SYNTHESIS OF SPHERICAL BACTERIAL NANOCELLULOSE AS A POTENTIAL SILVER ADSORPTION AGENT FOR ANTIMICROBIAL PURPOSES

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In this research, bacterial nanocellulose spheres (BNCs) were produced by *Gluconacetobacter xylinus* and employed as adsorbents of silver ions in aqueous solutions. Subsequently, the spheres were employed as bactericidal agents through the disk diffusion method in agar against *Escherichia coli* and *Staphylococcus aureus* bacteria. Kinetic data were adjusted to the Homogeneous Surface Diffusion Model, presenting an intraparticle diffusivity of silver ions (D_{int}) of 1.088 x 10^{-15} m² s^{-1}. The isotherm data were adjusted to the Langmuir and Freundlich models. Langmuir was the model that best described the adsorption isotherm, where the BNCs presented a maximum adsorption capacity of 55 mg g^{-1}. The results of the disk diffusion method in agar proved the antimicrobial properties of the spheres, with zones of inhibition of 3 mm for *E. coli* and 2 mm for *S. aureus*.

Keywords: bacterial cellulose, *Gluconacetobacter xylinus*, adsorption, silver, antimicrobial

INTRODUCTION

Cellulose is a long-chain polysaccharide whose molecular formula is $(C_6H_{10}O_5)_n$. Since cellulose is an important constituent of the plant cell wall and is widely available, it has wide application in the textile and paper industries.[1] In addition to the traditional methods of cellulose extraction, some microorganisms have the ability of synthesizing this polysaccharide, such as *Gluconacetobacter xylinus*, which excretes glycan chains that are added in microfibrils forming cellulose tapes.[2] Bacterial cellulose may be employed in biomedical engineering industries due to its biocompatibility and non-toxicity, in paper and packaging industries, since it presents characteristic properties, such as high crystallinity, biodegradability, great mechanical strength and high thermal stability.[3]

It is known that the adsorption technique is highly efficient for removing contaminants from liquid effluents due to its ease of operation, high efficiency and relatively low cost.[4] Several metals extremely harmful to humans and the environment can be removed by adsorption.[5] Silver is a metal widely used in industries owing to its ductility, malleability and electrical photosensitivity.[6] Besides that, it is toxic and has antimicrobial properties causing the death of several microorganisms.[7] Therefore, the synergistic effect between the removal of this contaminant by adsorption and consequent determination of the bactericidal potential of the adsorbent becomes a study of great relevance.

Several works addressing the preparation of silver-impregnated microbial cellulose have been

reported in the literature. Kim *et al.*[8] used the sodium borohydrate reduction method to impregnate silver in cellulose, which was then tested as a bactericidal agent against *Candida albicans*, *Micrococcus luteu*, *Pseudomonas putida* and *Escherichia coli*. Pal *et al.*[9] prepared a microbial cellulose membrane functionalized with silver nanoparticles by ultraviolet reduction and tested it against *Escherichia coli*. Wu *et al.*[10] synthesized multifunctional nanocellulose composite films with grape seed extracts and immobilized silver nanoparticles on them, testing the antimicrobial activity against *E. coli* and *S. aureus*. Jalili and Emtiazi[11] studied the production of bacterial cellulose impregnated with silver nanoparticles through *in situ* reduction of silver nitrate as precursor in the presence of sodium tripolyphosphate (TPP) and sodium hydroxide, and determined the antimicrobial activity against *E. coli* and *S. aureus*. Volova *et al.*[12] synthesized bacterial cellulose composites with silver nanoparticles and studied the antimicrobial activity against *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia* and *Staphylococcus aureus*. In these works, different methods to impregnate an antimicrobial agent on bacterial cellulose were developed, without addressing the adsorption potential of silver from contaminated aqueous solutions.

