Integrated Bioprocesses for Production of Bacterial Nanocellulose from Lignocellulosic Biomass

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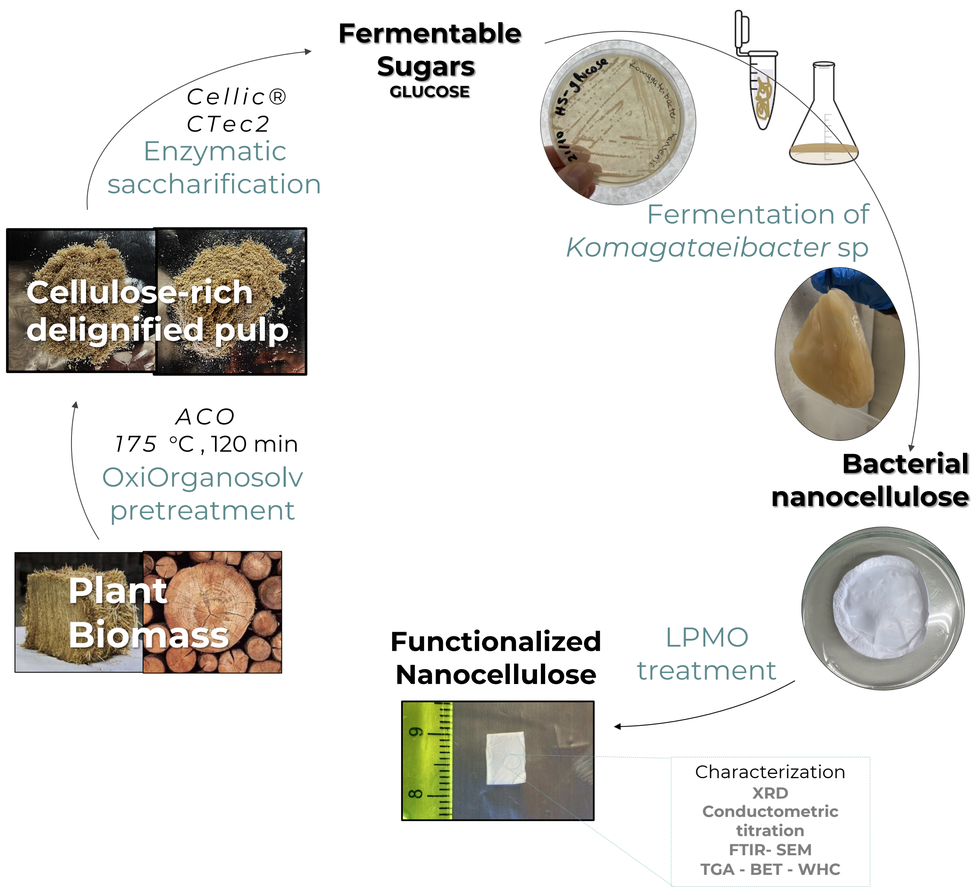
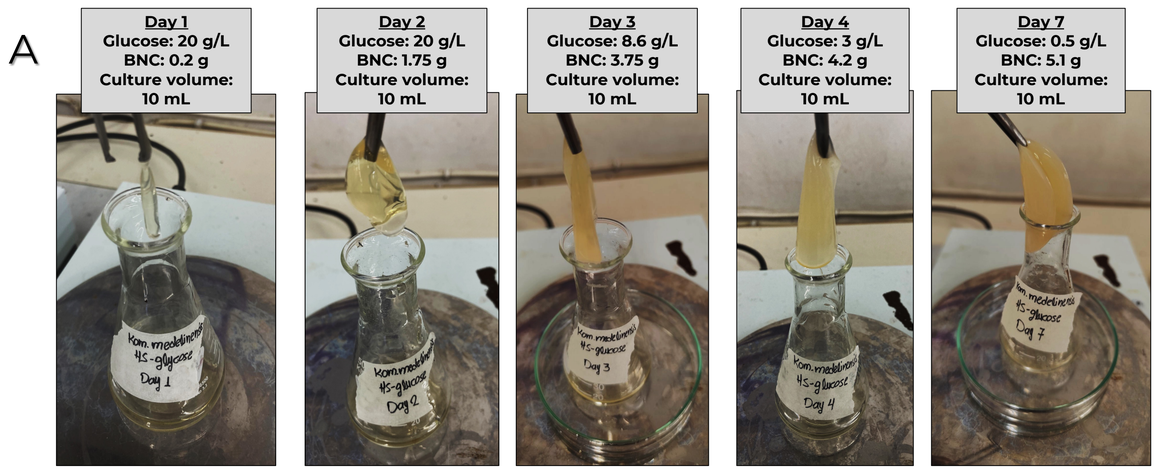
Introduction

