Bioprocessing Strategies for Sustainable Bacterial Nanocellulose Production from Lignocellulosic Residues

ECCS 2025 Conference

Koar Chorozian[1,2], Evdoxia Karpenisioti[1], Stylianos D. Stefanidis[3], Konstantinos G. Kalogiannis[4], Evangelos Topakas[1], Anthi Karnaouri[2*]

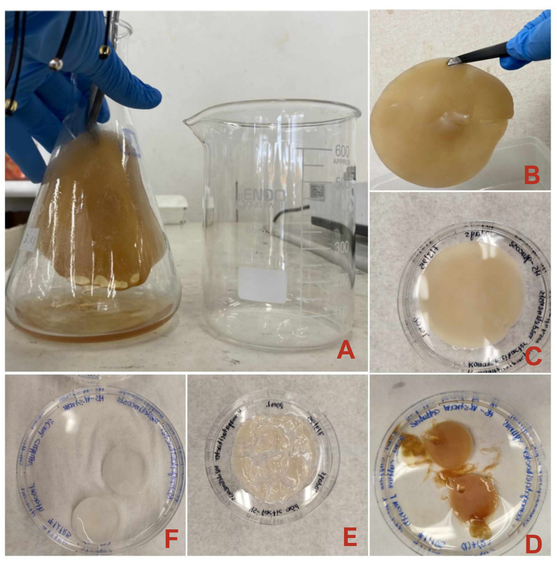
[1] InduBioCat Group, Biotechnology Laboratory, School of Chemical Engineering, National Technical University of Athens, Athens, Greece, [2] Laboratory of General and Agricultural Microbiology, Department of Crop Science, Agricultural University of Athens, Athens, Greece, [3] Chemical Process and Energy Resources Institute (CPERI), Centre for Research and Technology Hellas (CERTH), Thessaloniki, Greece, [4] Department of Chemical Engineering, University of Western Macedonia, Kozani, Greece

INTRODUCTION

The move toward a circular bioeconomy is driving the development of eco-friendly, bio-based materials from renewable resources and waste [1]. Among these, nanocellulose is a highly abundant natural polymer, mainly produced by plants. However, bacterial cellulose (BC), produced by certain bacteria such as *Komagataeibacter*, *Sarcina*, and *Agrobacterium* [2], is a highly pure and nanostructured form of cellulose, which offers superior physical and chemical properties compared to plant cellulose, including high crystallinity, hydrophilicity, and biocompatibility, free from lignin and hemicellulose [3]. BNC, is synthesized via a polymerization-based process, where bacteria convert sugars into cellulose [4]. Despite its promising properties and applications, BC production is expensive due to the high cost of traditional fermentation media. Therefore, finding cheaper, sustainable carbon sources is critical for commercial viability. Recent studies have explored using agricultural and industrial waste products, as alternative substrates for BC production. These efforts not only aim to reduce costs but also address environmental concerns by repurposing waste materials [2]. Bacterial nanocellulose (BNC) can be functionalized using lytic polysaccharide monooxygenases (LPMOs), oxidative enzymes that catalyze the cleavage of cellulose. LPMOs hydroxylate the C–H bond at the C1 and/or C4 positions, generating aldonic acids and ketoaldoses [5]. This enzymatic treatment enhanced BNC properties, including increased carboxylate content, offering new opportunities for advanced LPMO-assisted biorefinery processes [6]. In this study, agricultural and forest residual feedstocks were initially subjected to a mild OxiOrganosolv pretreatment process [7] , producing a cellulose-rich solid fraction and a hemicellulose rich aqueous liquor. Both streams underwent enzymatic hydrolysis and saccharification, with sugar release. The fermentable sugars were then used as a carbon source for microbial production of BNC by Komagataeibacter sp., and the resulting nanocellulose was characterized to confirm its properties. Finally, LPMOs (C1 and C4), used for differential modification of nanocellulose biofilm surface functionalization.

