanical properties of freeze-dried bacterial cellulose membranes produced at the various conditions were evaluated and are presented in Table 3. The data showed that ethanol addition increased the Young's modulus and tensile strength values for all the membranes produced by the bacterial strains using the diverse carbon sources. As a result, a higher force was required to break the material. The only exception was found with NFXK3 using glycerol where the material strength increased with 1.5% ethanol addition rather than 3.0% ethanol. Furthermore, both Young's modulus and tensile strength values for the various membranes increased in the following order: NFXK3 > LMG 18909 > FXV3. The cellulose concentration as well as the morphology of the fiber network were the key determinants factor that determine the overall strength of bacterial cellulose membranes [52]. In the case of strain FXV3, a high surface porosity resulted in membrane surface delamination and the membrane deformed more easily under low applied stress, therefore, a decline in both Young's modulus and tensile strength values were observed (Table 3). On the other hand, NFXK3 and LMG 18909 membranes showed the opposite trend due to more compact fiber network structure (**Tables 2 and 3**). In general, bacterial cellulose membranes produced were found to be mechanically stable, tough, stiff, and firm due to the strong hydrogen bonding and intermolecular interactions. The tensile strength of these bacterial cellulose membranes are comparable to commercially used synthetic polypropylene (30–40 MPa) [53].

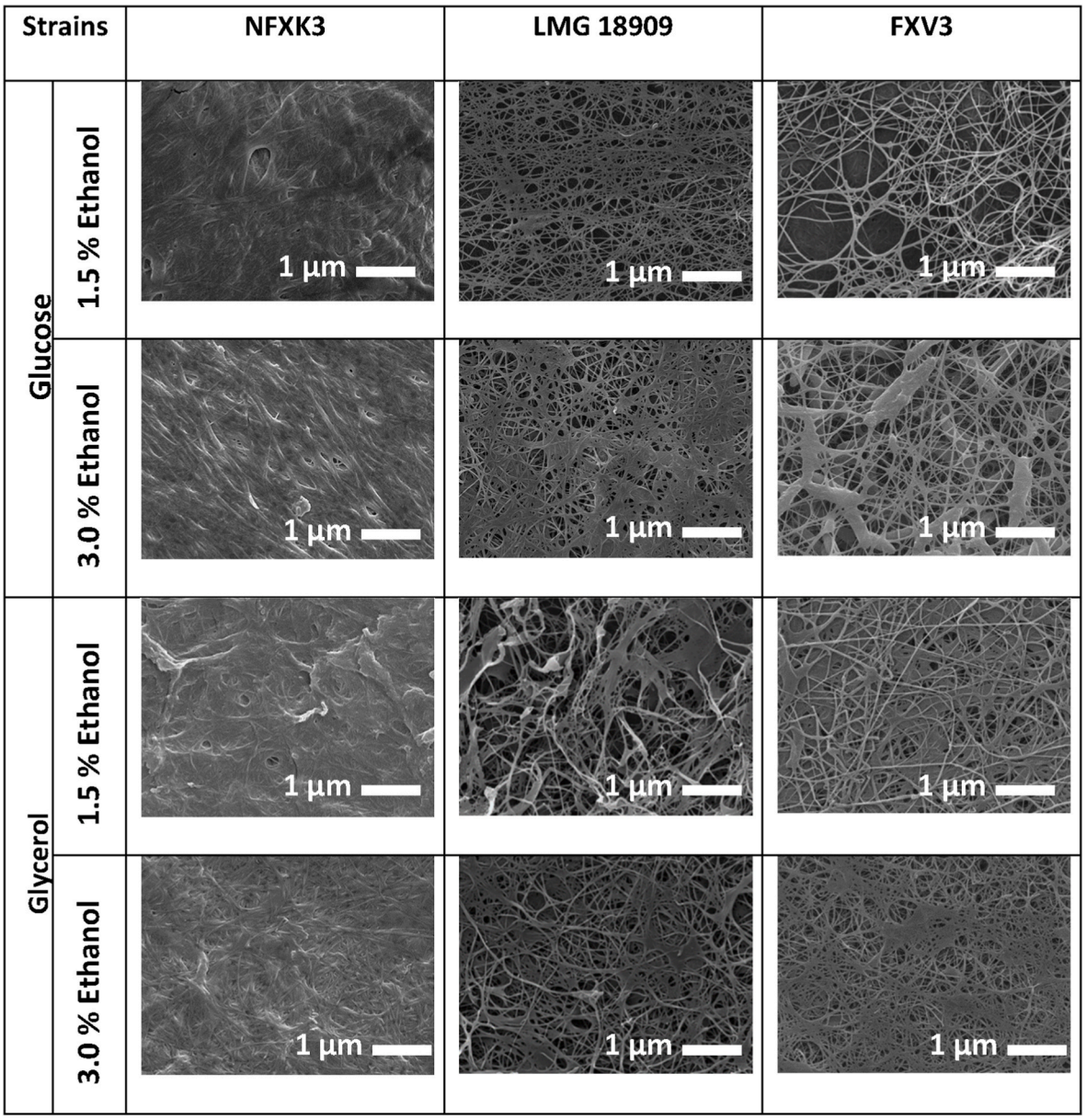

Fig. 5. SEM bottom surface morphology of bacterial cellulose membranes produced by NFXK3, LMG 18909, and FXV3 strains under different studied conditions. SEM images were otained with a magnification of 10,000.

