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Environment

Production of oat hulls or nanofibrillated cellulose incorporated with biological silver nanoparticles

Produção de casca de aveia ou celulose nanofibrilada impregnada com nanopartículas de prata biológica

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ABSTRACT

Agroindustrial residues, such as oat hulls (OH), represent an underexplored source of cellulose with great potential for conversion into high-value nanomaterials. At the same time, there is growing demand for sustainable antimicrobial composites to replace conventional materials containing synthetic or toxic agents. The central goal of this study was to integrate both strategies: the valorization of oat hull residues through the production of cellulose-based materials, and their functionalization with biologically synthesized silver nanoparticles (bio-AgNPs) to impart antimicrobial properties. Reactive extrusion combined with peracetic acid bleaching was employed to produce the NFC. Raw OH and NFC were incorporated with bio-AgNP and characterized according to their antibacterial activity against Escherichia coli(E. coli). Raw OH incorporated with bio-AgNP demonstrated antimicrobial activity against E. coli, whereas NFC incorporated with bio-AgNP did not exhibit such activity. Further research is required to enhance the antimicrobial properties of the NFC material. This could be achieved by increasing the concentration of bio-AgNP or refining the final washing process.

Keywords: Nanocellulose; Lignocellulosic residues; Antibacterial properties

RESUMO

Resíduos agroindustriais, como a casca de aveia, representam uma fonte pouco explorada de celulose com grande potencial para conversão em nanomateriais de alto valor agregado. Ao mesmo tempo, cresce a demanda por compósitos antimicrobianos sustentáveis que substituam materiais convencionais contendo agentes sintéticos ou tóxicos. O objetivo central deste estudo foi integrar ambas as estratégias: a valorização da casca de aveia por meio da produção de materiais celulósicos e sua funcionalização com nanopartículas de prata biológica (bio-AgNPs) para conferir propriedades antimicrobianas. A extrusão reativa combinada ao branqueamento com ácido peracético foi empregada para produzir a NFC. As cascas de aveia in natura e a NFC foram impregnadas com bio-AgNP e caracterizadas quanto à atividade antibacteriana frente à Escherichia coli (E. coli). As cascas de aveia in natura impregnadas com bio-AgNP apresentaram atividade antimicrobiana contra E. coli, enquanto a NFC impregnada com bio-AgNP não apresentou tal atividade. Pesquisas adicionais são necessárias para aprimorar as propriedades antimicrobianas do material nanofibrilado, seja pelo aumento da concentração de bio-AgNP ou pela melhoria do processo final de lavagem do material.

Palavras-chave: Nanocelulose; Resíduos lignocelulósicos; Propriedade antibacteriana

1 INTRODUCTION

The Brazilian agroindustry generates several lignocellulosic residues, such as oat hull, a residue from the oat milling process. Raw oat hulls (OH) are mainly composed of cellulose, hemicellulose and lignin. In this context, the use of this byproduct can be considered an excellent alternative to many applications, such as production of membranes (Parchami et al. 2023; Patel et al., 2023), hydrogels (Sulianto et al., 2024), cellulose, and nanocellulose (Mateo et al., 2021; Mantovan et al., 2022; Ahmad Khorairi et al., 2023).

The nanocellulose obtained from lignocellulosic residues has attracted the attention of researchers worldwide due to its large range of applications. Two types of nanocellulose can be obtained from lignocellulosic residues. Cellulose nanocrystals are needle-shaped with dimensions equal to or less than 100 nm and are naturally highly crystalline. Nanofibrillated cellulose (NFC) consists of long fibers with a diameter of less than 100 nm and a length of several micrometers, with alternating crystalline and amorphous regions (Debiagi et al., 2020; Mateo et al., 2021; Mehanny et al., 2021; Padhi et al., 2023; Ahmad Khorairi et al., 2023).

