Bentonite/chitosan/tetracycline as antimicrobial material

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INTRODUCTION

New antimicrobial materials have recently been reported, and clay minerals are interesting materials for this proposal (Santos et al., 2024). Bentonite (Bent) is a clay constituted predominantly by montmorillonite (≥50 %), which is a 2:1 phyllosilicate (Brigatti et al., 2013). Although the clay does not have significant antimicrobial activity, its interaction with antimicrobial agents can modify this action (Santos et al., 2024). In this study, the antibacterial properties of materials based on the incorporation of tetracycline (TC) into the chitosan/Bent composite were evaluated against Gram-positive and gram-negative bacteria. Chitosan (CS) is a biocompatibility polysaccharide derived from chitin composed of glucosamine and *N-acetyl-D-glucosamine* linked by β-(1,4)-glycosidic bonds, which exhibits antimicrobial properties, while TC is an antibiotic drug commonly used in medicine, livestock and agriculture (El-Alfy et al., 2020). Therefore, the effect of two different antimicrobial agents incorporated into the clay sample was investigated.

METHODS

Preparation of the CS/Bent nanocomposite and TC incorporation

Na-Bent was supplied by Bentonisa do Nordeste Company. The chemical composition of clay is SiO_2 (52.98%), Al_2O_3 (18.35%), Fe_2O_3 (3.96%), TiO_2 (0.18%), CaO (0.01%), MgO (2.47%), Na_2O (2.56%), and K_2O (0.22%). CS/Bent nanocomposite was obtained by reacting Na-Bent (74.6 cmol(+) kg^{-1} CEC) with CS solution in 100% CEC proportion at pH 5.0 under microwave heating at 50 °C for 30 min. For TC incorporation, Na-Bent or CS/NaBent samples were dispersed in 100 mg L^{-1} TC solution at pH 4.0 and the mixture reacted for 60 min at 25 °C under orbital agitation. The samples were characterized by X-ray diffractometry (XRD), Fourier transform infrared spectroscopy (FTIR), and thermogravimetry (TGA/DTG).

Antibacterial assays

The antibacterial properties of the materials were evaluated against *Escherichia coli* (*E. coli*, ATCC-25922) and *Staphylococcus aureus* (*E. aureus*, ATCC 25923) using the direct contact test, following previous procedures (CLSI, 2012).

RESULTS AND DISCUSSION

The incorporation of TC in the NaBent and CS/Bent samples was evaluated by X-ray diffraction (XRD), FTIR, and TG/DTG analysis. Na-Bent's XRD results indicated that the main phase was montmorillonite (Mt), with quartz, anorthite, and gypsum impurities, according to the literature (Santos et al., 2024). The reflection at 7.24° corresponded to the basal space (d_{001}) of 1.21 nm, characteristic of sodium Mt (Silva et al., 2021). The CS/Bent nanocomposite exhibited a d_{001} values of 1.84 and 1.25 nm. The higher 001 basal reflection is indicative of the CS intercalation in Mt. After drug incorporation the value of d_{001} changed to 1.49 nm for Bent-TC and 1.72 nm for

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CS/Bent-TC due to the TC intercalation in the interlayer space of the Mt. FT-IR spectra of the samples confirmed the presence of the biopolymer and drug in the clay. Na-Bent presented the characteristic bands of Mt, while the CS/Bent nanocomposite exhibited new bands at 2940 and 2890 cm^{-1} associated to -CH anti symmetrical and symmetrical vibrations, and at 1538 cm^{-1} (δNH_3^+) and at 1373 cm^{-1} (δCH_2 and δCH_3). TC loaded samples also showed characteristic bands of the drug in the range between 1540-1544, 1502-1509, 1457-1466, assigned to C=O stretching vibration in ring C, NH_2 amide, C=C stretching (Parolo et al., 2010), respectively. Furthermore, the bands at 1382 and 1308-1322 cm^{-1} were assigned to CH_3 and tertiary C-H bending, respectively (Parolo et al., 2010). TG/DTG curves showed that the Bent-TC and CS/Bent-TC hybrids exhibited higher total mass losses compared to those of Na-Bent and CS/Bent precursor materials, respectively, indicating the presence of the drug in the samples. Bent-TC sample exhibited mass losses of 10.4% in the range of 163-435 °C attributed to the thermal decomposition of the intercalated drug. The mass loss in the temperature range of 170-455 °C was attributed to the thermal degradation of the polysaccharide chains for CS/Bent (7.8%), and both drug and polysaccharide chains and drug for CS/Bent-TC (9.4%). These mass loss events were used to estimate the percentage of organic content in the samples and suggest the higher incorporation of TC in Na-Bent in relation to the CS/Bent nanocomposite. Antibacterial tests against *S. aureus* and *E. coli* strains are presented in Fig.1. TC-loaded samples exhibited higher antibacterial properties compared to those of the precursor materials. The inhibitory action of Ben-TC and CS/Bent-TC was 64 and 77% for *S. aureus*, respectively, and approximately 88% for *E. coli*. The results showed the potential of the prepared solids as antimicrobial materials and the best performance of Cs/Bent-TC for Gram-positive bacteria.

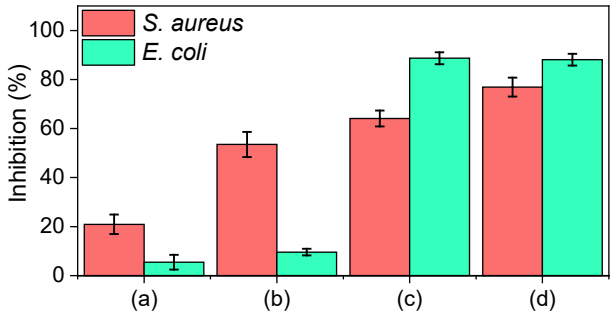

Fig. 1. *Inhibitory action for (a) Na-Bent, (b) CS/Bent, (c) Bent-TC, and (d) CS/BentTC against S. aureus and E. coli bacteria.*

CONCLUSIONS

Intercalation hybrids were obtained by incorporation of CS and TC molecules in clay sample. The loaded solids showed excellent antimicrobial activities against *S. aureus* and *E. coli* bacteria. The presence of the polysaccharide in Na-Bent improved the antimicrobial activity of Gram-positive bacteria.

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