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Valorization of Fermentation Waste: Eco-Friendly Synthesis of Antimicrobial Bacterial Cellulose-Silver Nanocomposites using Post-Fermentation Solution as a Reducing Agent

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This study presents a green approach for synthesizing antimicrobial bacterial cellulose-silver nanoparticles (BC/AgNPs) utilizing the residual sugar-containing post-fermentation solution (4.05 g/L) as a reducing agent. The treated post-fermentation solution effectively reduced silver ions into uniformly distributed crystalline AgNPs of approximately 100 nm size within the BC matrix, as confirmed by XRD, FTIR, and SEM analyses. FTIR revealed interactions between AgNPs and BC hydroxyl groups. The synthesized BC/AgNPs exhibited potent antimicrobial activity against *Escherichia coli*, with the 1:1 $AgNO_3$:BC ratio demonstrating superior efficacy. This valorization of the waste stream into a functional reducing agent aligns with sustainable principles, minimizing waste while enabling efficient resource utilization to produce antimicrobial nanocomposites with potential applications in biomedicine, food packaging, and environmental remediation.

1. Introduction

Nanocomposites comprising bacterial cellulose and silver nanoparticles (BC/AgNPs) have garnered significant interest due to their remarkable antimicrobial properties and robust physicochemical characteristics (Audtarat et al., 2022). Traditional synthesis methods for silver nanocomposites often employ chemical-reducing agents, which pose significant environmental and health risks. This study explores an innovative green synthesis approach utilizing post-fermentation solutions as a reducing agent to mitigate these challenges.

Bacterial cellulose (BC) is a high molecular weight polysaccharide biosynthesized by certain bacteria from various nutrient sources. BC exhibits a fine crystalline network structure, high purity, and exceptional mechanical properties, such as high tensile strength and superior water retention capacity (Amr and Ibrahim, 2023). When incorporated with silver nanoparticles (AgNPs), renowned for their potent antimicrobial activity, BC forms a nanocomposite (BC/AgNPs) that exhibits synergistic effects, including enhanced antibacterial, antifungal, and UV-resistant properties, while maintaining high durability and excellent mechanical performance.

Conventional methods for synthesizing BC/AgNPs typically involve chemical-reducing agents like sodium borohydride ($NaBH_4$) (Thiruvengadam and Bansod, 2020) or plant-derived polyphenols (Shaaban et al., 2023), which introduce environmental and economic challenges.

This research proposes a novel biogenic synthesis method for BC/AgNPs by exploiting the residual fermentation broth from BC production as a silver ion (Ag^+) reducing agent. This approach not only valorizes waste materials, reducing production costs, and enhances the sustainability of the BC production process. The post-fermentation solution, often containing significant amounts of unutilized sugars and typically discarded, offers a valuable resource for green synthesis, transforming waste into a functional reducing agent. The proposed green synthesis method hinges on the bioreduction of silver ions to silver nanoparticles (Ag^0) using the reducing sugars present in the post-fermentation solution (Volova et al., 2022). This biogenic approach integrates principles from biotechnology and materials science, providing a more sustainable and environmentally friendly pathway for nanocomposite production. This synthesis strategy mitigates the environmental impact of traditional chemical methods and offers a feasible and economically viable alternative.

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This paper aims to elucidate the theoretical foundation, detailed methodology, experimental results, and implications of utilizing post-fermentation solutions for the green synthesis of BC/AgNPs. By advancing a more sustainable synthesis route, this research contributes to the broader effort of developing eco-friendly materials with significant applications in healthcare, material science, and environmental engineering.

2. Materials and Methods

2.1 Materials

The bacterial cellulose (BC) used in this study was obtained from the Laboratory of the Biofuel and Biomass research at the University of Technology - Vietnam National University, Ho Chi Minh City. The BC was fermented from paper sludge using *Acetobacter xylium* and treated with NaOH solution.
The chemicals employed in this research: silver nitrate ($AgNO_3$), ammonium hydroxide (NH_4OH, 23-25 %) from China, potassium permanganate ($KMnO_4$, ethanol (C_2H_5OH, 99.9 %) and sodium hydroxide (NaOH) were purchased from Sigma-Aldrich, Merck KGaA, USA.