METHODS

- OxiOrganosolv pretreatment process of agricultural and forest residual feedstocks [7].
- Enzymatic hydrolysis in both cellulose-rich solid fraction and hemicellulose rich aqueous liquor with Cellic® CTec2 and Cellic® HTec2 respectively.
- Saccharification Analysis was performed with 3,5-Dinitrosalicylic acid (DNS) assay and GOD-POD (Glucose Oxidase-Peroxidase) test for sugar quantification and carbon source consumption.
- Bacterial strains used for cultures were *Komagataeibacter medelinensis* and *Komagataeibacter xylinus*.
- Carbon sources used for cultures were Glucose, Xylose, Lactic acid, Wheat straw derived cellulose, Wheat straw derived hemicellulose, Beechwood cellulose and Beechwood hemicellulose.
- Cultivation optimization involved varying incubation time and culture vessel type, while maintaining constant conditions of 25°C, a 1:5 air-to-culture medium ratio, and static incubation.
- BNC was harvested and treated with 5% (w/v) KOH overnight to remove medium components and attached bacteria. Then washed with deionized water until neutral pH (A to F).
- Nanocellulose functionalization was achieved through LPMO-mediated surface modification with C1- and C4-regioselective LPMOs.
- Nanocellulose characterization was conducted using FTIR and TGA analyses.



RESULTS

TABLE 1: BNC PRODUCTION FROM GLUCOSE IN DIFFERENT CULTURE VESSELS AND VOLUMES

MICROORGANISM	DAYS	CULTURE VOLUME (mL)	NANOCELLULOSE SHAPE	*WET* YIELD (mg/mL)
Komagataeibacter medelinensis	30	50	ERLENMEYER FLASK (50mL)	1180
	15			511.53
	30	20	CERTIFUGE TUBE (50mL)	162.825
Komagataeibacter xylinus	30	50	-	-
	15		ERLENMEYER FLASK (50mL	417.66
	30	20	CERTIFUGE TUBE (50mL)	171.4

TABLE 2: BNC PRODUCTION FROM GLUCOSE SUBSTRATE OVER 18 DAYS IN FLASKS (50 ML CULTURE VOLUME)

MICROORGANISM	WET YIELD (mg/mL)	DRY YIELD (%)	GLUCOSE CONSUMPTION (%)
Komagataeibacter medelinensis	511.53	27.1	94.43
Komagataeibacter xylinus	417.66	22.4	93.22

TABLE 3: BNC PRODUCTION FROM VARIOUS CARBON SOURCES AFTER 30 DAYS IN CENTRIFUGE TUBES (20 ML CULTURE)

MICROORGANISM	CARBON SOURCE	WET YIELD (mg/mL)	CARBON SOURCE CONSUMRTION (%)	*DRY* YIELD (%)
Komagataeibacter medelinensis	Glucose	162.825	62.30	13
	Xylose	74.9	18.80	19.8
	Wheat straw cellulose	151.45	44.65	17
	Beechwood cellulose	135.875	60.69	11.2
Komagataeibacter xylinus	Glucose	171.4	87.84	9.8
	Xylose	96.725	38.38	12.6
	Wheat straw cellulose	170.125	66.55	12.8
	Beechwood cellulose	148.3	70.7	10.4

