CHEME 7770/5440 - Problem Set 3

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File "srk_ps3_1.jl" contains the answers to a,b,c. Main points are summarized here. That Julia file references several subfunctions which are included in the repository as separate Julia files. To run, all files must be put in the same folder.

Open "srk_ps3_1.jl" to see answers about the stoichiometric and atom matrices.

Open "upper_bounds_math.jl" to see calculations for the upper bounds of v fluxes.

To see major answers, run the "srk_ps3_1.jl" file in PowerShell and follow instructions in this document about which variables to print in the command line.

1. **Answer to part a:**

   Assume everything in the system (the box) is at steady-state, and therefore that the time derivative is 0. "S original" is the stochimetric matrix below (in srk-ps3-1) In part b, the S will be rewritten.

$$\frac{d\mathbf{x}}{dt} = \begin{pmatrix} -1 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 \\ 1 & -1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & -1 & 0 \\ 0 & 1 & -1 & 0 & 1 & -1 & 0 & 0 & 0 & 0 \\ 0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & -1 \\ 0 & 0 & 1 & -1 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & -1 & 0 & 0 & 1 & 0 & 0 & 0 \\ -1 & 0 & 0 & 1 & -1 & 1 & 0 & 0 & 0 & 0 \end{pmatrix} \begin{pmatrix} v_1 \\ v_2 \\ v_3 \\ v_4 \\ v_{5,1} \\ v_{5,-1} \\ b_1 \\ b_2 \\ b_3 \\ b_4 \end{pmatrix} = 0 \tag{1}$$

$$S_{original} = \begin{pmatrix} -1 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 \\ 1 & -1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & -1 & 0 \\ 0 & 1 & -1 & 0 & 1 & -1 & 0 & 0 & 0 & 0 \\ 0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & -1 \\ 0 & 0 & 1 & -1 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & -1 & 0 & 0 & 1 & 0 & 0 & 0 \\ -1 & 0 & 0 & 1 & -1 & 1 & 0 & 0 & 0 & 0 \end{pmatrix} \tag{2}$$

   where we have the following k-cat (from KEGG and PS3),

$$k_{cat} for the following \begin{pmatrix} `v_1 \\ v_2 \\ v_3 \\ v_4 \\ v_{5,1} \\ v_{5,-1} \end{pmatrix} = \begin{pmatrix} 203s^{-1} \\ 34.5s^{-1} \\ 249s^{-1} \\ 88.1s^{-1} \\ 13.7s^{-1} \\ 13.7s^{-1} \end{pmatrix} \quad (3)$$

(Assume that $v_{5,1}$ is from citruline to arginine, and that $v_{5,-1}$ is arginine to citruline.)

and,

$$\mathbf{x} = \begin{pmatrix} Aspartate \\ Argininosuccinate \\ Fumarate \\ Arginine \\ Urea \\ Ornithine \\ CarbarmoylPhosphate \\ Citruline \end{pmatrix} \quad (4)$$

2. **Answer to part b:**

   Each of the different molecules has the following chemical formula:

   Aspartate = $C_4H_7NO_4$

   Argininosuccinate = $C_{10}H_{18}N_4O_6$

   Fumarate = $C_4H_4O_4$

   Arginine = $C_6H_{14}N_4O_2$

   Urea = CH_4N_2O

   Ornithine = $C_5H_{12}N_2O_2$

   Carbarmoyl Phosphate = CH_4NO_5P

   Citruline = $C_6H_{13}N_3O_3$

   An Atom Array with these formulas was put in srk-ps3-1, labeled "atom original" to denote the array that does not yet include additional metabolites."

   To check the original balance, calculate E original:

$$E_{original} = S_{orginial} * atom_{original} = () \quad (5)$$

   "E_original" can be gotten from the command line. The results of the E_original show non-zero values for all the rates v1 to v5,-1 (represented by the columns) except v2. As a result, research for all of the reactions was done to understand other species that might be involved. **Source: KEGG Pathway**

   Approach 1

   In this case assume all the new metabolite fluxes are irreversible. This has no effect for new metabolites for v1-v4. However, there will be separate fluxes for the forwards and backwards reactions for v5.

v1 (6.3.4.5): The cleavage of ATP probably added the energy for this reaction to occur, and is how the product got phosphorlyated (energized for future reactions.)