2.2 Bacterial Cellulose Fermentation and Post- Fermentation Solution Recovery

The paper waste sludge (PWS) was pretreated with 1 M HCl solution at a solid-to-liquid ratio of 1:10, incubated at 50 °C for 24 h with continuous shaking at 120 rpm. After incubation, the pretreated material was rinsed with water until neutral pH, dried in an oven, and stored below 15 % humidity. The enzymatic hydrolysis of pretreated PWS was performed using 1 g of dry PWS as the substrate and an enzyme concentration of 15 vol%. The reaction lasted 48 h.
The fermentation experiments were conducted in 100 mL flasks, each containing 20 mL of PWS solution supplemented with 0.3 % w/v $(NH_4)_2HPO_4$ and 0.5 % w/v peptone. After adding the nutrients, the medium was autoclaved at 121 °C for 5 min and cooled to 30 °C. Next, 10 vol% of *Acetobacter xylinum* strain with a bacterial density of 3.2 x 10^{11} CFU/mL was inoculated into the medium. The fermentation process was carried out statically at a stable temperature of 37 °C for 14 days.
The post-fermentation solution was collected, neutralized with NaOH to pH 7, heat-treated to eliminate bacteria, and filtered through a 110 nm filter paper to remove impurities.
The fermentation medium, post-fermentation solution, and treated solution were analyzed for reducing sugar content, pH, bacterial density, nitrogen content, biochemical oxygen demand (BOD), chemical oxygen demand (COD), and turbidity and suspendid solids (TSS). The reducing sugar content in the hydrolysate was measured using the DNS method with a spectrophotometer (Agilent Cary 60, USA). A calibration curve created with glucose solutions (0.1 to 1.0 mg/mL) yielded an R^2 value of 0.9938.

2.3 Green Synthesis of Antimicrobial Bacterial Cellulose-Silver Nanocomposite Using Post-fermentation Solution

Silver nitrate ($AgNO_3$) was weighed, and ammonium hydroxide (NH_3) solution was gradually added until the precipitate in the beaker dissolved completely. The solution was then diluted to 100 mL with water to form the $AgNO_3/NH_3$ solution. Dried bacterial cellulose (BC) was weighed and added to 100 mL of the $AgNO_3/NH_3$ solution in an Erlenmeyer flask, followed by sonication at room temperature for 20 min.
Subsequently, the treated post-fermentation solution was added to the mixture to initiate the reaction. The mixture was stirred at 60 °C for 2 h to form silver nanoparticles on the BC crystalline network. The reaction was carried out in a fume hood. After the reaction, the mixture was cooled to room temperature and filtered using filter with a vacuum filtration system. The solid material was retained, and the liquid was discarded. The filtered material was shaped and dried at 50 °C.
The mass ratios between $AgNO_3$ and BC were investigated and are presented in Table 1.

Table 1: Mass Ratio Analysis between $AgNO_3$ and BC

Sample ID	$AgNO_3$ (g)	BC (g)	Post-Fermentation Solution (mL)
BC/Ag 1: 1	0.25	0.25	62.5
BC/Ag 1: 0.5	0.125	0.25	31.25
BC/Ag 1: 0.2	0.05	0.25	12.5

2.4 Structural, Morphological, and Property Analysis

X-ray diffraction (XRD) analysis of BC and BC/AgNPs films was conducted using a B8 Bruker Advance instrument (Germany) with Cu-Kα radiation (λ = 0.1548 nm), 2θ scanning angle of 10-80°, voltage of 40 kV, current of 25 mA, scan rate of 1 °/s, and step size of 0.02°, measured at the Biofuel and Biomass research lab, University of Technology - VNU-HCM.

