Supplementary Information

Naphthols improve lignin separation and induce photoluminescent structural changes during glycolic acid/pentanol biphasic pretreatment of poplar

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1. Methods description

1.1 Cellulase hydrolysis of poplar solid residue

Enzymatic hydrolysis of poplar solid residue was performed in 50 mM sodium citrate buffer (pH 4.8) at 50 °C with continuous shaking at 150 rpm. The substrate loading was set at 5% (w/w) solids in a total working volume of 20 mL. Commercial cellulase was added at dosages of 5-20 FPU g^{-1} dry solid. The hydrolysis reaction was conducted for up to 72 h. Following the reaction, the mixture was heat-inactivated and then centrifuged to collect the supernatant containing the released sugars.

1.2 Monosaccharides and by-products analysis

Aqueous samples were centrifuged at 8,000 ×g for 10 min and subsequently filtered through 0.22 μm syringe filters prior to analysis. Monosaccharides (xylose, glucose, arabinose) and by-products (formic acid, acetic acid, 5-hydroxymethylfurfural, furfural) were quantified by high-performance liquid chromatography (Agilent 1200) equipped with a refractive index detector and an Aminex HPX-87H column (300 × 7.8 mm, Bio-Rad). The column was maintained at 45 °C with 5 mM H_2SO_4 as the mobile phase at a flow rate of 0.6 mL min^{-1} [21]. Quantification was performed using external calibration curves established with authentic standards.

1.3 Lignin isolation and pentanol recovery

Lignin was recovered from the organic phase following biphasic pretreatment. The pentanol-rich phase was evaporated under reduced pressure at 50–55 °C using a rotary evaporator, with the evaporated pentanol being condensed and collected for recycling. The resulting precipitate was washed repeatedly with deionized water until neutral pH was achieved, followed by freeze-drying to obtain the isolated lignin. The recovered lignin samples were subsequently subjected to chemical composition

analysis to assess their purity.

1.4 FTIR analysis and semi-quantitative index calculation

Fourier-transform infrared (FTIR) spectra of lignin samples were recorded on a FTIR spectrometer equipped with an attenuated total reflectance (ATR) accessory. Dried lignin powders were gently ground and pressed onto the ATR crystal, and spectra were collected in the range of 4000–600 cm^{-1} with a spectral resolution of 4 cm^{-1} by co-adding 32 scans. Background spectra of the clean crystal were recorded before each measurement and automatically subtracted. All samples were equilibrated at room temperature and measured under identical instrumental settings to enable semi-quantitative comparison.

The aromaticity index was defined as the integrated absorbance of the aromatic skeletal region (1608–1425 cm^{-1}), normalized to the O–H stretching band (3600–3200 cm^{-1}). The hydrophobicity index was calculated as the integrated absorbance of the aliphatic C–H region (3000–2800 cm^{-1}), normalized to the O–H band. The carbonyl conjugation index was derived by deconvoluting the baseline-corrected carbonyl region (1800–1600 cm^{-1}) into two components: non-conjugated C=O groups centered at 1703 cm^{-1} and conjugated/carboxylate C=O groups at 1662 cm^{-1}. The conjugation index was expressed as the ratio of the integrated area of conjugated/carboxylate C=O to the total carbonyl absorbance. All spectra were baseline-corrected and area-normalized using OriginPro, and peak deconvolution was performed using Gaussian fitting.

1.5 Elemental analysis of lignin samples

The contents of carbon (C), hydrogen (H), and nitrogen (N) in the lignin samples were determined using an elemental analyzer (e.g., Vario EL cube, Elementar, Germany) under a helium carrier gas flow. Approximately 2.0–3.0 mg of dried and

75 homogenized lignin powder was accurately weighed and combusted in an oxygen-rich
76 environment at 1150–1200 °C. The resulting gases (CO_2, H_2O, N_2) were separated and
77 detected via thermal conductivity detector (TCD), and the respective percentages of C,
78 H, and N were calculated. Oxygen content (O) was determined by difference.
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2. Figures and tables

