View metadata, citation and similar papers at core.ac.uk
brought to you by CORE
provided by CiteSeerX

Digest Journal of Nanomaterials and Biostructures Vol. 8, No. 4, October – December 2013, p. 1711 - 1717

SILVER GREEN SYNTHESIS ON BACTERIAL CELLULOSE MEMBRANES USING TANNIC ACID

S. I. JINGA[a], G. ISOPENCU[a*], A. STOICA- GUZUN[a], M. STROESCU[a], M. FERDES[b], B. OHREAC[a]
[a]*University Politehnica of Bucharest, Faculty of Applied Chemistry and Materials Science, Polizu 1-3, Bucharest, 011061, Romania*
[b] *University Politehnica of Bucharest, Faculty of Biotechnological Systems Engineering, Splaiul Independentei 313, Bucharest, 060042, Romania*

Silver nanoparticles were deposed on bacterial cellulose (BC) membranes using tannic acid as reducing agent. The synthesis of silver nanoparticles was confirmed by scanning electron microscopy (SEM), energy dispersive spectroscopy with X-ray (EDX) and UV-VIS spectroscopy. The antimicrobial activity of BC-silver films was tested against *E. coli* K12-MG1655, all the composites having a good antimicrobial activity. These composites could be used for antimicrobial wound dressings.

(Received October 4, 2013; Accepted November 21, 2013)

Keywords: Biomaterials, Composite materials, Bacterial cellulose, Silver, Tannic acid

1. Introduction

Cellulose is one of the most abundant natural biopolymer with a lot of applications in many fields of activity, i.e. textile industry, paper industry and medical field, due to its biodegradability and biocompatibility [1]. A growing interest has been also dedicated to microbial cellulose, produced by Gram-negative, acetic acid bacteria, *Gluconacetobacter xylinus (formerly Acetobacter xylinum)* being considered the most efficient strain for bacterial cellulose production. Even if bacterial cellulose (BC) has the same chemical composition as plant cellulose, it has also some remarkable properties, being superior due to its unique nanostructure, high crystallinity, large water holding capacity and enhanced mechanical properties [2].

This biopolymer is also a promising material particularly for medical applications. In a brief enumeration, bacterial cellulose was already tested for treating chronic wounds, burns, for skin repair, but also to replace blood vessels and for nerve and bone regeneration [3-6].

BC is characterized by an ultrafine 3-dimensional network structure and could be considered as an ideal matrix for metal incorporation. It is well known the fact that bacterial cellulose has not antimicrobial activity and this is a drawback when it is used as material for wound dressing. For this reason, bacterial cellulose was the subject of many investigations in order to obtain bionanocomposites with antimicrobial properties, especially with silver and its compounds [6-10].

For silver green synthesis many bacteria, fungi and plant extracts were already tested [11-16]. Tannic acid, which is a polyphenolic plant extract, was also used to obtain silver nanoparticles with different shapes [17, 18]. To the best of our knowledge a study of silver synthesis on bacterial cellulose membranes using tanning acid has not been conducted yet.

*Corresponding author: g_isopencu@chim.upb.ro

2. Materials and methods

2.1. Production and purification of bacterial cellulose membranes

Bacterial cellulose membranes were produced by *Acetobacter* sp. isolated from traditionally fermented apple vinegar in Microbiology Laboratory of Chemical and Biochemical Engineering Department of University Politehnica of Bucharest. The culture was grown in a modified Hestrin-Schramm (MHS) medium containing 2% fructose using a rotating biofilm contactor. The pellicles obtained were purified by treatment with 0.5 N NaOH aqueous solutions at 90 ºC for 1 h to eliminate the bacterial cells. The BC gel-like membranes were then washed with deionized water until the pH of water became neutral. BC pellicles were used as never dried membranes.

2.2. Composite membranes preparation

Silver particles were synthesized *in situ* into bacterial cellulose membranes using tannic acid as reducing agent. The films were prepared using the following procedure: a gel membrane of bacterial cellulose was immersed for 5 minutes into Tollens' reagent (film A) or in an $AgNO_3$ aqueous solution (0.01 M) (film B) and then into tannic acid solution (0.75 % w/w) for different periods of time: 3, 5 and 10 minutes. The films were then rinsed with distilled water and dried at room temperature for 48 hours. For the films identification, capital letters A or B are followed by a number which indicates the reaction time increase. For example, A3 means film A after 10 minutes of reaction time.