Fourier Transform Infrared (FT-IR) spectra of the samples were recorded in transmission mode from 4,000-400 cm^{-1} on a BRUKER TENSOR 27 (Germany) at the Institute of Chemical Technology, Ho Chi Minh City, with a resolution of 0.2 cm^{-1} and spectral accuracy of 0.1 % T.
The surface morphology was examined by Scanning Electron Microscopy (SEM) using a JSM-IT200 instrument at the Central Laboratory, HCMUS, with an operating voltage of 10 kV and magnifications ranging from 5,000 to 300,000 times.

2.5 Antibacterial Testing of BC/AgNPs

The antibacterial activity of BC/AgNPs against *Escherichia coli* (*E. coli*) was evaluated using agar well diffusion and liquid broth microdilution assays at the University of Medicine and Pharmacy, Ho Chi Minh City. For the agar assay, standardized *E. coli* cultures were swabbed on LB agar, wells made, samples added, and after incubation, inhibition zone diameters measured. For broth assay, *E. coli* was grown with samples, incubated, and OD_{610nm} measured to determine growth inhibition. Pristine BC and BC/AgNP with Ag/BC 1:1, 1:0.5, 1:0.2 ratios tested. Antimicrobial performance evaluated by agar inhibition zones and OD_{610nm} growth reduction in broth.

3. Results and discussions

3.1 Characterization of Fermentation Media and Solutions

The results of the analysis of various parameters for the fermentation medium, post-fermentation solution, and treated post-fermentation solution are presented in Table 2. These parameters provide crucial information regarding the feasibility of using the post-fermentation solution as a reducing agent for silver ions in the synthesis of BC/AgNPs materials.

Table 2: Composition of fermentation media and solutions post-treatment

Parameter	Unit	Fermentation Medium	Post-Fermentation Solution	Treated Post-Fermentation Solution
RSC	g/L	26.19	4.25	4.05
pH		5.5	3.44	7.5
Bacterial Density	CFU/mL	3.2 x 10^11	1.6 x 10^8	0
BOD	mgO_2/L	12,300	24,325	12,500
COD	mgO_2/L	5,600	26,200	14,800
TSS	mg/L	550	5,520	850

The post-fermentation solution contained RSC of 4.25 g/L, significantly lower than the initial 26.19 g/L in the fermentation medium due to bacterial consumption. Discarding this solution would waste 16.4 % of the initial sugar. Utilizing it as a reducing agent for silver ion minimizes waste and enhances raw material efficiency, as the remaining RSC adequately reduces Ag^+ to Ag^0 nanoparticles.
Neutralization with NaOH and mild heat treatment eliminated bacteria while only slightly reducing RSC from 4.25 to 4.05 g/L, preserving its reducing potential. The high BOD and COD values indicate a substantial organic load that would typically require extensive wastewater treatment before disposal. Using this solution to synthesize BC/AgNPs valorizes the organic compounds as reducing agents to form Ag^0 nanoparticles that integrate into the BC matrix. This approach transforms waste into a valuable nanocomposite while alleviating wastewater treatment needs. The simple neutralization and low-temperature treatment process does not require specialized equipment or significantly increase costs, offering environmental and economic benefits.

3.2 Green Synthesis and Characterization of Antimicrobial Bacterial Cellulose-Silver Nanocomposite Using Post-Fermentation Solution

