**Table III.** Influence of the NAD/NADH ratio on the thermodynamic feasibility of glycolysis.

CEN.PK 113-7A:mtlD, aerobic, glucose-limited,  $D = 0.1 \text{ h}^{-1}$ With total With cytosolic free Strain and conditions NAD/NADH NAD/NADH 0.23 FBP (mM) 3PG (mM)  $0.41^{a}$  $3.06^{b}$ ATP (mM)  $0.72^{b}$ ADP (mM) 43<sup>b</sup> Pi (mM) Total NAD/NADH 7.5 Cytosolic free NAD/NADH 101/320 assuming cytosolic pH 7.0/6.5  $\Delta_r G'$  (kJ/mol) at pH' 7.0/6.5 +12/+17-1.3/-1.4

Concentrations were converted to mM using the factor 2.38 mL<sub>cell</sub>/g<sub>DW</sub> (Theobald et al., 1997). The cytosolic free NAD/NADH was calculated from the steady-state NAD/NADH  $\times$  10<sup>pH-7.0</sup> ratio of 101, assuming the cytosolic pH is between 6.5 and 7.0 (see Results Section). Gibbs energies of formation of the metabolites at pH' 7.0 and 6.5,  $T=25^{\circ}$ C and I=0.25 M were obtained from Alberty (2003). The Gibbs energy of reaction was calculated for the overall reaction: FBP +2 NAD+2 ADP+2 Pi  $\rightarrow$  2 3PG+2 ATP+2 NADH. A reaction is feasible if  $\Delta_r G' \leq 0$ .

<sup>a</sup>Calculated from the concentration of 2PG + 3PG assuming equilibrium of phosphoglycerate mutase, with  $\Delta_{\rm r}G'^{\circ}=5.9$  kJ/mol (Alberty, 2003). <sup>b</sup>Taken from Wu et al. (2006).