

Support Information

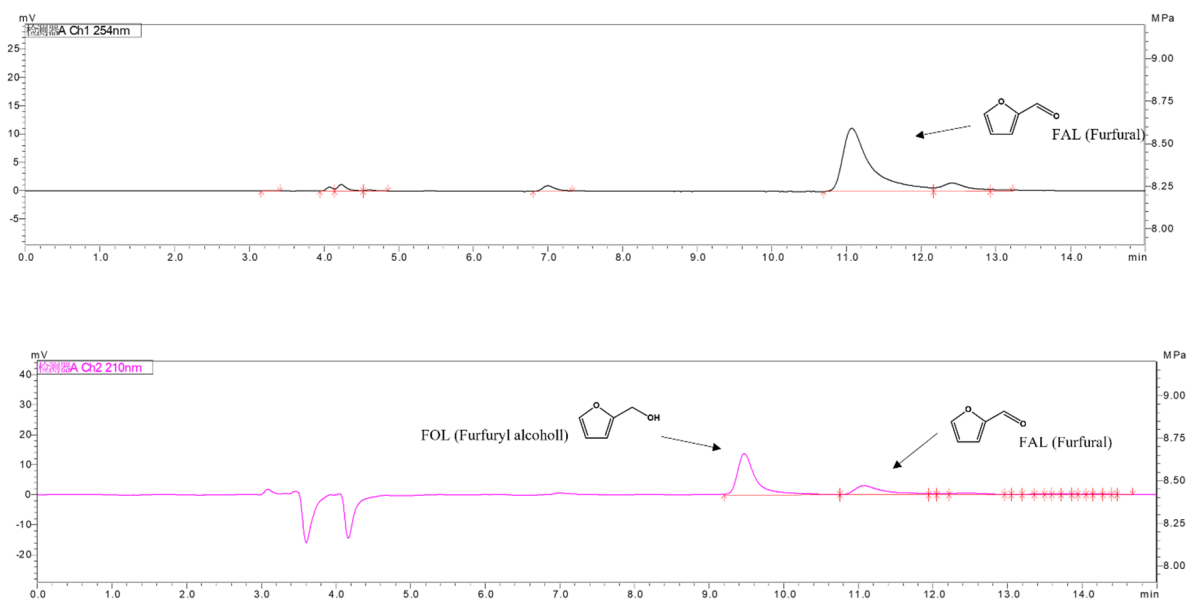


Figure S1. Representative HPLC chromatogram of the bioreduction of FAL by EutG–GDH whole cells under the indicated reaction conditions (40 °C, pH 7.5, glucose:FAL molar ratio = 2:1). Peaks corresponding to FAL and FOL were identified by comparison with authentic standards.

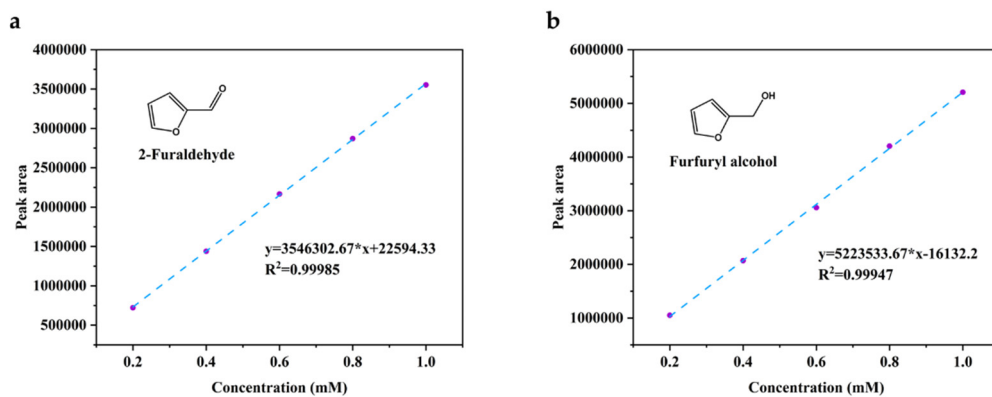


Figure S2. Calibration curves for the HPLC quantification of furfural (FAL) and furfuryl alcohol (FOL). (a) Calibration curve of FAL (2-furaldehyde) over the concentration range of 0.2–1.0 mM; (b) calibration curve of FOL over the concentration range of 0.2–1.0 mM. Peak area was plotted against analyte concentration, and linear regression equations and coefficients of determination (R^2) are shown in the corresponding panels.