To investigate the formation and distribution of AgNPs within the BC/AgNPs, XRD analysis was employed. Figure 1 presents the XRD patterns of the pristine BC sample and the BC/AgNPs synthesized at different BC:$AgNO_3$ mass ratios.
The XRD patterns confirmed successful formation of AgNPs within the BC matrix for the 1:1 and 1:0.5 BC:$AgNO_3$ ratios, exhibiting distinct BC peaks at 14.69° and 22.83° corresponding to cellulose Iα, and characteristic AgNP fcc peaks at 38.1°, 44.3°, 64.4°, and 77.4° (Prema et al., 2022). The lower AgNP peak intensities for 1:0.5 compared to 1:1 suggest the increased BC partially masks the AgNP signals. This masking effect is more pronounced for 1:0.2, where only BC peaks are visible. The results confirm successful Ag^+ reduction to Ag^0 nanoparticles by the reducing sugars like glucose present in the post-fermentation solution, proposed to occur via glucose oxidation to gluconic acid releasing electrons to reduce Ag^+. The varying AgNPs

peak intensities imply different AgNPs distributions and loadings within the BC matrix based on the initial $AgNO_3$:BC ratio used.

SEM analysis confirmed the successful synthesis of nanocomposites with ~100 nm AgNPs. The pristine BC appeared white (Figure 2a) and turned slightly darker upon AgNP incorporation (Figure 2b). The native BC exhibited an interconnected fibrillar network structure (Figure 2c). After synthesis, AgNPs were uniformly distributed throughout the BC fibril matrix, with the 1:1 $AgNO_3$:BC ratio having the highest AgNP density (Figure 2d), which gradually reduced with lower silver content (Figures 2e and 2f), corroborating the decreasing XRD AgNP peak intensities.

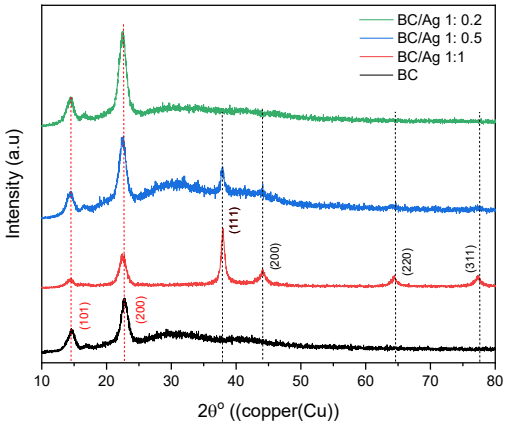

Figure 1: X-ray diffraction patterns of pristine BC, and BC/AgNPs synthesized at different $AgNO_3$: BC mass ratios

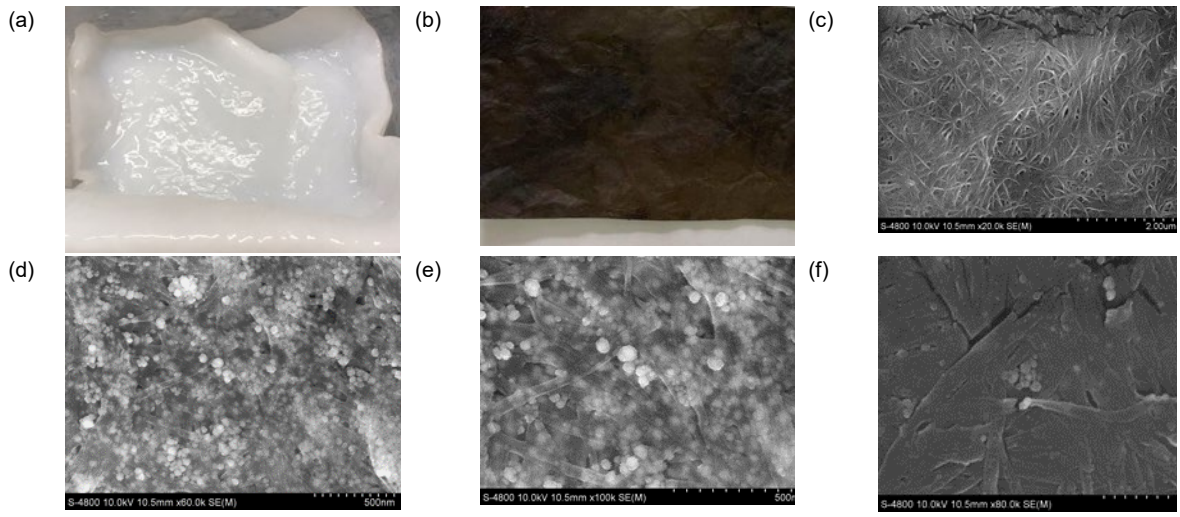

Figure 2: SEM images of (a) pristine BC, (b) BC/AgNPs, (c) fibrillar network of BC, and BC/AgNPs synthesized at (d) 1:1, (e) 1:0.5, and (f) 1:0.2 $AgNO_3$:BC mass ratios.