Table 2
Analysis of bottom surface of bacterial cellulose membranes: mean fiber diameter (nm), surface porosity (%), mean pore size (nm) measured by imageJ software. *Surface Area refers to the whole membrane and was measured by N_2 adsorption/desorption isotherms.

Bacterial Strains	Ethanol (%)	Mean Fiber Diameter (nm)		Surface Porosity (%)		Mean Pore Size (nm)		* Surface Area (m^2/g)	
		Glucose	Glycerol	Glucose	Glycerol	Glucose	Glycerol	Glucose	Glycerol
NFXK3	**1.5**	82.3 ± 3.28	86.7 ± 4.11	13.7 ± 0.03	13.6 ± 0.02	104.9 ± 0.12	81.8 ± 0.55	68.5	22.3
	3.0	88.6 ± 4.12	90.5 ± 2.22	13.2 ± 0.01	12.7 ± 0.08	52.3 ± 1.11	41.4 ± 1.31	53.9	21.6
LMG 18909	**1.5**	64.1 ± 5.11	73.8 ± 2.31	28.1 ± 0.01	23.7 ± 0.30	118.1 ± 2.11	148.3 ± 2.31	70.5	45.0
	3.0	75.4 ± 3.61	76.5 ± 4.41	26.3 ± 1.03	21.9 ± 0.07	109.1 ± 3.21	129.9 ± 2.56	48.6	32.4
FXV3	**1.5**	68.8 ± 4.31	71.1 ± 3.11	48.4 ± 1.52	29.5 ± 1.41	285.4 ± 4.21	174.8 ± 3.76	73.6	50.1
	3.0	71.6 ± 3.56	74.5 ± 2.11	40.1 ± 1.22	25.3 ± 1.04	128.2 ± 3.11	101.2 ± 4.77	54.3	38.7

3.5. Membranes gas permeation

The performance of all produced bacterial cellulose membranes was firstly evaluated with N_2 gas. The values of N_2 permeance were determined and reported in Table 4. N_2 permeance was higher for membranes prepared with 1.5% ethanol concentration. Furthermore, N_2 permeance was also found to be higher in membranes produced when glucose was employed as the bacterial feed.

The highest N_2 gas permeation behavior across FXV3 based membranes is clearly associated with the larger pore size and surface porosity

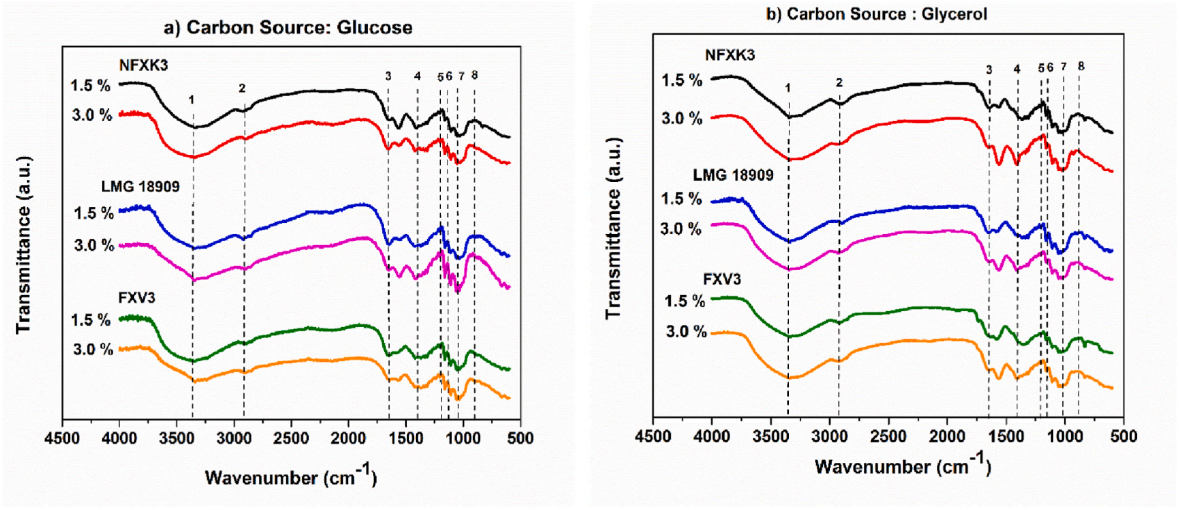

Fig. 6. Comparative Fourier transform infrared (FTIR) analysis of bacterial cellulose membranes produced by NFXK3, LMG 18909, and FXV3 strains using two carbon sources (a) glucose; (b) glycerol, at various ethanol concentrations (1.5%, 3.0%).