2.3. Characterization of BC films containing silver

The morphology of bacterial cellulose-silver composites was observed using a HITACHI S-2600N scanning electron microscope operating at 15-25 kV at a magnification of 5000-60000K. Silver particles formed and silver elemental distribution was relieved using energy dispersive X-ray spectroscopy (SEM-EDS). All specimens were coated with gold before SEM observation.

Possible interactions between bacterial cellulose and silver were studied by Fourier transform infrared (FT-IR) spectroscopy using a Jasco FT/IR 6200 with Intron μ Infrared Microscope with ATR-1000-VZ objective. The spectra were the average of 50 scans recorded at a resolution of 4 cm^{-1} in the range from 4000 to 500 cm^{-1} with a DLATGS detector.

The color of the films was also measured using a spectrophotometer UV-2450 with an integrating sphere (Shimadzu, Japan). Standard values considered were those of the white background. Calculations were made for D-65 illuminant and 2^0 observer. Color measurements were replicated three times for each type of film.

Hunter color scale of lightness (L), a (red–green), and b (yellow– blue) values were used to calculate the total color difference (ΔE) and the yellowness index (YI) using relations 1-2 [19].

$$\Delta E = \left(\Delta L^2 + \Delta a^2 + \Delta b^2\right)^{0.5} \quad (1)$$

$$YI = 142.86 \cdot b / L \quad (2)$$

where:

$\Delta L = L_s - L_{sample}$; $\Delta a = a_s - a_{sampke}$; $\Delta b = b_s - b_{sample}$

Reflectance spectra of obtained composites were also measured using the same spectrophotometer to evidence the presence of silver nanoparticles.

2.4. Antimicrobial activity

E. coli K12-MG1655 from Laboratory of Microbiology of Chemical and Biochemical Engineering Department of University Politehnica of Bucharest was used to test antimicrobial

activity of obtained films. Culture medium (Luria-Bertani) was inoculated with 1% suspension of microorganisms. The controlled release of antimicrobial agent from the composite membranes was examined in term of zone inhibition. Sterilized circular film pieces with constant weight were placed onto the surface of the microorganism – containing agar. After incubation at 37^0 C, the zone inhibition around the membrane samples (if any) was measured after 96 h.

3. Results and discussion

3.1. Characterization of BC-silver composites

The size and morphology of silver nanoparticles in the BC matrix are different from one film to another and are influenced by the pH of the medium and by reaction time. Figure 1 shows SEM images of the composite films A and B after different reaction time.

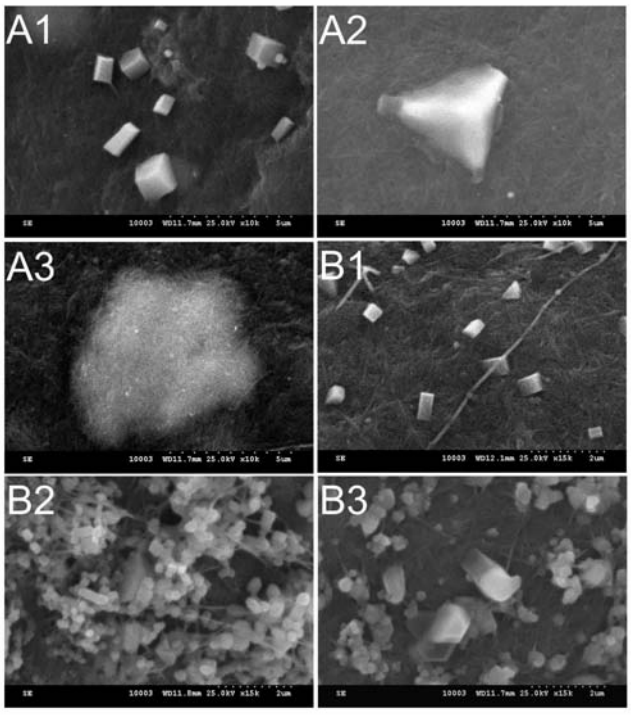

Fig. 1. SEM pictures of composite films BC-silver: A1-A3 and B1-B3.

