



Bioprocessing Strategies for Sustainable Bacterial Nanocellulose Production from Lignocellulosic Residues



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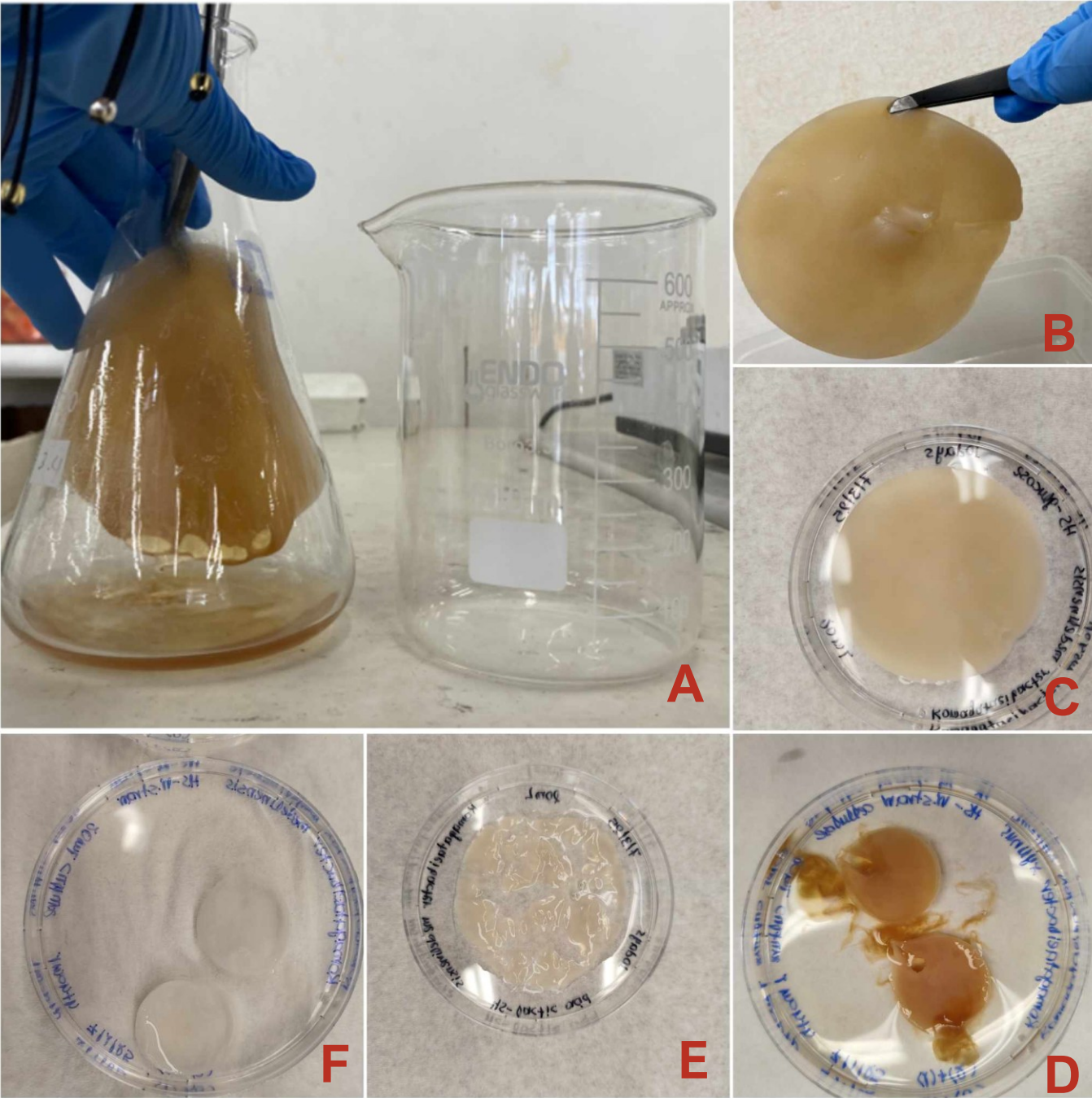
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INTRODUCTION

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 monoxygenases (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)
<i>Komagataeibacter medelinensis</i>	30	50	ERLENMEYER FLASK (50mL)	1180
	15			511.53
	30	20	CERTIFUGE TUBE (50mL)	162.825
<i>Komagataeibacter xylinus</i>	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 (%)
<i>Komagataeibacter medelinensis</i>	511.53	27.1	94.43
<i>Komagataeibacter xylinus</i>	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 CONSUMPTION (%)	DRY YIELD (%)
<i>Komagataeibacter medelinensis</i>	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
<i>Komagataeibacter xylinus</i>	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

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.

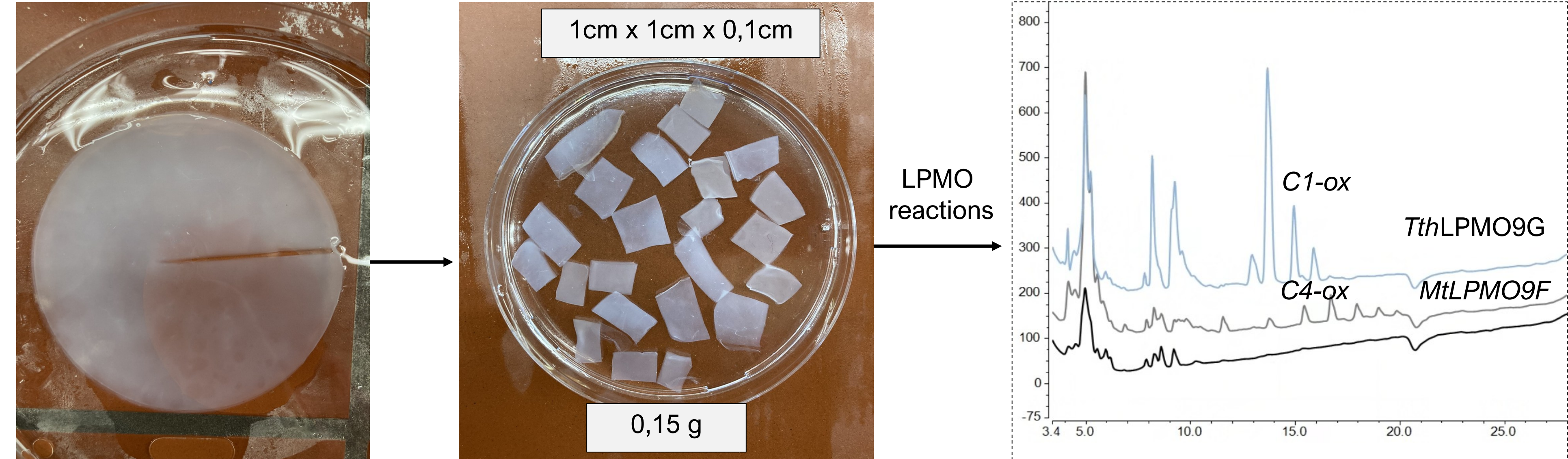


FIGURE 1: FUNCTIONALIZED BNC AFTER REACTION WITH LPMOs (DIONEX RESULTS)

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