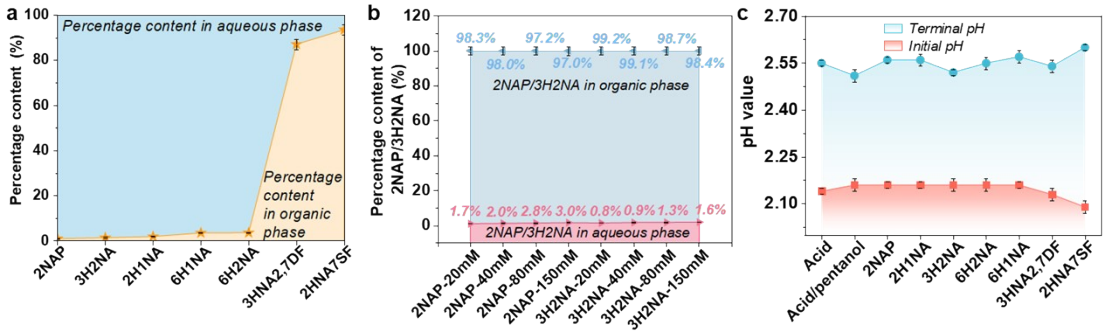


Fig. S1 (a) Distribution of naphthol derivatives between the aqueous and organic phases. (b) Effect of concentration on the aqueous/organic distribution of 2NAP and 3H2NA. (c) pH of the aqueous phase before and after acid/pentanol pretreatment of poplar with 20 mM naphthol derivatives at 180 °C for 30 min.

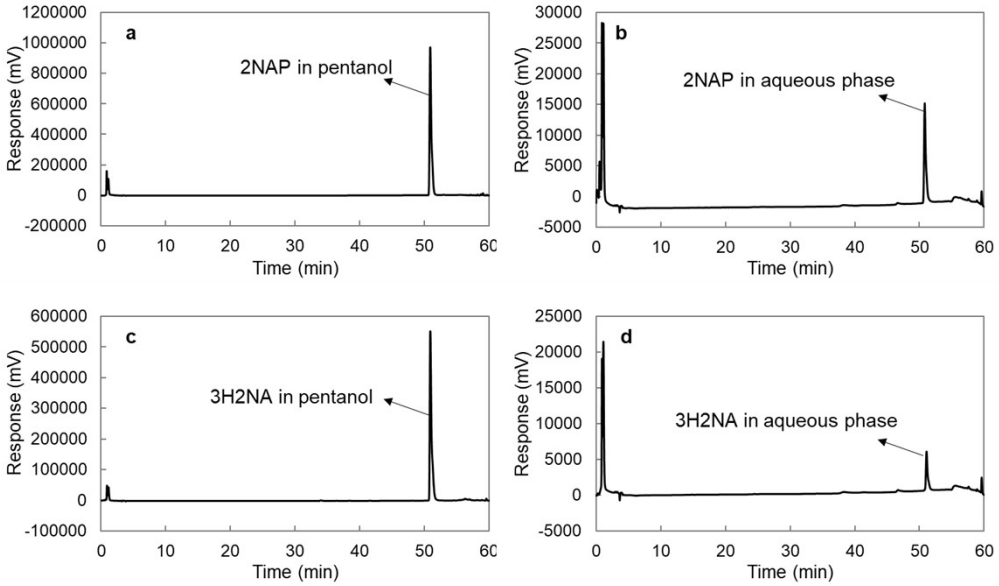
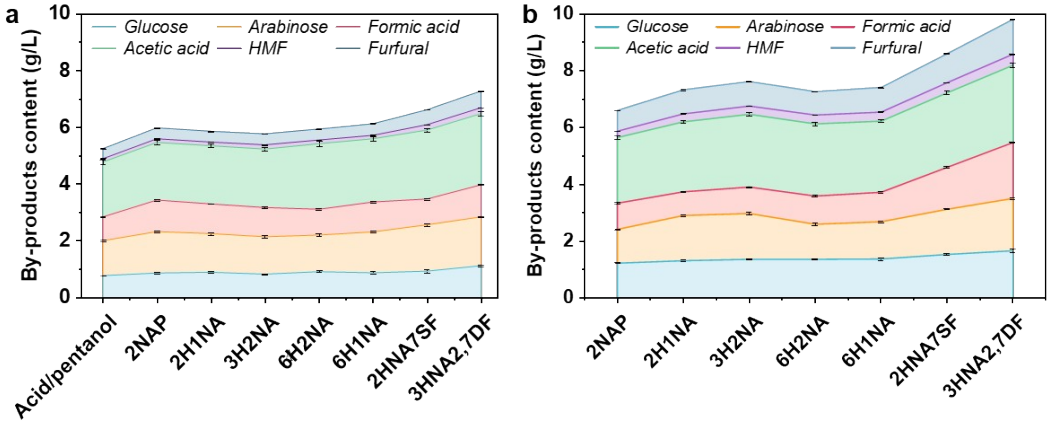


