CHARACTERIZATION OF COMPOSITE MATERIALS BASED ON BIOCELLULOSE MEMBRANES IMPREGNATED WITH SILVER PARTICLES AS ANTIMICROBIAL AGENT

Loredana – Mihaela DOBRE[1], Anicuța STOICA[2], Marta STROESCU[3], Sorin JINGA[4], Iuliana JIPA[5], Tănase DOBRE[6]

Lucrarea prezintă date experimentale privind sinteza şi caracterizarea unor materiale compozite pe bază de bioceluloză (BC) cu funcție antimicrobiană în vederea utilizării ca ambalaje alimentare. Ca agent antimicrobian s-a utilizat argint metalic. Membranele compozite obținute au fost caracterizate din punct de vedere morfologic şi structural utilizând tehnici de microscopie electronică de baleiaj (SEM), spectroscopie de dispersie a energiei cu raze X (EDS) şi spectroscopie în infraroşu cu transformată Fourier (FT-IR).

This work present data concerning synthesis and characterization of composite materials based on bacterial cellulose (BC) with antimicrobial properties that can be used as food packaging materials. As antimicrobial agent was used metallic silver. The composite membranes obtained were characterized using scanning electron microscope technique (SEM), energy dispersive spectroscopy with X-ray (EDS) and infrared spectroscopy with Fourier transform (FT-IR).

Keywords: bacterial cellulose, silver nanoparticles, food packaging

1. Introduction

Research into the area of bioactive materials has increased significantly during the last years. Antimicrobial packaging, an active packaging concept, can be considered an extremely challenging technology that could have a significant impact on shelf-life extension and food safety of different products [1, 2]. Materials with antimicrobial properties act to reduce, inhibit or retard the growth of microorganisms that may be present in the packed food [3]. There are two basic categories of antimicrobial films. One involves direct incorporation of the antimicrobial additive into the packaging film, while second type of film is coated

[1] PhD student, Dept. of Chemical Engineering, University POLITEHNICA of Bucharest, Romania, e-mail: Loredana.Dragne@gmail.com
[2] Reader, Dept. of Chemical Engineering, University POLITEHNICA of Bucharest, Romania
[3] Reader, Dept. of Chemical Engineering, University POLITEHNICA of Bucharest, Romania
[4] Reader, Dept. of Science and Engineering of Oxide Materials and Nanomaterials, University POLITEHNICA of Bucharest, Romania
[5] Lecturer, Dept. of Chemical Engineering, University POLITEHNICA of Bucharest, Romania
[6] Prof., Dept. of Chemical Engineering, University POLITEHNICA of Bucharest, Romania

with a material which acts as a carrier for the additive. It is important that the films and coatings are formulated to allow the controlled release of the antimicrobial additives into foodstuffs [4, 5].

Silver has been used since ancient times in the form of metallic silver, silver nitrate, silver sulfadiazine in medical field for antimicrobial applications. Due to the emergence of several antibiotics the use of these silver compounds has been declined in time. Nanotechnology is gaining tremendous impetus in the present century due to its capability of modulating metals into nanosize, which drastically changes their chemical, physical and optical properties. Metallic silver in form of silver nanoparticles has made remarkable comeback as a potential antimicrobial agent. It has been reported that the mode of antibacterial action of silver nanoparticles is similar to that of silver ion. The use of silver nanoparticles is also important, as several pathogenic bacteria have developed resistance against various antibiotics [6, 7].

Bacterial cellulose (BC) is produced by certain species of *Acetobacter*. It has various applications, because of its unique properties [8, 9]. BC shows high tensile strength and modulus, due to high crystalline structure and reduced fiber diameter. For instance, the BC microfibrils have a density of 1600 kg/m^3, Young's modulus of 138 GPa and tensile strength of at least 2 GPa, which are almost equal to those of aramid fibers [10]. BC has also medical applications such as wound dressings and artificial skins, artificial blood vessels and (bio) membranes. This material is commonly produced as a hydrated membrane pellicle at the air-medium interface. Membrane morphology is dependent on the shape of this interface, which may easily be manipulated or controlled. However, bacterial cellulose itself has no antimicrobial activity and that is why silver particles were impregnated into the polymer membranes. It has been reported that bacterial cellulose membranes present antimicrobial activity after immersion in silver nitrate solution and after treatment with different reducing agents as sodium borohydride [11, 12], ascorbic acid [13] or triethanolamine [14]. The total content as well as the release of silver from the impregnated cellulosic materials have also been studied [12, 15].