Bacterial nanocellulose (BNC) is a highly pure, crystalline, and biocompatible form of cellulose produced *by Komagataeibacter* sp. [1,2]. Its large-scale application is limited by the high cost of conventional fermentation media, while low-cost sugars from lignocellulosic residues represent a sustainable alternative [3,4]. In this work, beechwood residues and wheat straw waste were pretreated by OxiOrganosolv, enzymatically hydrolyzed, and used as carbon sources for *Komagataeibacter* fermentation, leading to BNC production. Yields and production values obtained from standard and alternative carbon sources were compared [3]. Functionalization with LPMOs was applied to enhance BNC surface properties, and the final materials were characterized in detail [5].

Process

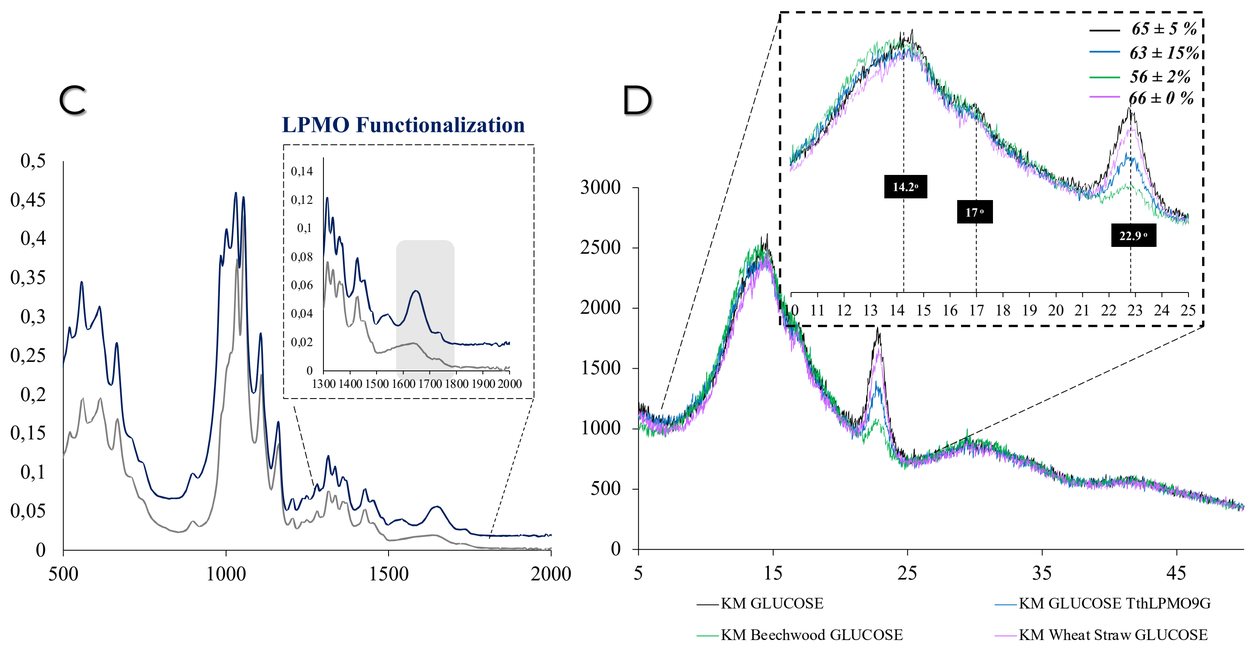


Results



B

Carbon Source	Microorganism	Wet mass BNC (g)	Final Carbon Source Concentration (g/L)	Yield (g of dry BNC/ 100 g of concumed sugars)	Production (g dry nanocellulose / L of culture)
Glucose	*K. medelinensis*	12.4 ± 0.1	0	31 ± 0.3	6.2 ± 0.1
Xylose	*K. medelinensis*	5.8 ± 0.1	8 ± 0.1	24 ± 0.2	2.9 ± 0.1
C6- hydrolysate-wheat straw	*K. medelinensis*	13.0 ± 0.5	0.7 ± 0	34 ± 1.4	6.5 ± 0.3
C6- hydrolysate-beechwood	*K. medelinensis*	10.6 ± 0.3	0.7 ± 0.1	27 ± 0.7	5.3 ± 0.2
Glucose	*K. xylinus*	8.8 ± 1.4	0	22 ± 3.4	4.4 ± 0.7
Xylose	*K. xylinus*	7.5 ± 0.4	6 ± 0.3	27 ± 0.7	3.8 ± 0.2
C6- hydrolysate-wheat straw	*K. xylinus*	12.3 ± 0.4	0.3 ± 0	31 ± 1.0	6.2 ± 0.2
C6- hydrolysate-beechwood	*K. xylinus*	11.6 ± 0.7	0.2 ± 0.1	29 ± 1.9	5.8 ± 0.4



Highlights from Figures

A. *Daily wet mass of BNC produced by Komagataeibacter medellinensis correlated with glucose consumption during the first 7 days.*

B. *BNC yields and production values were compared across glucose, xylose, and biomass-derived cellulose hydrolysates after 15 days.*

C. *FTIR spectra confirmed **successful BNC functionalization** with TthLPMO9G, showing differences from untreated samples.*

D. *XRD analysis demonstrated **high BNC crystallinity** (56–66%) across carbon sources, with no major loss after LPMO treatment.*

Major Findings

- *Both Komagataeibacter strains produced nanocellulose, with higher yield from K. medellinensis (5.12 g/L vs. 4.18 g/L), plateauing after day 7.*
- *Saccharification of beechwood and wheat straw biomass was successful, providing fermentable sugars (55.37 & 50.68 mg/mL respectively), for BNC production.*
