Problem Set #3
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1 Part A

To construct the stoichiometric array, I first identified 18 nodes or metabolites and 20 reactions or fluxes. Therefore, the stoichiometric array will be 18x20. The tables following define every meatbolite and flux needed for the reactions in the urea cycle.

$$\frac{dX_i}{dt} = \begin{bmatrix} -1 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 1 & -1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & -1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 1 & -1 & 0 & 1 & -1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & -1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 1 & -1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & -1 & 0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ -1 & 0 & 0 & 1 & -1 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ -1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & -1 & 0 & -2 & 2 & 0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & -1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & -1 & 0 & 0 & 0 & 0 & 0 & 0 \\ 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & -1 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & -1 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & -1 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 2 & -2 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & -1 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 1.5 & -1.5 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 \\ 0 & 0 & 0 & 0 & 1.5 & -1.5 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & 0 \\ 0 & 0 & 0 & 0 & -1.5 & 1.5 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & -1 \end{bmatrix} \begin{pmatrix} v_1 \\ v_2 \\ v_3 \\ v_4 \\ v_{5f} \\ v_{5r} \\ b_1 \\ b_2 \\ b_3 \\ b_4 \\ b_5 \\ b_6 \\ b_7 \\ b_8 \\ b_9 \\ b_{10} \\ b_{11} \\ b_{12} \\ b_{13} \\ b_{14} \end{pmatrix}$$

Table One: Defined Metabolites in S Consistent with PS3 Diagram

Row	Metabolite	Row	Metabolite
1	Aspartate	10	H_2O
2	Arginosuccinate	11	Pi
3	Fumarate	12	AMP
4	Arginine	13	PPi
5	Urea	14	NO
6	Ornithine	15	O_2
7	Carbamoyl Pi	16	H^+ Sink
8	Citruline	17	$NADPH$ Source
9	ATP	18	$NADP^+$ Source

Table Two: Defined Fluxes Consistent with PS3 Diagram

Variable	Flux	Variable	Flux
v_1	6.3.4.5 Catalyzed Reaction	b_5	ATP Source
v_2	4.3.2.1 Catalyzed Reaction	b_6	Water Source
v_3	3.5.3.1 Catalyzed Reaction	b_7	Phosphate Sink
v_4	2.1.3.3 Catalyzed Reaction	b_8	AMP Sink
v_{5f}	1.14.13.39 Forward Reaction (Cit to R)	b_9	Pyrophosphate Sink
v_{5r}	1.14.13.39 Reverse Reaction (R to Cit)	b_{10}	Nitric Oxide Sink
b_1	Carbamoyl Pi Source	b_{11}	O_2 Sink
b_2	Aspartate Source	b_{12}	H^+ Source
b_3	Fumarate Sink	b_{13}	$NADPH$ Source
b_4	Urea Sink	b_{14}	$NADP^+$ Sink

2 Part B

The code B_balanced.jl shows the balanced nature of the first 6 fluxes (the actual chemical reactions). An atom matrix with dimensions of # of elements by # of metabolites (A) is multiplied by the stoichiometric array (S). The rows of A are thus C,H,N,O,P, and S respectively. The product E has zero entries for the elementally balanced reactions or the first 6 fluxes v_{1-5r} (columns). The reaction and stoichiometric coefficients were taken from the reactions and conditions on the KEGG database for the urea cycle. When values are not zero in the v reactions, the exact element (row) and reaction (column) entry is shown to not be zero and thus unbalanced in **E**. We can thus edit the stoichiometric array until balanced.

$$\mathbf{A*S = E}$$

$$A = \begin{bmatrix} 4 & 10 & 4 & 6 & 1 & 5 & 1 & 6 & 10 & 0 & 0 & 10 & 0 & 0 & 0 & 0 & 21 & 21 \\ 7 & 18 & 4 & 14 & 4 & 12 & 4 & 13 & 16 & 2 & 3 & 14 & 4 & 0 & 0 & 1 & 30 & 29 \\ 1 & 4 & 0 & 4 & 2 & 2 & 1 & 3 & 5 & 0 & 0 & 5 & 0 & 1 & 0 & 0 & 7 & 7 \\ 4 & 6 & 4 & 2 & 1 & 2 & 5 & 3 & 13 & 1 & 4 & 7 & 7 & 1 & 2 & 0 & 17 & 17 \\ 0 & 0 & 0 & 0 & 0 & 0 & 1 & 0 & 3 & 0 & 1 & 1 & 2 & 0 & 0 & 0 & 3 & 3 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 3 & 3 \end{bmatrix}$$