In the present study, bacterial nanocellulose spheres (BNCs) were produced by *Gluconacetobacter xylinus* for two purposes. The first objective was determining the adsorption potential of the obtained BNCs for the treatment of silver-contaminated aqueous solutions. The second objective was the subsequent application of the BNCs for determining their antimicrobial activity against *Escherichia coli* and *Staphylococcus aureus*, which are the leading bacterial pathogens associated to bacteremia and healthcare-associated infections.[13]

EXPERIMENTAL

Bacterial nanocellulose spheres (BNC) were produced by *Gluconacetobacter xylinus*. The pre-inoculum was prepared (10% v/v) in Hestrin and Shramm (HS) liquid medium containing 25 gL^{-1} carbon source, 5 g L^{-1} peptone, 5 g L^{-1} yeast extract, 2.7 g L^{-1} Na_2HPO_4 and 1.2 g L^{-1} citric acid (pH 6.5); the growth proceeded under static condition for 5 days. The production of the spheres was carried out in 250 mL Erlenmeyer flasks, containing 100 mL of medium with 10% of pre-inoculum. The flasks were maintained at 26 °C, 160 rpm, for 5 days. After the incubation period, the BNC spheres were withdrawn from the

culture medium, washed with distilled water and purified. Purification was performed using 100 mM NaOH at 50 °C for 24 h. The spheres were washed with distilled water until the pH reached 7.4. Then, the spheres were autoclaved for 20 min at 121 °C.

Bacterial nanocellulose spheres were characterized by Fourier-transform infrared spectrophotometry (IR Prestige 21) to determine the functional groups present in the adsorbent. The spheres crystallinity was determined by X-ray diffraction (XRD, Rigaku Miniflex 300). Scanning electron microscopy with energy dispersive X-ray spectrometry (SEM/EDS, JEOL JSM-6390LV) was employed to observe the morphological characteristics and elemental mapping of the material.

The adsorption experiments were carried out in a batch system, using an aqueous solution of silver salt (20 mL) and an adsorbent dosage of 0.31 g L^{-1} under magnetic stirring. The adsorbent dosage to be used was determined by preliminary tests. Solutions of varied concentrations were obtained by dilution from a silver solution of 1000 mg L^{-1}, which was prepared by dissociating silver nitrate in deionized water. After each experiment, the BNCs were separated from the solution by filtration and the residual silver concentration was determined by atomic absorption spectroscopy (Agilent 240FS AA).

The models, parameters and equations used to describe the adsorption kinetics and adsorption isotherm are presented in a previous work.[14]

Antimicrobial activity was evaluated by the agar diffusion method. Tests were performed against gram-positive bacteria (*Staphylococcus aureus* ATCC 25923) and gram-negative bacteria (*Escherichia coli* ATCC 11229). For the diffusion tests in agar, the bacterial cultures were maintained in Mueller-Hinton agar, from which the colonies were isolated in order to select a turbidity of 0.5 on the McFarland scale in Mueller-Hinton agar. A sample of this bacterial solution was inoculated using a swab in Mueller-Hinton agar plates. The spheres were deposited aseptically in the Mueller-Hinton agar with the corresponding bacteria. All the plates were incubated at 37 °C during 24 h in a bacteriological incubator, and then, the zones of inhibition were evaluated.[15]

RESULTS AND DISCUSSION
Characterization of BNCs

The characterization results of the BNCs are shown in Figure 1. Figure 1 (a) shows that the bacterial nanocellulose spheres have a crystalline structure, with two strong peaks characteristic of cellulosic materials at 2θ of 14.4° and 22.7°, which are assigned to the diffraction planes of (101) and (002), respectively (JCPDS #03-0829).[1,9,16] This crystallinity comes from the establishment of the intra- and intermolecular hydrogen bonds formed between the cellulose

chains. The crystalline structure ensures good stability of the BNCs and renders them insoluble in water, making possible their use as an adsorbent in aqueous medium.

From Figure 1 (b), it is possible to observe the functional groups constituting the spheres. The band around 3400 cm^{-1} corresponds to the stretching vibration of the hydroxyl group in polysaccharides. The band at 2900 cm^{-1} is attributed to the C-H stretching vibration of cellulose, and the band at 1640 cm^{-1} is attributed to water molecules absorbed in cellulose.[17] The

bands at 1426, 1370 and 1160 cm^{-1} are attributed to CH_2 symmetric bending, C-H bending vibration and C-O-C asymmetrical stretching of cellulose, respectively.[18] The bands around 1050 and 900 cm^{-1} are due to the C-O-C stretching vibration of cellulose.[17] The band around 670 cm^{-1} is attributed to O-H out-of-phase bending.