For film A, obtained in alkaline conditions (pH=9), different shapes are observed for silver particles with the increasing of growing time, from cubic shape (A1) to triangular shapes (A2) and cluster containing spherical nanoparticles (A3). Small spherical particles are also observed in all three images (A1-A3). It was already demonstrated that tannic acid partially hydrolyses under mild acidic/basic conditions into gllucose and gallic acid. Such tannic acid could act as reducing agent under alkaline conditions at room temperature and it could accelerate the reaction process [17, 20]. Films B obtained in acidic conditions (pH=5) present a great polydispersity of silver particles, especially with the increase of reaction time. Highly agglomerated silver structures with a variety of morphologies are observed especially for film B2. With the increase of reaction time larger particles are also observed (film B3). The structure of the observed silver nanoparticles could not be explained only by the effect of pH upon tanninc acid, but also by considering BC nanostructure. It was already demonstrated that Ag^+ could be adsorbed on bacterial cellulose through their pores and also could interact with cellulose hydroxyl groups. The BC network could act as stabilizing template during the nucleation and growth of silver particles [10, 11]. For this reason, our results

are not identical with those obtained using tannic acid in solution without any solid support [17, 18]. All SEM-EDX spectra of the composite materials obtained confirm the presence of silver inside the films, as it can be observed in Figure 2. The silver distribution maps showed also a uniform assignment of the particles (the results are not presented).

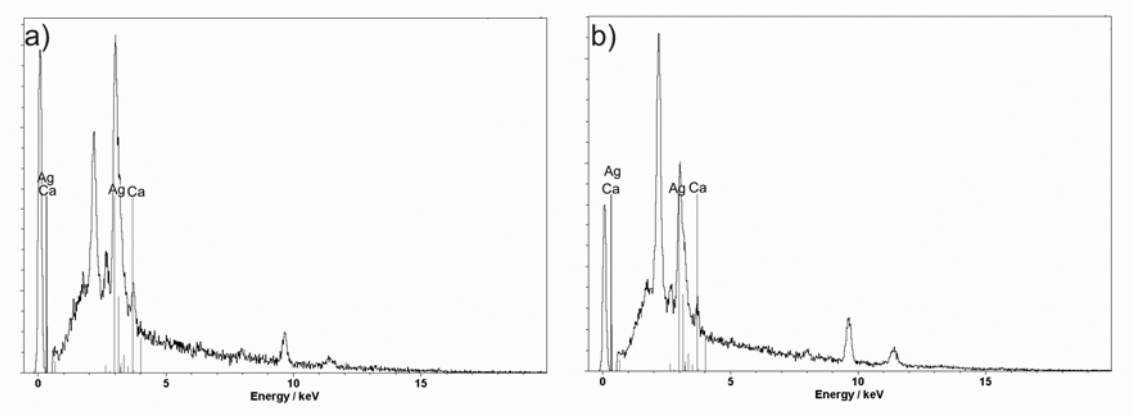

Fig 2. SEM-EDX spectra for composites films BC –silver: a) film A3 and b) film B3

Fig. 3 presents FT-IR spectra of a pure bacterial cellulose membrane and of films A3 and B3 for comparison. The characteristic bands of bacterial cellulose (Figure 3a) appeared at 3335 cm^{-1} for O-H stretching vibration, at 2895 cm^{-1} for C-H stretching vibration, at 1426 cm^{-1} for C-H bending vibration, at 1159 cm^{-1} for C-O-C asymmetric stretching vibration from the glycosidic ring [21]. The peak at 1632 cm^{-1} represents water molecules in the amorphous region. The spectra of films A3 (fig. 3b) and of film B3, which contains silver formed on bacterial cellulose matrix, presents the characteristic absorption peaks of bacterial cellulose. Only small peaks shifts were observed in comparison with BC spectrum. For example, the peak at 1632 cm^{-1} is found at 1638 cm^{-1} in the composite spectrum of film A3 and at 1644 cm^{-1} in the composite spectrum of film B3.

