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Functionalization of bacterial cellulose with antibiotics for use as a wound dressing

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ABSTRACT

Background: Presently, bacterial cellulose (BC) represents a promising biomaterial for biomedical applications, due to its exceptional properties. The capacity of BC to absorb and retain water, facilitating gas exchange, as well as its ability to serve as a scaffold for antimicrobial agents, makes it an optimal material for wound dressing that promotes healing and prevents infection.

Objective: The objective of the research was to obtain and study BC composites containing antibiotics with controlled release and significant antibacterial activity for potential use as wound dressings.

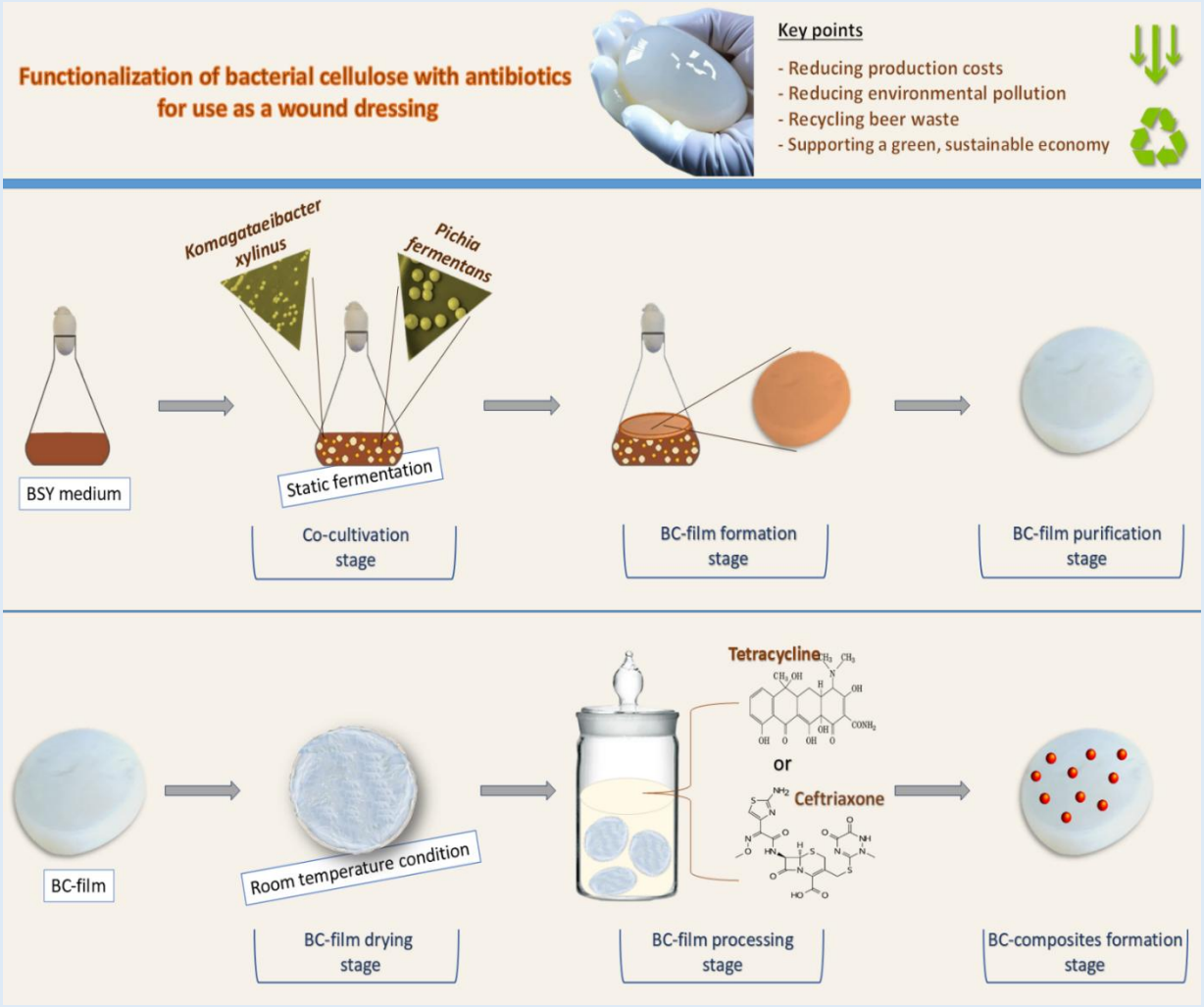
Methods: BC films were obtained by static co-fermentation of Komagataeibacter xylinus MS2530 strains with Pichia fermentans MDC 10169 on brewing waste (brewer's spent yeast (BSY)). The preparation of BC/antibiotic composites was achieved by immersing dry films in ceftriaxone and tetracycline solutions for 24 h in a sterile environment. The composites' morphology was analyzed by Fourier-transform infrared spectroscopy (FTIR) to evaluate their chemical structure and compatibility with ceftriaxone and tetracycline. The loading efficiency and release profile of ceftriaxone and tetracycline antibiotics were evaluated using a spectrophotometric method. The antimicrobial activity of BC

