

# Recombinant protein production in *E. coli* using the phoA expression system

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## Supplementary Material

**Table S1.** Extended strain physiological parameters for pET cultivations.

Cultivation	$\mu$ [h <sup>-1</sup> ]	Temp. [°C]	Induction [h]	DCW [g/L]	$\mu$ real [h <sup>-1</sup> ]	Glucose		Acetate		$Y_{X/S}$ [C-mol/C-mol]	$Y_{CO_2/S}$ [C-mol/C-mol]	C-balance
pET 1	0.1	35	4h	40.1	0.102	1.52	0.04	-	-	0.33	0.47	0.80
			8h	40.3 <sup>+</sup>	0.052	93.1	2.31	0.42	0.01	n.d.*	n.d.*	n.d.*
pET 2	0.1	30	4h	41.3	0.112	0.93	0.02	0.87	0.02	0.36	0.47	0.85
			8h	42.6	0.055	21.7	0.51	-	-	0.25	0.61	0.84
pET 3	0.05	35	4h	33.9	0.049	-	-	0.33	0.01	0.33	0.52	0.85
			8h	36.9	0.042	-	-	-	-	0.27	0.56	0.83
pET 4	0.05	30	4h	36.4	0.059	-	-	0.52	0.01	0.37	0.48	0.85
			8h	40.4	0.046	-	-	0.59	0.01	0.27	0.51	0.78

<sup>+</sup> biomass concentration was calculated from OD<sub>600</sub> due to unreliable values obtained from gravimetical determination resulting from cell lysis

(OD<sub>600</sub>/DCW correlation: DCW = 0.3077\*OD<sub>600</sub>; R<sup>2</sup> = 1)

\* not determined due to increased cell lysis

Abbr.: Y<sub>X/S</sub>, biomass / substrate yield; Y<sub>CO<sub>2</sub>/S</sub>, CO<sub>2</sub> / substrate yield

**Supplementary Table S2.** Extended Fab production data for pET cultivations.

Cult.	$\mu$ [h <sup>-1</sup> ]	Temp. [°C]	Ind. [h]	Fab insoluble (IBs)					Fab soluble					Ratio IB:SP*
				spec. titer [mg/g]	vol. titer [mg/L]	qp [mg/g/h]	rp [mg/L/h]	STY [mg/L/h]	spec. titer [mg/g]	vol. titer [mg/L]	qp [mg/g/h]	rp [mg/L/h]	STY [mg/L/h]	
pET 1	0.1	35	4h	19.5	782.6	5.13	191.8	33.4	2.2	89.0	0.31	10.58	3.8	8.8
			8h	22.2	896.6	0.65	26.13	32.2	1.9	76.5	-0.07	-2.95	2.8	11.8
pET 2	0.1	30	4h	14.9	613.0	4.14	148.7	26.0	2.9	119.5	0.51	18.39	5.1	5.1
			8h	22.1	942.4	1.85	77.70	33.9	2.4	102.9	-0.10	-3.86	3.7	9.1
pET 3	0.05	35	4h	20.4	693.6	5.30	168.2	29.3	2.4	80.6	0.28	9.01	3.4	8.6
			8h	24.6	907.7	1.64	58.80	33.5	2.2	82.0	0.01	0.49	3.0	11.2
pET 4	0.05	30	4h	12.0	436.5	3.14	105.5	18.3	2.8	102.9	0.40	13.32	4.3	4.2
			8h	20.9	841.0	2.67	102.4	30.4	2.5	101.5	-0.01	-0.26	3.7	8.3

\* ratio of insoluble (IB) Fab titer compared to soluble (SP) Fab titer

Abbr.: qp, specific product formation rate; rp, volumetric product formation rate; STY, space-time yield

**Supplementary Table S3.** Extended strain physiological parameters for pAT cultivations.

Cult.	$\mu$ [h <sup>-1</sup> ]	Temp. [°C]	Sample [-]	DCW [g/L]	$\mu$ real [h <sup>-1</sup> ]	qPO <sub>4</sub> [mmol/g/h]	Glucose		Acetate		Y <sub>X/S</sub> [C-mol/C-mol]	Y <sub>CO<sub>2</sub>/S</sub> [C-mol/C-mol]	C-balance [-]
pAT 1	0.1	35	> 1 mM PO <sub>4</sub>	42.8	0.126	0.112	-	-	0.28	0.01	0.45	0.42	0.87
			PO <sub>4</sub> starvation	53.2	0.093	0.008	1.70	0.03	0.99	0.02	0.30	0.49	0.80
pAT 2	0.1	30	> 1 mM PO <sub>4</sub>	47.6	0.121	0.097	-	-	0.46	0.01	0.44	0.52	0.96
			PO <sub>4</sub> starvation	52.0	0.078	0.018	-	-	0.69	0.01	0.27	0.85	1.13
pAT 3	0.05	35	> 1 mM PO <sub>4</sub>	31.8	0.033	0.052	-	-	-	-	0.20	0.65	0.85
			PO <sub>4</sub> starvation	47.1	0.057	0.008	-	-	0.34	0.01	0.32	0.57	0.89
pAT 4	0.05	30	> 1 mM PO <sub>4</sub>	43.0	0.062	0.055	-	-	0.28	0.01	0.47	0.49	0.97
			PO <sub>4</sub> starvation	52.6	0.042	0.008	-	-	0.58	0.01	0.29	0.70	0.99

Abbr.: qPO<sub>4</sub>, specific phosphate uptake rate; Y<sub>X/S</sub>, biomass / substrate yield; Y<sub>CO<sub>2</sub>/S</sub>, CO<sub>2</sub> / substrate yield