To increase applications of cellulose and nanocellulose, these materials can be modified by inserting chemical groups with antimicrobial activity. Silver nanoparticles (AgNP) presented low volatility, high thermal stability and high surface area, concerning their volume, providing greater contact with the microorganism and having a lasting antimicrobial effect (Deshmukh et al, 2019). Thus, AgNPs can be applied across a wide range of sectors, including biomedicine (e.g., wound dressings, surgical clothing, drug delivery systems), pharmacology (as antimicrobial and anticancer agents), environmental engineering (water and wastewater treatment, pool filters), agriculture (crop protection and food packaging), and materials science (nanocomposites and coatings) (Bruna et al., 2021; Deshmukh et al., 2019; Kaushal et al., 2023; Khan et al., 2023; Luceri et al., 2023; Nie et al., 2023; Zeng et al., 2023).

According to Iravani et al. (2014) chemical or biological methods can be employed to produce AgNP. Chemical routes generally involve the reduction of silver salts (e.g., $AgNO_3$) using reducing agents such as sodium borohydride, hydrazine, or citrate, often combined with stabilizing/capping agents to control particle size and prevent aggregation (Jiang et al., 2025). Biological processes are characterized by employing fungi (Pineda et al., 2023; Pourali et al., 2023), bacteria (Mbagwu et al., 2023; Shantkriti et al., 2023), plants (Ansari et al., 2023; Albert et al., 2023; Yontar; Çevik, 2023) and biomolecules (Chaudhari; Dwivedi, 2023; Faisal et al., 2023) to produce biological silver nanoparticles (bio-AgNP), without the presence of chemical reagents and, consequently, without the generation of effluents (Prasad et al., 2021; Ahmed; Ogulata 2022; More et al., 2023).

Therefore, the central goal of this study is to integrate both strategies: the valorization of oat hull residues through the production of cellulose-based materials, and their functionalization with biologically synthesized silver nanoparticles (bio-AgNPs) to impart antimicrobial properties. By combining waste valorization and nanotechnology, this work aims to develop innovative, eco-friendly, and cost-effective composites with potential applications across various industrial sectors.

2 MATERIALS AND METHODS

OH were donated by a local oat processing industry (SL Alimentos-Mauá da Serra, Paraná, Brazil), and the residue was dried (45 °C) and milled (particles < 0.30 mm). Sodium hydroxide PA (NaOH beads, Synth, Brazil), sulfuric acid PA (H_2SO_4 98%, Synth, Brazil), glacial acetic acid PA-ACS (99%, Synth, Brazil), and hydrogen peroxide PA (H_2O_2 35%, Synth, Brazil) were employed to extract nanocellulose from the residue. Gram-negative bacteria *Escherichia coli* (ATCC 8739) was obtained from the Laboratory of Basic and Applied Bacteriology and Laboratory of Medical Mycology of the State University of Londrina culture collections.

2.1 Production of NFC

OH were bleached using peracetic acid according to the method described by Nascimento et al. (2016), with 120 g immersed in 2 L of a peracetic acid solution (50 % acetic acid, 38 % hydrogen peroxide, and 12 % distilled water). Subsequently, the samples were vigorously stirred at 60 °C for 48 h. After this step, the sample was neutralized with a 10% (w/w) NaOH solution until a pH range of 6 - 7. Later, the sample was washed with 1 L of water while being vigorously stirred at 60 °C for 24 h, dried in a ventilated oven at 40 °C (035 Marconi MA, Brazil), and then milled (< particles 0.30 mm).

After bleaching, OH were extruded with H_2SO_4 (2 % w/w), employing a single screw extruder (AX Plastics, Brazil) with a diameter of 1.6 cm and length/diameter ratio (L/D) of 40, with four heating zones and a matrix of 0.8 cm in diameter. In all extruder zones, the temperature was fixed at 110 °C, and the screw speed was 100 rpm. The material obtained was neutralized with a 10 % NaOH solution (w/w) until a pH range of 5 to 7, and dried in a ventilated oven at 60 °C (035 Marconi MA, Brazil). The bleached and extruded sample was labeled as BEOH.