The aim of this work is to prepare and characterize bacterial cellulose membranes impregnated with silver particles using Tollens reaction.

2. Experimental

Reagents and apparatus

The chemicals used to prepare the modified Hestrin-Schramm medium are bacteriological peptone (from Sigma Aldrich), yeast extract (from Fluka) and fructose for food use. NaOH solution was prepared in laboratory from chemical

pure grade supplied from Merck Co. Tollens reagent was also prepared in laboratory using $AgNO_3$ and NH_3 from Merck Co. Also, glucose used was from Merck Co.

The morphology of bacterial cellulose was observed using a HITACHI S-2600N scanning electron microscope operating at 20 kV at a magnification of 500-5000 K. The structure of the membranes obtained was studied with a spectrophotometer FTIR Jasco 6200.

Production and purification of bacterial cellulose membranes

The membranes of BC were obtained in dynamic culture using a rotating disk contactor. *Acetobacter* sp. strain used in this study was isolated from the traditionally fermented vinegar in Microbiology Laboratory of Chemical Engineering Department of Politehnica University of Bucharest. Stock culture was inoculated into modified Hestrin-Schramm medium containing 3 % fructose and was incubated for 7 days in a rotating disk contactor. The obtained gel-like pellicles were purified by boiling in a 0.5 N aqueous solution of NaOH. The BC thin sheets were then washed with deionized water several times until pH of water became neutral. BC pellicles were used as gel membranes.

Impregnation of silver particles into biocellulose

Silver was incorporated into bacterial cellulose using the classical Tollens reaction. The biocellulose membranes were immersed for different period of times in Tollens reagent and glucose solution for silver ion's reduction, in order to achieve various silver concentrations. Membranes A1 and B1 were immersed into Tollens reagent for 2 hours and into glucose solution for 19 hours and membranes A2 and B2 were dipped into Tollens solution for 4 hours and into glucose for 38 hours. The difference between membranes A and B is that membrane A was first immersed in Tollens solution and after time expired it was immersed in glucose 10 % solution, while membrane B was first immersed in glucose solution and after that in Tollens reagent. For membranes A2 and B2 the cycle of immersing into solutions was repeated in order to obtain a larger quantity of impregnated silver and that is why the period of maintaining the membranes into solutions was double than for membranes A1 and B1.

Characterization of BC membranes impregnated with silver nanoparticles

The membranes morphology was studied with SEM technique. The formation of silver particles and silver elemental distribution on bacterial cellulose

samples were determined using energy dispersive X-ray spectroscopy (SEM-EDS). Also, silver identification could be noticed in the SEM-EDS spectra.

Fourier transform infrared (FT-IR) spectroscopy of composite materials was carried out in order to detect any peak shift that could result from interactions between silver and bacterial cellulose functional groups.

Swelling ability of composite materials was also studied. Membranes were cut into 2 cm × 2 cm square shapes and dried to constant weight. After the moisture content was removed, they were immersed in deionized water at room temperature. Swelling dynamics was obtained by measuring the initial weight (G_i) and the weight of sample in swollen state ($G_{s,\tau}$) using equation (1).

$$Swelling = \left(G_{s,\tau} - G_i\right) / G_i \tag{1}$$

3. Results and discussion

The SEM studies revealed the morphology of bacterial cellulose membranes impregnated with silver nanoparticles in comparison with the morphology of a simple bacterial cellulose membrane used as control sample. Fig. 1 presents the SEM image of simple bacterial cellulose membrane.

