Additional file 4

These tables provide the individual data points of the measured target protein concentration for the Figures 2, 3 and 4. For the explanation of the different abbreviations please refer to the original Figure legends.

Table 1: Measured protein concentrations during fed-batch cultivations of *Tr*CBH2 variants, optimized by different gene optimization methods; data as represented in Figure 2A.

Time (h)	P(AOX1)-*Tr*CBH2-CP-CN7±1 (g/l)	P(De)-*Tr*CBH2-CP-CN25±7 (g/l)	P(AOX1)-*Tr*CBH2-HM-CN3 (g/l)	P(De)-*Tr*CBH2-HM-CN7±1 (g/l)	P(De)-*Tr*CBH2-HM-CN1 (g/l)	P(GAP)-*Tr*CBH2-HM-CN4 (g/l)
0	0	0,01	0	0	0	0
18	0	0,22	0,05	0,14	0,01	0,35
47	0,18	0,73	0,81	1,20	0,05	1,11
74	0,69	2,42	1,70	2,74	0,35	1,70
90	1,26	3,81	2,78	4,37	0,43	2,43

Table 2: Measured protein concentrations during fed-batch cultivations of *Tr*CBH2 variants, optimized by different gene optimization methods; and normalized to regarding copy numbers, as represented in Figure 2B.

Time (h)	P(AOX1)-*Tr*CBH2-CP-CN7±1 (g/l)	P(De)-*Tr*CBH2-CP-CN25±7 (g/l)	P(AOX1)-*Tr*CBH2-HM-CN3 (g/l)	P(De)-*Tr*CBH2-HM-CN7±1 (g/l)	P(De)-*Tr*CBH2-HM-CN1 (g/l)	P(GAP)-*Tr*CBH2-HM-CN4 (g/l)
0	0	0	0	0	0	0
18	0	0,01	0,02	0,02	0,01	0,09
47	0,03	0,03	0,27	0,17	0,05	0,28
74	0,10	0,10	0,57	0,39	0,35	0,42
90	0,18	0,15	0,93	0,63	0,43	0,61

Table 3: Measured protein concentrations during fed-batch cultivations of *Tr*bMan and *Tl*XynA; data as represented in Figure 2A.

Time (h)	P(En)-*Tl*XynA-HM-CN10±3 (g/l)	P(En)-*Tl*XynA-HM-CN18±4 (g/l)
0	0	0
13	0,20	0
20	0,35	0,10
40	0,40	0,20
66,5	0,60	0,20

Time (h)	P(En)-*Tl*XynA-HM-CN6±1 (g/l)
0	0
21	0,26
30	0,37
52	0,61
75,5	0,86
92	0,85

Time (h)	P(En)-*Tr*bMan-HM-CN1 (g/l)
0	0
20	0,05
26	0,08
52	0,25
72	0,68
91	0,71

Table 4: Individual data points of the fed- batch cultivation of selected strains; data points at 90 h and the respective copy numbers and expression levels normalized to copy number; data as represented in Figure 3.

Strain	TrCBH2$_{t90}$ (g/l)	CN	TrCBH2$_{t90}$/CN (g/l)
P(AOX1)-CP-CN7±1	1,26	7	0,18
P(De)-CP-CN25±7	3,81	25	0,15
P(AOX1)-HM-CN3	2,78	3	0,93
P(De)-HM-CN7±1	4,37	7	0,63
P(De)-HM-CN1	0,43	1	0,43
P(GAP)-HM-CN4	2,43	4	0,61

Table 5: Relative ratios of the normalized expression levels at 90 h of the different gene optimization variants (HM/CP) under the control of P(AOX1) and P(De), data as represented in Figure 3B:

Promoter	HM$_{t90, norm}$ (g/l)		CP$_{t90, norm}$ (g/l)	HM$_{t90, norm}$/CP$_{t90, norm}$
P(De)	0,63	0,43	0,15	3,46 ±0,92
P(AOX1)	0,93		0,18	5,14

Table 6: Relative ratios of the normalized expression levels at 90 h of the different methanol inducible promoters (P(AOX1)/P(De)) expressing either TrCBH2-CP or TrCBH2-HM, data as represented in Figure 3C:

Gene optimization method	P(AOX1) (g/l)	P(De) (g/l)		P(AOX1)/P(De)
CP$_{t90, norm}$	0,18	0,15		1,18
HM$_{t90, norm}$	0,93	0,63	0,43	1,76

Table 7: Measured protein concentrations during fed-batch cultivations of TrCBH2 variants expressed under the control of strong methanol inducible promoters (P(AOX1), P(En)) compared to the weak methanol inducible promoter P(De), data as represented in Figure 4A

Time (h)	P(AOX1)-TrCBH2-HM-CN5±1 (g/l)
0	0
20,5	0,34
28,5	0,90
41,5	2,63
55,5	4,19
70	5,00
91,5	5,98

Time (h)	P(En)-TrCBH2-HM-CN6±1 (g/l)
0	0,05
19	0,31
27,5	2,08
44	5,11
51,5	4,90
67,5	6,55
90,5	5,55

Time (h)	P(De)-TrCBH2-HM-CN7±1 (g/l)
0	0
18	0,14
47	1,20
74	2,74
90	4,37

Table 8: Measured protein concentrations during fed-batch cultivation of a *Tr*bMan variant compared to previously obtained results, data as represented in Figure 4B:

Time (h)	P(De)-TrbMan-HM-CN5±1 (g/l)	P(En)-TrbMan-HM-CN1 (g/l)
0	0	0
20	0,10	0,05
26	0,40	0,08
52	0,60	0,25
72	0,90	0,68
91	1,14	0,72