composites was determined using test cultures Escherichia coli and Staphylococcus aureus.

Results: The process of co-fermentation using the BSY medium made it possible to produce BC films at a low cost. Based on BC films, BC/antibiotic composites with controlled release and significant antibacterial activity were obtained, which is a novelty of this study.

Conclusion: The co-fermentation of K. xylinus MS2530 and P. fermentans MDC 10169 on BSY medium simultaneously increase the BC yield and reduce fermentation time, leading to a substantial reduction in production costs. BC composites obtained by modifying BC films with antibiotics, exhibit significant antibacterial activity against E. coli and S. aureus. Therefore, the BC composites obtained have a possible potential for application as dressings for wound surface treatment.

Keywords: BC composite, tetracycline, ceftriaxone, controlled release, alternative wound dressing



Graphical Abstract: Functionalization of bacterial cellulose with antibiotics for use as a wound dressing

INTRODUCTION

Bacterial cellulose is one of various promising materials for a variety of technological and biomedical applications [1-2]. Aerobic bacteria such as *Achromobacter*, *Alcaligenes*, *Aerobacter*, *Agrobacterium*, *Azotobacter*, *Komagataeibacter* (formerly *Gluconacetobacter*), *Pseudomonas*, *Rhizobium*, *Sarcina*, *Dickeya*, and *Rhodobacter* produce BC in the form of exopolysaccharide [3-5]. Among them, the most widely studied bacteria belong to the genus *Komagataeibacter*, which can tolerate a wide range of carbon and nitrogen sources [13]. The relatively large variety of microorganisms that produce cellulose, as well as the wide range of cultivation methods, create excellent opportunities for modifying and adjusting the properties of the material and finding new areas of application for BC.

BC has a similar chemical composition to plant cellulose, but is synthesized in its pure form, without lignin, hemicellulose, or pectin [14]. Excellent physical properties such as high purity, exceptional mechanical strength, crystallinity, nanostructured network, significant water retention capacity, biodegradability, and biocompatibility make BC a promising biomaterial for therapeutic use [1-2, 15-16].

Presently, the incorporation of BC as a component in functional products is of significant pertinence. As is well known, functional products are foods and/or their components that are not part of the basic diet and have a beneficial effect on the health of living organisms by providing them with the nutrients necessary for normal growth and development. The potential benefits of BC, including its ability to enhance food products and deliver functional substances as a biologically active carrier, have led to a range of considerations for its application in the food industry. Research has demonstrated that BC in functional foods exerts a regulatory effect on blood glucose levels and exhibits a beneficial impact on gastrointestinal health [10-11].

However, the use of BC is dependent on the economic viability of its production processes. Although BC is in high demand in various fields, the production process remains costly. The cost of synthetic nutrient media used for the microbiological synthesis of BC alone can account for up to 65% of the total cost of the process [4, 21]. Consequently, the expansion of the scale and scope of bacterial cellulose usage is contingent upon the availability of productive strains that ensure high yields of this valuable biotechnological product while allowing for the use of more accessible substrates [22-25]. Among the approaches for cost-effective BC production are the use of waste-based substrates [26-27] from industrial and agricultural sources and the co-fermentation of the BC producer bacteria with various microorganisms [28-29].

Our previous research demonstrated that co-fermentation of the highly efficient BC-producing strain *K. xylinus* MS 2530 with the yeast strain *P. fermentans* led to a significant increase in BC yield. Moreover, the use of brewing waste instead of expensive classical media significantly reduced the cost of synthesized BC [30].