2.2 Incorporation with bio-AgNP

The bio-AgNPs were produced by several strains of the fungus *Fusarium oxysporum*, according to Durán et al. (2005). The incorporation process was performed using 5 g of each sample (OH and BEOH), which were immersed in 100 mL of a distilled water solution containing 1 mM bio-AgNP, and subjected to 30 min of sonication, operating at 20 kHz with a maximum output power of 500 W (Fisher Scientific tip, model FB 4219, USA). The samples were incubated in a shaker (Bunker, model NI 1715, Brazil) for 24 h at 300 rpm at 25 °C. Afterwards, they were centrifuged (9000 rpm, 25 °C, 30 min) to remove the solid fraction, dried in a ventilated oven at 60 °C for 24 h (035 Marconi MA, Brazil), and finally milled (particles < 0.30 mm). Once the incorporation process was complete, the samples were labeled OH_{Ag} and $BEOH_{Ag}$.

2.3 Atomic force microscopy (AFM)

The morphology and dimension of nanocellulose were observed through an AFM, employing a NanoSurf FlexAFM instrument (Nanosurf AG, Switzerland) and using silicon AFM probes under air. The NFC dimension was determined by AFM micrographs utilizing an image analysis program (ImageJ 1.37v®), with 20 measurements used for this assessment.

2.4 Antimicrobial activity *against E. coli*

Initially, the samples underwent sterilization via moist heat. Subsequently, a quantitative antimicrobial activity analysis against *E. coli* ATCC 25922 was conducted. The bacterial density suspension was measured using McFarland's 0.5 turbidity scale, suspending colonies in sterile saline (0.85 % NaCl in water w/v), corresponding to approximately 1 x 10^8 UFC/mL of the *E. coli*. Then, 0.25 g of each sample was mixed with an aliquot of 20 µL of this bacterial suspension and 3.980 µL of Mueller-Hinton broth (MHB, Difco). Samples not incorporated with bio-AgNP were used as negative controls for antibacterial activity. Volumes of 10 µL from both treated and untreated samples were transferred to Petri plates containing nutrient agar. The plates were incubated at 37 °C for 24 h and CFU/mL were performed. Each treatment was performed three times.

Mueller-Hinton broth (MHB, Difco) plus 0.25 g of each sample were tested as sterility controls, and untreated bacteria inoculated on MHB alone were tested as bacterial viability controls. Then, all samples (OH, OH_{Ag}, BE_{OH} and $BEOH_{Ag}$, sterility controls and bacterial viability controsl) were incubated at 37 ºC, under agitation at 150 rpm for 24 h.

3 RESULTS AND DISCUSSION

The synthesis of NFC and OH incorporating biological silver nanoparticles was successfully achieved. The nanocellulose shape and dimension were obtained from atomic force microscopy (AFM) images (Figure 1). It is important to highlight that the bio-AgNPs displayed a spherical shape with nanometric size. Other authors employed *Fusarium oxysporum* in the synthesis of bio-AgNPs and observed a similar microstructure, with spherical nanoparticles of nanometric size (Srivastava et al., 2019; Sumera et al., 2021; Liu et al., 2021; Nasr Azadani et al., 2024).

As shown in Figure 1, the $BEOH_{Ag}$ structure was composed of interconnected webs of tiny nanofibers with diameters on the nanometric scale (100 ± 25 nm) and lengths of several micrometers, which is characteristic of NFC. It is important to highlight that the silver nanoparticles were deposited on the surfaces of both samples (OH_{Ag} and $BEOH_{Ag}$), suggesting a possible interaction between the samples and the bio-AgNP.

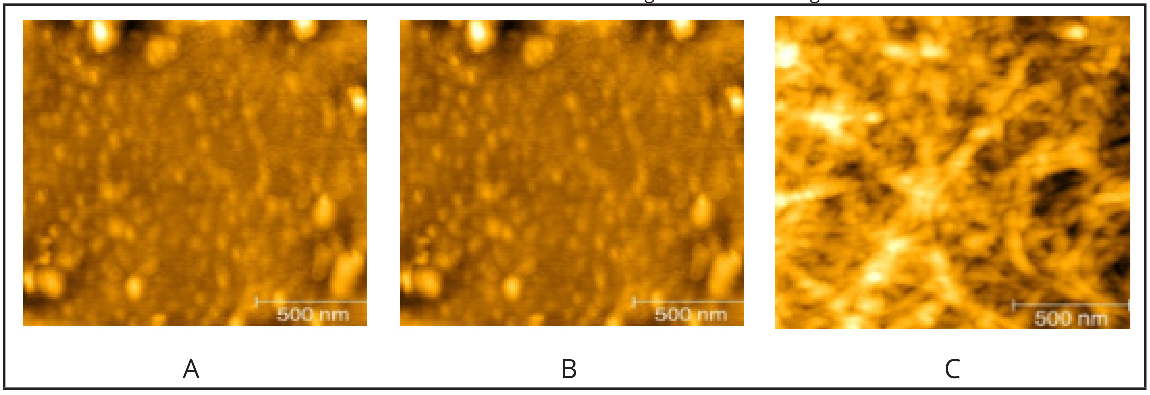
The samples were incubated in a culture medium for 24 h, showing no bacterial growth, assuring the effectiveness of the sterilization process in eliminating any potential contamination that may have occurred before the procedure (Table 1).

