[Supplementary information]

Engineering of *Corynebacterium glutamicum* for Consolidated Conversion of Hemicellulosic Biomass into Xylonic Acid

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Figure S1. Secretory production of endoxylanase and xylosidase in the supernatant. Supernatant of the culture was 30-fold concentrated by acetone precipitation method and analyzed through (A) SDS-PAGE and (B) Western blot. Lane 1 (Culture supernatant of *C. glutamicum* harboring pCES208), Lane 2 (pUXE), Lane 3 (pUXED). Black arrow indicates endoxylanase (XlnA, 48 kDa), and White arrow indicates xylosidase (XynB, 62 kDa). Cterminal His-tag on endoxylanase and C-terminal FLAG-tag on xylosidase were used for detection with anti-His antibody-horseradish peroxidase (HRP) conjugate and anti-FLAG M2 antibody-HRP conjugate (Sigma-Aldrich) together.



Figure S2. Optimization of xylose dehydrogenase (*xdh*) expression with different synthetic promoters. (A) SDS-PAGE analysis of *xdh* gene expression under three different strength promoters. Lanes 1 to 4: *C. glutamicum* harboring pCES208, pUX-L10, pUX-I16, and pUX, respectively; Lanes T and S: total and soluble protein fractions, respectively. An arrowhead indicates the band of Xdh. (B, C) Time profiles of (B) cell density (optical density (OD) at 600 nm) and (C) xylose and xylonic acid concentration (g/L) in flask cultivation. Triangles, squares, and circles indicate pUX-L10, pUX-I16, and pUX, respectively. (In C) Open and closed symbols represent xylose and xylonic acid, respectively. Results are the mean of duplicate experiments and error bars indicate standard deviations.



Figure S3. Optimization of *xylE* gene expression with different synthetic promoters. Time profiles of (A) cell density (OD at 600 nm) and (B) xylose and xylonic acid concentration in flask cultivation. Circles, squares, and triangles represent pUXE, pUXE-I12, and pUXE-H72, respectively. (In B) Open and closed symbols represent xylose and xylonic acid, respectively. Results are the mean of duplicate experiments and error bars indicate standard deviations.

Table S1. pH of the culture at the beginning and end of the cultivation. All are average values of duplicate experiments.

Plasmid	pH at 0 h	pH at 48 h
nCES208	6.9	74
pelb200	0.9	7.7
рХ	6.91	4.0
nUX	6.92	3 72
port	0.72	5.12
pUXC	6.91	3.89
nUXE	6.92	3 65
pont	0.72	5.05

Cell cultures for conversion of xylose to xylonic acid (set in Fig. 4)

Cell cultures for conversion of xylan to xylonic acid (set in Fig. 5)

Plasmid	pH at 0 h	pH at 48 h
pCES208	6.51	6.51
pUXE	6.61	7.28
pUXED	6.60	5.00