In recent years, there has been a significant increase in research focusing on the BC biopolymer and its composites in the domain of biomedicine, particularly in the context of wound dressings [31-32]. The primary function of traditional wound dressings is to isolate wounds from the external environment, thereby protecting them from damage. However, the slow self-healing of wounds and the occurrence of infection during the healing process remain unresolved problems. High doses of systemic or oral antibiotics are commonly prescribed, which can lead to the development of bacterial resistance and environmental pollution with pharmaceuticals [34]. Consequently, there has been a growing interest in the development of alternative

methods, such as the treatment of wounds with topical antibiotics.

The unique properties of BC can make it possible to create an improved wound dressing that controls wound exudates and provide a moist environment during the healing process [35]. In addition, wound dressings loaded with antibiotics can be used for treatment of infected wounds, which significantly decreases the dosage of antibiotics compared to intravenous or oral administration and may also reduce the development of bacterial resistance [36]. The introduction of various antimicrobials, anti-inflammatories, antiseptics, and other drugs into wound dressings will facilitate the treatment of difficult-to-heal wounds [37-39]. Unlike traditional gauze and bandage dressings, which cause secondary damage when reapplied, the uniqueness of BC lies in its ability to prevent adhesion to the wound surface while maintaining the necessary moisture balance, thereby eliminating secondary damage to the wound when changing the dressing. Moreover, BC has a microfibrillar structure, which promotes gas exchange [40-41].

These qualities make it possible to use BC-based wound dressings for wounds located in difficult areas of the body such as the groin, neck, etc., where it is necessary for the dressing to fit snugly to the wound and not traumatize the surface during frequent dressing changes [42-43]. Studies have shown that BC-based wound dressings enriched with antimicrobial agents exhibit strong antimicrobial effects and significant healing activity [46]. The highly porous structure of BC makes it possible to load antimicrobial agents [44].

To produce BC with antibacterial activity BC films are immersed in antibiotic solutions. The most used antibiotics are ciprofloxacin, ceftriaxone (CEF), tetracycline hydrochloride, amoxicillin, etc. It has been shown that ciprofloxacin, amikacin, and ceftriaxone can be incorporated into BC to provide bioactivity for dressing materials and tissue engineering [43-44, 45-46].

The objective of the research was to obtain and study BC composites exhibiting controlled release of antibiotics and significant antibacterial activity for potential use as wound dressings.

MATERIALS AND METHODS

Production and purification of bacterial cellulose film: Bacterial cellulose films were synthesized by our mutant strain *K. xylinus MS2530* co-cultured with *P. fermentans* MDC 10169 in a ratio 1:1 as described in our previous work. Co-cultivation was carried out in BSY medium under static conditions for 5-7 days at 30 °C, pH 5.5. Then, the BC films obtained were first washed with distilled water to remove cell debris and medium components. Subsequently, the films were immersed in a 0.5% NaOH solution at room temperature (25 °C) for 24 hours to ensure complete removal of the attached bacterial cells. To eliminate alkali residues, the films were subjected to repeated washings with deionized water until a neutral pH was attained [30]. Then, the purified BC films were dried on a silicone substrate at room temperature.

Production of BC composites with antibiotics: In order to impart bactericidal properties to bacterial cellulose, BC composites with antibacterial drugs were prepared. The antibiotics tetracycline (TC) and ceftriaxone (CEF) were used (OOO Sintez, Russia). The dried BC films were die-cut into 1 cm^2 squares and immersed in antibiotic solutions of various concentrations (0.2%, 0.5%, 1%). After 24 hours of immersion, the films were removed from the antibiotic solutions, washed in distilled water, and dried at room temperature [42].

TC and CEF contents were calculated according to the original concentrations of CEF and TC (C_0) and the concentration of unloaded CEF and tetracycline hydrochloride (C_1) quantified using a UV/VIS

spectrophotometer (T8DCS, Persee, Beijing, China). TC was measured at a wavelength of 357 nm, while CEF was measured at 304 nm.

Antibiotics contents (W) in the composite films were determined with the following equation:

$$W = (C_0 - C_1) \times \frac{V}{A},$$

where V – the volume of total CEF or TC equal to 10 mL in this calculation, and A – the area of BC/CEF or BC/TC composite films.

In vitro release of antibiotics from BC composites: Antibiotic release experiments were conducted in 50 ml PBS at pH 7.0 and 37 °C. Samples of 1 ml were taken at regular intervals (1 h) for measurement using a UV/VIS spectrophotometer.

Fourier transform infrared (FTIR) spectroscopy: The functional groups and chemical bonds of BC and BC/antibiotic composites were studied by IRTracer-100 FTIR spectrometer (Shimadzu, Kyoto, Japan) equipped with a KBr prism in single reflection mode in the range of 500–4000 cm^{-1} with a resolution of 4 cm^{-1}.