The results of the antimicrobial test against the *E. coli* bacteria are presented in Table 2. The OH_{Ag} sample demonstrated inhibitory effects on *E. coli* growth, which is likely attributable to a bactericidal mechanism. The antibacterial action of the silver nanoparticles on the occurrence of gram-negative bacteria is attributable to the composition of their cell walls. The peptidoglycan present in *E. coli* is in the form of a thin layer, which allows for the entry of nanoparticles into the bacterial cell (Ibrahim, 2015; Durán et al., 2016; Srivastava et al., 2019; Alavi; Hamblin, 2023; Vasiliev et al., 2023; Vidyasagar et al., 2023).

Ahmed and Ogulata (2022) reported that some mechanisms may elucidate the effect of silver nanoparticles against Gram-negative bacteria. The nanoparticles may attach to disrupt cell membranes, causing physical and chemical changes, resulting in changes in basic functions such as permeability, osmoregulation, electron transport, and cellular respiration. In addition, nanoparticles can also damage cells by interacting with the microorganism DNA and proteins. According to More et al. (2023), the nanoparticles bactericidal effect can be associated with its silver ions release.

The results illustrated in Table 2 reveal that OH, BEOH and $BEOH_{Ag}$ did not show antimicrobial activity against *E. Coli*. The microbial count increased from 5 x 10^5 CFU/mL (initial bacterial inoculum) to 10^8 CFU/mL in OH and BEOH samples, proving the absence of antimicrobial activity. Zhang et al. (2019) synthesized silver nanoparticles on nanocellulose using the hydrothermal method and observed that the resulting composite exhibited high bactericidal efficiency against bacteria and fungi. However, in the present study, the absence of antimicrobial activity in the NFC material can be attributed to three factors: (i) the type and lowest concentration of bio-AgNP used, (ii) chemical residues present in the nanocellulose due to deficient washing, and (iii) the immobilization of bio-AgNP on the nanocellulose, thereby reducing the likelihood of direct contact with bacteria.

Figure 1 – AFM images of bio-AgNP (A), OH_{Ag} (B) $BEOH_{Ag}$ (C)



Source: Authors (2024)

Table 1 – Sterility testing of sample control

Dilution	Sterility Control (CFU/mL)			
	OH	BEOH	OHAg	BEOHAg
0	0	0	0	0
-2	0	0	0	0
-4	0	0	0	0

Source: Authors (2024)

4 CONCLUSIONS

The production of OH and NFC incorporated with bio-AgNPs was successfully accomplished. The study demonstrated that only the OH incorporated with Bio-AgNPs exhibited antimicrobial activity against E. coli. Further research is needed to enhance the antimicrobial efficacy of the NFC material, potentially by increasing the concentration of Bio-AgNPs or improving the final material wash process. The key contribution of this work is to demonstrate that an agro-industrial residue (oat hulls) can be valorized into antimicrobial composites through biogenic silver nanoparticle.

Moreover, it is necessary to explore the nature of the chemical interactions among Ag nanoparticles, OH, and nanocellulose. FT-IR analysis should be suggested to characterize OH, nanocellulose, and the synthesized materials. XRD analysis must also be included to evaluate the crystallinity of nanocellulose and the formation of AgNPs within the materials. In addition, dynamic light scattering (DLS) and zeta potential measurements should be performed to provide more robust results regarding AgNP size, surface charge, and stability.

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