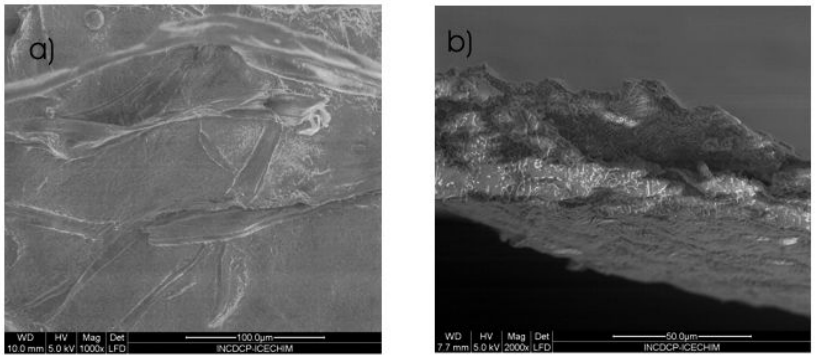

Fig. 1. Biocellulose membrane – (a) surface (x2.0k) and (b) transversal section (x1.5k)

In Figs. 2 and 3 are presented the SEM images of membrane A1, respectively B1 at different magnifications. It can be observed the silver particles deposited on the surface and into the bacterial cellulose membranes.

Figs. 4 and 5 reveal SEM micrographs for membranes A2 and B2. It is also observed the metallic silver impregnated into biocellulose membranes. By comparing the two membranes type it can be observed that more silver particles were formed in membranes 2 and these particles has aggregated into insular formations. Also, it can be observed that the quantity of silver deposed onto the

BC membrane is very large and the BC fibrils cannot be seen any more because of that.

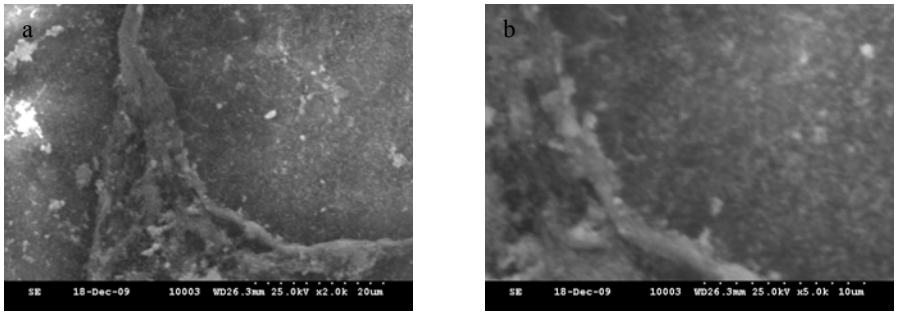

Fig. 2. SEM images of membrane A1 (a – x2.0k; b – x5.0k)

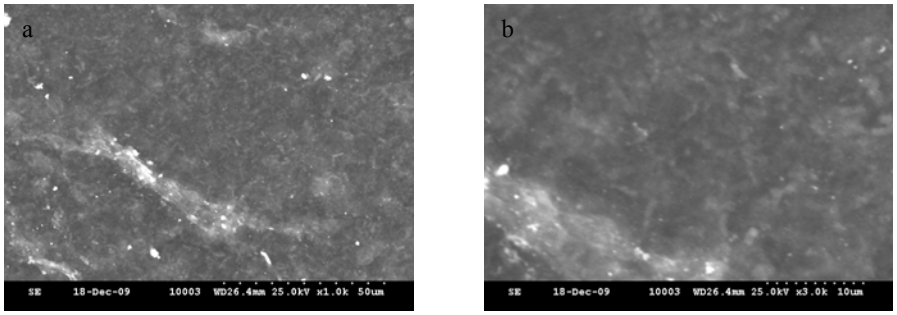

Fig. 3. SEM images of membrane B1 (a – x1.0k; b – x3.0k)

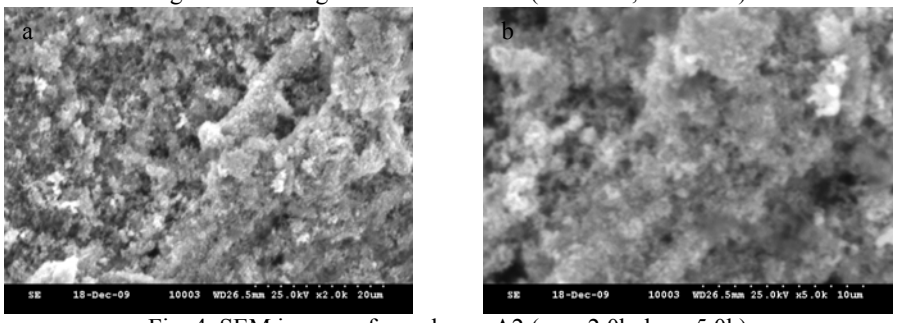

Fig. 4. SEM images of membrane A2 (a – x2.0k; b – x5.0k)