Fig. S2 Distribution of 20 mM 2NAP (a, b) and 3H2NA (c, d) in the organic (pentanol) and aqueous phases.



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92 Fig. S3 Glycolic acid/pentanol pretreatment of poplar at 180 °C for 30 min with 20

93 mM naphthol derivatives. By-products content in aqueous phase at 20 mM (a) and 40

94 mM (b).

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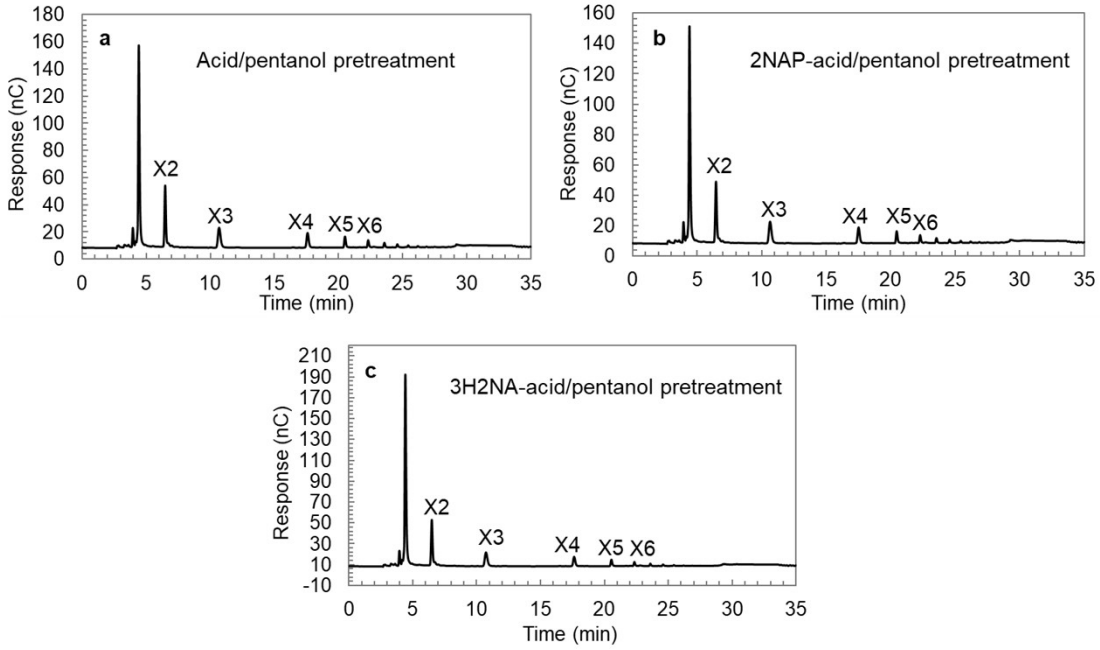

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97 Fig. S4 HPAEC spectra of XOS obtained from poplar by acid/pentanol pretreatment

98 without (a) and with 20 mM 2NAP (b) /3H2NA (c) at 180 °C for 40 min.

