

Supplementary Information

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Bioconversion of an untapped lignin monomer into a new pyridine-dicarboxylic acid building-block

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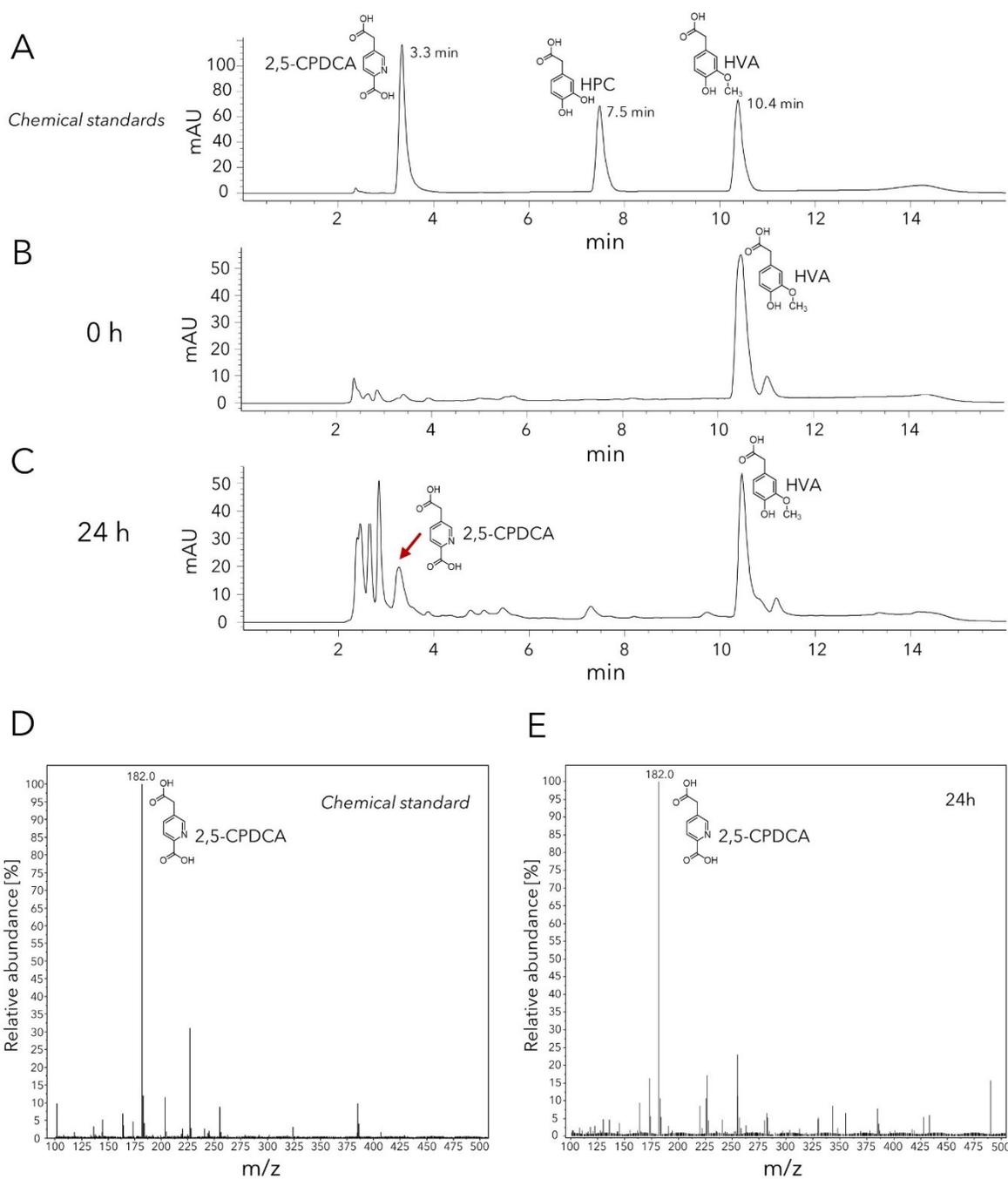
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Supplementary Figures