In vitro antibacterial tests: The antibacterial activity of BC-antibiotic composites was examined on the test *E. coli* ATCC 25922 and *S. aureus* ATCC 25923, using the disk diffusion method. The bacterial suspension with a final concentration of 0.5 McFarland's standard density (equivalent to 1.5 x 10^8 CFU/mL) was added to Petri dishes (with a diameter of 90 mm) containing 20 ml of the LB agar. Subsequently, the dishes were then left to solidify at room temperature. Then BC composite 1 cm^2 squares, containing tested concentrations of antibiotics, were placed on the agar surface. The BC square, devoid of any antibiotic treatment, was used as a control. The Petri dishes were incubated at a constant temperature of 35°C for a duration of 18-24 hours. The antibacterial activity was evaluated by measuring the diameter of the growth inhibition zones [47].

Statistical analysis: All experiments were performed in triplicate, and results were expressed as the mean ± standard deviation. The data were analyzed using a One-way ANOVA with RStudio software (version 1.4.1106) employing Student's *t*-test and Dunnett's test. Statistical significance was defined as $p<0.05$.

RESULTS AND DISCUSSION

The BC films obtained as a result of co-cultivation were analyzed using scanning electron microscopy (SEM) and FTIR methods. Certain physical properties (Young's modulus, tensile strength, elasticity, etc.) were also investigated. The results of previous studies demonstrated the effectiveness of the co-cultivation method for increasing the production of BC with optimal mechanical and chemical characteristics [30].

Table 1. Antibiotic contents in BC composites membrane

Samples	Thickness of wet BC (mm)	Antibiotic content (mg/dm²)
BC/CEF$_{0,2}$	1.967 ± 0.04	2.27 ± 0.09
BC/CEF$_{0.5}$	1.971 ± 0.02	6.15 ± 0.08
BC/CEF$_1$	1.973 ± 0.03	9.34 ± 0.12
BC/TC$_{0,2}$	1.970 ± 0.04	2.17 ± 0.11
BC/TC$_{0.5}$	1.972 ± 0.04	7.15 ± 0.07
BC/TC$_1$	1.973 ± 0.02	10.04 ± 0.08

Obtaining BC/antibiotic composites: Composites obtained by immersing dry BC films in solutions of CEF and TC antibiotics (0.2%, 0.5%, and 1.0%) were designated BC/$CEF_{0.2}$, BC/$CEF_{0.5}$, BC/CEF_1, BC/$TC_{0.2}$, BC/$TC_{0.5}$, BC/TC_1, respectively.

Quantitative determination of antibiotics in BC composites: TC and CEF contents in BC composites were determined using a UV/VIS spectrophotometer and the results were presented in Table 1. The data from the table demonstrate a direct correlation between the antibiotic loading in composite membranes and the initial antibiotic concentration. The large surface area and porosity of BC, as well as its water-holding capacity, lead to higher drug loading efficiency [48].

FTIR spectroscopy analysis: The morphology of the prepared composites was analyzed by FTIR to analyze their chemical structure and compatibility with ceftriaxone and tetracycline. As shown in Figure 1, the IR spectra of BC/CEF and BC/TC composite membranes have the same peaks with pure BC film (without antibiotic). The stretching vibration of O–H bond of hydroxyl group of cellulose was detected at 3200–3300 cm^{-1}. This finding is consistent with results reported by Volova et al. (2022) and Feng et al. (2024) [35, 41]. The absorption band centered at 2854–2885 cm^{-1} corresponds to the stretching vibrations of the C–H bond, which is consistent with the findings published by Bai et al. (2023) [43]. The band at 1157 cm^{-1} is due to the stretching vibrations of the C–O bond, and the bands in the range of 1026–1049 cm^{-1} are due to the stretching vibrations of the C–O–C pyranose ring, which is due to the structure of cellulose [44]. After the introduction of ceftriaxone, the BC/CEF composite showed new peaks in the range of 3340–3271 cm^{-1}, which corresponds to the stretching vibrations of the amino group NH2, and at 1728 cm−1, which corresponds to the stretching vibrations of the C=C bond [45-46]. The BC-TC composite showed a peak at 1535 cm 1, which corresponded to the deformation of the NH_2 amino group of tetracycline [47].

