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ANTIMICROBIAL ENZYME IMMOBILIZATION IN BACTERIAL CELLULOSE

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ABSTRACT

This work studied the physical immobilization of laccase on bacterial nanocellulose (BNC) aiming to identify the enzyme antibacterial properties suitable for wound dressings. The pH optimum and activation energy of free laccase depends on the substrate employed. The Michaelis-Menten constant for the immobilized laccase was found to be almost double of that of the free enzyme. However, the specific activities of immobilized and free laccase are similar suggesting that entrapped laccase on BNC maintain some flexibility and favour substrate accessibility. The results clearly show the antimicrobial effect of laccase and cytotoxicity acceptable for wound dressing applications.

INTRODUCTION

BNC is a nontoxic, noncarcinogenic and biocompatible biopolymer synthesized by bacteria that displays unique structural and mechanical properties as compared with plant cellulose including higher purity, higher crystallinity, higher capacity for water retention, and a three-dimensional nanoscale arrangement of the cellulose fibrils allowing its utilization in the biomedical field for wound dressings, burn treatments, tissue regeneration and as temporary skin substitutes. (Römling and Galperin 2015). However, BNC per se, lacks antibacterial properties. Laccase enzymes are known to catalyze reactions that lead to the generation of antimicrobial species in the presence of wide range of substrates (Grover, Dinu et al. 2013). Direct antimicrobial activity of crude laccase against was also observed (Sampaio, Padrao et al. 2016). Effective enzyme immobilization on BNC was reported, however, the use of BNC as a support for laccase immobilization is still poorly studied (Frazão, Silva et al. 2014).

RESULTS AND CONCLUSIONS

Laccase activity characterization in function of pH, activation energy, temperature and thermal stability showed that the optimum pH depends on the employed substrate with higher thermal stability for the phenolic 2,6-dimethylphenol (DMP) than for 2,2′-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) ABTS. The activation energies suggest a better conformational accommodation in the active site for DMP. Thermal inactivation rate constant and observed half-life values show that, thermal stability of laccase is not improved after immobilization procedure. The enzyme kinetics parameters of free and immobilized laccase suggest a lower substrate affinity for the immobilized enzyme as compared to the free enzyme due to diffusional substrate limitations of the laccase penetrated in the BNC structure.

However, the specific activity of immobilized laccase is close to the free enzymes suggesting that the cage-like structure of BNC allows entrapped laccase to maintain some flexibility and favour mass transport and accessibility of the substrate (Fig. 1).

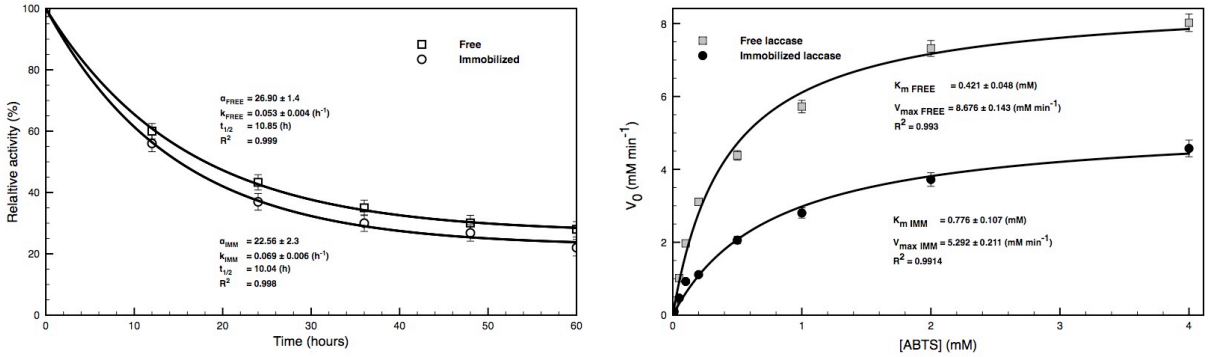

Fig.1 Thermal inactivation and initial reaction rates of free and immobilized laccase on BCN at 37 ºC.

The laccase immobilized onto BNC clearly show and antimicrobial effect, being more sensitive for Gram-positive bacteria than Gram-negative ones due to the different electrochemical mode of action of laccase to penetrate the different structure and composition of the Gram-positive and Gram-negative cell walls. Laccase exerted a small cytotoxic effect on fibroblasts but is considered acceptable for wound dressing applications (Fig. 2).

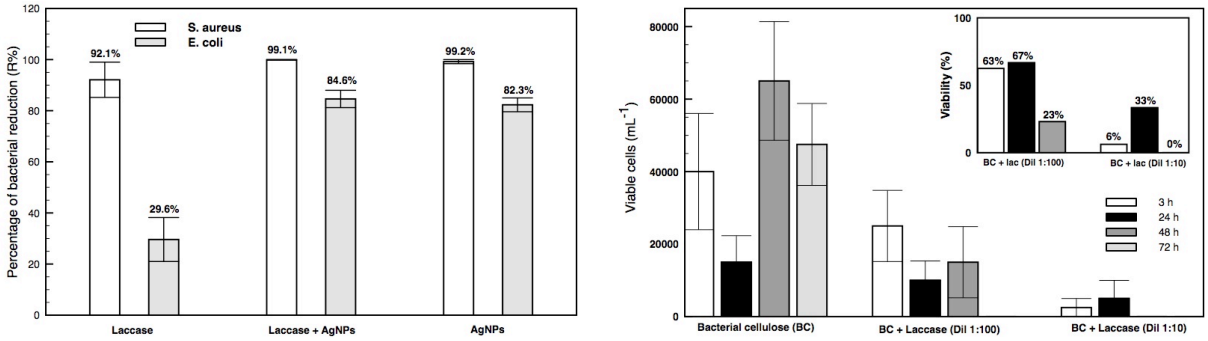

Fig.2 Escherichia coli and Staphylococcus aureus growth reduction of BNC immobilized laccase and BNC cytotoxicity and viability (inset) with different laccase concentrations.

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