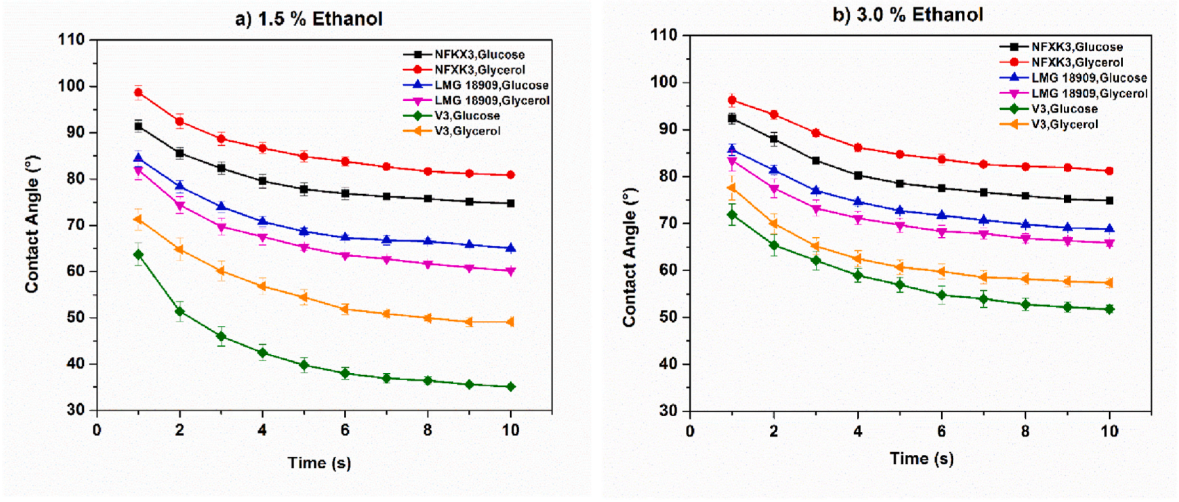

Fig. 7. Wettability of bacterial cellulose membranes produced by NFXK3, LMG 18909, and FXV3 strains using two carbon sources (glucose, glycerol). a) 1.5% ethanol; b) 3.0% ethanol c) Contact angle measurements at two different moments using water, and glycerol as a probe liquid.

Table 3
Mechanical Properties of bacterial cellulose membranes produced by NFXK3, LMG 18909, and FXV3 strains using two carbon sources (glucose, glycerol) at various ethanol concentrations (1.5%, 3.0%).

Bacterial Strains	Ethanol (%)	Tensile Strength (MPa)		Young's Modulus (MPa)	
		Glucose	Glycerol	Glucose	Glycerol
NFXK3	**1.5**	18.6 ± 2.9	34.1 ± 4.3	93.1 ± 6.2	210.8 ± 4.7
	3.0	23.8 ± 3.7	17.4 ± 2.1	161.2 ± 3.3	145.1 ± 3.1
LMG 18909	**1.5**	3.6 ± 1.9	5.5 ± 2.8	40.8 ± 5.3	51.3 ± 3.8
	3.0	8.5 ± 2.6	9.2 ± 3.5	133.5 ± 2.9	68.6 ± 5.2
FXV3	**1.5**	2.2 ± 3.3	1.3 ± 4.5	18.1 ± 4.2	17.4 ± 4.4
	3.0	6.3 ± 4.1	12 ± 3.6	48.8 ± 3.7	121.4 ± 2.9

depicted in Table 2. NFXK3 based membranes, on the other hand, had the lowest N_2 gas permeability of all the strains examined, owing to the fact that they were more compact and less porous. Apart from pore size, fiber entanglement/bonding, fiber diameter, and the pore shape inside the membrane matrix all had a significant impact on the resulting membrane gas transport.

From the obtained data (Fig. 8) it can be concluded that the permeability of N_2 through the various membranes prepared correlates clearly with the mean pore size of the membranes. Also, it shows that the mean pore size can be modulated by selecting the appropriate strain and cultivation conditions (carbon source and concentration of ethanol in the media). The membranes minimum and maximum pore size values are summarized in Table S3. This feature offers the possibility to design the membrane according to a particular target application.