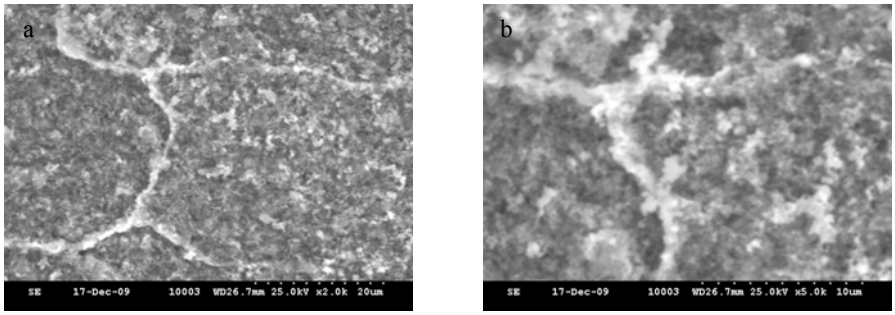

Fig. 5. SEM images of membrane B2 (a – x2.0k; b – x5.0k)

The explanation of this is that in repeated cycle of immersing, the already constituted silver became a physical barrier to diffusion of solutions through the material and a major part of silver was formed onto the surface of the membranes.

Fig. 6 shows the EDS spectrum for elemental silver found in membrane A1 and the distribution of silver particles in the analyzed zone of the material. The white background represents the bacterial cellulose substrate and the red points illustrate the silver particles.

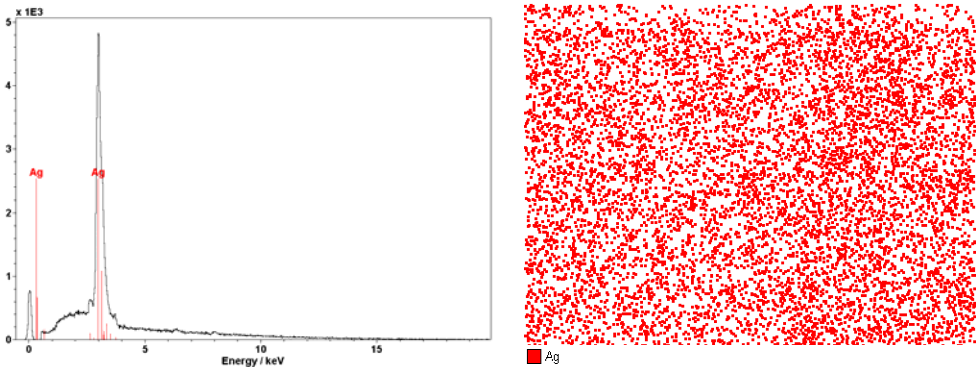

Fig. 6. SEM-EDS images of membrane A1

The SEM-EDS spectrum of composite material A2 presented in Fig. 7 reveals silver particles distribution in this material. This distribution map confirms the larger quantity of silver found in membrane A2.

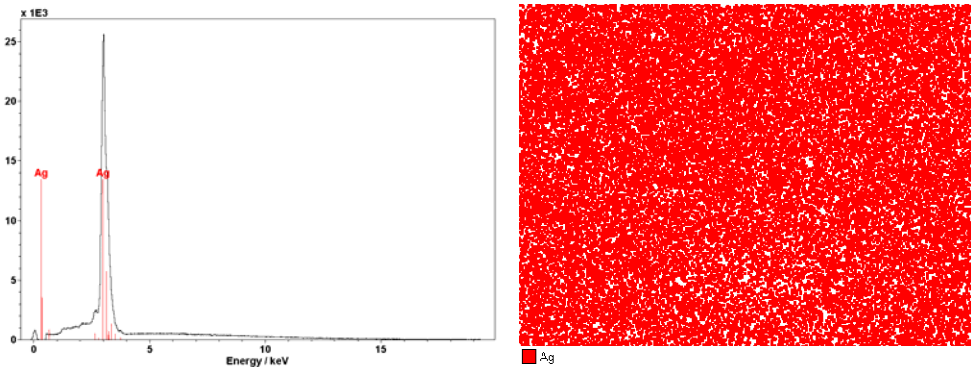

Fig. 7. SEM-EDS images of membrane A2

All the SEM-EDS spectra for the composite materials analyzed confirmed the presence of silver in membranes and showed a relative uniform distribution of

the impregnated particles. There were not been observed major differences between membranes A and B.