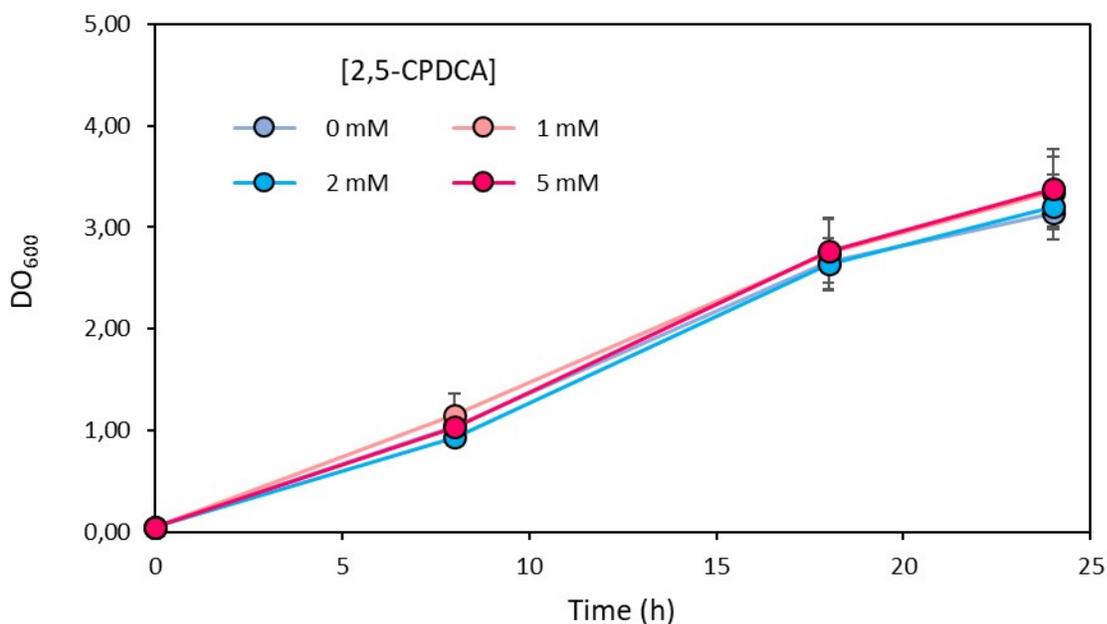


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8 **Figure S1.** HPLC analysis of the conversion of HVA into 2,5-CPDCA. (A) HPLC
9 chromatogram of chemical standards. (B, C) HPLC chromatograms of a *P. putida* KT2440

1 recombinant strain harboring pIZ2-hpaD and pSEVA224-hmv plasmids in resting cell
2 conditions after 0h (B) and 24h (C) incubation with 1mM HVA. (D, E) Mass spectra of 2,5-
3 CPDCA standard (D) and 2,5-CPDCA produced after 24h resting cells conditions (E).

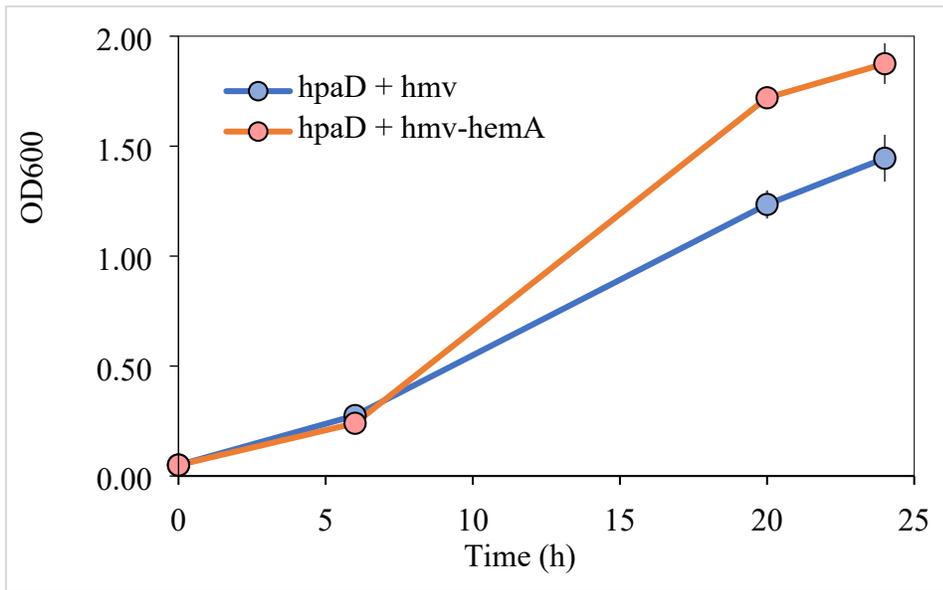
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Figure S2. Growth of *P. putida* KT2440 in the presence of increasing concentrations of 2,5-CPDCA.