From Fig. 9 it is confirmed, as expected, that the cellulose membranes do not exhibit selectivity between the various gases tested (N2, O_2 and CO_2). The ideal selectivity values obtained, defined for each pair of gases as the ratio between their individual permeabilities are very close to 1 and, when the values deviate from the unity, that results from a higher relative error associated with low absolute values of permeability (for the cases where glycerol was used). The results obtained

Table 4
N_2 permeance of prepared membranes considering different cultivation conditions. Deviation in N_2 permeance and permeability was calculated as the deviation from membrane replicas. *GPU = Gas Permeation Units, 1 GPU = 10^{-6} cm^3 (STP).cm^{-2}.s^{-1}.$cmHg^{-1}$ and 1 Barrer = 10^{-10} cm^3 (STP).cm.cm^{-2}.s^{-1}.$cmHg^{-1}$.

Bacterial Strain	Carbon Source	Ethanol (%)	N_2 Permeance (GPU*)	Thickness (μm)	N_2 Permeability (Barrer)
NFXK3	**Glucose**	1.5	2.9 ± 1.1	61.8	185.5 ± 0.01
		3	3.0 ± 0.4	165.0	491.6 ± 0.04
	Glycerol	1.5	0.3 ± 0.5	37.1	14.8 ± 0.02
		3	0.1 ± 0.3	32.6	1.6 ± 0.01
LMG 18909	**Glucose**	1.5	1.4 ± 1.1	28.1	41.0 ± 0.03
		3	1.2 ± 0.2	92.2	32.6 ± 0.01
	Glycerol	1.5	12.9 ± 0.2	74.1	957.2 ± 0.04
		3	6.0 ± 0.3	28.0	168.0 ± 0.01
FXV3	**Glucose**	1.5	249.9 ± 0.5	29.5	7247.9 ± 4.01
		3	313.4 ± 7.2	29.1	9247.5 ± 3.02
	Glycerol	1.5	78.1 ± 0.2	28.0	2188.7 ± 2.11
		3	40.0 ± 0.5	22.2	880.0 ± 2.78

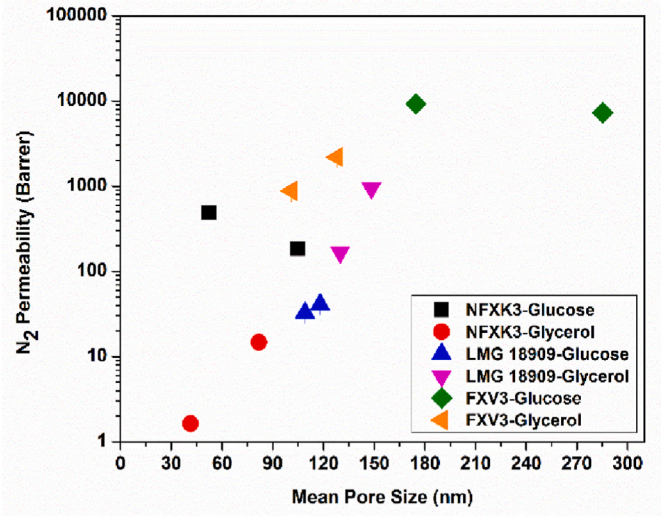

Fig. 8. N_2 permeability plotted against mean pore size of prepared membranes considering different cultivation conditions.

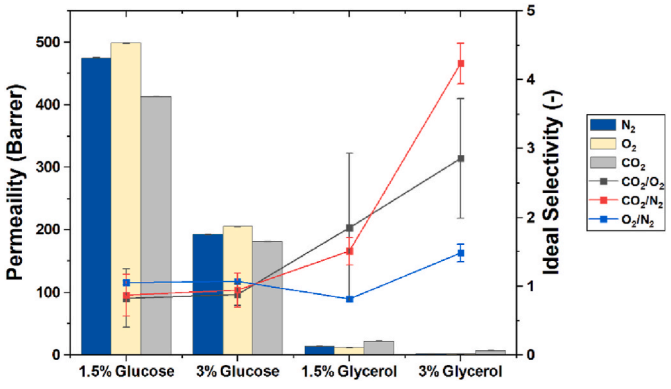

Fig. 9. Gas permeation data for membranes prepared with NFXK3 using as carbon source: (a) glucose; (b) glycerol with different ethanol contents for N_2, O_2 and CO_2 at 30 °C.

during the gas permeation studies showed that the membranes produced are excellent candidates for use as air/gas filtration, allowing for the retention of particles larger than 0.2 μm including biological agents, such as bacteria (from the supplementary material it can be seen that it is possible to produce membranes where even the largest pore size is below 200 nm). The retention of virus would require a detailed study but the membranes produced in the low range of pore size could be referred as potential candidates [54].

