Solution E3

The flux balances including biomass formation are:

01 G6P: $0 = 1v_{Glu} + 0v_{Pyr} + 0v_{Ace} + 0v_{CO2} - 0.00506v_{BM} - 1v1 - 1v2 + 0v3 + 0v4 + 0v5 + 0v6 + 0v7 + 0v8 + 0v9 + 0v10 + 0v11 + 0v12 + 0v13 + 0v14 + 0v15 + 0v16 + 0v17 + 0v18$

02 F6P: $0 = 0v_{Glu} + 0v_{Pyr} + 0v_{Ace} + 0v_{CO2} - 0.00175v_{BM} + 1v1 + 0v2 + 0v3 + 0v4 + 1v5 + 0v6 + 1v7 - 1v8 + 0v9 + 0v10 + 0v11 + 0v12 + 0v13 + 0v14 + 0v15 + 0v16 + 0v17 + 0v18$

03 Ru5P: $0 = 0v_{Glu} + 0v_{Pyr} + 0v_{Ace} + 0v_{CO2} + 0.0000v_{BM} + 0v1 + 1v2 - 1v3 - 1v4 + 0v5 + 0v6 + 0v7 + 0v8 + 0v9 + 0v10 + 0v11 + 0v12 + 0v13 + 0v14 + 0v15 + 0v16 + 0v17 + 0v18$

04 R5P: $0 = 0v_{Glu} + 0v_{Pyr} + 0v_{Ace} + 0v_{CO2} - 0.02220v_{BM} + 0v1 + 0v2 + 0v3 + 1v4 + 0v5 - 1v6 + 0v7 + 0v8 + 0v9 + 0v10 + 0v11 + 0v12 + 0v13 + 0v14 + 0v15 + 0v16 + 0v17 + 0v18$

05 X5P: $0 = 0v_{Glu} + 0v_{Pyr} + 0v_{Ace} + 0v_{CO2} + 0.0000v_{BM} + 0v1 + 0v2 + 1v3 + 0v4 - 1v5 - 1v6 + 0v7 + 0v8 + 0v9 + 0v10 + 0v11 + 0v12 + 0v13 + 0v14 + 0v15 + 0v16 + 0v17 + 0v18$

06 S7P: $0 = 0v_{Glu} + 0v_{Pyr} + 0v_{Ace} + 0v_{CO2} + 0.0000v_{BM} + 0v1 + 0v2 + 0v3 + 0v4 + 0v5 + 1v6 - 1v7 + 0v8 + 0v9 + 0v10 + 0v11 + 0v12 + 0v13 + 0v14 + 0v15 + 0v16 + 0v17 + 0v18$

07 E4P: $0 = 0v_{Glu} + 0v_{Pyr} + 0v_{Ace} + 0v_{CO2} - 0.00892v_{BM} + 0v1 + 0v2 + 0v3 + 0v4 - 1v5 + 0v6 + 1v7 + 0v8 + 0v9 + 0v10 + 0v11 + 0v12 + 0v13 + 0v14 + 0v15 + 0v16 + 0v17 + 0v18$

08 FDP: $0 = 0v_{Glu} + 0v_{Pyr} + 0v_{Ace} + 0v_{CO2} + 0.0000v_{BM} + 0v1 + 0v2 + 0v3 + 0v4 + 0v5 + 0v6 + 0v7 + 1v8 - 1v9 + 0v10 + 0v11 + 0v12 + 0v13 + 0v14 + 0v15 + 0v16 + 0v17 + 0v18$

09 GlP: $0 = 0v_{Glu} + 0v_{Pyr} + 0v_{Ace} + 0v_{CO2} + 0.0000v_{BM} + 0v1 + 0v2 + 0v3 + 0v4 + 0v5 + 0v6 + 0v7 + 0v8 + 1v9 - 1v10 + 0v11 + 0v12 + 0v13 + 0v14 + 0v15 + 0v16 + 0v17 + 0v18$

10 G3P: $0 = 0v_{Glu} + 0v_{Pyr} + 0v_{Ace} + 0v_{CO2} - 0.00319v_{BM} + 0v1 + 0v2 + 0v3 + 0v4 + 1v5 + 1v6 - 1v7 + 0v8 + 1v9 + 1v10 - 1v11 + 0v12 + 0v13 + 0v14 + 0v15 + 0v16 + 0v17 + 0v18$