3 Part C

CHEME5440HW3_C.jl calls the Flux.jl function to complete the linear programming problem of FBA. We need bounds and an objective. the objective is to maximize urea flux:

$$Objective = Maximize\, b_4$$

Subject To:

$$0 \leq v_j \leq k_{cat,j} * E * \theta * \prod_{i=1}^{M} \frac{X_i}{K_{Mij} + X_i}$$

$$0 \leq b_{1-4} \leq 10 mmol/gDW - hr$$

$$-10 \leq b_{5-14} \leq 10 mmol/gDW - hr$$

Where M is the number of metabolites acted on by that enzyme in v_j. E, $k_{cat,j}$, and θ are given in the problem statement. X_i and K_{Mij} are the metabolite concentrations and K_M values for each enzyme and substrate. These are all from the BRENDA enzyme database and Park et al[1]. A value for human cells was chosen first, then *Mus musculus* if needed, and finally a yeast value if none of those existed. When no metabolite value or K_M value could be found, the reaction had to be assumed to act at saturation. The saturation term was set to be 1. This was especially true for metabolites like water or H^+ whose concentrations are not really measurable and are quite abundant anyway. b_j refers to fluxes in and out of the system and the bound is given. The metabolites (b_{5-14}) have reversible transport and thus have both bounds. The stoichiometric matrix reveals the assumed direction (+ for into the cell). There are no metabolite bounds on this problem. All values were converted to $umol/gDW - hr$

via the conversion in Appendix A. The solution or returned flux vector in $umol/gDW - hr$:

$$\mathbf{v} = \begin{bmatrix} 777.9 \\ 777.9 \\ 1271.1 \\ 1271.1 \\ 493.2 \\ 0.0 \\ 1271.1 \\ 777.9 \\ 777.9 \\ 1271.1 \\ 777.9 \\ 2257.5 \\ 1271.1 \\ 777.9 \\ 777.9 \\ -493.2 \\ 986.4 \\ -739.8 \\ -739.8 \\ -739.8 \end{bmatrix}$$

Therefore the maximum urea flux b_4 is:

$$UreaFlux = 1271.1 umol/gDW - hr$$

All zero fluxes are reactions or transports that are not used. It is also worth noting that **S*v=0** so the system checks itself.

4 Appendix A: Conversion

Molar values were converted to $umol/gDW$ with the following conversion factors. The mass of a mammalian cell ($2.3e-9g$ from Park[2]) was converted to dry mass via the water fraction (0.798 from Savitz[3]). The molar value is divided by this and multiplied by the volume of a mammalian cell ($1e-12L$ from Sims[4]). This is done via the internal function "convertfactor" in CHEME5440HW3_C.jl.

References

[1] J. O. Park, S. A. Rubin, Y.-F. Xu, D. Amador-Noguez, J. Fan, T. Shlomi, and J. D. Rabinowitz, "Metabolite concentrations, fluxes and free energies imply efficient enzyme usage," *Nature chemical biology*, vol. 12, no. 7, p. 482, 2016.

[2] K. Park, J. Jang, D. Irimia, J. Sturgis, J. Lee, J. P. Robinson, M. Toner, and R. Bashir, "living cantilever arrays for characterization of mass of single live cells in fluids," *Lab on a Chip*, vol. 8, no. 7, pp. 1034–1041, 2008.

[3] D. Savitz, V. W. Sidel, and A. Solomon, "Osmotic properties of human red cells," *The Journal of general physiology*, vol. 48, no. 1, pp. 79–94, 1964.

[4] C. E. Sims and N. L. Allbritton, "Analysis of single mammalian cells on-chip," *Lab on a Chip*, vol. 7, no. 4, pp. 423–440, 2007.