Supplementary Information

Heterologous Lignan Production in Stirred-Tank Reactors—Metabolomics-Assisted Bioprocess Development for an In Vivo Enzyme Cascade

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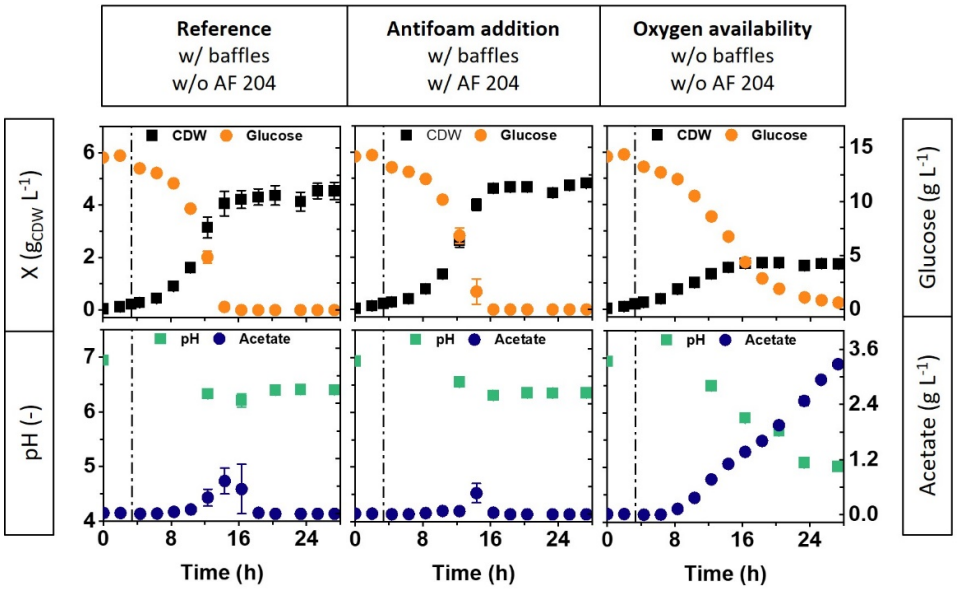

Figure S1. Influence of antifoam addition and oxygen availability on growth parameters in shake flasks. *Escherichia coli* (*E. coli*) C43(DE3) pCDFDuet_syfiPLR was cultivated at 37 °C, 180 rpm, in modified Riesenberg medium (15 g L^{-1} glucose). At an optical density at 600 nm (OD_{600}) of 0.6, 0.75 mM isopropyl-β-D-thiogalactopyranoside (IPTG) were added for induction. 50 µM $CuSO_4$ and 0.5 g L^{-1} coniferyl alcohol were added at the same time (indicated by pointed-dashed line). After induction, cultivation temperature was decreased to 30 °C. Cultivations were ended 24 h after induction. Cultivations were performed in baffled (w/ baffles) or unbaffled shake flasks (w/o baffles). For some shake flask cultivations, 0.01% (v/v) antifoam 204 (w/ AF204) was added.

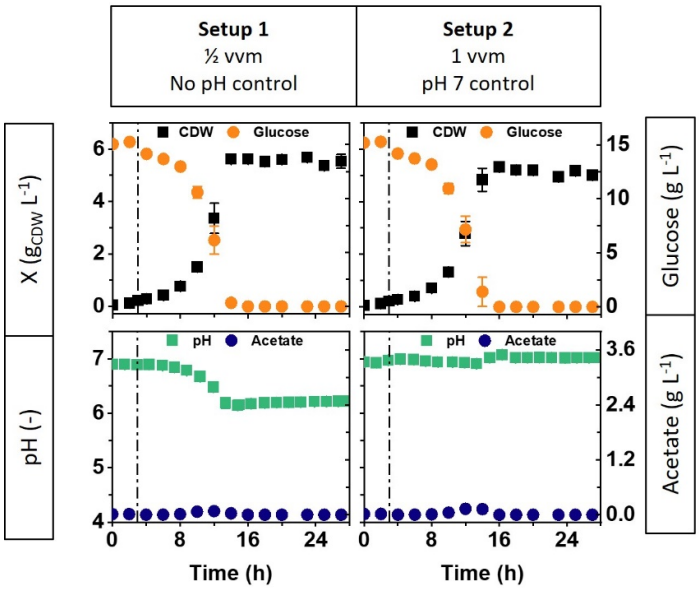

Figure S2. Effect of various STR process conditions on growth parameters. *E. coli* C43(DE3) pCDFDuet_syfiPLR was cultivated at 37 °C, 1000 rpm, in modified Riesenberg medium (15 g L^{-1} glucose, 0.01% (v/v) AF204). At OD_{600} 0.6, 0.75 mM IPTG, 0.5 g L^{-1} coniferyl alcohol and 50 µM $CuSO_4$ were added (indicated by pointed-dashed line). After induction, cultivation temperature was decreased to 30 °C. Cultivations were ended 24 h after induction. Bioreactor cultivations were aerated with 6 L h^{-1} (0.5 volume gas per volume liquid per minute, vvm) or 12 L h^{-1} (1 vvm) compressed air. pH of cultivations was either not regulated or maintained at pH 7.0.