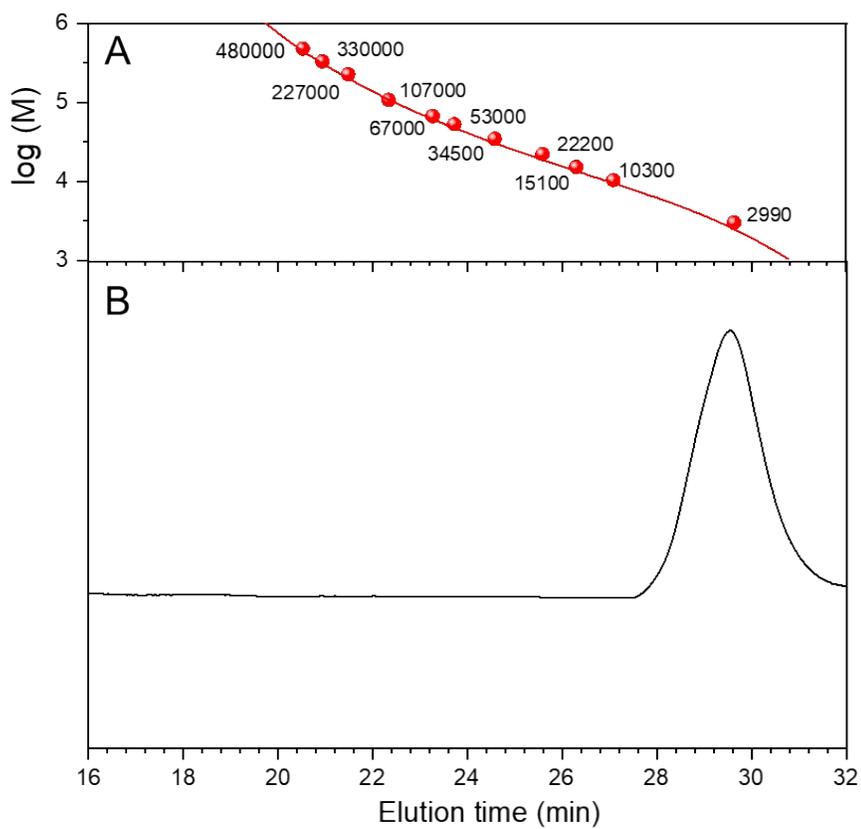
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Figure S3. Recombinant *P. putida* KT2440 strains harboring the pIZ2-hpaD and pSEVA224-hmv (blue line) or pSEVA224-hmv-hemA (expressing the *hemA* gene) (orange line) plasmids after growing for 24 h in M63 minimal media supplemented with FeSO_4 1 mM. Error bars represent standard deviation of the duplicates.