Fig. 8 and 9 presents FT-IR spectra of membranes A1 and A2. The characteristic bands of biocellulose appeared at 3351.68 cm^{-1} for hydroxyl groups stretching vibration, at 2896.56 cm^{-1} for C-H stretching vibration, at 1427.07 cm^{-1} for C-H bending vibration, at 1161.9 cm^{-1} for C-O-C asymmetric stretching vibration from the glycosidic ring and 900.59 cm^{-1} for C-H bending vibration from the β-anomeric link [16]. The peak from 1646.91 cm^{-1} represents water molecules in the amorphous region. From these spectra it can be observed that for membrane A2 (which has more silver formed onto the surface of the BC membranes) the intensity of the peaks is lower because of higher opacity of the sample.

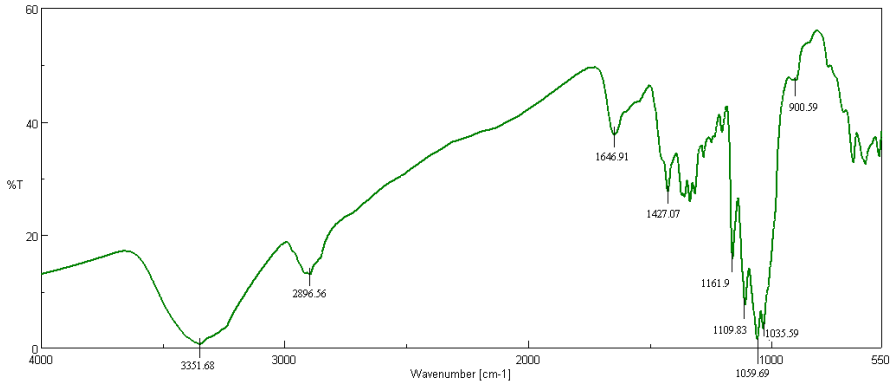

Fig. 8. FT-IR spectra of membrane A1

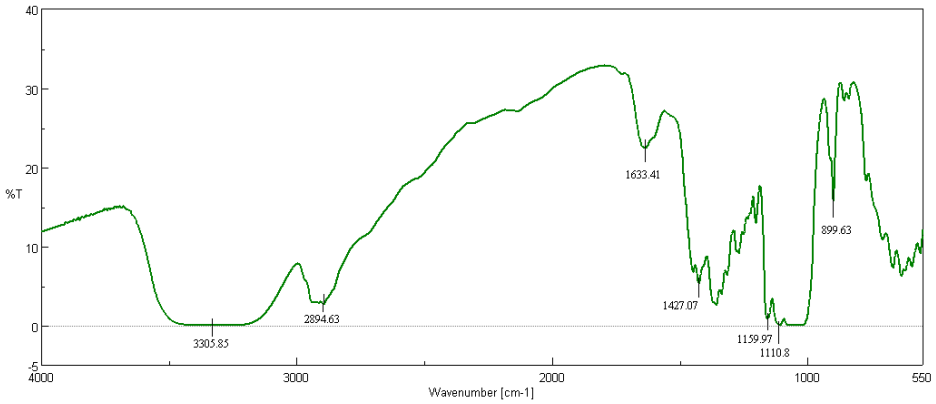

Fig. 9. FT-IR spectra of membrane A2

The swelling ability of the composite materials obtained is presented in Figs. 10 and 11.