4. Conclusions

A systematic approach has been carried out to produce bacterial cellulose from *Komagataeibacter* strains: *Komagataeibacter* sp. FXV3, *Komagataeibacter* sp. NFXK3, and *K. intermedius* LMG 18909. Optimal fermentation conditions were identified to convert a carbon source (glucose and glycerol) into cellulose. All the strains exhibited higher potential to produce cellulose membranes from the carbon sources tested. It was observed that the NFXK3 strain produced the highest cellulose mass, amounting to 9.6 g/L using glucose and 11.46 g/L using glycerol. Contrarily, the LMG 18909 strain produced more cellulose (5.93 g/L) using glucose whereas the cellulose production by FXV3 strain remained the same (~5 g/L) using both the carbon sources. To assess the potential of bacterial cellulose for air filter applications, flat sheet membranes were produced. The usage of ethanol had a significant impact on the bacterial cellulose yield and on the morphology of the membranes produced. The synthesized bacterial cellulose membranes possessed remarkably distinctive physical properties such as surface porosity, fiber diameter, pore size distribution and specific surface area. These pristine membranes also offered desirable characteristics like biocompatibility, biodegradability and mechanical stability. Amongst the plethora of applications reported for bacterial cellulose membranes, gas permeation was explored in this study by conducting permeability and selectivity measurements for N_2, CO_2 and O_2. Lastly, this work potentiate the application of bacterial cellulose membranes as eco-friendly air filters or facemasks.

Author contributions

Arooj Fatima: Conceptualization, Methodology, Validation, Investigation, Writing - original draft, Visualization. **Paloma Ortiz-Albo:** Investigation, Writing - review & editing, Visualization. **Luísa A. Neves:** Investigation, Writing - review & editing, Visualization, Supervision. **Francisco X. Nascimento:** Conceptualization, Methodology, Investigation, Writing - review & editing, Visualization, Supervision. **J.G. Crespo:** Conceptualization, Writing - review & editing, Supervision, Project administration, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgements

This work was supported by the Associate Laboratory for Green Chemistry — LAQV, iNOVA4Health and the Associate Laboratory LS4FUTURE which are financed by Portuguese national funds from the Fundação para a Ciência e Tecnologia (FCT/MCTES, Portugal) (UIDB/50006/2020; UIDP/50006/2020; UIDB/04462/2020, UIDP/04462/2020; and LA/P/0087/2020; respectively). Arooj Fatima acknowledges FCT for PhD grant reference 2021.07557. BD. The authors acknowledge

Professor Vítor D. Alves, from Instituto Superior de Agronomia, Universidade de Lisboa, for the support in the analysis of membrane mechanical properties.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.memsci.2023.121509.

References

[1] F. Galiano, K. Briceño, T. Marino, A. Molino, K.V. Christensen, A. Figoli, Advances in biopolymer-based membrane preparation and applications, J. Membr. Sci. 564 (2018) 562–586, https://doi.org/10.1016/j.memsci.2018.07.059.

[2] D. Klemm, B. Heublein, H.P. Fink, A. Bohn, Cellulose: fascinating biopolymer and sustainable raw material, Angew. Chem. Int. Ed. 44 (2005) 3358–3393, https://doi.org/10.1002/anie.200460587.

[3] M. Iguchi, S. Yamanaka, A. Budhiono, Bacterial cellulose - a masterpiece of nature's arts, J. Mater. Sci. 35 (2000) 261–270, https://doi.org/10.1023/A:1004775229149.

[4] A.J. Brown, On an acetic ferment which forms cellulose, J. Chem. Soc. Trans. 49 (1886) 432–439, https://doi.org/10.1039/CT8864900432.

[5] S.S. Wang, Y.H. Han, J.L. Chen, D.C. Zhang, X.X. Shi, Y.X. Ye, D.L. Chen, M. Li, Insights into bacterial cellulose biosynthesis from different carbon sources and the associated biochemical transformation pathways in Komagataeibacter sp. W1, Polymers 10 (2018), https://doi.org/10.3390/polym10090963.

[6] R. Naomi, R.B.H. Idrus, M.B. Fauzi, Plant-vs. Bacterial-derived cellulose for wound healing: a review, Int. J. Environ. Res. Publ. Health 17 (2020) 1–25, https://doi.org/10.3390/ijerph17186803.

[7] J. Wang, J. Tavakoli, Y. Tang, Bacterial cellulose production, properties and applications with different culture methods – a review, Carbohydr. Polym. 219 (2019) 63–76, https://doi.org/10.1016/j.carbpol.2019.05.008.

[8] G. Helenius, H. Bäckdahl, A. Bodin, U. Nannmark, P. Gatenholm, B. Risberg, In vivo biocompatibility of bacterial cellulose, J. Biomed. Mater. Res., Part A 76 (2006) 431–438, https://doi.org/10.1002/jbm.a.30570.