Figure 1 (c) shows the well-organized cellulose fibers, which are responsible for the crystalline character of the BNCs. In addition, the BNCs have a fibrillated and interlaced nanometric structure.
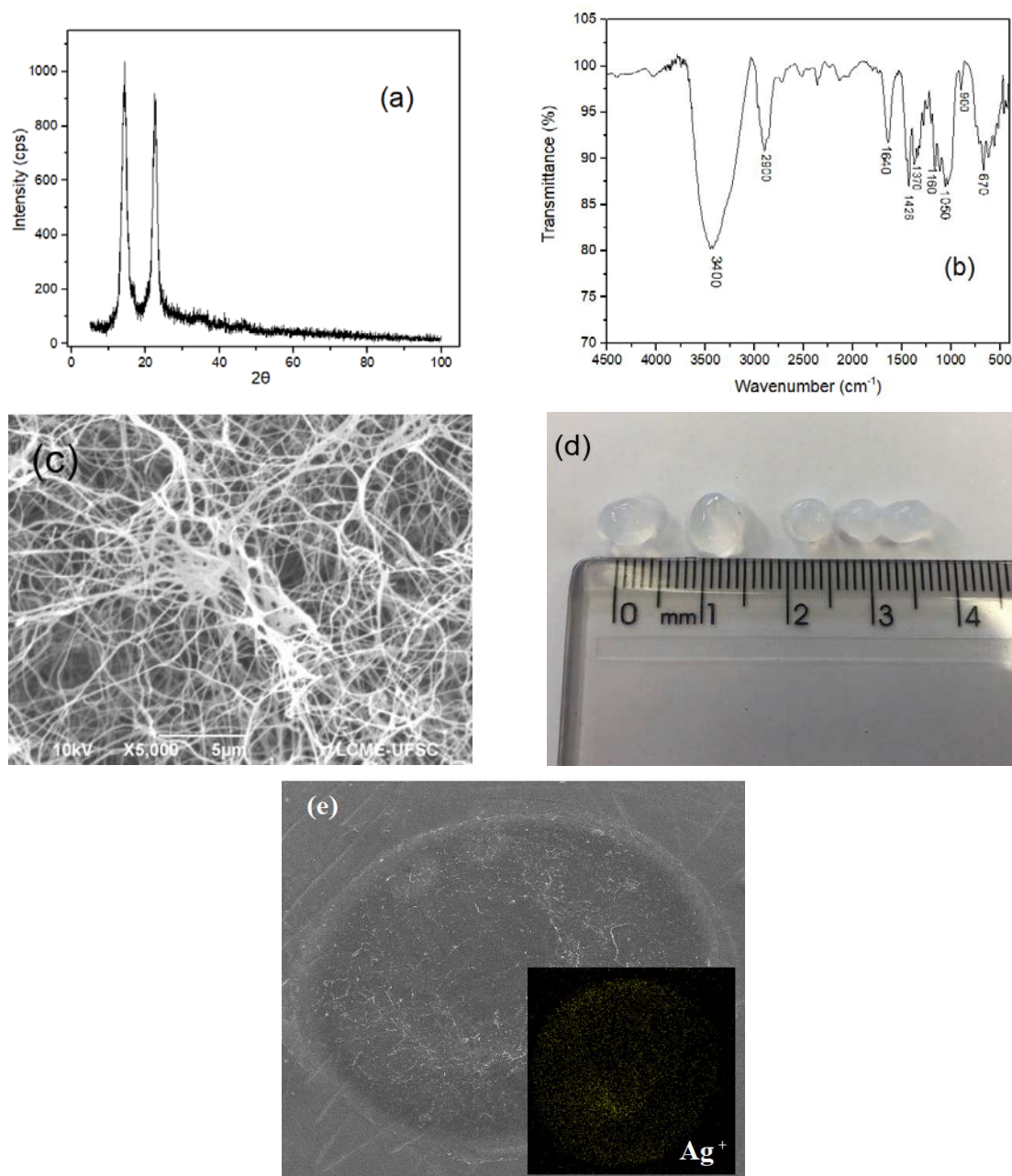

Figure 1: Characterization of bacterial nanocellulose spheres; (a) XRD, (b) FTIR, (c) MEV, and (d) photograph of BNCs; (e) elemental mapping before and after (inset) adsorption of Ag^+