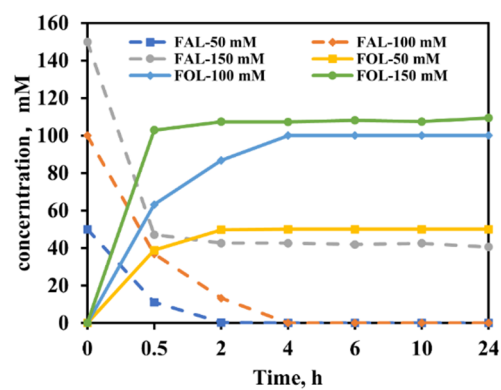


Figure S3. Time courses for the bioreduction of commercial FAL at different initial concentrations (50–150 mM) by EutG-expressing *E. coli* whole cells under the same reaction conditions used for comparison with the EutG–GDH system.

Table S1. The nucleotide sequences of EutG and GDH encoding genes.

Name	Sequence
EutG	ATGCAGAATGAACTGCAGACCGCCCTGTTTCAGGCATTTGATACCCTGAATCTGCAGCGTG TTAAACCTTTAGTGTTCCGCCGGTGACCCTGTGCGGTCCGGGTTCA GTGAGTAGTTGCGG CCAGCAGGCCCAGACCCGTGGTCTGAAACATCTGTTTGTGATGGCAGATAGTTTCTGCAT CAGGCCGGCATGACCGCAGGCCTGACCCGTAGCCTGACCGTGAAAGGCATTGCAATGACC CTGTGGCCGTGCCCCGGTTGGTGAACCGTGCATTACCGATGTGTGTGCCGCCGTTGCACAGC TGCGTGAAAGTGGCTGTGATGGCGTGATTGCATTTGGCGGCGGCAGCGTTCTGGATGCCGC CAAAGCAGTTACCCTGCTGGTGACCAATCCGGATAGTACCCTGGCAGAAATGAGCGAAAC CAGCGTGCTGCAGCCGCGCCTGCCGTTAATTGCAATTCCGACCACCGCAGGCACCGGTAGT GAAACCACCAATGTGACCGTTATTATTGATGCCGTTAGTGGTCGCAAACAGGTGCTGGCAC ATGCAAGTCTGATGCCGGATGTTGCAATTCTGGATGCAGCACTGACCGAAGGTGTGCCGAG TCATGTGACCGCCATGACCGGTATTGATGCCCTGACCCATGCAATTGAAGCCTATAGCGCAC TGAATGCAACCCCGTTTACCGATAGTCTGGCCATTGGCGCCATTGCAATGATTGGTAAAAGC CTGCCGAAAGCAGTTGGTTATGGTCATGATCTGGCCGCACGCGAAAGCATGCTGCTGGCCA GTTGCATGGCAGGCATGGCATTTAGCAGCGCCGGCCTGGGCCTGTGTCATGCCATGGCACA TCAGCCGGGCGCCGCACTGCATATTCCGCATGGTCTGGCCAATGCAATGCTGCTGCCGACC GTGATGGAATTTAATCGTATGGTGTGTCGGAACGTTTTAGCCAGATTGGTCGCGCCCTGCG CACCAAAAAAAGTGATGATCGTGATGCCATTAATGCCGTTAGTGAACTGATTGCAGAAGTG GGCATTGGCAAACGCCTGGGTGATGTTGGCGCAACCAGTGCACATTATGGTGCATGGGCAC AGGCCGCCCTGGAAGATATTTGTCTGCGTAGCAATCCGCGTACCGCAAGTCTGGAACAGAT TGTTGGTCTGTATGCAGCCGCACAGTAA