[9] P.R. Brandão, M.T.B. Crespo, F.X. Nascimento, Phylogenomic and comparative analyses support the reclassification of several Komagataeibacter species as novel members of the Novacetimonas gen. nov. and bring new insights into the evolution of cellulose synthase genes, Int. J. Syst. Evol. Microbiol. 72 (2022) 1–17, https://doi.org/10.1099/ijsem.0.005252.

[10] P. Singhsa, R. Narain, H. Manuspiya, Physical structure variations of bacterial cellulose produced by different Komagataeibacter xylinus strains and carbon sources in static and agitated conditions, Cellulose 25 (2018) 1571–1581, https://doi.org/10.1007/s10570-018-1699-1.

[11] T. Naritomi, T. Kouda, H. Yano, F. Yoshinaga, Effect of ethanol on bacterial cellulose production from fructose in continuous culture, J. Ferment. Bioeng. 85 (1998) 598–603, https://doi.org/10.1016/S0922-338X(98)80012-3.

[12] Y.E. Agustin, K.S. Padmawijaya, Effect of acetic acid and ethanol as additives on bacterial cellulose production by acetobacter xylinum, IOP Conf. Ser. Earth Environ. Sci. 209 (2018), https://doi.org/10.1088/1755-1315/209/1/012045.

[13] S. Yunoki, Y. Osada, H. Kono, M. Takai, Role of ethanol in improvement of bacterial cellulose production: analysis using13C-labeled carbon sources, Food Sci. Technol. Res. 10 (2004) 307–313, https://doi.org/10.3136/fstr.10.307.

[14] S. Gorgieva, J. Trček, Bacterial cellulose: production, modification and perspectives in biomedical applications, Nanomaterials 9 (2019) 1–20, https://doi.org/10.3390/nano9101352.

[15] S.M. Santos, J.M. Carbajo, J.C. Villar, The effect of carbon and nitrogen sources on bacterial cellulose production and properties from gluconacetobacter sucrofermentans CECT 7291 focused on its use in degraded paper restoration, Bioresources 8 (2013) 3630–3645, https://doi.org/10.15376/biores.8.3.3630-3645.

[16] Y. Hu, M. Yue, F. Yuan, L. Yang, C. Chen, D. Sun, Bio-inspired fabrication of highly permeable and anti-fouling ultrafiltration membranes based on bacterial cellulose for efficient removal of soluble dyes and insoluble oils, J. Membr. Sci. 621 (2021), 118982, https://doi.org/10.1016/j.memsci.2020.118982.

[17] A.M. Sokolnicki, R.J. Fisher, T.P. Harrah, D.L. Kaplan, Permeability of bacterial cellulose membranes, J. Membr. Sci. 272 (2006) 15–27, https://doi.org/10.1016/j.memsci.2005.06.065.

[18] F.G. Blanco Parte, S.P. Santoso, C.C. Chou, V. Verma, H.T. Wang, S. Ismadji, K. C. Cheng, Current progress on the production, modification, and applications of bacterial cellulose, Crit. Rev. Biotechnol. 40 (2020) 397–414, https://doi.org/10.1080/07388551.2020.1713721.

[19] S. Swingler, A. Gupta, H. Gibson, M. Kowalczuk, W. Heaselgrave, I. Radecka, Recent advances and applications of bacterial cellulose in biomedicine, Polymers 13 (2021) 1–29, https://doi.org/10.3390/polym13030412.

[20] C. Zhong, Industrial-scale production and applications of bacterial cellulose, Front. Bioeng. Biotechnol. 8 (2020) 1–19, https://doi.org/10.3389/fbioe.2020.605374.

[21] C.J.S. Galdino, A.D. Maia, H.M. Meira, T.C. Souza, J.D.P. Amorim, F.C.G. Almeida, A.F.S. Costa, L.A. Sarubbo, Use of a bacterial cellulose filter for the removal of oil from wastewater, Process Biochem. 91 (2020) 288–296, https://doi.org/10.1016/j.procbio.2019.12.020.

[22] G. Li, A.G. Nandgaonkar, Q. Wang, J. Zhang, W.E. Krause, Q. Wei, L.A. Lucia, Laccase-immobilized bacterial cellulose/TiO2functionalized composite membranes: evaluation for photo- and bio-catalytic dye degradation, J. Membr. Sci. 525 (2017) 89–98, https://doi.org/10.1016/j.memsci.2016.10.033.

[23] S. Zhang, J. Luo, F. Zhang, M. Du, H. Hui, F. Zhao, X. He, Z. Sun, A porous, mechanically strong and thermally stable zeolitic imidazolate framework-8@ bacterial cellulose/aramid nanofibers composite separator for advanced lithium-ion batteries, J. Membr. Sci. 652 (2022), 120461, https://doi.org/10.1016/j.memsci.2022.120461.