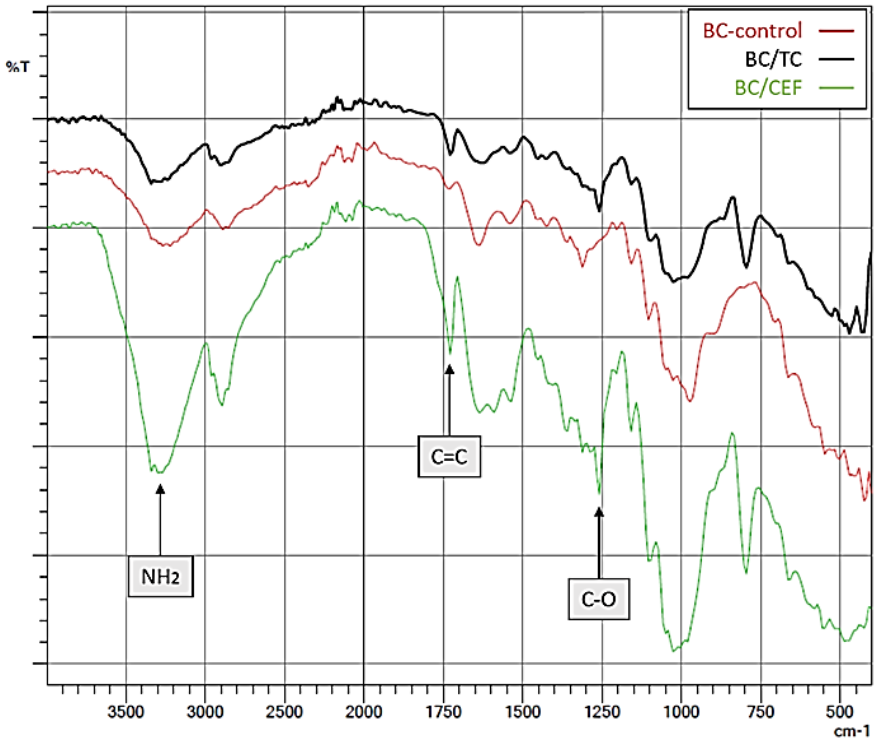


Figure 1. FTIR analysis of BC, BC/CEF and BC/TC composite membranes

Antibacterial activity: Two antibiotics, ceftriaxone and tetracycline, were selected to impart antibacterial properties. The selection of these antibiotics was predicated on a number of factors. Ceftriaxone, classified as a third-generation antibiotic, demonstrates a broad spectrum of activity against most Gram-negative and Gram-positive microbes. Ceftriaxone has been shown to inhibit the synthesis of peptidoglycan, the structural component of microbial cell walls. Tetracycline is a broad-spectrum antibiotic suitable for both oral and topical use. Tetracycline has been shown to have a bacteriostatic effect, which is achieved through the suppression of protein synthesis.

The antibacterial activity effect of BC/antibiotic composites was tested on cultures E. coli ATCC 25922 and S. aureus ATCC 25923, using the disk diffusion method. As seen in Figure 2 and Figure 3, the obtained BC/TC and BC/CEF composites have pronounced antibacterial activity. The largest inhibition zone was observed for the BC/CEF composite in both test cultures. The inhibition zones at different concentrations of CEF did not differ significantly, showing that the lower concentrations of the antibiotic are also effective.

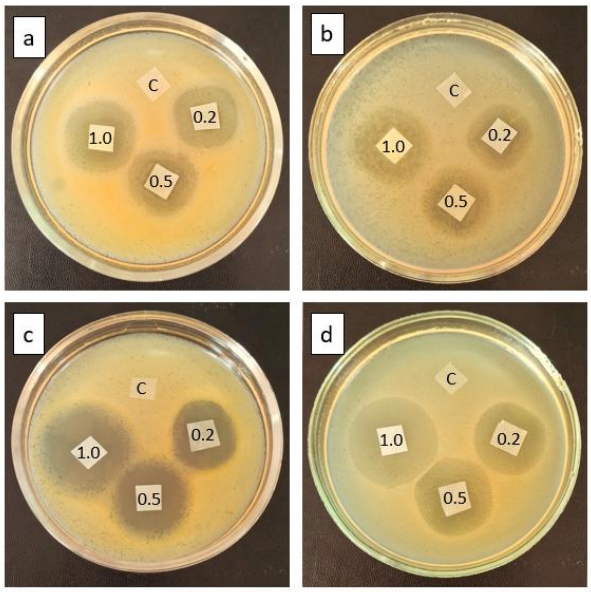


Figure 2. Antibacterial activity of BC/TC composite in the cases of S. aureus (a) and E. coli (b), and BC/CEF composite in the cases of S. aureus (c) and E. coli (d). $p<0.05$

