Supplementary material to Synthetic Biology and Engineering

Nitrogen-controlled valorization of xylose-derived compounds by metabolically engineered *C. glutamicum*

Table S1 List of oligonucleotides used in this work.

Name	Sequence (5'-3')	Purpose
gfp_{UV}-sg-fw	GCCAAGCTTGCATGCCTGCACATCTAATTC AACAAGAATT	Generation of sgRNA for gfp_{UV} knockdown
gfp_{UV}-sg-rv	GCTATTTCTAGCTCTAAAACAATTCTTGTT GAATTAGATG	Generation of sgRNA for gfp_{UV} knockdown
P_{amtA}-fw	CGTCTCATTTTCGCCAGATATCGACGTGAT TTCGGCAAAGTGGTGGTC	Amplification of the 314 bp promoter region of *amtA* from genomic DNA of *C. glutamicum*
P_{amtA}-rv	GAGTATTTCTTATCCATAGATCCTTTCTCCT CTTTAGATCTTTTGAATTCTTTTCGTTAAAG GCCTTTCAGCTAAGTGAT	Amplification of the 314 bp promoter region of *amtA* from genomic DNA of *C. glutamicum*
P_{amtB}-fw	CGTCTCATTTTCGCCAGATATCGACGTCCG ATAAGCGAGAACCACCTG	Amplification of the 284 bp promoter region of *amtB* from genomic DNA of *C. glutamicum*
P_{amtB}-rv	GAGTATTTCTTATCCATAGATCCTTTCTCCT CTTTAGATCTTTTGAATTCTTTTCAGCGTG GATGACCTCCTTTGAC	Amplification of the 284 bp promoter region of *amtB* from genomic DNA of *C. glutamicum*
$xylB_{Cc.}$-fw	CATGGAATTCGAGCTCGGTACCCGGGACT CTAAATAAAAGGAGGTAAGAGTATGTCCT CAGCCATCTATCCCAG	Amplification of $xylB_{Cc.}$ from pEKEx3_$xylXABCD_{C.c.}$
$xylB_{Cc.}$-rv	GCTTGCATGCCTGCAGGTCGACTCTAGAG TCAACGCCAGCCGGCGTC	Amplification of $xylB_{Cc.}$ from pEKEx3_$xylXABCD_{C.c.}$
pE_$xr_{R.m}$-fw	CATGGAATTCGAGCTCGGTACCCGGGCCC GAAAAGTCGAAAGGAGGTATTTAATG	Amplification of $xr_{R.m.}$ from pMA-RQ_XR_*R-mucilaginosa* for expression plasmids
pE_$xr_{R.m}$-rv	GCTTGCATGCCTGCAGGTCGACTCTAGAG CTACTGGATCTTCACCTGGTACTTTGC	Amplification of $xr_{R.m.}$ from pMA-RQ_XR_*R-mucilaginosa* for expression plasmids
pE_P_{amtA}-fw	CTGTGCGGTATTTCACACCGCAGATTTCGG CAAAGTGGTGGTC	Amplification of $P_{amtA.}$ from genomic DNA of *C. glutamicum* for expression plasmids
pE_P_{amtA}-rv	CCGGGTACCGAGCTCGAATTCCATGGTTA AAGGCCTTTCAGCTAAGTGAT	Amplification of $P_{amtA.}$ from genomic DNA of *C. glutamicum* for expression plasmids
pE_P_{amtB}-fw	CTGTGCGGTATTTCACACCGCACCGATAAG CGAGAACCACCTG	Amplification of $P_{amtB.}$ from genomic DNA of *C. glutamicum* for expression plasmids
pE_P_{amtB}-rv	CCGGGTACCGAGCTCGAATTCCATGAGCG TGGATGACCTCCTTTGAC	Amplification of $P_{amtB.}$ from genomic DNA of *C. glutamicum* for expression plasmids
gfp_{UV}-for-P_{amtA}-fw	GCTGAAAGGCCTTTAACGAAAAGAATTCA AAAGAAAGGAGGCCCTTCAGATGAGTAA AGGAGAAGAACTTTTCACTGG	Amplification of gfp_{UV} for replacement of the dCas9 gene in pS_P_{amtA}-dCas9-gfp_{UV}
gfp_{UV}-for-P_x-rv	GCCTTTCGTTTTATTTGATGCCTGGAGATC CTTACTTATTTGTAGAGCTCATCCATGCCAT GTG	Amplification of gfp_{UV} for replacement of the dCas9 gene in pS_P_{amtA}-dCas9-gfp_{UV}
gfp_{UV}-for-P_{amtB}-fw	GGTCATCCACGCTGAAAAGAATTCAAAAG AAAGGAGGCCCTTCAGATGAGTAAAGGA GAAGAACTTTTCACTGG	Amplification of gfp_{UV} for replacement of the dCas9 gene in pS_P_{amtA}-dCas9-gfp_{UV}