[24] V. Dubey, L.K. Pandey, C. Saxena, Pervaporative separation of ethanol/water azeotrope using a novel chitosan-impregnated bacterial cellulose membrane and chitosan-poly(vinyl alcohol) blends, J. Membr. Sci. 251 (2005) 131–136, https://doi.org/10.1016/j.memsci.2004.11.009.

[25] C.G. Eggensperger, M. Giagnorio, M.C. Holland, K.M. Dobosz, J.D. Schiffman, A. Tiraferri, K.R. Zodrow, Sustainable living filtration membranes, Environ. Sci. Technol. Lett. 7 (2020) 213–218, https://doi.org/10.1021/acs.estlett.0c00019.

[26] M.C. Holland, C.G. Eggensperger, M. Giagnorio, J.D. Schiffman, A. Tiraferri, K. R. Zodrow, Facile postprocessing alters the permeability and selectivity of microbial cellulose ultrafiltration membranes, Environ. Sci. Technol. 54 (2020) 13249–13256, https://doi.org/10.1021/acs.est.0c00451.

[27] Z. Sun, Y. Kuang, M. Ahmad, Y. Huang, S. Yin, F. Seidi, S. Wang, Enhanced osmotic energy conversion through bacterial cellulose based double-network hydrogel with 3D interconnected nanochannels, Carbohydr. Polym. 305 (2023), 120556, https://doi.org/10.1016/j.carbpol.2023.120556.

[28] E. Trovatti, C.S.R. Freire, P.C. Pinto, I.F. Almeida, P. Costa, A.J.D. Silvestre, C. P. Neto, C. Rosado, Bacterial cellulose membranes applied in topical and transdermal delivery of lidocaine hydrochloride and ibuprofen: in vitro diffusion studies, Int. J. Pharm. 435 (2012) 83–87, https://doi.org/10.1016/j.ijpharm.2012.01.002.

[29] A. Figoli, T. Marino, S. Simone, E. Di Nicolò, X.M. Li, T. He, S. Tornaghi, E. Drioli, Towards non-toxic solvents for membrane preparation: a review, Green Chem. 16 (2014) 4034–4059, https://doi.org/10.1039/c4gc00613e.

[30] D.A. Gregory, L. Tripathi, A.T.R. Fricker, E. Asare, I. Orlando, V. Raghavendran, I. Roy, Bacterial cellulose: a smart biomaterial with diverse applications, Mater. Sci. Eng. R Rep. 145 (2021), 100623, https://doi.org/10.1016/j.mser.2021.100623.

[31] R.M. Ashour, A.F. Abdel-Magied, Q. Wu, R.T. Olsson, K. Forsberg, Green synthesis of metal-organic framework bacterial cellulose nanocomposites for separation applications, Polymers 12 (2020), https://doi.org/10.3390/POLYM12051104.

[32] L.C. Tomé, L. Brandão, A.M. Mendes, A.J.D. Silvestre, C.P. Neto, A. Gandini, C.S. R. Freire, I.M. Marrucho, Preparation and characterization of bacterial cellulose membranes with tailored surface and barrier properties, Cellulose 17 (2010) 1203–1211, https://doi.org/10.1007/s10570-010-9457-z.

[33] D. Martins, B. Estevinho, F. Rocha, F. Dourado, M. Gama, A dry and fully dispersible bacterial cellulose formulation as a stabilizer for oil-in-water emulsions, Carbohydr. Polym. 230 (2020), 115657, https://doi.org/10.1016/j.carbpol.2019.115657.

[34] F.X. Nascimento, C.A.V. Torres, F. Freitas, M.A.M. Reis, M.T.B. Crespo, Functional and genomic characterization of Komagataeibacter uvaceti FXV3, a multiple stress resistant bacterium producing increased levels of cellulose, Biotechnol. Reports. 30 (2021), e00606, https://doi.org/10.1016/j.btre.2021.e00606.

[35] P. Zikmanis, S. Kolesovs, M. Ruklisha, P. Semjonovs, Production of bacterial cellulose from glycerol: the current state and perspectives, Bioresour. Bioprocess. 8 (2021) 1–14, https://doi.org/10.1186/s40643-021-00468-1.

[36] L. Greenspan, Humidity fixed points of binary saturated aqueous solutions, J. Res. Natl. Bur. Stand. - A. Phys Ics Chem. 81A (1977) 89–96, https://doi.org/10.6028/jres.081A.011.

[37] C.T. Rueden, J. Schindelin, M.C. Hiner, B.E. DeZonia, A.E. Walter, E.T. Arena, K. W. Eliceiri, ImageJ2: ImageJ for the next generation of scientific image data, BMC Bioinf. 18 (2017) 1–26, https://doi.org/10.1186/s12859-017-1934-z.

