

Supplementary Information (SI)

Carboxylation of lyocell fabrics using C-1 lytic polysaccharides monooxygenases (DcLPMO9A) for enhanced affinity to cationic dye.

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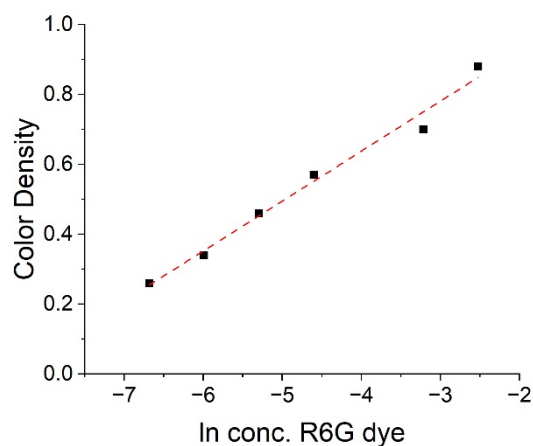
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Fig. S1. Calibration curve for dye quantification. The curve represents the logarithmic dye concentration on fabric, ranging from 0.00125 μg to 0.08 μg , plotted against the corresponding measured signal



Equation S1. Final equation used to calculate the dye amount on fabric based on the measured color density

$$\text{dye amount} = e^{\frac{(\text{density measured} - 1.2089)}{0.1427}}$$

Method S1. Soluble product analysis with HPAEC-PAD

The liquid fractions obtained from the LPMO reaction were analyzed using high-performance anion-exchange chromatography coupled with pulsed amperometric detection (HPAEC-PAD). A 25 μL sample was injected into a Dionex ICS-5000 system (Thermo Scientific, Sunnyvale, CA, USA) equipped with a CarboPac PA100 analytical column. The system was operated at a flow rate of 0.5 mL/min, following a 60-minute gradient protocol adapted from Tuveng et al. (2020).

Fig. S2. HPAEC-PAD chromatograms showing the effect of varying DcLPMO9A loadings. The analysis was performed following the protocol detailed in Method S1

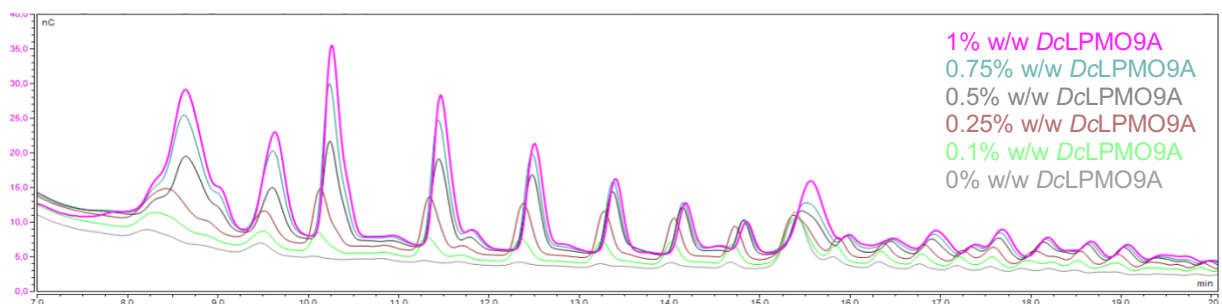
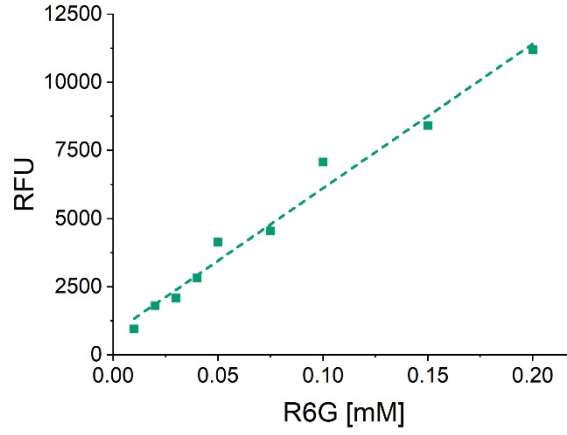


Fig.S3. Calibration curve for C1-COOH via NED signal. NED concentrations from 0.01 mM and 0.20 mM were measured in triplicates and plotted against the RFU at $\lambda_{ex} = 350$ nm, $\lambda_{em} = 420$ nm. The slope of the linear calibration is $5.95 \pm 0.60 \times 10^4$ RFU, and the intercept is $4.57 \pm 2.55 \times 10^2$.