C — BC as a control; values 0.2, 0.5, 1.0 — tested concentrations of antibiotics in percentage

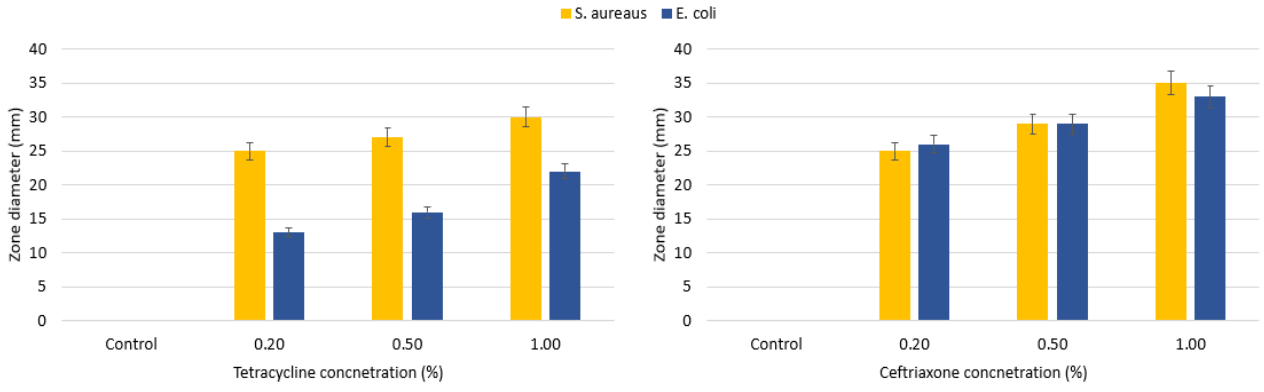


Figure 3. Comparative analysis of inhibition zones of BC composites. $p<0.05$

The strongest inhibition of BC/TC was observed on *S. aureus*; on the *E. coli* culture, the zones were smaller, which may be due to the fact that *E. coli* is a Gram-negative bacterium with a more complex cytoplasmic membrane structure which prevents TC from penetrating into the periplasmic space of the cell.

With regard to the release of antibiotics from BC composites, which demonstrate maximal activity within the initial 10 hours, our research has indicated that ceftriaxone exhibits a more rapid release profile (61.5% within 72 hours) in comparison to tetracycline (48.5% within 72 hours). The difference in the rate of antibiotic release may be related to the strength of adhesion, which depends on the charge of the antibiotic, since BC has a negative charge. As a result of electrostatic interaction between BC and positively charged tetracycline, the release of the antibiotic is slowed down, while the negative charge of ceftriaxone, on the contrary, promotes faster release of the antibiotic from BC. Our results correlate with the results obtained by Volova et al. (2018), Breijaert et al. (2025) [47-48].

Thus, the novelty of this study lies in obtaining BC/antibiotic composites with controlled release and significant antibacterial activity. These composites are based on BC obtained from the processing of brewing waste, which can ensure cost-effective production in accordance with the principles of a green sustainable economy.

CONCLUSION

The current study showed that BC films obtained by co-cultivation of *K. xylinus* MS2530 with *P. fermentans* MDC 10169 on brewing waste medium can be used as carriers of antibiotics, such as tetracycline and ceftriaxone, and exhibit significant antibacterial effects against *S. aureus* and *E.coli*. Consequently, our results show that BC composites filled with antibiotics can be used as wound dressings with antimicrobial action.

List of Abbreviations: BC: bacterial cellulose, BSY: brewing waste, CFU: colony forming unit, SEM: scanning electron microscopy, FTIR: Fourier-transform infrared spectroscopy, PBS: phosphate-buffered saline, CEF: ceftriaxone, TC: tetracycline, BC/CEF: ceftriaxone loaded bacterial cellulose, lBC/TC: tetracycline loaded bacterial cellulose

Competing Interests: The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Author's Contributions: SA: supervision, methodology, writing – original draft preparation, writing – review and editing, data curation. AH: investigation, methodology, data curation. MP: investigation, methodology, writing – original draft preparation, writing – review and editing. ATs: data curation, methodology. SH: methodology, data curation. MA: methodology, investigation. AT: investigation, data curation. HK: investigation, methodology, writing – review and editing.

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