[38] L.A. Neves, J.G. Crespo, I.M. Coelhoso, Gas permeation studies in supported ionic liquid membranes, J. Membr. Sci. 357 (2010) 160–170, https://doi.org/10.1016/j.memsci.2010.04.016.

[39] D. Mikkelsen, B.M. Flanagan, G.A. Dykes, M.J. Gidley, Influence of different carbon sources on bacterial cellulose production by Gluconacetobacter xylinus strain ATCC 53524, J. Appl. Microbiol. 107 (2009) 576–583, https://doi.org/10.1111/j.1365-2672.2009.04226.x.

[40] A. Basu, S.V. Vadanan, S. Lim, A novel platform for evaluating the environmental impacts on bacterial cellulose production, Sci. Rep. 8 (2018) 1–8, https://doi.org/10.1038/s41598-018-23701-y.

[41] F. Mohammadkazemi, M. Azin, A. Ashori, Production of bacterial cellulose using different carbon sources and culture media, Carbohydr. Polym. 117 (2015) 518–523, https://doi.org/10.1016/j.carbpol.2014.10.008.

[42] S.K. Padmanabhan, F. Lionetto, R. Nisi, M. Stoppa, A. Licciulli, Sustainable production of stiff and crystalline bacterial cellulose from orange peel extract, Sustainability 14 (2022) 2247, https://doi.org/10.3390/su14042247.

[43] E. Trovatti, L.S. Serafim, C.S.R. Freire, A.J.D. Silvestre, C.P. Neto, Gluconacetobacter sacchari: an efficient bacterial cellulose cell-factory, Carbohydr. Polym. 86 (2011) 1417–1420, https://doi.org/10.1016/j.carbpol.2011.06.046.

[44] Y. Huang, C. Zhu, J. Yang, Y. Nie, C. Chen, D. Sun, Recent advances in bacterial cellulose, Cellulose 21 (2014) 1–30, https://doi.org/10.1007/s10570-013-0088-z.

[45] F. Mohammadkazemi, K. Doosthoseini, M. Azin, Effect of ethanol and medium on bacterial cellulose (BC) production by Gluconacetobacter xylinus (ptcc 1734), Cellul. Chem. Technol. 49 (2015) 5–6.

[46] A. Mautner, A. Bismarck, Bacterial nanocellulose papers with high porosity for optimized permeance and rejection of nm-sized pollutants, Carbohydr. Polym. 251 (2021), 117130, https://doi.org/10.1016/j.carbpol.2020.117130.

[47] E.E. Al-Shamary, A.K.A. Darwash, Influence of fermentation condition and alkali treatment on the porosity and thickness of bacterial cellulose membranes, Online J. Sci. Technol. 3 (2013) 194–203.
[48] C. Molina-Ramírez, M. Castro, M. Osorio, M. Torres-Taborda, B. Gómez, R. Zuluaga, C. Gómez, P. Gañán, O.J. Rojas, C. Castro, Effect of different carbon sources on bacterial nanocellulose production and structure using the low pH resistant strain Komagataeibacter medellinensis, Materials 10 (2017), https://doi.org/10.3390/ma10060639.
[49] Y. Hu, J.M. Catchmark, Y. Zhu, N. Abidi, X. Zhou, J. Wang, N. Liang, Engineering of porous bacterial cellulose toward human fibroblasts ingrowth for tissue engineering, J. Mater. Res. 760 (2014) 2682–2693, https://doi.org/10.1557/jmr.2014.315.
[50] N. Halib, M.C.I.M. Amin, I. Ahmad, Physicochemical properties and characterization of nata de Coco from local food industries as a source of cellulose, Sains Malays. 41 (2012) 205–211.
[51] R.E.A. Nascimento, J. Monte, M. Cadima, V.D. Alves, L.A. Neves, Rendering banana plant residues into a potentially commercial byproduct by doping cellulose films with phenolic compounds, Polymers 13 (2021) 1–15, https://doi.org/10.3390/polym13050843.
[52] S.Q. Chen, P. Lopez-Sanchez, D. Wang, D. Mikkelsen, M.J. Gidley, Mechanical properties of bacterial cellulose synthesised by diverse strains of the genus Komagataeibacter, Food Hydrocolloids 81 (2018) 87–95, https://doi.org/10.1016/j.foodhyd.2018.02.031.
[53] I. Betlej, K.J. Krajewski, P. Boruszewski, S. Zakaria, Bacterial cellulose-properties and its potential application, Sains Malays. 50 (2021) 493–505, https://doi.org/10.17576/jsm-2021-5002-20.
[54] M. Lippi, L. Riva, M. Caruso, C. Punta, Cellulose for the production of air-filtering systems: a critical review, Materials 15 (2022), https://doi.org/10.3390/ma15030976.