

Problem 5

Zhu et al. (2008)[†] conducted chemostat experiments with *E. coli* having a knockout in genes coding enzymes of the pyruvate dehydrogenase complex. Thus, the strain is unable to convert pyruvate into acetate. The strain furthermore accumulates pyruvate and consumes acetate. The measurements are shown in Table 1:

Table 1. Observed fluxes of *Escherichia coli* ALS929 under acetate-limited steady-state conditions at a growth rate of 0.15 h^{-1} .

Parameter	rate (mmol/gh)
glucose consumption	8.89
acetate consumption	1.10
pyruvate generation	12.75
CO ₂ generation	1.10
biomass generation	6.07

Use the flux map on the next page, which is typically used for wild-type *E. coli*, and which considers the glyoxylate shunt pathway to be insignificant. Note that a growth rate of 0.15 h^{-1} corresponds to 0.15 g cells/gh and the unit carbon molecular weight of *E. coli* is 24.70 g/mol . That is,

$$(0.15 \text{ g/gh}) \times (1000 \text{ mmol/mol}) \times (\text{mol}/24.70 \text{ g}) = 6.07 \text{ mmol cells/gh}.$$

- 1) Write the 19 equations representing material balances for the 18 “nodes” (abbreviated G6P, F6P, Ru5P, R5P, X5P, S7P, E4P, FDP, GIP, G3P, 3PG, PEP, PYR, ACA, OAA, CIT, AKG, SUC) and CO₂. The equations should contain the unknown fluxes and the five known fluxes (see Table 1). Remember to include biomass, with non-integer stoichiometric coefficients, as one of the the known fluxes.
- 2) Is this set of equations determined or overdetermined?
- 3) Solve the set of equations, finding the unknown fluxes, and write a flux map (showing the values for all the fluxes in units of mmol/gh. Highlight the known fluxes to distinguish them from the calculated fluxes.
- 4) Contemplate the difference between any observed and calculated fluxes, and any negative fluxes. What insights has this model given you regarding the carbon fluxes during acetate-limited growth of this strain?

[†]Y. Zhu, M. A. Eiteman, R. Altman, E. Altman, “High glycolytic flux improves pyruvate production by a metabolically engineered *Escherichia coli* strain,” *Appl. Environ. Microbiol.*, 74(21):6649-6655 (2008). Note that Y. Zhu was the Ph.D. student of M. A. Eiteman, and that these are indeed his reported results.