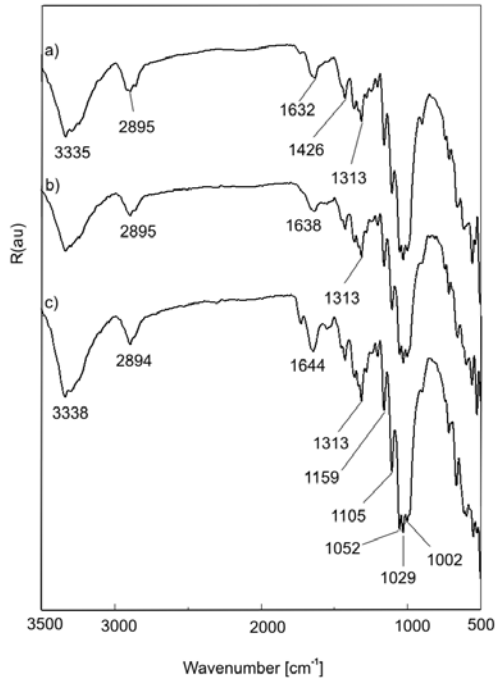

Fig.3. FT-IR spectra of BC dry membrane and films A3 and B3: (a) control sample BC, (b) Film A3 and c) film B3.

In conclusion, FT-IR spectra of all the composites are similar with the infrared spectrum of bacterial cellulose, which means that no interactions exists between silver particles and functional groups from bacterial cellulose. Due to its 3-dimensional structure BC allows quest molecules to penetrate in its network and, probably, the silver particles are such guest molecules in the obtained BC-silver membranes. We suppose that there are too much weaker interactions between silver and bacterial cellulose chains.

The reflectance UV-VIS spectra of all films are presented in figure 4. The characteristic band of the silver surface plasmon was detected for all the films at 440 nm, indicating the formation of larger size nanoparticles [22]. It is also possible that the excessive silver-ammonia complex be adsorbed on the nanoparticles surfaces for films A1-A3 and contribute also to a broad size distribution.

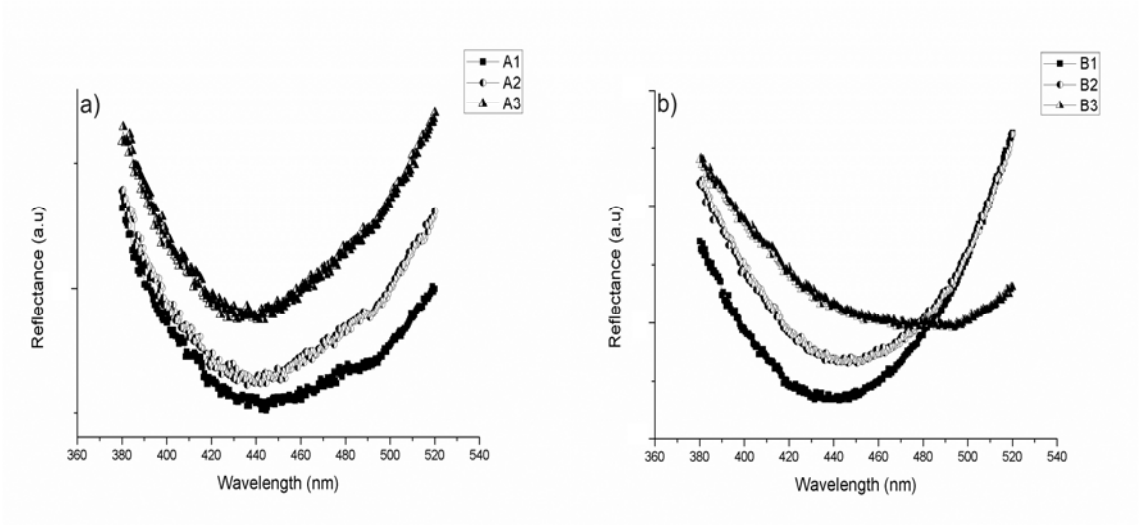

Fig. 4. (a) Reflectance spectra of films A1-A3; (b) Reflectance spectra of films B1-B3.