- Protein binding assays showed stronger interaction of *Tth*LPMO9G with BNC (62% decrease in free enzyme) compared to PASC (44% decrease).
- *HPAEC-PAD verified release of oxidized cello-oligosaccharides after LPMO treatment of wet BNCs. Also, shows that the type of carbon source doesn't affect the structural features of BNC relevant to enzymatic functionalization.*
- *FTIR spectral mapping demonstrated that BNC films were chemically uniform across their surfaces.*
- *Surface carboxylate groups on BNC increased by ~58% after LPMO treatment (0.414 to 0.654 mmol/g).*
- *LPMO treatment altered BNC structure, increasing BET surface area (145.6 to 442.1 m²/g) and reducing average pore diameter (121.8 to 61.8 Å).*
- *Water holding capacity (WHC) was highest in glucose-derived BNC (132.8 g/g), followed by beechwood (104.5 g/g) and wheat straw (97.3 g/g).*

Future Directions

- Utilize hemicellulose-rich hydrolysates as alternative carbon sources for BNC production.
- Expand the range of substrates (e.g., lactic acid, PLA hydrolysates, acid whey) for *Komagataeibacter* fermentation.
- Explore scale-up strategies with low-cost hydrolysates in bioreactor systems for industrial BNC production.
- Integrate lignocellulosic biomass decomposition to isolate plant nanocellulose and use saccharification products for *Komagataeibacter* fermentation.
- Investigate binding of functionalized BNC to selected chemical compounds for advanced material applications.

Acknowledgments

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References

1. Donini, Í.A.N., Salvi, D.T.B., Fukumoto, F.K., Lustri, W.R., Barud, H.S., Marchetto, R., et al. (2010). Biosynthesis and recent advances in production of bacterial cellulose. Eclética Química, 35, 165–178.
2. Castro, C., Zuluaga, R., Álvarez, C., Putaux, J.L., Caro, G., Rojas, O.J., et al. (2012). Bacterial cellulose produced by a new acid-resistant strain of Gluconacetobacter genus. Carbohydrate Polymers, 89(4), 1033–1037.Costa, A.F.S., Almeida, F.C.G., Vinhas, G.M., & Sarubbo, L.A. (2017).
3. Production of bacterial cellulose by Gluconacetobacter hansenii using corn steep liquor as nutrient source. Frontiers in Microbiology, 8, 2027.Wang, J., Tavakoli, J., & Tang, Y. (2019).
4. Bacterial cellulose production, properties and applications with different culture methods – A review. Carbohydrate Polymers, 219, 63–76.Kaczmarek, M., & Białkowska, A.M. (2025).
5. Enzymatic functionalization of bacterial nanocellulose: current approaches and future prospects. Journal of Nanobiotechnology, 23, 82.