Equation S2. Final equations to determine the NED concentration in LPMO-treated lyocell fabrics and the amount of carboxylate ($\mu\text{mol/g}$) based on the measured RFU signal.

$$c_{NED}(mM) = \frac{(RFU - \text{intercept})}{\text{slope} \left(\frac{1}{mM} \right)} * \text{diltution factor}$$

$$NED (\mu\text{mol}) = c_{NED}(mM) * V(mL)$$

$$C1 - COOH \left(\frac{\mu\text{mol}}{g} \right) = \frac{NED (\mu\text{mol})}{m_{fabric} (g)}$$

Method S2. Pairwise t-test for significance testing of dry weight change after DcLPMO9A treatment

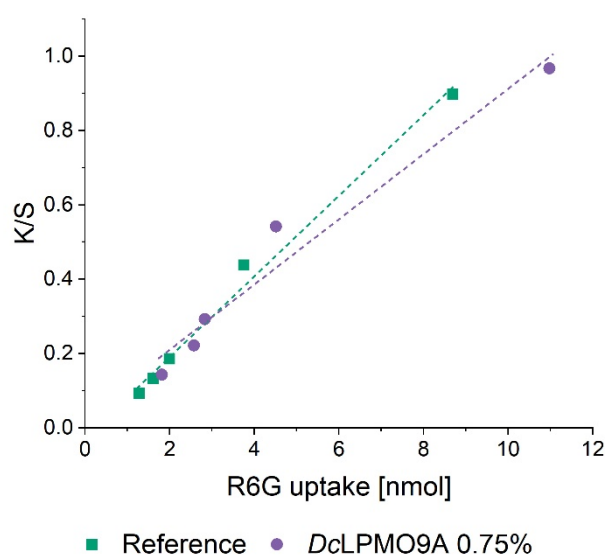
Statistical significance was assessed using a pairwise t-test, where the calculated t-values were compared to the critical t-value (**4.303**) obtained from a t-distribution table at a significance level of $\alpha = 0.05$ and degrees of freedom (**df**) = 2.

Table S1. Dry weight before and after *DcLPMO9A* treatment (performed in triplicates) and the corresponding t-values for significance testing (with $t_{crit} > 4.303$, $\alpha = 0.05$)

<i>DcLPMO9A</i> conc. (% w/w)	fabric weight t=0h (mg)	fabric weight t=24h (mg)	standard deviation	t- values	Significance
0	99.4	99.7	0.9	0.184	n.s.
0.1	98.8	99.4	0.7	0.565	n.s.
0.25	99.2	99.6	0.5	0.660	n.s.
0.5	99.6	100.4	0.5	1.085	n.s.
0.75	99.8	100.4	0.4	1.217	n.s.
1	100.2	101.1	0.2	3.103	n.s.

‘n.s.’ indicate not significant

Fig.S4. Kubelka–Munk color depth K/S at 520 nm versus R6G uptake for reference and *DcLPMO9A*-treated fabrics. Dashed lines are linear fits.



Equation 3. Kubelka–Munk equation at the reflectance minimum of R6G (520 nm)

$$K/S_{520} = \frac{(1 - R_{520})^2}{2R_{520}}$$

Table S2. R6G dye dosage, uptake, and Kubelka–Munk color depth at 520 nm for reference and *DcLPMO9A*-treated fabrics.

R6G conc. (% w/w)	R6G uptake (nmol) reference	K/S₅₂₀ reference	R6G uptake (nmol) <i>DcLPMO9A</i>	K/S₅₂₀ <i>DcLPMO9A</i>
0.25%	1.28	0.093	1.82	0.143
0.5%	1.62	0.133	2.58	0.222
1.0%	1.99	0.186	2.83	0.293
2.5%	3.75	0.438	4.52	0.542
5.0%	8.69	0.898	10.98	0.967

Linear fit:

Reference fabrics: $K/S = -0.026 + 0.1086 (R6G \text{ uptake}) (R^2 = 0.990)$

DcLPMO9A fabrics: $K/S = 0.035 + 0.0878 (R6G \text{ uptake}) (R^2 = 0.961)$

References:

Tuveng TR, Jensen MS, Fredriksen L, Vaaje-Kolstad G, Eijsink VGH, Forsberg Z (2020) A thermostable bacterial lytic polysaccharide monooxygenase with high operational stability in a wide temperature range. *Biotechnol Biofuels* **13** (1):194. <https://doi.org/10.1186/s13068-020-01834-5>