Supporting Information

Lytic polysaccharide monooxygenases (LPMOs) mediated production of ultra-fine cellulose nanofibres from delignified softwood fibres

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Table S1. Carbohydrate composition of the original holocellulose, the control (holocellulose treated with autoclave), the nanofibres prepared by treatment with the LPMOs, NcLPMO9E and NcLPMO9F, followed by mild homogenisation.

<table>
<thead>
<tr>
<th></th>
<th>Arabinose (%)</th>
<th>Galactose (%)</th>
<th>Glucose (%)</th>
<th>Mannose (%)</th>
<th>Xylose (%)</th>
<th>Hemicellulose wt (%)</th>
<th>Cellulose wt (%)</th>
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</thead>
<tbody>
<tr>
<td>Original holocellulose</td>
<td>0.8</td>
<td>1.0</td>
<td>75.4</td>
<td>8.7</td>
<td>14.0</td>
<td>19.7</td>
<td>9.5</td>
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<tr>
<td>Control holocellulose</td>
<td>0.1</td>
<td>0.1</td>
<td>84.4</td>
<td>3.8</td>
<td>11.5</td>
<td>15.4</td>
<td>3.9</td>
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<tr>
<td>NcLPMO9E treated holocellulose</td>
<td>0.1</td>
<td>0.1</td>
<td>76.1</td>
<td>3.5</td>
<td>10.7</td>
<td>14.4</td>
<td>3.6</td>
</tr>
<tr>
<td>NcLPMO9F treated holocellulose</td>
<td>0.1</td>
<td>0.1</td>
<td>71.6</td>
<td>3.2</td>
<td>10.5</td>
<td>14.2</td>
<td>3.3</td>
</tr>
</tbody>
</table>

Table S2. Physical and average mechanical properties of the nanopapers prepared from the control holocellulose without LPMO-treatment and the LPMO-oxidised nanofibres.\(^a\)

<table>
<thead>
<tr>
<th>Material</th>
<th>Porosity (%)</th>
<th>Density (g/cm(^3))</th>
<th>Young's modulus (GPa)</th>
<th>Tensile strength (MPa)</th>
<th>Strain-to-failure (%)</th>
<th>Work to fracture (MJ/m(^3))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>7.0</td>
<td>1.39</td>
<td>12.5 (1.1)</td>
<td>148.7 (4.2)</td>
<td>2.6 (0.3)</td>
<td>2.2 (0.4)</td>
</tr>
<tr>
<td>NcLPMO9E</td>
<td>5.0</td>
<td>1.42</td>
<td>15.1 (2.3)</td>
<td>257.0 (6.2)</td>
<td>4.2 (0.7)</td>
<td>6.4 (0.7)</td>
</tr>
<tr>
<td>NcLPMO9F</td>
<td>5.7</td>
<td>1.41</td>
<td>16.3 (2.1)</td>
<td>262.2 (10.1)</td>
<td>3.7 (0.4)</td>
<td>6.4 (0.6)</td>
</tr>
</tbody>
</table>

\(^a\) The values in parentheses are the sample standard deviations.
Figure S1. Codon-optimized gene sequence for NcLPMO9E (1028 bp).

Figure S2. Codon-optimized gene sequence for NcLPMO9F (731 bp).
**Figure S3.** Restriction pattern analysis of the pPICZB vector construct containing the genes of *N. crassa* LPMOs, where lane 1=Generuler 1kb DNA ladder, Thermo Scientific (5 µL) and lanes 2-6=10 µL of the EcoRI-NotI restriction reactions.

**Figure S4.** Colony PCR of *P. pastoris* X33 transformed with *N. crassa* LPMOs. A) NcLPMO9E, and B) NcLPMO9F, where lane 1=DNA ladder, 2-6=transformant DNA, 7=negative control untransformed *P. pastoris* X33.
Figure S5. Production of *N. crassa* NcLPMO9E in *P. pastoris*. A) SDS-PAGE and B) western-blot, where lane 1=PageRuler prestained protein ladder (10 µL), and lanes 2-5 contain *P. pastoris* culture supernatant from 1 to 4 days of methanol induction.

Figures S6. Production of *N. crassa* NcLPMO9F in *P. pastoris*. A) SDS-PAGE and B) western-blot, where lane 1=PageRuler prestained protein ladder (10 µL), and lanes 2-5 contain *P. pastoris* culture supernatant from 1 to 4 days of methanol induction.
Figure S7. AFM height images and histograms showing the width distributions of CNFs produced from NcLPMO9E-oxidised and NcLPMO9F-oxidised holocellulose.