The presence of silver particles affected the appearance of the composite films in both color and transparency. Hunter color parameters (L, a, and b), color difference (ΔE), and yellowness index (YI) for the studied samples are presented in Table 1. The redness value (a^*) is increasing from film A1 to film A3, indicating that the films became dark brown due to the presence of silver in the films. In the case of films B1-B3, the redness value is slightly decreasing, being at high values from the beginning. The presence of silver deposed on bacterial cellulose sheets increase the yellowness, as evidenced by high b^* and YI values. These parameters are increasing from film B1 to B3.

Table 1 Hunter color parameters and yellowness index for all the studied samples

Sample	L	a	b	ΔE	YI
A1	33.58 ± 0.03	0.562 ± 0.06	8.08 ± 0.07	67.05 ± 0.08	23.57 ± 0.07
A2	39.43 ± 0.80	2.37 ± 0.34	11.31 ± 0.59	61.65 ± 0.44	40.98 ± 0.77
A3	39.54 ± 0.81	13.00 ± 0.88	16.24 ± 0.87	64.40 ± 0.35	58.681 ± 0.80
B1	44.16 ± 0.89	13.15 ± 0.07	11.41 ± 0.43	59.44 ± 0.08	27.22 ± 0.05
B2	47.75 ± 0.15	12.31 ± 0.69	12.95 ± 0.84	55.97 ± 0.84	38.75 ± 0.08
B3	55.46 ± 0.86	10.22 ± 0.06	25.14 ± 0.57	51.39 ± 0.08	64.75 ± 0.49

[#] Color parameters for white standard were: L_s= 100.32; a_s= -5.41 and b_s=5.5

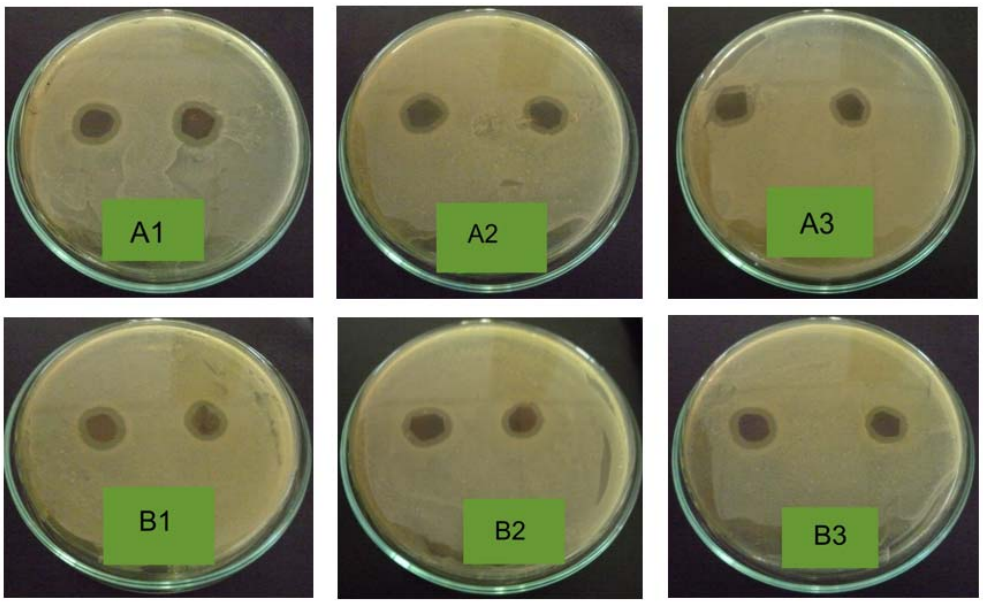

Fig. 5. Images showing the inhibition zone of BC-silver composites against E. coli: films A1-A3 and films B1-B3.

3.2. Antimicrobial activity

The results obtained for antimicrobial activity against *E. coli* K12-MG1655 of BC-silver composites are presented in Figure 5. All the composites containing silver particles showed mild inhibition of bacterial growth, without notable differences between the two series of films A and B.