11 3PG: $0 = 0v_{Glu} + 0v_{Pyr} + 0v_{Ace} + 0v_{CO2} - 0.03700v_{BM} + 0v1 + 0v2 + 0v3 + 0v4 + 0v5 + 0v6 + 0v7 + 0v8 + 0v9 + 0v10 + 1v11 - 1v12 + 0v13 + 0v14 + 0v15 + 0v16 + 0v17 + 0v18$

12 PEP: $0 = -1v_{Glu} + 0v_{Pyr} + 0v_{Ace} + 0v_{CO2} - 0.01280v_{BM} + 0v1 + 0v2 + 0v3 + 0v4 + 0v5 + 0v6 + 0v7 + 0v8 + 0v9 + 0v10 + 0v11 + 1v12 - 1v13 - 1v14 + 0v15 + 0v16 + 0v17 + 0v18$

13 PYR: $0 = 1v_{Glu} - 1v_{Pyr} + 0v_{Ace} + 0v_{CO2} - 0.0700v_{BM} + 0v1 + 0v2 + 0v3 + 0v4 + 0v5 + 0v6 + 0v7 + 0v8 + 0v9 + 0v10 + 0v11 + 0v12 + 1v13 + 0v14 + 0v15 + 0v16 + 0v17 + 0v18$

14 ACA: $0 = 0v_{Glu} + 0v_{Pyr} + 1v_{Ace} + 0v_{CO2} - 0.09260v_{BM} + 0v1 + 0v2 + 0v3 + 0v4 + 0v5 + 0v6 + 0v7 + 0v8 + 0v9 + 0v10 + 0v11 + 0v12 + 0v13 + 0v14 - 1v15 + 0v16 + 0v17 + 0v18$

15 OAA: $0 = 0v_{Glu} + 0v_{Pyr} + 0v_{Ace} + 0v_{CO2} - 0.04410v_{BM} + 0v1 + 0v2 + 0v3 + 0v4 + 0v5 + 0v6 + 0v7 + 0v8 + 0v9 + 0v10 + 0v11 + 0v12 + 0v13 + 1v14 - 1v15 + 0v16 + 0v17 + 1v18$

16 CIT: $0 = 0v_{Glu} + 0v_{Pyr} + 0v_{Ace} + 0v_{CO2} + 0.0000v_{BM} + 0v1 + 0v2 + 0v3 + 0v4 + 0v5 + 0v6 + 0v7 + 0v8 + 0v9 + 0v10 + 0v11 + 0v12 + 0v13 + 0v14 + 1v15 - 1v16 + 0v17 + 0v18$

17 AKG: $0 = 0v_{Glu} + 0v_{Pyr} + 0v_{Ace} + 0v_{CO2} - 0.02660v_{BM} + 0v1 + 0v2 + 0v3 + 0v4 + 0v5 + 0v6 + 0v7 + 0v8 + 0v9 + 0v10 + 0v11 + 0v12 + 0v13 + 0v14 + 0v15 + 1v16 - 1v17 + 0v18$

18 SUC: $0 = 0v_{Glu} + 0v_{Pyr} + 0v_{Ace} + 0v_{CO2} + 0.0000v_{BM} + 0v1 + 0v2 + 0v3 + 0v4 + 0v5 + 0v6 + 0v7 + 0v8 + 0v9 + 0v10 + 0v11 + 0v12 + 0v13 + 0v14 + 0v15 + 0v16 + 1v17 - 1v18$

19 CO2: $0 = 0v_{Glu} + 0v_{Pyr} + 0v_{Ace} - 1v_{CO2} + 0.0000v_{BM} + 0v1 + 1v2 + 0v3 + 0v4 + 0v5 + 0v6 + 0v7 + 0v8 + 0v9 + 0v10 + 0v11 + 0v12 + 0v13 - 1v14 + 0v15 + 1v16 + 1v17 + 0v18$

This set of equations is overdetermined.

