

*Supplementary Information*

**Lignin Valorization for Protocatechuic Acid Production in Engineered**

***Saccharomyces cerevisiae***

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Table S1 Constructed plasmids used in the present study.

Plasmids	Description	Source
pRS415	pRS415 Single copy plasmid with LEU2 and Amp <sup>R</sup> marker	This lab
pRS416	pRS416 Single copy plasmid with URA3 and Amp <sup>R</sup> marker	This Lab
pRS413	pRS413 Single copy plasmid with HIS3 and Amp <sup>R</sup> marker	This Lab
pRS415- <i>SsfcS-Ssech</i>	pRS415 $P_{TP11}$ - <i>SsfcS</i> - $T_{GPM1}$ - $P_{FBA1}$ - <i>Ssech</i> - $T_{PG11}$	This study
pRS415- <i>Ppfcs-Ppech</i>	pRS415 $P_{TP11}$ - <i>Ppfcs</i> - $T_{GPM1}$ - $P_{FBA1}$ - <i>Ppech</i> - $T_{PG11}$	This study
pRS415- <i>4CL-Ppech</i>	pRS415 $P_{TP11}$ - <i>4CL</i> - $T_{GPM1}$ - $P_{FBA1}$ - <i>Ppech</i> - $T_{PG11}$	This study
pRS415- <i>vdh</i>	pRS415 $P_{TP11}$ - <i>4CL</i> - $T_{GPM1}$ - $P_{FBA1}$ - <i>Ppech</i> - $T_{PG11}$ - $P_{ENO2}$ - <i>vdh</i> - $T_{ENO2}$	This study
pRS415- <i>pobA</i>	pRS415 $P_{TP11}$ - <i>4CL</i> - $T_{GPM1}$ - $P_{FBA1}$ - <i>Ppech</i> - $T_{PG11}$ - $P_{ENO2}$ - <i>vdh</i> - $T_{ENO2}$ - $P_{PGK1}$ - <i>pobA</i> - $T_{HXT7}$	This study
pRS413- <i>pobA</i>	pRS413 $T_{ENO2}$ - $P_{PGK1}$ - <i>pobA</i> - $T_{HXT7}$	This study
pRS415- <i>PdvanAB</i>	pRS415 $T_{HXT7}$ - $P_{TEF1}$ - <i>PdvanA</i> - $T_{CYC1}$ - $P_{TDH3}$ - <i>PdvanB</i> - $T_{TDH3}$	This study
pRS415- <i>RjvanAB</i>	pRS415 $T_{HXT7}$ - $P_{TEF1}$ - <i>RjvanA</i> - $T_{CYC1}$ - $P_{TDH3}$ - <i>RjvanB</i> - $T_{TDH3}$	This study
pRS415- <i>ligM</i>	pRS415 $T_{HXT7}$ - $P_{TEF1}$ - <i>ligM</i> - $T_{CYC1}$	This study
pRS413- <i>Met6</i>	pRS413 $T_{PG11}$ - $P_{GPM1}$ - <i>MET6</i> - $T_{TEF1}$	This study
pRS416- <i>Ssmetf1</i>	pRS416 $T_{TDH3}$ - $P_{ACT1}$ - <i>Ssmetf1</i> - $T_{ADH1}$	This study
pRS416- <i>Rdmetf1</i>	pRS416 $T_{TDH3}$ - $P_{ACT1}$ - <i>Rdmetf1</i> - $T_{ADH1}$	This study

Table S2 Engineered *S. cerevisiae* strains used in the present study.

