

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. To run, run the "srk-ps3-1.jl" file in PowerShell.

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 stoichiometric matrix below (in srk-ps3-1) In part b, the S will be rewritten.

$$\frac{dx}{dt} = \begin{pmatrix} -1 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 & 0 \\ 1 & -1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & -1 & 0 \\ 0 & 1 & -1 & 0 & 1 & -1 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & -1 \\ 0 & 0 & 1 & -1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & -1 & 0 & 0 & 1 & 0 & 0 & 0 & 0 \\ -1 & 0 & 0 & 1 & -1 & 1 & 0 & 0 & 0 & 0 & 0 \end{pmatrix} \begin{pmatrix} v_1 \\ v_2 \\ v_3 \\ v_4 \\ v_{5,1} \\ v_{5,-1} \\ b_1 \\ b_2 \\ b_3 \\ b_4 \end{pmatrix} = 0 \quad (1)$$

$$S_{original} = \begin{pmatrix} -1 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 & 0 \\ 1 & -1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & -1 & 0 \\ 0 & 1 & -1 & 0 & 1 & -1 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & -1 \\ 0 & 0 & 1 & -1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & -1 & 0 & 0 & 1 & 0 & 0 & 0 & 0 \\ -1 & 0 & 0 & 1 & -1 & 1 & 0 & 0 & 0 & 0 & 0 \end{pmatrix} \quad (2)$$

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

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

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

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

2. Answer to part b:

Each of the different molecules has the following chemical formula:

Aspartate = $\text{C}_4\text{H}_7\text{NO}_4$

Argininosuccinate = $\text{C}_{10}\text{H}_{18}\text{N}_4\text{O}_6$

Fumarate = $\text{C}_4\text{H}_4\text{O}_4$

Arginine = $\text{C}_6\text{H}_{14}\text{N}_4\text{O}_2$

Urea = $\text{CH}_4\text{N}_2\text{O}$

Ornithine = $\text{C}_5\text{H}_{12}\text{N}_2\text{O}_2$

Carbarmoyl Phosphate = $\text{CH}_4\text{NO}_5\text{P}$

Citruline = $\text{C}_6\text{H}_{13}\text{N}_3\text{O}_3$

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 v_1 to $v_{5,-1}$ (represented by the columns) except v_2 . 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 v_1 - v_4 . However, there will be separate fluxes for the forwards and backwards reactions for v_5 .

v_1 (6.3.4.5): The cleavage of ATP probably added the energy for this reaction to occur, and is how the product got phosphorylated (energized for future reactions.)

- (a) ATP substrate (enters) (rate b_5 , $\text{C}_{10}\text{H}_{16}\text{N}_5\text{O}_{13}\text{P}_3$),
- (b) ADP product (leaves) ($\text{C}_{10}\text{H}_{16}\text{N}_5\text{O}_{13}\text{P}_3$, rate b_6),
- (c) diphosphate product (leaves) (rate b_7 , $\text{H}_4\text{P}_2\text{O}_7$);

v2 (4.3.2.1): no extra substrates or products

v3 (3.5.3.1): Arginine was probably cleaved into urea and ornithine 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 stoichiometric 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 v_{5,1} and v_{5,-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, $b_i \geq -10 \text{ mmol}/(\text{gDW} \cdot \text{hr})$ if $i = \{8, 10, 11, 12, 13, 14\}$

Upper Bounds: (both APPROACH 1 and APPROACH 2)

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

$$v_{max} = k_{cat} * \frac{e_j}{e} * \theta * \sum_i^N \frac{x_i}{K_m + x_i} \quad (6)$$

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

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

and theta is the control element

$$\theta = \theta_{max} = 1. \quad (8)$$

- I could not find the concentrations of Arginosuccinate, Fumarate, Urea, Ornithine, and Carbamoyl 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, ($x_i \gg K_m$), you go from the original saturation term equation:

$$\frac{x_i}{K_m + x_i} \quad (9)$$

to

$$\frac{x_i}{x_i} = 1 \quad (10)$$

All bounds are defined in "upper_bound_math.jl". The flux function is used with the objective of maximizing b_4 (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 $b_4 = 0.26$ $\mu\text{mol/gDW-s}$
- (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 $\mu\text{mol/gDW-s}$ (the order is $v_1 - v_4$, $v_{5,1}$, $v_{5,-1}$, $b_1 - b_4$)
- (d) print "calculated_flux_array_converted" : all the fluxes are listed in mmol/gDW-hr (the order is $v_1 - v_4$, $v_{5,1}$, $v_{5,-1}$, $b_1 - b_4$)

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

$$S_{balanced,1} * v_{calculated_flux_array1} = \mathbf{0} \quad (11)$$

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 $\mu\text{mol/gDW-s}$). 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 $b_4 = 0.26$ $\mu\text{mol/gDW-s}$
- (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 $\mu\text{mol/gDW-s}$ (the order is $v_1 - v_4$, $v_{5,1}$, $v_{5,-1}$, $b_1 - b_4$)
- (d) print "calculated_flux_array_converted2" : all the fluxes are listed in mmol/gDW-hr (the order is $v_1 - v_4$, $v_{5,1}$, $v_{5,-1}$, $b_1 - b_4$)

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

$$S_{balanced,2} * v_{calculated_flux_array2} = \mathbf{0} \quad (12)$$

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

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.