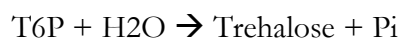


Thermodynamic analysis of the T6P-phosphatase reaction

The T6P-phosphatase reaction catalyze the conversion of trehalose-6-phosphate to trehalose:



The standard Gibbs free energy for this reaction ($\Delta_r G^\circ$) is $-14.8 \text{ kJ mol}^{-1}$ or the $K'_{\text{eq}} = 400$, which makes this reaction irreversible at standard conditions. In the following, we estimate the Gibbs free energy of this reaction considering the measured metabolite concentrations during the stepwise experiment, assuming an intracellular pH of 7.0 and a ionic strength of 0.2 M. For unit conversion a factor of $1.7 \text{ mL g}_{\text{DW}}^{-1}$ was used. All the calculations were made using the web-based interface eEquilibrator at <http://equilibrator.weizmann.ac.il>

Table A6-1. Estimation of Gibbs free energy of the T6P-phosphatase reaction for the different growth rates studied

D (h⁻¹)	0.054	0.101	0.207	0.307
T6P ($\mu\text{mol g}_{\text{DW}}^{-1}$)	0.34	0.37	0.04	0.02
T6P (μM)	197.36	220.28	22.84	9.17
Trehalose ($\mu\text{mol g}_{\text{DW}}^{-1}$)	145.90	162.16	1.74	0.29
Trehalose (mM)	85.82	95.39	1.02	0.17
Pi ($\mu\text{mol g}_{\text{DW}}^{-1}$) ^a	25.50	25.50	25.50	25.50
Pi (mM)	15	15	15	15
H ₂ O (M)	55	55	55	55
Gibbs free energy (kJ mol^{-1})	-8.6	-8.6	-14.2	-16.4

^a Cytosolic orthophosphate concentration was assumed to be the same as in other chemostat experiments with a mutant strain of CEN.PK113-7D (personal communication Zhang J., 2014)

Under all the growth conditions tested in the present work, T6P-phosphatase reaction must operate irreversibly. Reversing this situation would demand extremely large concentration of orthophosphate, which would be in the order of $850 \mu\text{mol g}_{\text{DW}}^{-1}$. Due to experimental evidence suggesting the existence of trehalose phosphorylation and that ATP hydrolysis accounts for approximately $30\text{-}50 \text{ kJ mol}^{-1}$ under the conditions encountered here, the existence of a kinase reaction is a sound assumption.