4. Conclusions

Green synthesis of metallic silver deposed on bacterial cellulose membranes using tannic acid as reducing agent was conducted. The energy dispersive X-ray and UV-VIS spectra demonstrated that all composite materials obtained contain elemental silver. The morphology of nanoparticles was determined from SEM images. A variety of silver nanoparticles was observed: from spherical, to cubic and triangular morphologies considering pH variation and also BC presence as solid support. The composite materials obtained on a basis of BC-silver showed inhibitory activity against *Escherichia Coli* K12-MG1655. The advantages of the proposed method are: eco-friendly materials used and the possibility of obtaining silver composite materials at room temperature in a short reaction time.

Acknowledgement

Financial support from UEFSCDI–Romania (Research Grant–SABIOM-No. 114/2012) is gratefully acknowledged.

References

[1] G. Siqueira, J. Bras, A. Dufresne, Polymers, **2**, 728 (2010).
[2] P. Chen, S. Y. Cho, H-J. Jin, Macromol. Res., **18**, 309 (2010).
[3] W. Czaja, A. Krystynowicza, S. Bielecki, R. M. Brown Jr., Biomaterials, 27, 145 (2006).
[4] H. Bäckdahl, B. Risberg, P. Gatenholm, Mat. Sci. Eng. C, **31,** 14 (2011).

[5] M. Zaborowska, A. Bodin, H. Bäckdahl, J. Popp, A. Goldstein, P. Gatenholm, Acta Biomater., **6**, 2540 (2010).
[6] L. Fu, J. Zhang,G. Yang, Carbohydr. Polym., **92,** 1432, (2013).
[7] Maneerung T, Tokura S, Rujiravanit R, Carbohydr. Polym., **72**, 43 (2008).
[8] H.S. Barud, C. Barrios, T. Regiani, R. F. C. Marques, M. Verelst, J. Dexpert-Ghys, Y. Messaddeq, S. J. L. Ribeiro, Mat. Sci. Eng. C, **28**, 515 (2008).
[9] L. C. de Santa Maria, A. L. C. Santos, P. C. Oliveira, H.S. Barud, Y. Messaddeq, S.J.L. Ribeiro, Mat Lett, **63**, 797 (2009).
[10] G. Yang, J. Xie, F. Hong, Z. Cao, X. Yang, Carbohydr. Polym., **87**, 839 (2012).
[11] G. Yang, J. Xie, Y. Deng, Y. Bian, F. Hong, Carbohydr Polym, **87**, 2482 (2012).
[12] V. K. Sharma, R. A. Yngard, Y. Lin, Adv. Colloid Interf. Sci., **145**, 83 (2009).
[13] R. Vaidyanathan, K. Kalishwaralal, S. Gopalram, S. Gurunathan, Biotech. Adv., **27**, 924 (2009).
[14] K. Vijayaraghavan, S.P. Kamala Nalini, Biotechnol. J., **5**, 1098 (2010).
[15] A. Singh, S. Mittal, R. Shrivastav, S. Dass, J.N. Srivastava, Dig. J. Nanomater. Bios., **7**, 1157 (2012).
[16] Y. Y. Loo, B. W. Chieng, M. Nishibuchi, S. Radu, Int. J. Nanomedicine, **7**, 4263 (2012).
[17] Z. Yi, X. Li, X. Xu, B. Luo, J. Luo, W. Wu, Y. Yi, Y. Tang, Colloids Surfaces A: Physicochem. Eng. Aspects, **392**, 131 (2011).
[18] Dadosh T. Mat Lett, **63**, 2236 (2009).
[19] J. W. Rhim, Y. Wu, C. L. Weller, M. Schnepf, J. Food. Sci, **64**, 149 (1999).
[20] S.K. Sivaraman, I. Elango, S. Kumar, V. Santhanaman, Current Science, **97**, 1055 (2009).
[21] S. Gea, C. T. Reynolds, N. Roohpour, B. Wirjosentono, N. Soykeabkaew, E. Bilotti, T. Peijs, Bioresource Technol., **102**, 9105 (2011).
[22] X. Wu, T. Wang, H. Wu, J. Wuhan Univ .Technol. Mater. Sci. Ed., **26**, 38 (2011).