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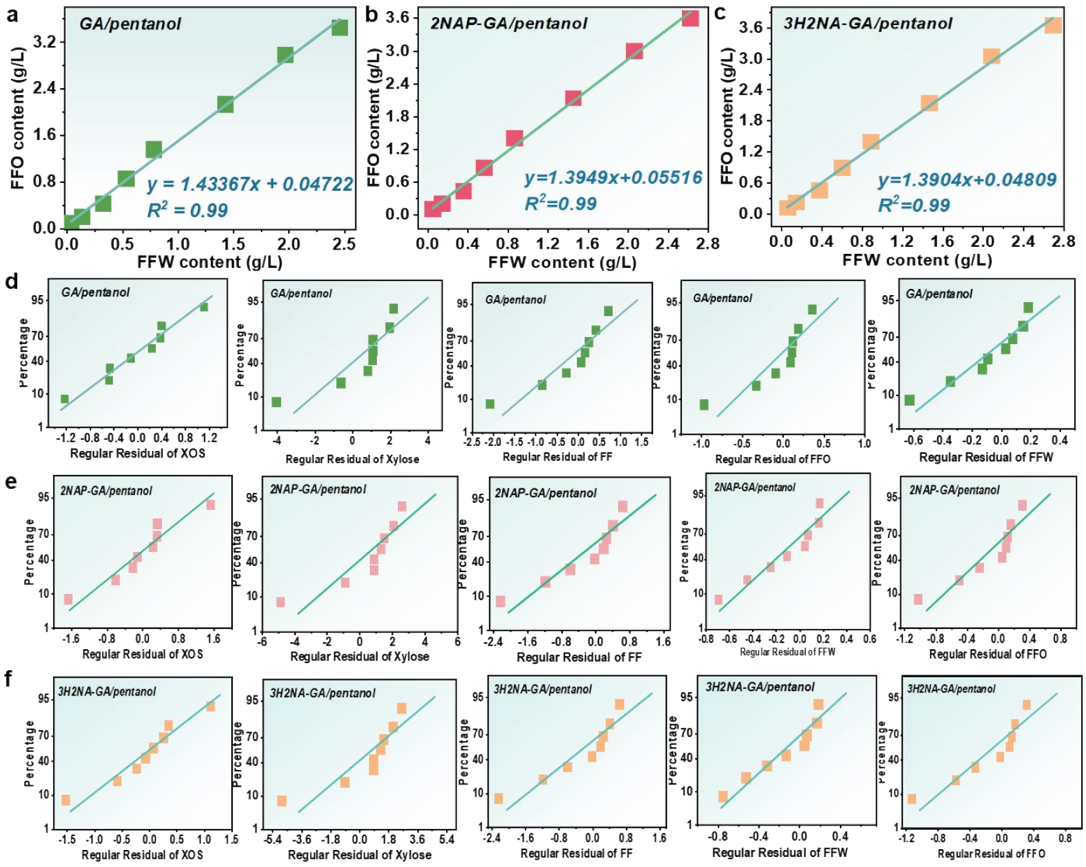
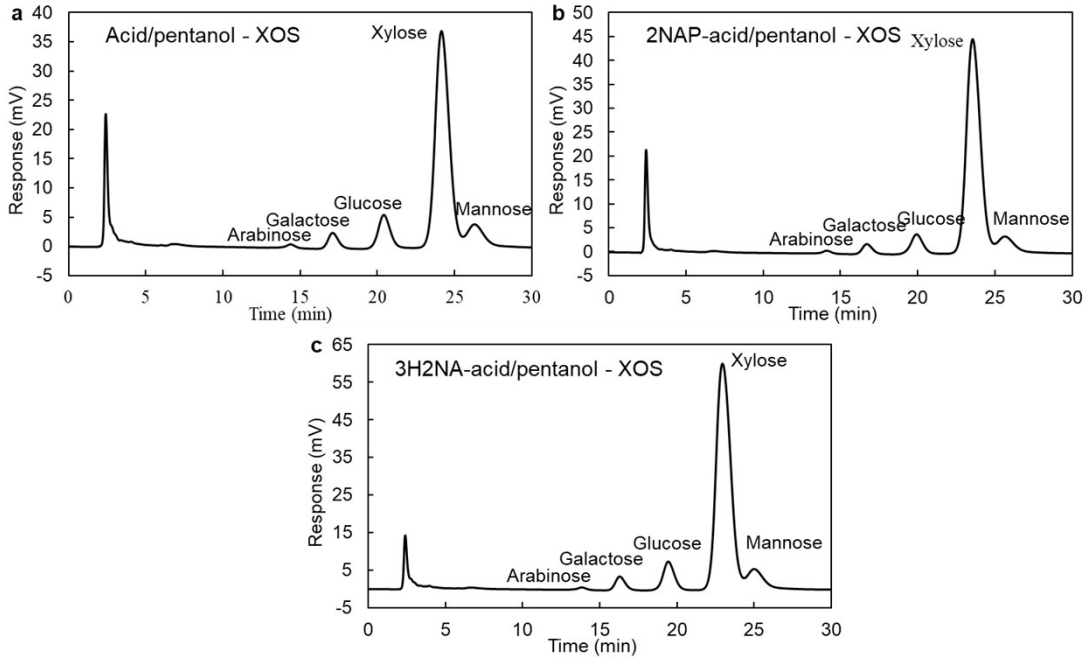