**Supplementary Table S4.** Extended Fab production data for pAT cultivations.

Cult.	$\mu$ [h <sup>-1</sup> ]	Temp. [°C]	Sample	Fab insoluble (IBs)					Fab soluble					Ratio IB:SP*
				spec. titer [mg/g]	vol. titer [mg/L]	qp [mg/g/h]	rp [mg/L/h]	STY [mg/L/h]	spec. titer [mg/g]	vol. titer [mg/L]	qp [mg/g/h]	rp [mg/L/h]	STY [mg/L/h]	
pAT 1	0.1	35	> 1 mM PO <sub>4</sub>	-	-	-	-	-	2.28	97.30	0.21	4.36	4.36	-
			PO <sub>4</sub> starvation	-	-	-	-	-	3.21	170.9	0.31	14.6	6.24	-
pAT 2	0.1	30	> 1 mM PO <sub>4</sub>	-	-	-	-	-	2.95	140.4	0.26	6.27	6.27	-
			PO <sub>4</sub> starvation	-	-	-	-	-	2.91	150.1	0.07	3.68	5.98	-
pAT 3	0.05	35	> 1 mM PO <sub>4</sub>	-	-	-	-	-	2.53	80.62	0.11	1.86	1.86	-
			PO <sub>4</sub> starvation	8.16	385.0	0.85	33.2	7.02	2.54	119.5	0.08	3.38	2.18	3.2
pAT 4	0.05	30	> 1 mM PO <sub>4</sub>	-	-	-	-	-	4.63	198.8	0.25	5.37	5.37	-
			PO <sub>4</sub> starvation	7.88	414.2	0.85	40.3	8.76	6.09	321.1	0.25	11.8	6.77	1.3

\* ratio of insoluble (IB) Fab titer compared to soluble (SP) Fab titer

Abbr.: qp, specific product formation rate; rp, volumetric product formation rate; STY, space-time yield

**Supplementary Table S5.** Extracellular DNA contents of pET and pAT cultivations.

Cultivation	$\mu$ [h <sup>-1</sup> ]	Temp. [°C]	Induction [h]	DNA <sup>+</sup> [mg/L]	Cultivation	$\mu$ [h <sup>-1</sup> ]	Temp. [°C]	Sample [-]	DNA <sup>+</sup> [mg/L]
pET 1	0.1	35	4h 8h	1,275 1,811	pAT 1	0.1	35	> 1 mM PO <sub>4</sub> PO <sub>4</sub> starvation	1,159 1,222
pET 2	0.1	30	4h 8h	931 1,190	pAT 2	0.1	30	> 1 mM PO <sub>4</sub> PO <sub>4</sub> starvation	1,125 1,221
pET 3	0.05	35	4h 8h	887 1,040	pAT 3	0.05	35	> 1 mM PO <sub>4</sub> PO <sub>4</sub> starvation	1,716 1,957
pET 4	0.05	30	4h 8h	976 1,215	pAT 4	0.05	30	> 1 mM PO <sub>4</sub> PO <sub>4</sub> starvation	1,067 1,184

<sup>+</sup> Extracellular DNA contents were determined in cell-free culture medium

**Supplementary Table S6.** Extended overview of strain physiological parameters of *E. coli* W3110 harboring the phoA expression system at different extracellular PO<sub>4</sub> concentrations.

process time	DCW	OD <sub>600</sub>	μ	cPO <sub>4</sub>	qPO <sub>4</sub>	Glucose	Acetate	Y <sub>CO<sub>2</sub>/S</sub>	Y <sub>X/S</sub>	C-balance
[h]	[g/L]	[-]	[h <sup>-1</sup> ]	[mM]	[mmol/g/h]	[g/L]	[g/L]	[C-mol/C-mol]	[C-mol/C-mol]	[-]
27.4	29.0	60.4	-	33.5	-	n.d.	n.d.	0.54	0.55	1.09
29.3	30.7	64.8	0.040	27.5	0.090	n.d.	n.d.	0.58	0.38	0.98
31.3	32.0	67.6	0.036	24.3	0.039	n.d.	n.d.	0.59	0.32	0.89
33.3	34.3	68.6	0.049	20.2	0.053	n.d.	n.d.	0.58	0.42	0.99
35.3	37.0	74.4	0.054	14.6	0.070	n.d.	n.d.	0.57	0.45	1.01
37.3	39.0	80.7	0.045	8.7	0.071	n.d.	n.d.	0.56	0.36	0.91
39.3	41.4	83.8	0.049	3.6	0.061	n.d.	n.d.	0.56	0.38	0.93
41.3	45.5	92.7	0.070	1.9	0.018	n.d.	n.d.	0.54	0.54	1.07
43.3	48.7	98.7	0.057	0.13	0.018	n.d.	n.d.	0.56	0.43	0.99
45.0*	48.9	101.5	0.021	< 0.10	0.0003	n.d.	n.d.	0.82	0.19	0.99

n.d. not detectable

\* time point of harvest

Abbr.: DCW, dry cell weight; μ, specific growth rate; cPO<sub>4</sub>, extracellular phosphate concentration; qPO<sub>4</sub>, specific PO<sub>4</sub> uptake rate; Y<sub>CO<sub>2</sub>/S</sub>, CO<sub>2</sub> / substrate yield; Y<sub>X/S</sub>, biomass / substrate yield

**Figure S1.** Time courses of extracellular PO<sub>4</sub> concentration in the culture broth (black circles) and intracellular P content of the *E. coli* W3110 biomass (blue squares) during fed-batch cultivation until PO<sub>4</sub> starvation.