(a) ATP substrate (enters) (rate b5, C10H16N5O13P3),

(b) ADP product (leaves) (C10H16N5O13P3, rate b6),

(c) diphosphate product (leaves) (rate b7, H4P2O7);

v2 (4.3.2.1): no extra substrates or products

v3 (3.5.3.1): Arginine was probably cleaved into urea and orinithine through a hydrolysis reaction.

(a) H2O substrate (enters, rate b8);

v4 (2.1.3.3): The cleavage of a phosphate group probably provided energy for this reaction)

(a) phosphate product (leaves, rate b9, H3PO4);

v5,1 (1.14.13.39, citruline to arginine):

(a) nitric oxide substrate (enters, b10, NO),

(b) NADP+ substrate (enters b11, C21H29N7O17P3),

(c) H2O substrate (enters, b12, H2O),

(d) NADPH product (leaves, rate b13, C21H30N7O17P3),

(e) H+ product (leaves, rate b14, H)

(f) O2 product (leaves, rate b20, O2)

v5,-1 (1.14.13.39, arginine to citruline):

(a) NADPH substrate (enters, rate b15, C21H30N7O17P3),

(b) H+ substrate (enters, rate b16, H)

(c) O2 substrate (enters, rate b21, O2)

(d) nitric oxide product (leaves, rate b17, NO),

(e) NADP+ product (leaves, b18, C21H29N7O17P3),

(f) H2O product (leaves, b19, H2O)

Changes were made the stoichiometeric matrix. Please see "stoichiometric_matrix_balanced" for the new values for all reactions and the coefficients of reactants. The "E_balanced" shows zero values for all reactions v1 - v5. These variables can be printed from the command line.

Approach 2

Another option is to use Approach 1 for v1-v4, but make all the extra metabolites for v5,1 and v5,-1 in/out rates represented by the same fluxes. (Essentially make the fluxes for v5 reversible.) This is done in "stoichiometric_matrix_balanced2" and the forward reaction of citruline to arginine is used as the reference direction:

v5,1 (1.14.13.39, citruline to arginine) AND v5,-1: b8 and b11-14 are reversible.

(a) nitric oxide substrate (enters, b10, NO),

(b) NADP+ substrate (enters b11, C21H29N7O17P3),

(c) H2O substrate (enters, b8, H2O),

(d) NADPH product (leaves, rate b12, C21H30N7O17P3),

(e) H+ product (leaves, rate b13, H)

(f) O2 product (leaves, rate b14, O2)

If printed from the command line, "stoichiometric_matrix_balanced2" shows the stoichiometric matrix for Approach 2.

"E_balanced2" shows zero values for all reactions v1 - v5. These variables can be printed from the command line.

3. **Answer to part c:**

Assume:

-there is negligible consumption of metabolites for biomass growth

-there is negligible dilution of metabolites during biomass growth

-allosteric control function is 1

-continue to use terminology of APPROACH 1 and APPROACH 2 from part b.

Bounds

Lower Bounds:

(a) APPROACH 1: Since v5 was split into v5,1 and v5,-1 (forwards/backwards respectively as defined in a,) the minimum rate for all v's was 0 for all since they were assumed irreversible. Since all b's were also split into forwards/backwards, they all had a minimum of 0 as well.

(b) APPROACH 2: Since v5 was split into v5,1 and v5,-1 (forwards/backwards respectively as defined in a,) the minimum rate for all v's was 0 for all since they were assumed irreversible. Since b's for v5 were not split into forwards/backwards, $b_i \geq -10$ mmol/(gDW*hr) if $i = \{8, 10, 11, 12, 13, 14\}$. For all other b that were either in/out fluxes for irreversible v, $b_i \geq 0$ mmol/(gDW*hr) if $i = \{1, 2, 3, 4, 5, 6, 7, 9\}$.