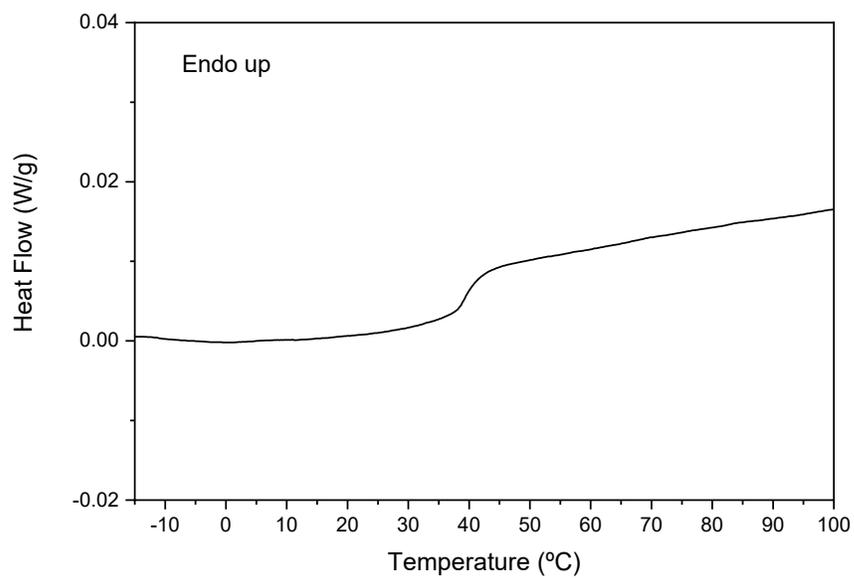
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Figure S4. SEC curve of the synthesized PBCP polymer (B). Calibration curve with PMMA standards (A).

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6 **Figure S5.** DSC thermogram of the synthesized PBCP polymer.

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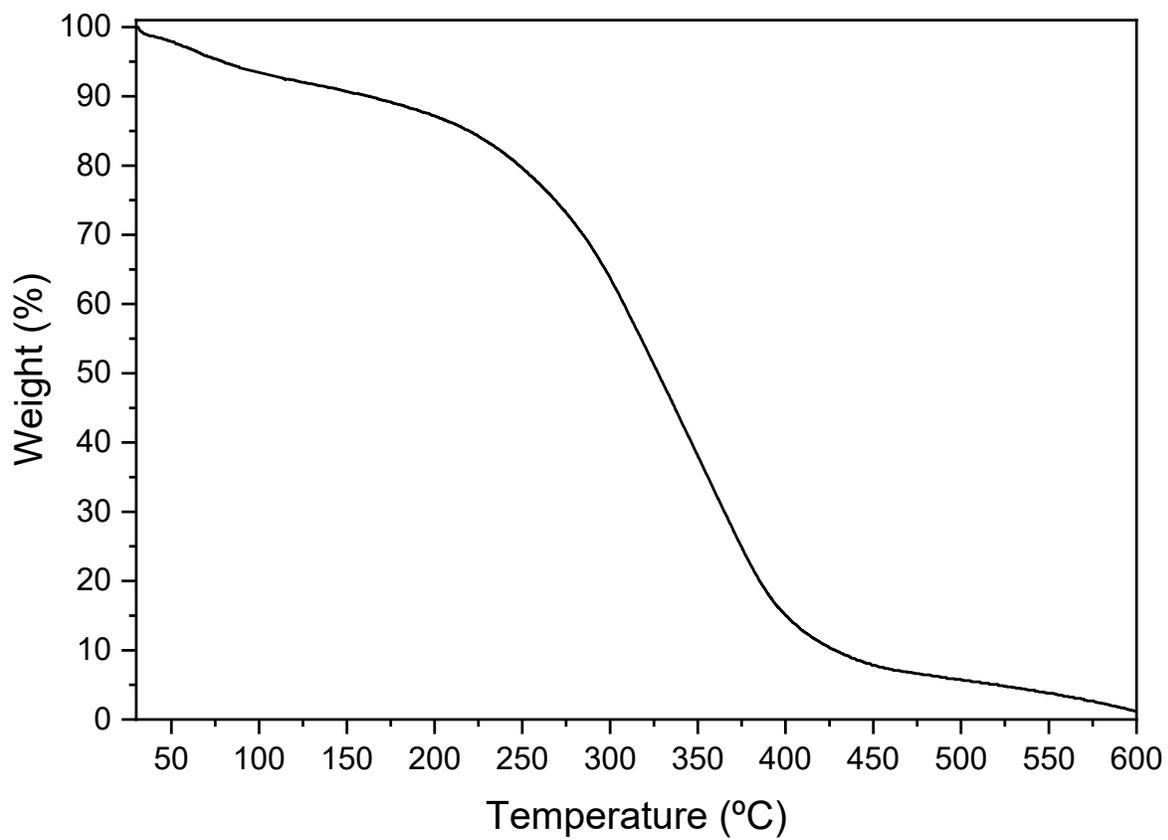
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Figure S6. TGA of synthesized PBCP polymer.