Figure 1 (d) shows the macromorphology of the spheres, revealing their white and translucent aspect and rounded shape, with sizes around 5 mm. Figure 1 (e) shows a BNC used as a silver adsorbent in aqueous solution, and the inset image – mapping of the adhered silver on the BNC sample, exhibiting good distribution of the silver on the surface of the BNC.
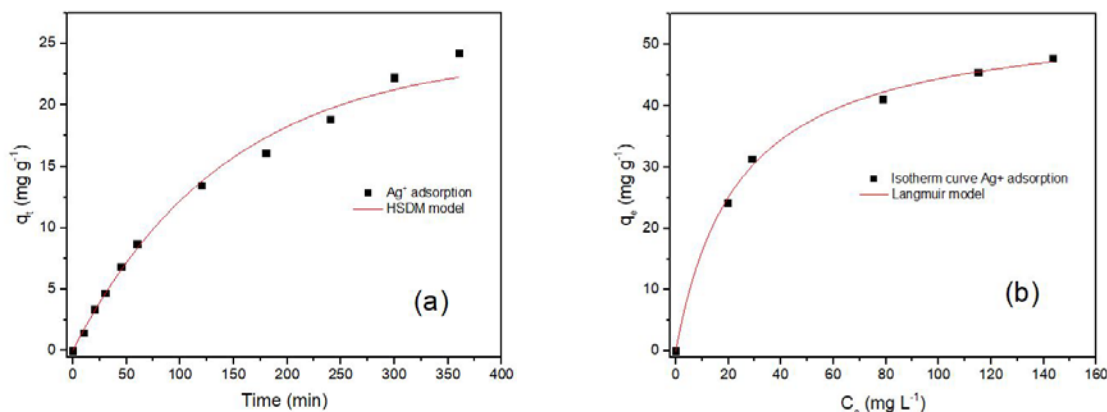

Figure 2: Adsorption experiments, (a) kinetic curve and (b) isotherm curve of silver adsorption onto bacterial nanocellulose spheres

Table 1
Isotherm parameters for Ag$^+$ adsorption on BNCs

Model			
Langmuir		Freundlich	
R^2	0.998	R^2	0.994
R^2_{adj}	0.997	R^2_{adj}	0.993
ARE (%)	1.797	*ARE* (%)	3.02
q_m	55.05	k_f	10.39
k_l	0.042	n_f	3.21

R^2 = coefficient of determination; R^2_{adj} = adjusted coefficient of determination; *ARE* = average relative error; q_m = maximum adsorption capacity (mg g^{-1}); k_l = equilibrium constant of Langmuir (L mg^{-1}); k_f = Freundlich constant ((mg g^{-1}) (mg L^{-1})$^{-1/nf}$); nf = exponent of Freundlich

Adsorption kinetics and isotherm

The kinetic data were adjusted to the HSDM model to estimate the silver ion diffusivity in BNCs. The values of the coefficient of determination (R^2) of 0.989, the adjusted coefficient of determination (R^2_{adj}) of 0.988 and the average relative error (*ARE* %) of 5.39 indicate a good fit of the experimental data to the model, as can be seen in Figure 2 (a). The value of D_{int} was 1.088×10^{-15} m^2 s^{-1}, corroborating in terms of order de magnitude with those reported in other works involving metals adsorption from aqueous solutions.[19,20]

The isotherm data were fitted to the Freundlich and Langmuir models, and the results are shown in Figure 2 (b) and Table 1. Due to the high values of R^2, R^2_{adj} and low value of *ARE* (%), the data were considered to fit better to the Langmuir model, where the maximum theoretical adsorption capacity of silver by BNCs was 55 mg g^{-1}. This satisfactory adsorption capacity of Ag$^+$ ions can be associated with the presence of negatively charged functional groups on the cellulose surface, such as carboxyl groups (as observed by FTIR analysis, Fig. 1 (b)), as the positively charged silver interacts with the electrons of carboxyl by electrostatic attraction.[21] In addition, the BNCs can be considered as a potential adsorbent for silver removal, when compared to other adsorbents, such as clinoptilolite,[22] cellulose and chitin nanomaterials,[21] montmorillonite,[23] verde-lodo bentonite[6] and chitosan/bamboo charcoal composite beads,[24] which achieved maximum capacities of Ag$^+$ adsorption of 33.33 mg g^{-1}, 34.4 mg g^{-1}, 42.06 mg g^{-1}, 9.63 mg g^{-1} and 52.09 mg g^{-1}, respectively.