GDH ATGTATAAAGATTTAGAAAGGAAAAGTAGTTGTCATAACAGGTCATCTACCGGTTTAGGAA

AAGCAATGGCGATTTCGTTTTGCGACAGAAAAAGCTAAAGTAGTTGTGAACTATCGTTCGAA

AGAAGAAGAAGCTAACAGCGTTTTAGAAAGAAATTAAAAAAGTGGGCGGAGAGGCTATTGC

CGTCAAAGGTGATGTAACAGTTGAGTCTGATGTGATCAATTTAGTTCAATCTGCTATTAAAG

AATTTGGAAAGCTAGACGTTATGATTAATAACGCAGGAATGGAAAATCCGGTTTCGTCTCAT

GAAATGTCTTTAAGTGATTGGAATAAAGTCATTGATACGAACTTAACGGGAGCATTTTTAGG

CAGCCGTGAAGCGATTAAATATTTTGTGGAAAATGATATTAAGGGAACAGTTATTAACATGT

CGAGTGTTACACGAGAAAATTCCTTGGCCATTATTTGTTTCATTACGCAGCAAGTAAAGGCGG

AATGAAGCTCATGACCGAAACACTTGCATTAGAATACGCTCCAAAAGGTATTCGTGTAAAT

AACATTGGACCGGGAGCGATTAATACACCGATTAACGCTGAGAAATTTGCTGATCCTGAGC

AGCGTGCAGATGTAGAAAGCATGATTCCAATGGGATACATTGGAGAGCCGGAAGAAATTGC

AGCGGTTGCTGCATGGCTAGCTTCTTCAGAGGCAAGTTATGTAACAGGGATTACACTCTTT

GCTGACGGCGGTATGACACAGTACCCATCATTCCAAGCAGGACGCGGATAA

Table S2. Primer sequence of EutG and GDH.

Primer sequence	
EutG-F	GCCATCACCATCATCACCACATGCAGAATGAACTGCAGACCGC
EutG-R	GATATATCTCCTTAGGTACCTTACTGTGCGGCTGCATACAG
GDH-F	GGTACCTAAGGAGATATATCATGTATAAAGATTTAGAAGGAAAAGTAGTTGTC
GDH-R	CTTAAGCATTATGCGGCCGCTTATCCGCGTCCTGCTTGG
Duet-F	GCGGCCGCATAATGCTTAAG
Duet-R	GTGGTGATGATGGTGATGGCTG

Table S3. Comparison of the present sequential *D*-xylose (or biomass)-to-furfuryl alcohol process with representative previously reported chemo-biocatalytic systems.

Ref.	DES composition / catalytic medium	Feedstock / xylose loading	FAL yield / titer	FOL yield / titer	Dilution before bioreduction	Reaction (dehydration bioreduction)	time /	Catalyst / whole-cell loading
[10]	ChCl:Gly-H ₂ O (7.5 vol% DES); HCOOH 3.0 wt%	<i>D</i> -Xylose, 60.0 g/L	58.3% / ~233 mM	89.5% yield / < 50 mMv	Yes / pH adjustment and medium adaptation required	30 min / 12 h		HCOOH, 3.0 wt%; recombinant <i>E. coli</i> CF (FDH-CgCR), 0.050 g wet cells/mL
[20]	CA:Betaine-H ₂ O (10:90, wt/wt)	Corncob, 60 g/L	48.2% based on xylan / 74.6 mM	95.8% yield / < 60 mM	No additional FAL isolation reported	30 min / 2 h		CA:Betaine, 10 wt%; recombinant <i>E. coli</i> SF21
[31]	BE:BA-H ₂ O, BE:BA = 1:2 (mol/mol), 2.5 wt%	<i>D</i> -Xylose, 30 g/L	51.1% / 95.7 mM	99.0% yield / < 95.7 mM	pH adjustment; 95.7 mM liquor used directly; two-fold dilution also examined	30 min / 3 h		BE:BA, 2.5 wt%; recombinant <i>E. coli</i> DCF, 0.10 g wet cells/mL
[33]	LA:Bet-H ₂ O; LA:Bet 10 vol% for dehydration	<i>D</i> -xylose, 0.75 g/50 mL	81.6% / 81.6 mM	95.8% yield; overall xylose-to-FOL yield 78.2%; ~ 68 mM FOL	Yes; pH adjusted to 7.0 and FAL diluted from 81.6 to 71.35 mM	30 min / 24 h		LA:Bet, 10 wt%; recombinant <i>E. coli</i> HMFOMUT, 2.859 g wet cells; glucose 0.8 mol/mol FAL
[46]	No DES; aqueous system for dehydration; MIBK-H ₂ O biphasic system for bioreduction	Corncob, 75.0 g/L	40.9% / 72.1 mM	100% yield; FOL productivity 0.304 g/g xylan; ~ 72 mM	Not mentioned	30 min / 2 h		UST-Sn-RH, 1.5 wt%; recombinant <i>E. coli</i> KPADH; optimized bioreduction conditions: 0.10 g wet cells/mL and glucose/FAL = 2.5 mol/mol

This study	Bet:FA:MA–H ₂ O, 15 wt%	<i>D</i> -Xylose, 22.5 g/L (150 mM)	65.8% / 98.7 mM	Essentially complete conversion / ~30.0 mM FOL	Yes; adjusted and diluted 98.7 to 30.0 mM	pH 7.5 FAL from 30.0	30 min / 24 h	Bet:FA:MA, 15 wt%; recombinant <i>E. coli</i> EutG–GDH, 0.10 g wet cells/mL; glucose 2 mol/mol FAL
------------	------------------------------------	-------------------------------------	-----------------	--	---	----------------------	---------------	--