<i>S. cerevisiae</i> strains	Description	Source
BY4742	MAT $\alpha$ his3 $\Delta$ 1 leu2 $\Delta$ 0 lys2 $\Delta$ 0 ura3 $\Delta$ 0	This lab
yZR1	BY4742, $\Delta$ ADH6, $\Delta$ ADH7, $\Delta$ BDH2, $\Delta$ FDC	This study
yZR2	BY4742, $\Delta$ ADH6, $\Delta$ ADH7, $\Delta$ BDH2, $\Delta$ FDC, $\Delta$ MHT1	This study
yZR3	BY4742, $\Delta$ ADH6, $\Delta$ ADH7, $\Delta$ BDH2, $\Delta$ FDC, $\Delta$ MHT1, $\Delta$ SAM4	This study
yZR4	yZR1, pRS415	This study
yPHNA1	yZR1, pRS415- <i>Ssfcs-Ssech</i>	This study
yPHNA2	yZR1, pRS415- <i>PpfcS-Ppech</i>	This study
yPHNA3	yZR1, pRS415- <i>4CL-Ppech</i>	This study
yPHCA1	yZR1, pRS415- <i>vdh</i>	This study
yPCA1	yZR1, pRS415- <i>pobA</i> , pRS413	This study
yPCA2	yZR1, pRS415- <i>pobA</i> , pRS413- <i>PobA</i>	This study
yPCA3	yZR1, pRS415- <i>PpvanAB</i>	This study
yPCA4	yZR1, pRS415- <i>RjvanAB</i>	This study
yPCA5	yZR1, pRS415- <i>ligM</i> , pRS413	This study
yPCA6	yZR1, pRS415- <i>ligM</i> , pRS413- <i>MET6</i>	This study
yPCA7	yPCA5, $\Delta$ MHT1	This study
yPCA8	yPCA6, $\Delta$ SAM4	This study
yPCA9	yZR3, pRS415- <i>ligM</i> , pRS413- <i>MET6</i> , pRS416- <i>Ssmetf1</i>	This study
yPCA10	yZR3, pRS415- <i>ligM</i> , pRS413- <i>MET6</i> , pRS416- <i>Rdmetf1</i>	This study
yPCA11	yPCA10, <i>HO::P<sub>TPH1</sub>-4CL-T<sub>GPM1</sub>-P<sub>FBA1</sub>-P<sub>pech</sub>-T<sub>PGH1</sub>-P<sub>ENO2</sub>-vdh-T<sub>ENO2</sub>-P<sub>PGK1</sub>-pobA-T<sub>HXT7</sub>-P<sub>TEF1</sub>-P<sub>dvanA</sub>-T<sub>CYC1</sub>-P<sub>TDH3</sub>-P<sub>dvanB</sub>-T<sub>TDH3</sub></i>	This study
yPCA12	yZR3, <i>HO::P<sub>TPH1</sub>-4CL-T<sub>GPM1</sub>-P<sub>FBA1</sub>-P<sub>pech</sub>-T<sub>PGH1</sub>-P<sub>ENO2</sub>-vdh-T<sub>ENO2</sub>-P<sub>PGK1</sub>-pobA-T<sub>HXT7</sub>-P<sub>TEF1</sub>-P<sub>dvanA</sub>-T<sub>CYC1</sub>-P<sub>TDH3</sub>-P<sub>dvanB</sub>-T<sub>TDH3</sub>, YPRCdelta15::P<sub>PGK1</sub>-pobA-T<sub>HXT7</sub>-P<sub>TEF1</sub>-<i>ligM</i>-T<sub>CYC1</sub>-P<sub>GPM1</sub>-<i>MET6</i>-T<sub>TEF1</sub>-P<sub>ACT1</sub>-<i>Rdmetf1</i>-T<sub>ADH1</sub></i>	This study

Table S3 Different concentrations of APL media were used in this study.

APL Concentration (X)	0.2	0.5	0.8
Initial APL (mL)	4	10	16
10X YPD (mL)	2	2	2
Seed culture (mL)	~2	~2	~2
ddH <sub>2</sub> O (mL)	~12	~6	~0
Total volume (mL)	20	20	20

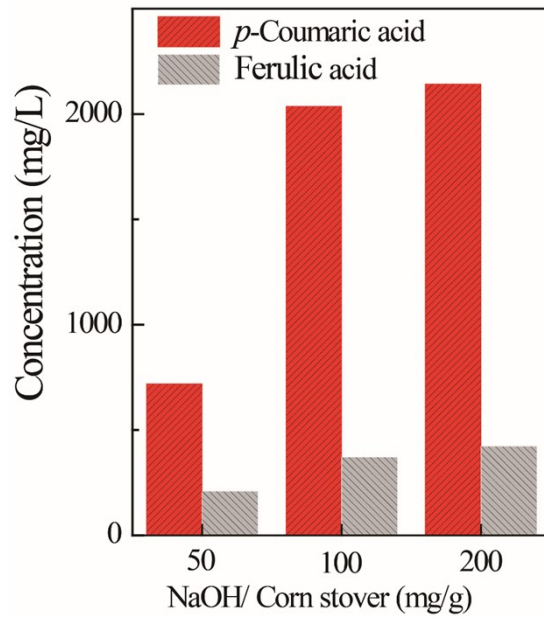


Figure S1 The concentration of *p*-coumaric acid and ferulic acid in alkaline pretreated liquor (APL) produced from different conditions of alkaline pretreatment.