Antimicrobial activity of BNCs

The results of the antimicrobial activity of the spheres used as adsorbents of silver ions against *Escherichia coli* and *Staphylococcus aureus* bacteria are shown in Figure 3 (a) and (b), respectively. In both figures, it is possible to spot the presence of a zone of inhibition in the growth of the bacteria, proving the antimicrobial properties of the spheres. The magnitude of the inhibition zone for *E. coli* was 3 mm, while for *S. aureus*, it was 2 mm. These values are, in general, in good agreement with previous findings from the literature, where zones of inhibition of 2 mm for *E. coli* and 3.5 mm for *S. aureus* were found by Maeerung *et al.*,[2] who used bacterial cellulose loaded with silver prepared by the impregnation method. Meanwhile Wu *et al.*[10] obtained zones of inhibition of 1.24 mm for *E. coli* and of 3.38 mm for *S. aureus*, using multifunctional nanocellulose composite films impregnated with silver. Jalili and Emtiazi[11] obtained zones of inhibition of 2 mm for *E. coli* and 5 mm for *S. aureus*, using bacterial cellulose impregnated with silver nanoparticles through *in situ* reduction of silver nitrate. Wu *et al.*[25] used silver-nanoparticles/bacterial cellulose composites and obtained zones of inhibition of 1.25 mm for *E. coli*, 2.2 mm for *Pseudomonas aeruginosa* and 2.34 mm for *S. aureus*. These results prove the efficient antimicrobial activity of the bacterial nanocellulose spheres synthesized in this work and used as silver ion adsorbent in aqueous solution.
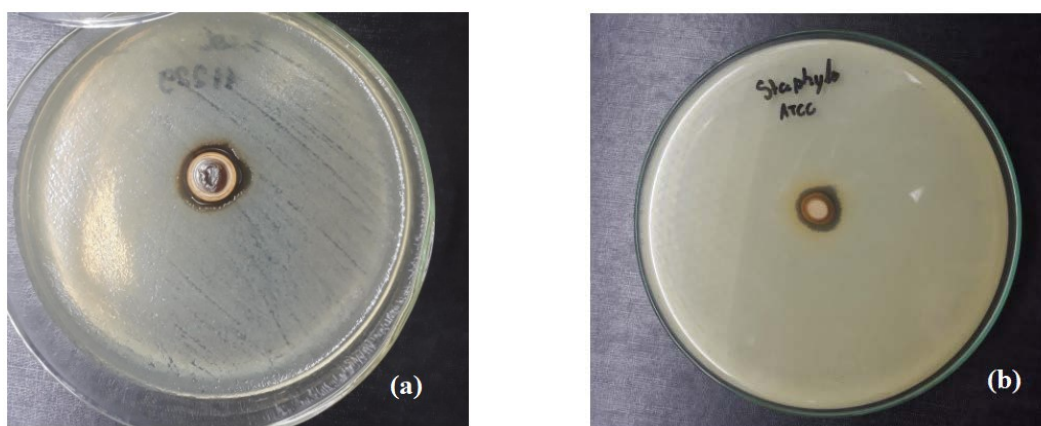

Figure 3: Determination of antimicrobial activity against (a) *E. coli* and (b) *S. aureus*

CONCLUSION

The utilization of cellulose spheres as silver ion adsorbent from aqueous solution, followed by determining the antimicrobial activity of the BNCs, was successfully achieved in this work. The kinetic study showed a relatively high value of intraparticle diffusivity (D_{int}) of silver ions inside the spheres. The Langmuir model described more accurately the adsorption isotherm, where the BNCs presented a remarkable value of adsorption capacity – of 55 mg g^{-1} of silver ions. The antimicrobial property of the BNCs used as adsorbents was demonstrated by means of the inhibition zones of 2 and 3 mm for the growth of *S. aureus* and *E. coli*, respectively. The remarkable adsorption capacity towards silver ions, together with the antimicrobial activity demonstrated against the microorganisms *S. aureus* and *E. coli*, recommends the BNCs as potential adsorbents for the removal of silver from aqueous solution and as remarkable antimicrobial agents.

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