

Supplementary Materials

Biosynthesis of Bacterial Nanocellulose from Low-Cost Cellulosic Feedstocks: Effect of Microbial Producer

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Table S1. Absorption band assignments for functional groups of the BNC samples obtained from oat hulls and *Miscanthus* using individual *Komagataeibacter xylinus* strains and symbiotic culture.

Absorption band assignment*	Absorption band peak, cm ⁻¹								
	<i>Komagataeibacter xylinus</i>			<i>Komagataeibacter xylinus</i>			<i>Medusomyces gisevii</i> Sa-12		
	B-12429			B-12431					
	MH	OHH	Control	MH	OHH	Control	MH	OHH	Control
v OH groups involved in intermolecular and intramolecular H-bonding	3413	3435	3368	3411	3415	3379	3335	3432	3429
v bonds in CH and CH ₂ groups	2916	2916	2916	2914	2917	2915	2919	2921	2920
δ HOH bonds due to tightly bound water present	1645	1639	1647	1640	1639	1641	1644	1643 1532	1654 1638
δ CH ₂ groups	1426	1426	1426	1426	1426	1426	1429	1431	1430
δ OH groups in CH ₂ OH	1372	1371	1369	1369	1369	1369		1375	1375
	1335	1335	1335	1335	1336	1335	1316	–	–
	1315	1315		1315	1316	1315			
δ CH ₂ groups in CH ₂ OH	1278			1279	1279	1279		1281	1282
	1232	1278	1279	1247	1248	1248	1280	1249	1235
				1233	1233	1233			
δ OH groups	–	–	–	–	1203	1203	1203	1204	–
v C-O bonds (bands typical of polysaccharides due to present C-O-C acetyl bonds and C-O bonds in alcohols)	1161	1162	1160	1162	1162	1162	1102		1165
	1057	1058	1109	1057	1055	1060	992	1102	1060
			1057	1032	1030	1033			
β-1,4 linkages	894	894	850	896	895	896	899	899	899

1)* v – stretching, δ – bending; MH – *Miscanthus* hydrolyzate; OHH – oat-hull hydrolyzate; 2) the controls were the Hestrin-Schramm medium for *Komagataeibacter xylinus* B-12429 and B-12431, and the synthetic medium supplemented with black tea extract for *Medusomyces gisevii* Sa-12 ; 3) some of data on BNCs synthesized by *Medusomyces gisevii* Sa-12 were reported previously [25,26].

Chemical pretreatment of plant feedstocks [25]

The pretreatment was carried out under pilot production conditions at atmospheric pressure and 90–96 °C. A 250-L standard vessel was employed, and all the equipment and its piping are made of stainless steel.

The single feedstock loading was 10 kg. The pretreatment time was 14 h. The chopped *Miscanthus* or pristine oat hulls were pre-hydrolyzed with a 1% HNO₃ solution (AO KhimReaktiv, CAS No. 7697-32-7, Russia) at 96 °C for 1 h. Afterwards, the feedstocks were treated with a 4 % HNO₃ solution, washed with water, treated with a 4 % NaOH solution (OAO Kaustik, CAS No.1310-73-2, Russia), and then washed with water until natural wash waters. The resultant pulps were squeezed in a vacuum filter and used as substrates in the wet state for enzymatic hydrolysis.

Table S2 lists the chemical compositions of the feedstocks and the resultant pulps.

Table S2. Chemical compositions of *Miscanthus*, oat hulls and pulps [69].

Measures	<i>Miscanthus</i>	<i>Miscanthus</i> pulp	Oat hulls	Oat-hull pulp
α -Cellulose, %	50.3±0.2	95.8±0.3	44.9±0.2	94.2±0.2
Pentosans, %	22.7±0.1	2.5±0.1	31.4±0.1	3.8±0.1
Acid-insoluble lignin, %	21.4±0.1	1.5±0.1	18.8±0.1	0.8±0.1
Ash content, %	5.6±0.05	0.2±0.05	4.9±0.05	1.2±0.05
Total hydrolyzables, %	72.3±0.3	98.3±0.3	76.3±0.3	97.8±0.3

Enzymatic hydrolysis of pulps [69]

The enzymatic hydrolysis of the resultant substrates was run in an 11-L fermentor in an aqueous medium at $47\pm 2^\circ\text{C}$ for 72 h by using the enzymes CelloLux-A (Sibbiopharm Ltd, Russia) and BrewZyme BGX (Tarchomin Pharmaceutical Works Polfa S.A., Poland) standardized against cellulase, xylanase and β -glucanase in compliance with their analytical datasheets. The enzyme cocktail was injected as follows: CelloLux-A 40 FPU/g solid and BrewZyme BGX 15 FPU/g solid. FPU was determined by the international procedure. The pH was maintained at 4.7 ± 0.2 with ammonium hydroxide (OOO KhimPostavka, CAS No. 1336-21-6, Russia) and orthophosphoric acid (OOO KhimSpetsializatsia, CAS No. 7664-38-2, Russia). The initial solid loading was 30 g/L.

The yield of reducing sugars (RS) was calculated by Eq. (1):

$$\eta_{\text{RS}} = \frac{C_{\text{F}} \cdot V_{\text{H}}}{m_{\text{S}}} \cdot 0.9 \cdot 100 \quad (1)$$

where η_{RS} is the RS yield on a solid weight basis (%);

C_{F} is the final RS concentration in the hydrolyzate (g/L);

V_{H} is the enzymatic hydrolyzate volume (L);

0.90 is the coefficient attributed to the water molecule addition to anhydroglucose residues of the corresponding monomer units as a result of enzymatic hydrolysis;

m_{S} is the weight of the substrate for fermentation (g).

Table S3 summarizes enzymatic hydrolysis results for the feedstocks and pulps.

Table S3. Enzymatic hydrolysis results for *Miscanthus*, oat hulls and pulps.

Measures	<i>Miscanthus</i>	<i>Miscanthus</i> pulp	Oat hulls	Oat-hull pulp
RS concentration, g/L	4.1±0.1	26.7±0.2	4.5±0.1	24.5±0.2
RS yield on a substrate weight basis, %	11.1±0.3	72.1±0.3	15.8±0.3	73.5±0.3
Glucose concentration, g/L	4.0±0.1	26.5±0.2	4.4±0.1	22.3±0.2
Xylose concentration, g/L	0.10±0.05	0.20±0.05	0.10±0.05	2.20±0.05
Once standardized against reducing sugars				
RS concentration, g/L	–	20.0±0.2	–	20.0±0.2
Xylose concentration, g/L	–	0.15±0.05	–	1.80±0.05