ANTIMICROBIAL PERFORMANCE OF LIGNIN EMBEDDED IN BACTERIAL NANOCELLULOSE MEMBRANES

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ABSTRACT

The development of bio-based antimicrobial polymeric composites has never been so urgent. Novel antimicrobial fibrous-based biocomposites will certainly allow the development of important solutions to fight the present and future Pandemics, while reducing the dependence of petrochemical based polymers and fibers. Lignin has a pivotal function in preventing the invasion of phytopathogens, thus, this work explores the antimicrobial potential of lignin when embedded in a biosynthesized fibrous nanomatrix with superior mechanical properties: bacterial nanocellulose (BNC). Lignin was subjected to alkali treatment to promote the inclusion of lignin within BNC which comprises pores ranging from 20 to 300 nm. Both alkali treatment efficiency, bactericidal and antiviral activities were investigated

INTRODUCTION

The inclusion of lignin within a bacterial nanocellulose (BNC) matrix generates a fully bio-based composite encompassing the excellent properties of both components. BNC is synthesized by bacteria and exhibits mechanical properties roughly equivalent to Kevlar® and an impressive biocompatibility (Padrão, 2021). Lignin has a satisfactory stiffness, good thermal stability and antioxidant activity (Melro, 2018). Lignin antimicrobial activity per se and when embedded within a BNC membrane was assessed. Two model bacteria: Staphylococcus aureus and Escherichia coli, and MS2 bacteriophage, which contains a RNA genome in addition to a capsid which roughly has a similar architecture as SARS-CoV-2. Lignin was obtained from the black liquor of a Kraft pulp mill (ENCE, Pontevedra, Spain) using Eucalyptus globulus as raw material. The black liquor was subjected to lignin precipitation with sulfuric acid and then the lignin was extensively washed with acidic solution and water, dried and milled with a mortar and pestle was submitted to two alkali treatments: 0.05 M and 0.1 M of sodium hydroxide. After neutralization, minimal bactericidal/virucidal concentration was evaluated. Once embedded within a BNC matrix, the composites were analyzed to test their efficacy against bacteria and the MS bacteriophage.

RESULTS AND CONCLUSIONS

The alkali treatment has an obvious impact on the size of the lignin fragments, becoming smaller as the alkali concentration increases (Fig. 1). The minimal bactericidal/virucidal activity (Table 1) confirms the potential antibacterial activity of all lignin samples, by displaying a relevant Log reduction against S. aureus. As for the minimal virucidal activity, the Log reduction is low with the exception of 10 mg mL-1 of lignin treated with 0.1 M of NaOH. Once the different lignins are incorporated into BNC (Fig. 2), the BNC with the highest zone of inhibition against S. aureus contains lignin 0.1 M of NaOH. Nevertheless, the bactericidal activity against E. coli and the antiviral activity are negligible (Table 2).

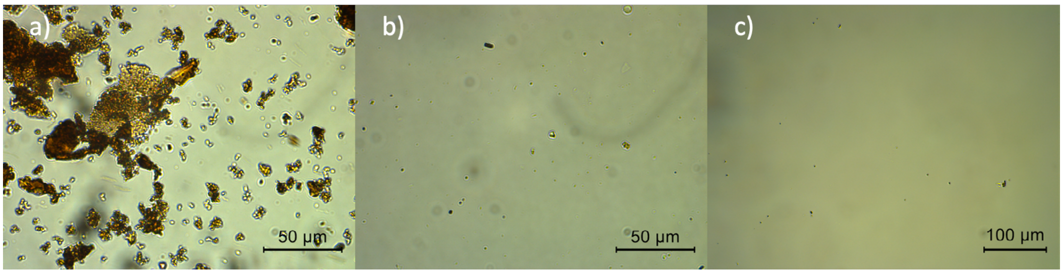

Fig. 1 Microscopy images of: a) lignin, b) lignin 0.05M NaOH and c) lignin 0.1M NaOH

Table 1 – Minimal bactericidal/virucidal concentration

		Lignin			Lignin 0.05 M NaOH			Lignin 0.1 M NaOH		
Concentration (mg mL^{-1})		**1**	**0.1**	**0.01**	**10**	**1**	**0.01**	**10**	**1**	**0.01**
S. aureus	**Log reduction (CFU mL^{-1})**	3.8	2.9	0	2.4	1.7	0	1.8	1.9	0
E. coli		0.9	0	0	1.2	0	0	0.6	0	0
MS2	**Log reduction (PFU mL^{-1})**	1.8		1.4	1.68		1.39	3.0		1.2

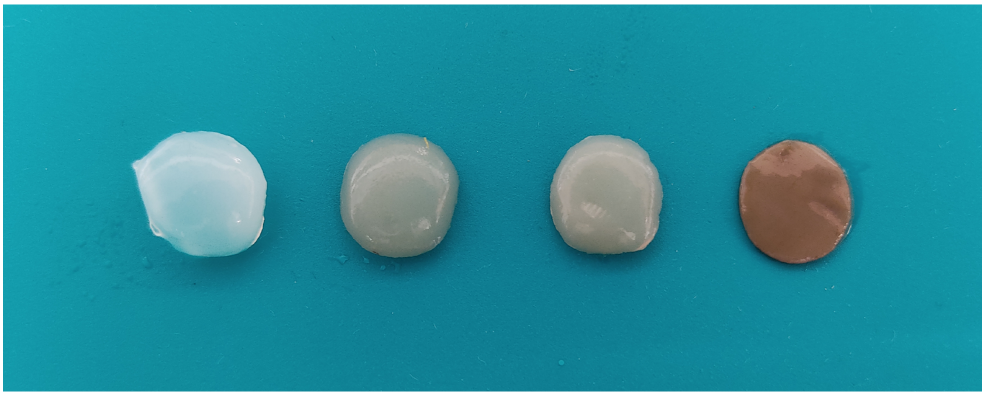

Fig. 2 BNC membranes embedded with lignin. From left to right: BNC, lignin 0.05M NaOH, lignin 0.1M NaOH and lignin.

Table 2 – Minimal bactericidal/virucidal concentration

Sample		**BNC**	**Lignin**	**Lignin 0.05 M NaOH**	**Lignin 0.1 M NaOH**
S. aureus	**ZoI (mm)**	0	8.5	7.7	13.2
E. coli		0	0	0	0
MS2	**Log reduction (PFU mL^{-1})**	0	0.3	0.1	0.3

Further antimicrobial analysis is required to provide further insights of the bactericidal activity of the composites.

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REFERENCES

[1] Padrão J, Zille A. Surface chemistry of nanocellulose and its composites. In: Kumar D. (ed) Nanocellulose and its composites for water treatment applications. CRC Press, Boca Raton, 2021, in press

[2] Melro E, Alves LA, Antunes FE, Medronho B. A brief overview on lignin dissolution. Journal of Molecular Liquids, 2018, 265, p. 578-584