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Supplementary Tables

Table S1. Codon-optimized genes list

Gene	Nucleotide sequences of codon optimized genes
<i>hpaD</i>	<p>ATGGGAAAGTTAGCGTTAGCAGCAAAAACCACTCACGTACCGTCGATGTATCTTTC TGAAGTGCCGGGTA AAAATCACGGCTGTCGTCAGGGAGCCATCGACGGGCACAAG GAAATCGGCAAGCGTTGCCGCGAACTCGGCGTCGATACCTTCATCGTCTTTGATAC CCACTGGCTGGTCAACAGCGCTTACCACATTA ACTGCGCCGACCATTTCCAGGGC GTCTATAACCAGCAACGAGCTGCCGCATTTTATCCGCGACATGACCTACGACTACGA CGGTAACCCGGAGCTGGGCCACCTGATCGCCGACGAAGCGGTCAAGCTCGGCGT CCGCGCTAAAGCGCACAACATCCCGAGCCTTAAGCTGGAGTACGGCACGCTGGTG CCGATGCGCTACATGAACGCCGATAAGCACTTCAAAGTGGTGTGATCTCCGCCTT CTGTACCGTTCACGACTTCGCCGACAGCCGCAAGCTGGGCGAAGCCATTTCGAAA GCTATCGAGAAATATGACGGCACCGTAGCGGTTCTGGCCAGCGGCTCGCTCTCAC ATCGCTTTATCGATGACCAGCGCGCAGAAGAAGGCATGAACAGCTATACCCGCGA GTTTCGATCATCAGATGGATGAGCGTGTGCTGAAGCTGTGGCGCGAGGGTAAATTC AAAGAGTTCTGCACCATGCTGCCGGAGTACGCCGACTACTGCTACGGCGAAGGCA ATATGCACGACACGGTGATGCTGCTGGGCCCTGCTGGGCTGGGATAAATACGACGG CAAAGTGGAAATTTATCACCGAACTGTTCCGCCAGCTCCGGCACCGGCCAGGTCAAC GCCGTGTTCCCGCTGCCGAAACGGCTTAA</p>
<i>hmvA</i>	<p>ATGACCGCAGGATTACTTGAAGCACCCACCGCAGATTGGGTACATCTAGCAGATCT ATACCAGAACCCATTTCCGATTTTCGAGCGACTTCGCTCGGAGTCACCTGTGCGCCT GGGTTCCCGAGGCTGGGCGCTATCTGATCACCAGCTATAGCGGGGTTCTGGCCG CCGATGTGGACCAAACCAGTTCGCGCAATGAAAAGAAATCTCTCATGCTACGT GCCATGGGGCATTCCATGCTTCGAAAGGATGACCCTGATCACCAGGTGGAACGTC GTGCTTGGCAACCGTCCCTGAAACCAGGGACGGTAAAAAGGTATGGAAGCAAAA GTTTGCTGAGAACGCTGACCGTTATTTGGACGCTTACATCGACGCAGGGTCAGGC TCAGACTTCATGCAGGGTTTCGCCGCTCCTTCGTCGCCGAAAATCTCCGAGCCCT CATAGGTTTCGAGAACGCTTCCGAGGCAGATTTGCAACGTTGGTCTCAGACTCTCA TCGATGGCGCAGGCAATTACCCCGATGACCCTGACGTTTGGGCGAAAAGCCAAACA GTCCTCTGACGAGATCGACGCAGCGCTCGAGGAGATGATCCAGTGGCACTCCGGA CGACCTGAGACTCACTCTTTTCATACCTTCTCCGTAGCGCTGACTACCAGATGCC TCTGGAGTCCATCCGTTTCGAACATAAAGATGACGATCGGGGGAGGACTTAACGAA CCGCGAGACGTTTTGGGAGTCTCAACGCTTGCCCTCCTGAGTTCGTCCAAACAATT GGAACTCGTCTTGCGAGATCCCAAAGTGTGGGGAGCGGTATTTGAGGAGTCCATC CGTTGGGTGGCGCCATTGGAATGGTGCCTCGCCAGACAGTCGTAGACACGGAAC TGGATGGATATTTATCCCTCGTGGAGCCAAACTTGGGCTTTGCATTTTGTGCGGC AACCGTGATCGTAGCGTTTGGTCAGATCCCGACCGTTTCGACATCGAGCGCGGTT CTGAGGCTCACCTCGCTTTTCGGCAAGGGTGTGCACGTTTGCCTGGGAGCCTGGGC CGCCGTTCCCAAGTAGCGGACGTGGGTCTTCCGGCACTTTTCTCCCGCTTGAAG GGTTTACGTCTCGACCCGAATCAGGAAGCTACACATGGGGGATGGGTCTTTTCGTG GCCCGTTGTCGTTGCCTTTAGTTTGGGACAAAGCAGTTCGTTCCGAACTCGCATGA</p>
<i>hmvB</i>	<p>ATGGGAGACCATGTGTCAGTGCCTCAGATAGCCGTCGTCGGCGCCGGTCCATCCG GATGCTACGCGGCACTTGCGATTTCAAAGAAGTTTCCCGGAGCCGAAATTGTGATT TTTGATCAGCTCCCTGTGCCTTTTCGGGCTTATTGTTACGGAGTGGCAGCGGACCA TCAGGGAACGAAGGCTGTTACCGCGCAGTTTGAACGAATGTTCTCCTCGGGCACA GCCCGCTTCGTGGGGGCATCCAAATTGGAGGAAGTCTCGAACTGGAAGACATTTC TTGATGCCTTCGATGTCGTCATCTGGGCTGTTGGTCTTGCTCGTGATCGTAACCTT GGGATTCCGGGCGAGGACACCCTGGTGTATATGGCTCAGGTGTCGTGAGCCGT</p>