The first working matrix is:

A =

v_{Glu}	v_{Pyr}	v_{Ace}	v_{CO2}	v_{BM}	v1	v2	v3	v4	v5	v6	v7	v8	v9	v10	v11	v12	v13	v14	v15	v16	v17	v18	
1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
1	0	0	0	-0.00506	-1	-1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	G6P
0	0	0	0	-0.00175	1	0	0	0	1	0	1	-1	0	0	0	0	0	0	0	0	0	0	F6P
0	0	0	0	0	0	1	-1	-1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	Ru5P
0	0	0	0	-0.0222	0	0	0	1	0	-1	0	0	0	0	0	0	0	0	0	0	0	0	R5P
0	0	0	0	0	0	0	1	0	-1	-1	0	0	0	0	0	0	0	0	0	0	0	0	X5P
0	0	0	0	0	0	0	0	0	0	1	-1	0	0	0	0	0	0	0	0	0	0	0	S7P
0	0	0	0	-0.00892	0	0	0	0	-1	0	1	0	0	0	0	0	0	0	0	0	0	0	E4P
0	0	0	0	0	0	0	0	0	0	0	0	1	-1	0	0	0	0	0	0	0	0	0	FDP
0	0	0	0	0	0	0	0	0	0	0	0	0	1	-1	0	0	0	0	0	0	0	0	GlP
0	0	0	0	-0.00319	0	0	0	0	1	1	-1	0	1	1	-1	0	0	0	0	0	0	0	G3P
0	0	0	0	-0.037	0	0	0	0	0	0	0	0	0	0	1	-1	0	0	0	0	0	0	3PG
-1	0	0	0	-0.0128	0	0	0	0	0	0	0	0	0	0	0	1	-1	-1	0	0	0	0	PEP
1	-1	0	0	-0.07	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	PYR
0	0	1	0	-0.0926	0	0	0	0	0	0	0	0	0	0	0	0	0	0	-1	0	0	0	ACA
0	0	0	0	-0.0441	0	0	0	0	0	0	0	0	0	0	0	0	0	1	-1	0	0	1	OAA
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	-1	0	0	CIT
0	0	0	0	-0.0266	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	-1	0	AKG
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	-1	SUC
0	0	0	-1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	-1	0	1	1	0	CO2

The second working matrix is:

	v_{Glu}	v_{Pyr}	v_{Ace}	v_{CO2}	v_{BM}	v1	v2	v3	v4	v5	v6	v7	v8	v9	v10	v11	v12	v13	v14	v15	v16	v17	v18	
T11	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	T12
	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
	1	0	0	0	-0.00506	-1	-1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
	0	0	0	0	-0.00175	1	0	0	0	1	0	1	-1	0	0	0	0	0	0	0	0	0	0	
	0	0	0	0	0	0	1	-1	-1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
	0	0	0	0	-0.0222	0	0	0	1	0	-1	0	0	0	0	0	0	0	0	0	0	0	0	
	0	0	0	0	0	0	0	1	0	-1	-1	0	0	0	0	0	0	0	0	0	0	0	0	
T21	0	0	0	0	0	0	0	0	0	0	1	-1	0	0	0	0	0	0	0	0	0	0	0	T22
	0	0	0	0	-0.00892	0	0	0	0	-1	0	1	0	0	0	0	0	0	0	0	0	0	0	
	0	0	0	0	0	0	0	0	0	0	0	0	1	-1	0	0	0	0	0	0	0	0	0	
	0	0	0	0	0	0	0	0	0	0	0	0	0	1	-1	0	0	0	0	0	0	0	0	
	0	0	0	0	-0.00319	0	0	0	0	1	1	-1	0	1	1	-1	0	0	0	0	0	0	0	
	0	0	0	0	-0.037	0	0	0	0	0	0	0	0	0	0	1	-1	0	0	0	0	0	0	
	-1	0	0	0	-0.0128	0	0	0	0	0	0	0	0	0	0	0	1	-1	-1	0	0	0	0	
	1	-1	0	0	-0.07	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	
	0	0	1	0	-0.0926	0	0	0	0	0	0	0	0	0	0	0	0	0	0	-1	0	0	0	
	0	0	0	0	-0.0441	0	0	0	0	0	0	0	0	0	0	0	0	0	1	-1	0	0	1	
	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	-1	0	0	
	0	0	0	0	-0.0266	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	-1	0	
	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	-1	
	0	0	0	-1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	-1	0	1	1	0	