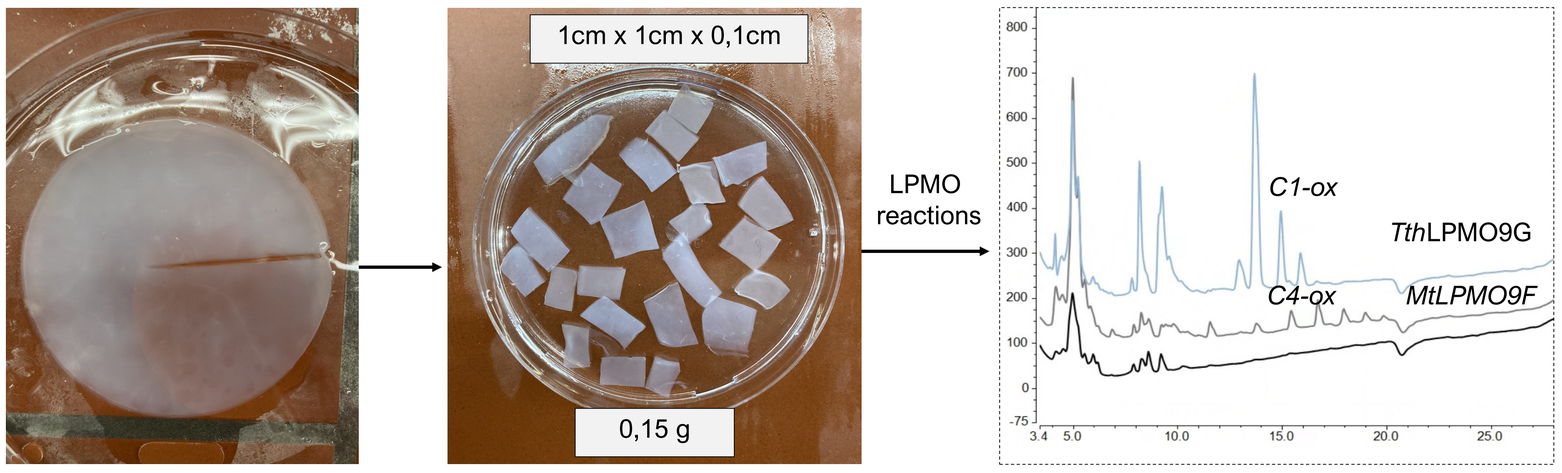


FIGURE 1: FUNCTIONALIZED BNC AFTER REACTION WITH LPMOs (DIONEX RESULTS

KEY FINDINGS

- Both bacterial strains produced successfully nanocellulose from standard carbon sources (glucose and xylose).
- Nanocellulose produced from solid fractions of agricultural and forest feedstocks. The production yields were comparable to those obtained using glucose and xylose as carbon sources.
- The geometry of the cultivation vessel and the culture volume, significantly influences the yield of nanocellulose production.
- Distinct regioselectivity of LPMO enzymes allowed precise and differential functionalization of nanocellulose biofilm surfaces.

REFERENCES

[1] Karnaouri, A., Chorozian, K., Zouraris, D., Karantonis, A., Topakas, E., Rova, U., & Christakopoulos, P. (2022). Lytic polysaccharide monooxygenases as powerful tools in enzymatically assisted preparation of nano-scaled cellulose from lignocellulose: A review. *Bioresource Technology*, *345*, 126491. https://doi.org/10.1016/j.biortech.2021.126491

[2] Costa, A. F. S., Almeida, F. C. G., Vinhas, G. M., & Sarubbo, L. A. (2017). Production of Bacterial Cellulose by Gluconacetobacter hansenii Using Corn Steep Liquor As Nutrient Sources. *Frontiers in Microbiology*, *8*. https://doi.org/10.3389/fmicb.2017.02027

[3] Fan, X., Gao, Y., He, W., Hu, H., Tian, M., Wang, K., & Pan, S. (2016). Production of nano bacterial cellulose from beverage industrial waste of citrus peel and pomace using Komagataeibacter xylinus. *Carbohydrate Polymers*, *151*, 1068–1072. https://doi.org/10.1016/j.carbpol.2016.06.062

[4] Castro, C., Zuluaga, R., Álvarez, C., Putaux, J.-L., Caro, G., Rojas, O. J., Mondragon, I., & Gañán, P. (2012). Bacterial cellulose produced by a new acid-resistant strain of Gluconacetobacter genus. *Carbohydrate Polymers*, *89*(4), 1033–1037. https://doi.org/10.1016/j.carbpol.2012.03.045

[5] Kosinas, C., Chorozian, K., Sandgren, M., Topakas, E., & Dimarogona, M. (2025). Mutational study of a lytic polysaccharide monooxygenase from Myceliophthora thermophila (MtLPMO9F): Structural insights into substrate specificity and regioselectivity. *International Journal of Biological Macromolecules*, *288*, 138574. https://doi.org/10.1016/j.ijbiomac.2024.138574

[6] Chorozian, K., Karnaouri, A., Karantonis, A., Souli, M., & Topakas, E. (2022). Characterization of a Dual Cellulolytic/Xylanolytic AA9 Lytic Polysaccharide Monooxygenase from *Thermothelomyces thermophilus* and its Utilization Toward Nanocellulose Production in a Multi-Step Bioprocess. *ACS Sustainable Chemistry & Engineering*, *10*(27), 8919–8929. https://doi.org/10.1021/acssuschemeng.2c02255

[7] Kalogiannis, K. G., Karnaouri, A., Michailof, C., Tzika, A. M., Asimakopoulou, G., Topakas, E., & Lappas, A. A. (2020). OxiOrganosolv: A novel acid free oxidative organosolv fractionation for lignocellulose fine sugar streams. *Bioresource Technology*, *313*, 123599. https://doi.org/10.1016/j.biortech.2020.123599

ACKNOWLEDGMENTS

The research project NanoHybrid is implemented in the framework of H.F.R.I's call "Basic research Financing (Horizontal support of all Sciences)" under the National Recovery and Resilience Plan "Greece 2.0" funded by the European Union – NextGenerationEU (H.F.R.I. Project Number: 015795).