CTTTGGAATGCACATCCGGGAACGACGGGCGCCCAAGTACGTATAGGGCGTCGTG
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CCGCACGCTCAGCTAGCCGGCTCCGATCTCGATGAAACTTTTCGACGAATCGGAAC
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CCGTAATAAATTCGTGACATTTCTCCATTGACTCACTACTCACCAGTACACGTAG
CGCCCTAACATGA

hmvC

ATGAAAGTATCAATCCTCTACGGAACCTGAGAGCGGCAATGCCGAACCTCGTTGCCGA
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CGCCAGCGAAGTCGCTGTTGCCTGGGCAGCGGGCGTCTTGCCAATTCTTACGAA
ACAACACCCGCCTAA

hemA

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GTGGAACCGCCCCGATGGCGGCAAGCAGGACATCACCGTCTGGTGCGGCAACGA
CTATCTGGGCATGGGCCAGCACCCGGTCTGTGCTGGCCGCGATGCATGAGGCGCT
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CCTATCACCGCCGTCTGGAAGCCGAGATCGCCGATCTGCACGGCAAGGAAGCGG
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TGCGCTGAATCGCGCCGAGGCCTCGGCCTGA

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Table S2. Quantified monomeric compounds in depolymerized alkaline lignin

Compound	Concentration (mg/L)	Molecular Weight (g/mol)	Concentration (mM)
Vanillin	386.6	152.15	2.54
Acetovanillone	201.65	166.17	1.21
Guaiacol	132.76	124.14	1.07
Homovanillic acid (HVA)	120.24	182.18	0.66
Vanillic acid	85.58	168.14	0.51
Other trace compounds (e.g., syringol, phenol, cresol, <i>m</i> - xylene)	-	-	-

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