FTIR spectra (Figure 3) revealed characteristic functional groups of BC and the BC/AgNPs. For BC, a 3,500-3,543 cm^{-1} broad band corresponded to O-H stretching, 1,060.23 cm^{-1} indicated glycosidic -CH-O-CH_2- linkages, 2,891 cm^{-1} arose from C-H and CH_2 stretching, with 2,891.58 cm^{-1} assigned to C-H stretching and 1430.08 cm^{-1} to C-H bending (Pham Ngoc et al., 2022). The 1,668.31 cm^{-1} band related to C=O stretching. Intensity changes in the O-H region for BC/AgNPs suggest interactions between Ag^0 nanoparticles and BC hydroxyl groups (Pal et al., 2017).

For the BC/AgNP nanocomposites, the O-H absorption band shifted position and changed intensity compared to pristine BC. The peak at 3,543.88 cm^{-1}, which had the largest area for BC, decreased in intensity for BC/AgNPs. This change suggests interactions between the Ag^0 nanoparticles and hydroxyl groups in the BC matrix, potentially due to adsorption or surface bonding between Ag^0 and BC functional groups (Pal et al., 2017). The AgNP incorporation appeared to influence the vibrational modes of BC functional groups, indicating successful silver nanoparticle integration into the cellulose structure. The shifts in position and intensity of the

O-H stretching region support the hypothesis that Ag^0 nanoparticles may form surface bonds or van der Waals interactions with BC hydroxyl groups. Such interactions could strengthen the BC-nanosilver bonding in the composites, enhancing durability and preventing silver particle exfoliation.

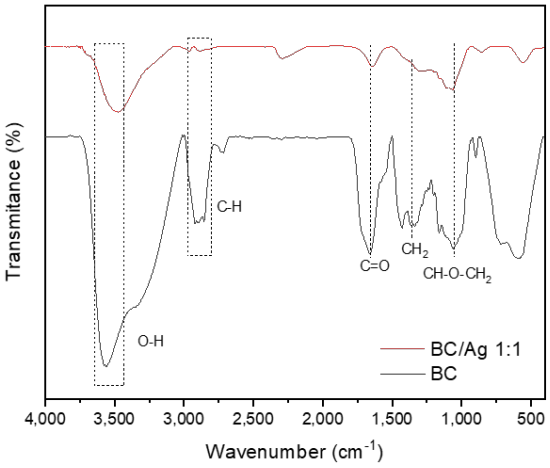

Figure 3: FTIR spectra of BC and BC/AgNP nanocomposites

3.3 Antimicrobial Activity Results

The antimicrobial data clearly demonstrates the potent bactericidal effects of the Ag/BC nanocomposites against *E. coli* in both solid agar (Figure 4a) and liquid broth (Figure 4b) tests.

