Supplementary material

Integrated process development for grass biomass utilization through enzymatic saccharification and upgrading hydroxycinnamic acids via microbial funneling

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Supplemental table captions

Table S1. Comparative yields and ratios of aromatic compounds in the grass biomass extracts obtained by mild alkaline pretreatment under the optimal conditions (bamboo, 80°C, 4% NaOH; rice straw, 60°C, 4% NaOH). The yields were calculated on the basis of Klason lignin (bamboo, 22.8% w/w; rice straw, 14.84% w/w).

Table S2. Comparative titers and molar yields of the fed-batch production from aromatic compounds and biomass derivatives. [a] Maximum value from reference. [b] Inferred from reference. [c] Qualitative only. [d] A yield higher than 100 may be the result of conversion of non-detected aromatic compounds or the soluble lignin-derived oligomers.

Table S3. Comparative pretreatment methods and glucose yields in bamboo, rice straw, and other grass biomass. [a] Maximum value from reference (respect to cellulose w/w%). [b] Respect to dry biomass w/w%. [c] Abbreviation of total titratable alkali.

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	Bamboo extract	Rice straw extract
Yield (w/w)		
CA	9.1%	4.7%
FA	0.6%	2.8%
OAs	0.4%	0.5%
Ratio (w/w)		
CA : FA : OAs	90 : 6 : 4	59 : 35 : 6

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Bacterium/relevant characteristic	Feedstock	Product	Titer (g/L)[a]	Molar yield (%)[a]	Reference
	Aromatic compound				
P. putida KT2440-CJ251/Δ*pcaHG*::Ptac:*ligABC*	HBA	PDC	58.0	80.7	(Johnson et al., 2019)
P. putida KT2440/Δ*pcaHG*::Δ*crc*::Ptac:*ligABC*	CA	PDC	22.7	100.0[b]	(Lee et al., 2022)
P. putida PpY1100/Plac:*ligABC*	PCA	PDC	11.5[b]	-[c]	(Otsuka et al., 2006)
Novosphingobium aromaticivorans DSM12444 /Δ*ligI*::Δ*desCD*	VL and VA	PDC	4.9	-	(Perez et al., 2019)
PpY1100-dHG/pJF-X	CA	PDC	10.1	98.3	This study
PpY1100-dHG/pJF-X	FA	PDC	8.0	96.2	This study
P. putida PpY1100/Plac:*pcaHG*:*CMLE*	PCA	4*S*-3CML	-[c]	-[c]	(Kondo et al., 2016)
PpY1100/pJF-HB	CA	4*S*-3CML	10.5	99.3	This study
PpY1100/pJF-HB	FA	4*S*-3CML	8.2	97.7	This study
	Biomass derivative				
P. putida PpY1100/Plac:van*AB*:*pobA*:*ligV*:*ligABC*	Depolymerized lignosulfonate	PDC	9.3	-[c]	(Suzuki et al., 2020)
P. putida PpY1100/Δ*pcaHG*::Plac:*bzaA*:*desZ*:*ferBA* ::Plac:van*AB*:*pobA*:*ligV*:*ligABC*	Depolymerized cedar lignin	PDC	0.69[b]	156.0[d]	(Qian et al., 2016)
P. putida PpY1100/Δ*pcaHG*::Plac:*bzaA*:*desZ*:*ferBA* ::Plac:van*AB*:*pobA*:*ligV*:*ligABC*	Depolymerized birch lignin	PDC	0.52[b]	130.1[d]	(Qian et al., 2016)
PpY1100-dHG/pJF-X	Bamboo extract	PDC	8.7	93.6	This study
PpY1100-dHG/pJF-X	Rice straw extract	PDC	3.9	92.4	This study
PpY1100/pJF-HB	Bamboo extract	4*S*-3CML	9.3	99.1	This study
PpY1100/pJF-HB	Rice straw extract	4*S*-3CML	4.0	94.6	This study

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Pretreatment	Plant species	Reaction condition	Effect on lignocellulosic biomass	Glucose yield (%)[a]	Reference
Mild alkaline pretreatment	Rice straw	4% NaOH, 60°C, 2 h	HCAs extraction	73.8	This study
Mild alkaline pretreatment	Rice straw	1% NaOH, 50°C, 2 h	Lignin and hemicellulose extraction	70.0	(Li et al., 2018)
Mild alkaline pretreatment	Bamboo	4% NaOH, 80°C, 2 h	HCAs extraction	29.5	This study
Steam explosion/ green liquor pretreatment	Bamboo	213.3°C, 5 min/ 31.01%[b] TTA[c], 166.41°C, 28.01 min	Lignin and hemicellulose extraction	100.0	(Gao et al., 2021)
Green liquor pretreatment	Miscanthus	32.77%[b] TTA[c], 150.00°C, 23.18 min	Lignin and hemicellulose extraction	86.6	(Alam et al., 2020)