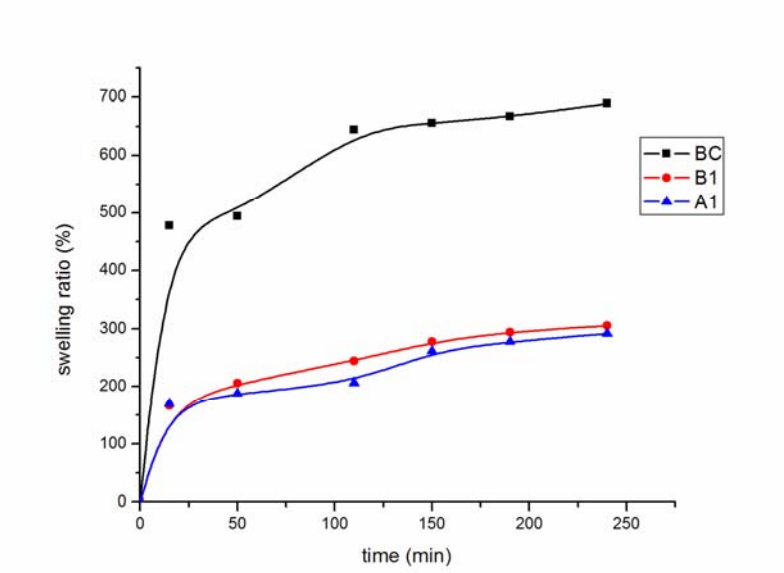

Fig. 10. Swelling behavior of membranes A1, B1 and simple BC membrane

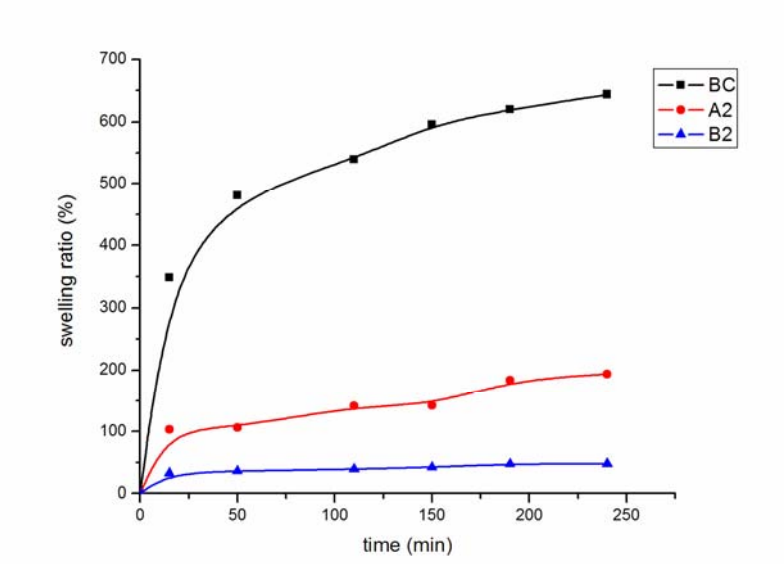

Fig. 11. Swelling behavior of membranes A2, B2 and simple BC membrane

Swelling studies revealed that simple bacterial cellulose membranes had more capacity to absorb water, until 600 % from their initial weight, than the composite materials impregnated with silver, which absorbed water up to 300 % of their dry weight. Also, it is easily to observe that the composite materials with more silver deposed onto the surface of the BC membrane had lower swelling ability, respectively membranes A2 and B2. This phenomenon is explained by the rigid structure of these materials, owing to metallic silver particles, which does not permit water molecules to diffuse through the membrane and to form intermolecular hydrogen bonds. For membranes A1 and B1 the rate of swelling was very rapid such that more than 100 % weight gain was achieved within 10 minutes of the test. For all the composite materials with impregnated silver the swelling phenomenon reached steady state after 15 minutes. The antimicrobial activity of BC membranes impregnated with silver against *Candida sp*. yeast and *Pseudomonas aeruginosa* has been tested, the results being presented elsewhere. The growth of tested microorganisms was inhibited in the presence of elemental silver in comparison with untreated BC membrane [17].

6. Conclusions

Composite materials based on bacterial cellulose membranes impregnated with metallic silver were obtained using Tollens reaction. The surface morphology of BC membranes with and without silver was characterized using SEM images. The energy dispersive X-ray spectra demonstrated that all composite materials obtained contain elemental silver and that the silver particles are uniform distributed in the material. The FT-IR spectra revealed the characteristic groups of bacterial cellulose which did not interact with silver. The swelling studies show a lower ability to retain water for the composite materials obtained comparing with simple BC pellicles. This weak swelling property does not allow microorganisms to develop at the food product surface. Bacterial cellulose membranes containing silver could be promising materials for food packaging due to their morphological characteristics. More studies are necessary to investigate also optical properties of the obtained films and their biodegradation properties.

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