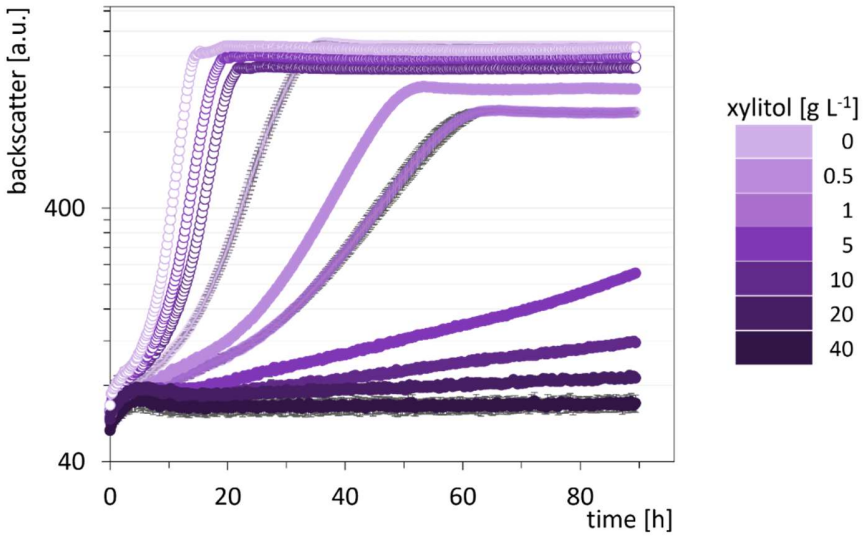

Figure S1 Effect of xylitol on growth of *C. glutamicum* gX with xylose or glucose as carbon source. gX was cultivated in a BioLector microcultivation system in CgXII, supplemented with 40 g L^{-1} xylose (closed symbols) or glucose (open symbols) and 0-40 g L^{-1} xylitol for 96 h. Growth was followed by the backscatter signal, given in arbitrary units (a.u.). Values of means with standard deviations refer to triplicate cultivations.

Table S2 Titers of xylitol or xylonate, produced by gX derived strains with P_{trc}, P_{amtA}, or P_{amtB}. Xylitol and xylonate producer strains gX-P_{trc}-*xr*, gX-P_{amtA}-*xr*, gX-P_{amtB}-*xr*, gX- P_{trc}-*xylB*, gX-P_{amtA}-*xylB*, and gX-P_{amtB}-*xylB* were cultivated with 40 g L^{-1} xylose in shake flasks for 144 h either with 100% nitrogen (CgXII) or with 5% nitrogen (N-CgXII). Strains gX-P_{trc}-*xr*, and gX- P_{trc}-*xylB* were cultivated in CgXII with/without IPTG induction of P_{trc}. Values of means with standard deviations refer to triplicate cultivations.