The MATLAB program is:

```
%% This MATLAB program provides a computational solution to Problem E3
%% It solves a set of 19 linear equations with 18 unknowns
(overdetermined).
%%

%define matrices

T11 = [1,0,0,0;
      0,1,0,0;
      0,0,1,0;
      0,0,0,1;
      0,0,0,0];

T21 = [1,0,0,0;
      0,0,0,0;
      0,0,0,0;
      0,0,0,0;
      0,0,0,0;
      0,0,0,0;
      0,0,0,0;
      0,0,0,0;
      0,0,0,0;
      0,0,0,0;
     -1,0,0,0;
     1,-1,0,0;
      0,0,1,0;
      0,0,0,0;
      0,0,0,0;
      0,0,0,0;
      0,0,0,0;
      0,0,0,1];

T12 = [0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0;
      0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0;
      0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0;
      0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0;
      1,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0];

T22 = [-0.00506,-1,-1,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0;
      -0.00175,1,0,0,0,1,0,1,-1,0,0,0,0,0,0,0,0,0;
      0,0,1,-1,-1,0,0,0,0,0,0,0,0,0,0,0,0,0;
      -0.0222,0,0,0,1,0,-1,0,0,0,0,0,0,0,0,0,0,0;
      0,0,0,1,0,-1,-1,0,0,0,0,0,0,0,0,0,0,0;
      0,0,0,0,0,0,1,-1,0,0,0,0,0,0,0,0,0,0;
      -0.00892,0,0,0,0,-1,0,1,0,0,0,0,0,0,0,0,0,0;
      0,0,0,0,0,0,0,0,1,-1,0,0,0,0,0,0,0,0;
      0,0,0,0,0,0,0,0,0,1,-1,0,0,0,0,0,0,0;
      -0.00319,0,0,0,0,1,1,-1,0,1,1,-1,0,0,0,0,0,0;
      -0.037,0,0,0,0,0,0,0,0,0,0,1,-1,0,0,0,0,0;
      -0.0128,0,0,0,0,0,0,0,0,0,0,0,1,-1,-1,0,0,0;
      -0.07,0,0,0,0,0,0,0,0,0,0,0,0,1,0,0,0,0;
      -0.0926,0,0,0,0,0,0,0,0,0,0,0,0,0,-1,0,0,0;
      -0.0441,0,0,0,0,0,0,0,0,0,0,0,0,0,1,-1,0,0,1;
      0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,1,-1,0,0;
      -0.0266,0,0,0,0,0,0,0,0,0,0,0,0,0,0,1,-1,0;
      0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,1,-1;
      0,0,1,0,0,0,0,0,0,0,0,0,0,-1,0,1,1,0];

vM = [8.89;12.75;1.10;1.10;6.07];
T = T11 - T12*pinv(T22)*T21;
TT = transpose(T);
v1 = pinv(TT*T)*TT*vM
v2 = -pinv(T22)*T21*v1
```

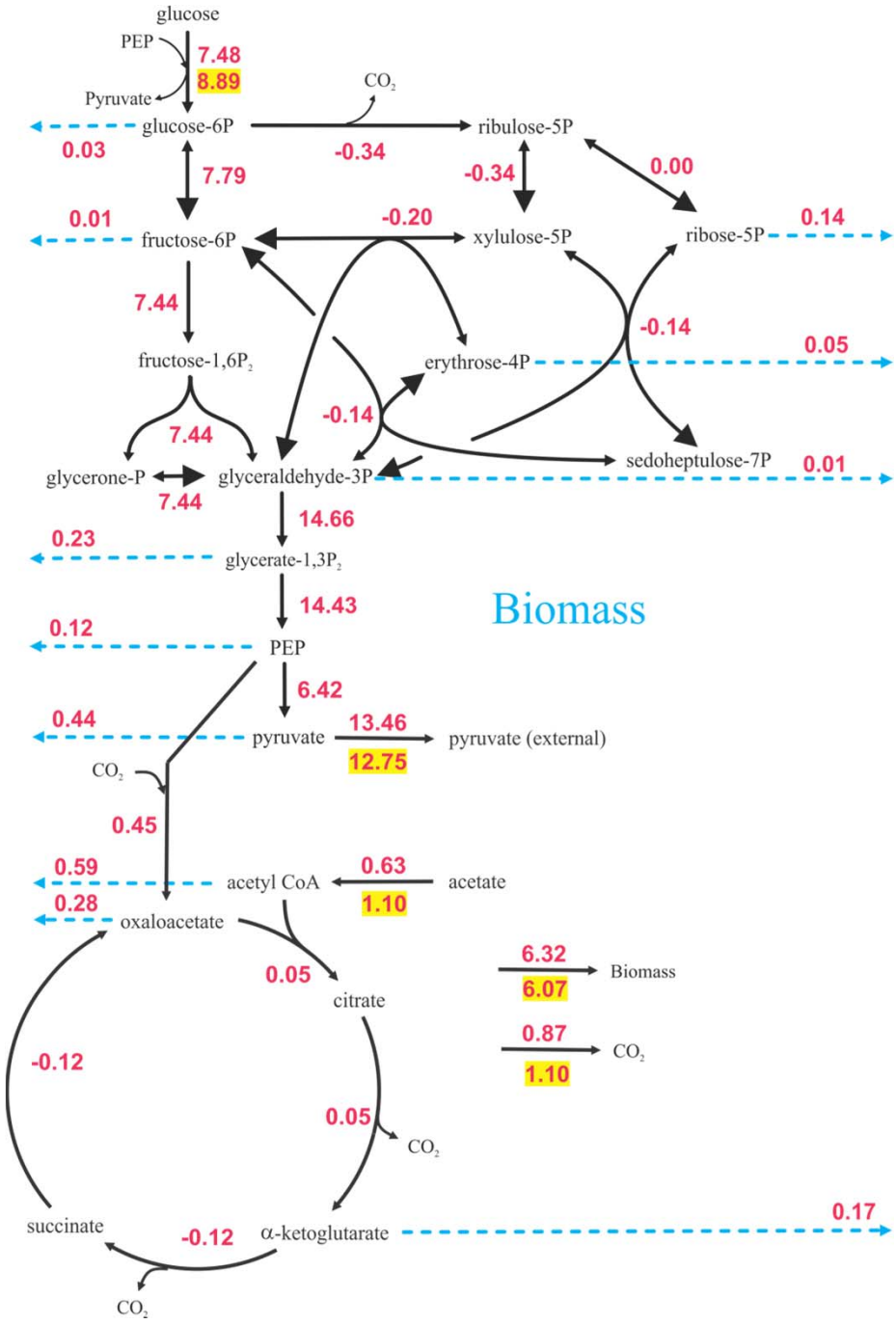
The MATLAB program output is:

$$vM = \begin{bmatrix} v_{Glu} \\ v_{Pyr} \\ v_{Ace} \\ v_{CO2} \\ v_{BM} \end{bmatrix} = \begin{bmatrix} 8.89 \\ 12.75 \\ 1.10 \\ 1.10 \\ 6.07 \end{bmatrix}$$

$$v1 = \begin{bmatrix} v_{Glu} \\ v_{Pyr} \\ v_{Ace} \\ v_{CO2} \end{bmatrix} = \begin{bmatrix} 7.48 \\ 13.46 \\ 0.63 \\ 0.87 \end{bmatrix}$$

$$v2 = \begin{bmatrix} v_{BM} \\ v1 \\ v2 \\ v3 \\ v4 \\ v5 \\ v6 \\ v7 \\ v8 \\ v9 \\ v10 \\ v11 \\ v12 \\ v13 \\ v14 \\ v15 \\ v16 \\ v17 \\ v18 \end{bmatrix} = \begin{bmatrix} 6.32 \\ 7.79 \\ -0.34 \\ -0.34 \\ 0.00 \\ -0.20 \\ -0.14 \\ -0.14 \\ 7.44 \\ 7.44 \\ 7.44 \\ 14.66 \\ 14.42 \\ 6.42 \\ 0.45 \\ 0.05 \\ 0.05 \\ -0.12 \\ -0.12 \end{bmatrix}$$

So, the flux map becomes
(observed values highlighted)



Several fluxes are negative, and the difference between observed and caluclated flux for glucose and pyruvate seem large.

Thoughts:

1. The flux through the pentose phosphate pathway is very low. The cells might be using the non-oxidative pathway exclusively to generate erythrose-4P and ribose-5P.
2. The flux through the TCA cycle is very low.
3. If the fluxes in both the TCA cycle and the pentose phosphate pathway are low, then how is the cell obtaining NAPDH?
    a. We have no ability to examine transhydrognease with this analysis. Measuring the activity of this enzyme might clear this question.
    b. The acetate consumption is underestimated....perhaps our assumption about the glyoxylate shunt being inactive is poor.
    c. Also, and similarly, acetate might be directed toward malate, and then the malic enzyme might be a means for the cells to obtain NADPH.
    d. The CO_2 flux was underestimated by over 20%. An activity in malic enzyme would also mean additional CO_2 generated.
    e. We cannot include malic enzyme in this model, as this flux would cause a singularity in the resulting matrix. That is, with this methodology we woul dnot be able to dsitinguish between two or more pathways, leading to an infinite number of solutions. In order to test whether malic enzyme flux might be relevant, measure enzyme activity, and then conduct an different experiment: for example use labeled acetate or glucose.