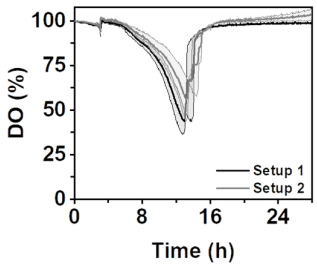

Figure S3. DO during testing of various STR process conditions. E. coli C43(DE3) pCDFDuet_syfiPLR was cultivated at 37 °C, 1000 rpm in modified Riesenberg medium (15 g L^{-1} glucose, 0.01% AF204). At OD_{600} 0.6, 0.75 mM IPTG, 0.5 g L^{-1} coniferyl alcohol and 50 µM $CuSO_4$ were added. After induction, cultivation temperature was decreased to 30 °C. Cultivations were ended 24 h after induction. For Setup 1, 6 L h^{-1} (0.5 vvm) compressed air and no pH regulation were used. Setup 2 was run with 12 L h^{-1} (1 vvm) compressed air and regulated at pH 7.

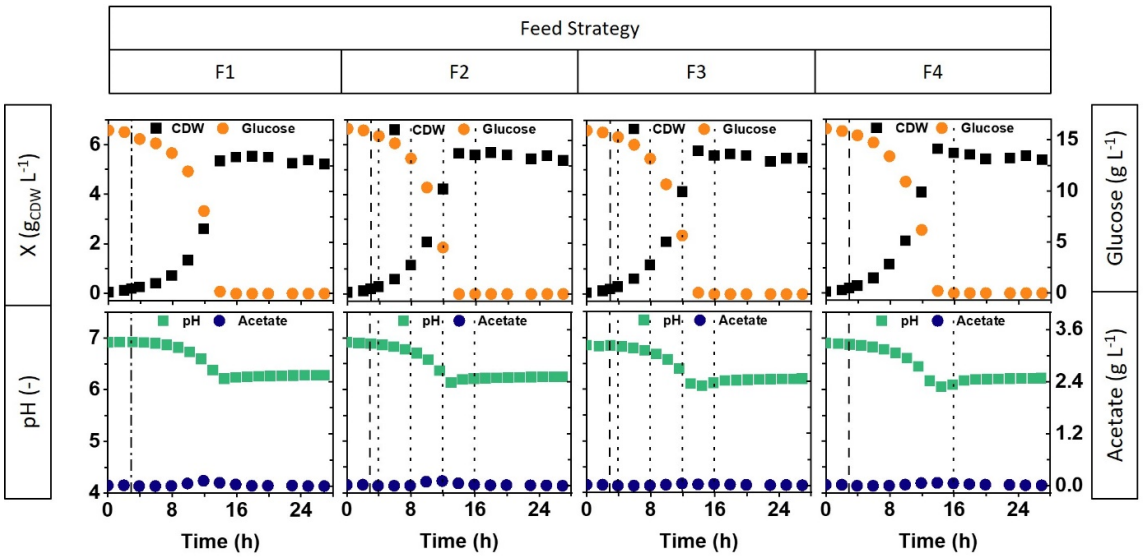

Figure S4. Growth parameters for comparison of substrate feeding strategies during STR cultivation. *E. coli* C43(DE3) pCDFDuet_syfiPLR was cultivated at 37 °C, 1000 rpm in modified Riesenberg medium (15 g L^{-1} glucose, 0.01% AF204). At OD_{600} 0.6, 0.75 mM IPTG and 50 µM $CuSO_4$ were added (pointed-dashed or dashed line). After induction, cultivation temperature was decreased to 30 °C. For F1, 0.5 g L^{-1} coniferyl alcohol was added at the time of induction (pointed-dashed line). Regarding F2 and F3, the amount of substrate corresponding to a final concentration of 0.5 g L^{-1} coniferyl alcohol was split and supplemented at 1, 5, 9, and 13 h after induction (pointed lines). For F2, substrate was added in a linear pulsed feed of 25% (corresponding to 125 mg L^{-1}) or for F3 in an exponential pulsed feed profile with 1.25% (6.25 mg L^{-1}), 5% (25 mg L^{-1}), 18.75% (93.75 mg L^{-1}) and 75% (375 mg L^{-1}). For F4, 0.5 g L^{-1} coniferyl alcohol was added at 13 h after induction (pointed line). Cultivations were ended 24 h after induction.