			time [h]			
		medium	24	48	72	144
xylitol [mM]	gX-P_{trc}-*xr*	CgXII	7 ± 1	16 ± 1	19 ± 1	16 ± 1
		CgXII + IPTG	15 ± 1	18 ± 1	27 ± 1	42 ± 4
	gX-P_{amtA}-*xr*	CgXII	6 ± 1	11 ± 1	13 ± 1	9 ± 1
		N-CgXII	16 ± 1	33 ± 1	37 ± 1	86 ± 5
	gX-P_{amtB}-*xr*	CgXII	6 ± 1	13 ± 1	15 ± 1	13 ± 1
		N-CgXII	16 ± 1	34 ± 2	38 ± 1	83 ± 10
xylonate [mM]	gX-P_{trc}-*xylB*	CgXII	19 ± 4	77 ± 3	97 ± 18	86 ± 4
		CgXII + IPTG	44 ± 3	170 ± 19	185 ± 13	217 ± 7
	gX-P_{amtA}-*xylB*	CgXII	10 ± 1	46 ± 3	51 ± 8	53 ± 2
		N-CgXII	60 ± 3	138 ± 1	173 ± 7	209 ± 7
	gX-P_{amtB}-*xylB*	CgXII	10 ± 1	45 ± 2	57 ± 1	48 ± 4
		N-CgXII	50 ± 1	119 ± 13	160 ± 13	171 ± 9

Table S3 Biomass formation of xylitol and xylonate producer strains with P_{trc}, P_{amtA}, or P_{amtB}. Xylitol and xylonate producer strains gX-P_{trc}-*xr*, gX-P_{amtA}-*xr*, gX-P_{amtB}-*xr*, gX- P_{trc}-*xylB*, gX-P_{amtA}-*xylB*, and gX-P_{amtB}-*xylB* were cultivated with 40 g L^{-1} xylose in shake flasks for 144 h either with 100% nitrogen (CgXII) or with 5% nitrogen (N-CgXII). Strains gX-P_{trc}-*xr*, and gX- P_{trc}-*xylB* were cultivated in CgXII with/without IPTG induction of P_{trc}. Values of means with standard deviations refer to triplicate cultivations.

			time [h]			
		medium	24	48	72	144
Biomass [g L^{-1}]	gX-P_{trc}-*xr*	CgXII	3.9 ± 0.1	8.6 ± 0.3	8.0 ± 0.1	8.4 ± 0.2
		CgXII + IPTG	0.7 ± 0.1	0.1 ± 0.1	0.2 ± 0.1	0.3 ± 0.1
	gX-P_{amtA}-*xr*	CgXII	3.1 ± 0.1	9.5 ± 0.3	9.0 ± 0.2	8.9 ± 0.3
		N-CgXII	2.1 ± 0.7	2.2 ± 0.1	2.1 ± 0.0	2.5 ± 0.2
	gX-P_{amtB}-*xr*	CgXII	3.2 ± 0.1	9.0 ± 0.2	8.7 ± 0.4	9.1 ± 0.9
		N-CgXII	1.9 ± 0.1	2.5 ± 0.2	2.4 ± 0.2	2.6 ± 0.2
	gX-P_{trc}-*xylB*	CgXII	3.3 ± 0.1	8.6 ± 0.2	7.6 ± 0.1	7.9 ± 0.1
		CgXII + IPTG	1.4 ± 0.1	4.0 ± 0.1	3.8 ± 0.1	3.8 ± 0.1
	gX-P_{amtA}-*xylB*	CgXII	3.7 ± 0.1	9.3 ± 0.2	8.7 ± 0.3	9.0 ± 0.4
		N-CgXII	2.3 ± 0.2	2.6 ± 0.1	2.5 ± 0.1	2.6 ± 0.1
	gX-P_{amtB}-*xylB*	CgXII	4.4 ± 0.2	9.6 ± 0.2	8.8 ± 0.1	9.0 ± 0.1
		N-CgXII	2.6 ± 0.4	2.7 ± 0.1	2.5 ± 0.2	2.7 ± 0.2