Fig. S5 Fitting of the phase partition coefficient for GA/pentanol (a), 2NAP-GA/pentanol (b), 3H2NA-GA/pentanol (c) pretreatments. Normal probability plot of residuals from the kinetic fitting of hemicellulose degradation for GA/pentanol (d), 2NAP-GA/pentanol (e), 3H2NA-GA/pentanol (f) pretreatments.



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107 Fig. S6 HPAEC spectrum of monosaccharide composition in XOS obtained via

108 acid/pentanol pretreatment of poplar: untreated (a) and treated with 20 mM 2NAP

109 (b)/3H2NA (c) at 180 °C for 40 min.

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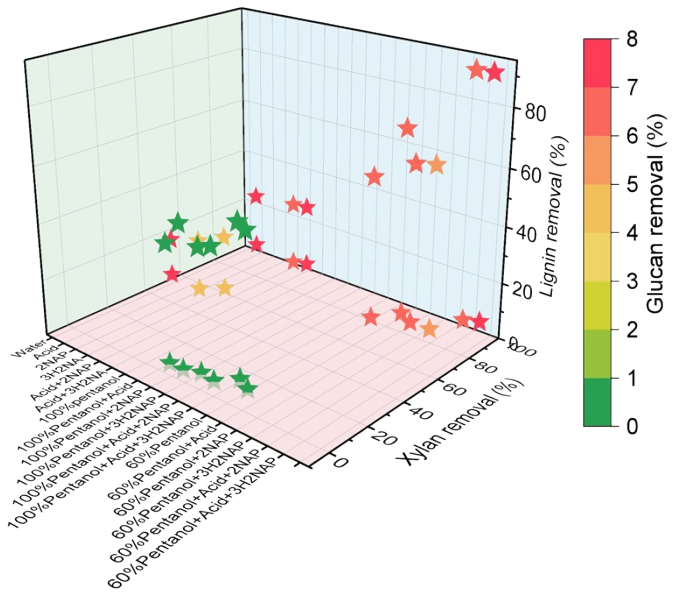

111 Fig. S7 Solvation effects in naphthol-assisted acid/pentanol biphasic pretreatment at

112 180 °C for 30 min.

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Table S1 Semi-quantitative FTIR analysis of residual lignin from naphthol-assisted pretreatment of poplar: relative band intensities for the aromaticity, hydrophobicity, and conjugation indices.

	Aromaticity index	Hydrophobicity index	Conjugation index
Poplar-MWL	0.48±0.02	0.47±0.03	0.24±0.01
Pentanol-MWL	0.52±0.05	0.35±0.02	0.30±0.02
2NAP-MWL	0.55±0.06	0.28±0.02	0.39±0.02
3H2NA-MWL	0.59±0.03	0.28±0.04	0.40±0.02

119 Table S2 Qualitative comparison of solvent polarity and viscosity.

Solvent	Protic/aprotic	Polarity	Viscosity
Dimethyl sulfoxide (DMSO)	Aprotic	✦✦✦✦	++
Acetonitrile (ACN)	Aprotic	✦✦✦	+
Tetrahydrofuran (THF)	Aprotic	✦✦	+
Toluene (PhMe)	Aprotic	✦	+
Methanol (MeOH)	Protic	✦✦✦	+
Ethylene glycol (EG)	Protic	✦✦✦	++++

Polarity: more ✦ = higher polarity;
Viscosity: more + = higher viscosity

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