Upper Bounds: (both APPROACH 1 and APPROACH 2)

For v's, the upper bound was determined with:

$$v_{max} = k_{cat} * \frac{e_j}{e} * \theta * \sum_{i}^{N} \frac{x_i}{K_m + x_i} \tag{6}$$

where N is the # of metabolites, E is the steady state concentration

$$E = \frac{e_j}{e} = 0.01(assume)[\mu mol/gDW], \tag{7}$$

and theta is the control element

$$\theta = \theta_{max} = 1. \tag{8}$$

- I could not find the concentrations of Arginosuccitate, Fumarate, Urea, Orinithine, and Carbarmoyl Phosphate. I could also not find Km for all substrates in all reactions. In these cases where Km or concentration could not be found, the saturation terms for those in the upper bound calculation was

assumed to be 1 (the max.) This is because if you assume concentration is much greater than Km, ($x_i >> K_m$), you go from the original saturation term equation:

$$\frac{x_i}{K_m + x_i} \tag{9}$$

to

$$\frac{x_i}{x_i} = 1 \tag{10}$$

All bounds are defined in "upper_bound_math.jl". The flux function is used with the objective of maximizing b4 (urea). The results are converted to the needed units.

For b's, the upper bounds was $b_i \geq 10$ mmol/(gDW*hr) for $i = [1, 14]$

Calculate Optimal Flux Distribution See "srk_ps3_1.jl" for inputs to flux function to calculate flux distribution for maximizing urea production b_4 mmol/gDW-hr

Results from "Approach 1" in Part B

After opening "srk_ps3_1.jl" in the command line:

(a) print "objective_value" : the maximum flux of urea is $b_4 = 0.26$ μmol/gDW-s

(b) print "objective_value_converted" : the maximum flux urea is $b_4 = 0.99$ mmol/gDW-hr

(c) print "calculated_flux_array" : all the fluxes are listed in μmol/gDW-s (the order is $v_1 - v_4$, $v_{5,1}$, $v_{5,-1}$, $b_1 - b_{21}$)

(d) print "calculated_flux_array_converted" : all the fluxes are listed in mmol/gDW-hr (the order is $v_1 - v_4$, $v_{5,1}$, $v_{5,-1}$, $b_1 - b_{21}$)

As a check that the fluxes balance (print "check1"):

$$S_{balanced,1} * v_{calculated\_flux\_array1} = \mathbf{0} \tag{11}$$

As can be seen, the flux for all the $v_1 - v_4$ and $b_1 - b_9$ are non-zero and the same number (0.26 μmol/gDW-s). This is reasonable since as much as products are formed in one step of the cycle, they are begin taken up by the next step. The $v_{5,1}, v_{5,-1}$, and $b_{10} - b_{21}$ were 0: these are all representative of the reversible v_5, so this result means v_5 does not occur.

Results from "Approach 2" in Part B

(a) print "objective_value2" : the maximum flux of urea is $b_4 = 0.26$ μmol/gDW-s

(b) print "objective_value_converted2" : the maximum flux urea is $b_4 = 0.99$ mmol/gDW-hr

(c) print "calculated_flux_array2" : all the fluxes are listed in μmol/gDW-s (the order is $v_1 - v_4$, $v_{5,1}$, $v_{5,-1}$, $b_1 - b_{14}$)

(d) print "calculated_flux_array_converted2" : all the fluxes are listed in mmol/gDW-hr (the order is $v_1 - v_4$, $v_{5,1}$, $v_{5,-1}$, $b_1 - b_{14}$)

As a check that the fluxes balance (print "check2"):

$$S_{balanced,2} * v_{calculated\_flux\_array2} = \mathbf{0} \tag{12}$$

As can be seen, the flux for all the $v_1 - v_4$ and $b_1 - b_{20}$ are non-zero. This means all the fluxes $v_1 - v_5$ all occur in the system. Notice that $b_{16} - b_{20}$, the metabolites for v_5 are negative: since the

reference direction was that of $v_{5,1}$, this means that the backwards reaction flux (arginine to citruline) was faster than the forwards reaction (citruline to arginine). Indeed, the flux array does show that $v_{5,1} < v_{5,-1}$.

Comparing APPROACH 1 and APPROACH 2 answers

Approach 1 shows v_5 does not occur; Approach 2 shows that it does. Both results are metabolically feasible. I am inclined to believe that Approach 2 is more physically relevant however, since v_5 is observed in the wild bio-synthesis pathway.