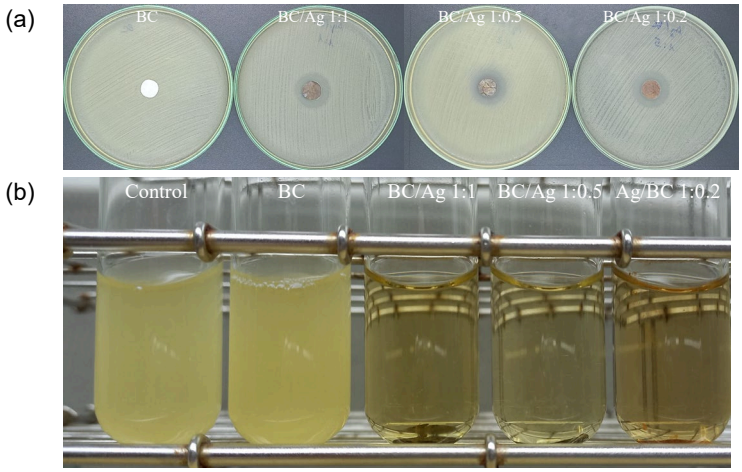

Figure 4: Antimicrobial Activity by (a) Agar Well Diffusion and (b) Liquid Broth

Table 3: Antibacterial Activity and Optical Density in Agar Well Diffusion and Liquid Broth Assays

Sample	Antibacterial Zone Diameter (mm)	$OD_{610\ nm}$
Bacterial Culture + Medium	-	2.032
BC	-	2.214
BC: Ag 1:1	18.9	0.012
BC: Ag 1:0.5	19.2	0.005
BC: Ag 1:0.2	19.8	0.014

In the agar diffusion tests, pristine BC did not exhibit any zone of inhibition (0 mm), and its high $OD_{610\ nm}$ value of 2.214 confirmed the lack of inherent antibacterial properties (Table 3). The incorporation of silver nanoparticles endowed the BC/Ag nanocomposites with potent antimicrobial activity. The 1:1 Ag/BC nanocomposite displayed an exceptionally large inhibition zone diameter of 18.9 mm. Even at lower silver loadings of 1:0.5 and 1:0.2 Ag/BC ratios, the inhibition zones remained substantial at 19.2 mm and 19.8 mm (Table 3). These sizable clearance zones indicate effective diffusion and sustained release of bactericidal silver

species from the BC matrix into the surrounding agar medium, preventing surface bacterial growth (Homwan et al., 2023). The liquid broth tests further corroborated the antimicrobial efficacy against planktonic *E. coli* cells. The control bacterial culture showed an $OD_{610\ nm}$ of 2.032, indicating uninhibited growth. In contrast, the 1:1 Ag/BC nanocomposite drastically reduced the $OD_{610\ nm}$ to 0.012, demonstrating almost complete inhibition of bacterial proliferation in the broth. The 1:0.5 and 1:0.2 Ag/BC samples also showed very low OD610nm values of 0.005 and 0.014 (Table 3), indicating effective suppression of bacterial growth even at lower silver nanoparticle loadings.

These findings highlight the dual antimicrobial mechanisms of the Ag/BC nanocomposites – the sustained release of silver ions/nanoparticles creating an anti-bacterial zone on surfaces, coupled with direct bactericidal action against suspended bacterial cells facilitated by the intimate nanoparticle-bacteria interactions. The nanocomposite form enhances antimicrobial performance by increasing bioavailable silver while being supported by the BC matrix.

4. Conclusions

This study recorded significant results in the successful green synthesis of antimicrobial BC/AgNPs materials using the post-fermentation solution from bacterial cellulose production as the reducing agent. The obtained silver nanoparticles were around 100 nm in size and uniformly distributed on the bacterial cellulose fibril surface, as confirmed by XRD and SEM analyses. The reduction mechanism of Ag^+ ions to Ag^0 nanoparticles is proposed to occur via residual reducing sugars like glucose present in the post-fermentation solution. The bonding between the silver nanoparticles and the cellulose hydroxyl groups through hydrogen bonding or van der Waals interactions contributes to the enhanced durability of the material, preventing the leaching of silver particles. This uniform distribution of AgNPs within the BC matrix also enhances the antimicrobial properties, particularly against *E. coli*, with antimicrobial zone diameters reaching 18.9-19.8 mm for different BC/Ag samples in the agar well diffusion assay. In the liquid broth, the antimicrobial efficacy was also remarkably high, with OD_{610nm} values ranging only from 0.005-0.014, indicating substantial inhibition of bacterial growth.

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