# PS 3 Solution

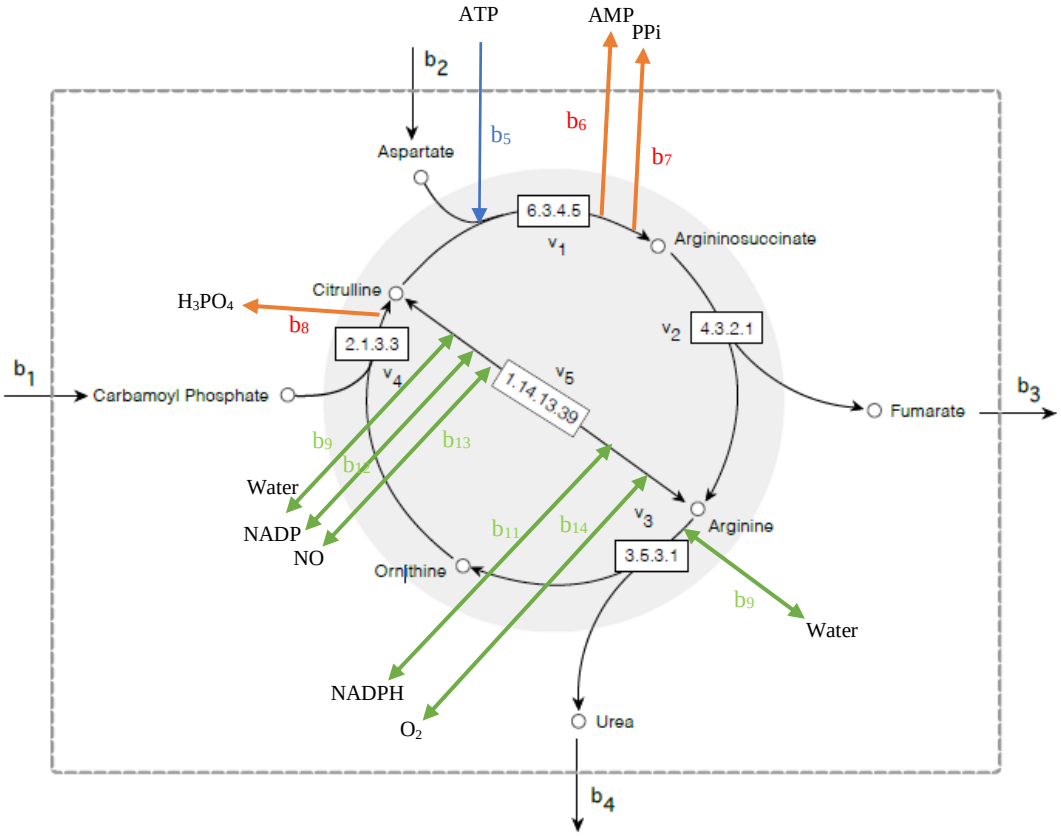


**Figure 1:** Schematic of the Urea cycle.

Solution 1 (a)

- Table 1 Metabolite ID

| # | Metabolite | Symbol | # | Metabolite | Symbol |
|---|---|---|---|---|---|
| M.1 | Arginine (arg) | $C_6H_{14}N_4O_2$ | M.10 | ATP | $C_{10}H_{16}N_5O_{13}P_3$ |
| M.2 | Ornithine (orn) | $C_5H_{12}N_2O_2$ | M.11 | AMP | $C_{10}H_{14}N_5O_7P$ |
| M.3 | Urea (u) | $CH_4N_2O$ | M.12 | Diphosphate (PPi) | $H_4PO_7$ |
| M.4 | Carbamoyl Phosphate (camp) | $CH_4NO_5P$ | M.13 | Orthophosphate | $H_3PO_4$ |
| M.5 | L-aspartate (asp) | $C_4H_7NO_4$ | M.14 | NADPH | $C_{21}H_{30}N_7O_{17}P_3$ |
| M.6 | Citrulline (cit) | $C_6H_{13}N_3O_3$ | M.15 | NADP | $C_{21}H_{29}N_7O_{17}P_3$ |
| M.7 | Arginine succinate (arg_s) | $C_{10}H_{18}N_4O_6$ | M.16 | Oxygen | $O_2$ |
| M.8 | Fumarate (fum) | $C_4H_4O_4$ | M.17 | Nitric Oxide | NO |
| M.9 | Water | $H_2O$ | M.18 | $H^+$ | H |

- Reactions involved in the urea cycle (Source: KEGG)

**E.C. 6.3.4.5**

$C_4H_7NO_4 + C_6H_{13}N_3O_3 + C_{10}H_{16}N_5O_{13}P_3 ===> C_{10}H_{18}N_4O_6 + C_{10}H_{14}N_5O_7P + H_4PO_7$

asp + cit + ATP ===> arg_s + AMP + PPi

**E.C. 4.3.2.1**

$C_{10}H_{18}N_4O_6 ===> C_6H_{14}N_4O_2 + C_4H_4O_4$

arg_s ===> arg + fum

**E.C. 3.5.3.1**

$C_6H_{14}N_4O_2 + H_2O ===> C_5H_{12}N_2O_2 + CH_4N_2O$

Arg + water ==> orn + urea

**E.C. 2.1.3.3**

$C_5H_{12}N_2O_2 + CH_4NO_5P ===> C_6H_{13}N_3O_3 + H_3PO_4$

orn + camp ===> cit + orthophosphate

**E.C. 1.14.13.39**

$2\ C_6H_{14}N_4O_2 + 3\ C_{21}H_{30}N_7O_{17}P_3 + 3\ H^+ + 4\ O_2 <===> 2\ C_6H_{13}N_3O_3 + 2\ NO + 3\ C_{21}H_{29}N_7O_{17}P_3 + 4\ H_2O$

2*Arg + 3*NADPH + 3*H + 4*oxygen <==> 2* cit + 2*NO + 3*NADP + 4*$H_2O$

- Table 2 Reaction ID

| # | **Reaction** | # | **Reaction** |
|---|---|---|---|
| $v_1$ | *e.c.6.3.4.5* asp-->arg_s | $b_5$ | [ ]-->ATP |
| $v_2$ | *e.c.4.3.2.1* arg_s-->arg | $b_6$ | AMP-->[ ] |
| $v_3$ | *e.c.3.5.3.1* arg-->orn | $b_7$ | PPi-->[ ] |
| $v_4$ | *e.c.2.1.3.3* orn-->cit | $b_8$ | $H_3PO_4$-->[ ] |
| $v_5$ | *e.c.1.14.13.39* arg-->cit | $b_9$ | [ ]<-->$H_2O$ |
| $v_6$ | rev. $v_5$ *e.c.1.14.13.39* cit-->arg | $b_{10}$ | NADPH<-->[ ] |
| $b_1$ | [ ]-->camp | $b_{11}$ | H<-->[ ] |
| $b_2$ | [ ]-->asp | $b_{12}$ | NADP<-->[ ] |
| $b_3$ | fum-->[ ] | $b_{13}$ | NO<-->[ ] |
| $b_4$ | u--> [ ] | $b_{14}$ | $O_2$<-->[ ] |

**(answer)** Table 3 **Stoichiometric Matrix, S**

| | $v_1$ | $v_2$ | $v_3$ | $v_4$ | $v_5$ | $v_6$ | $b_1$ | $b_2$ | $b_3$ | $b_4$ | $b_5$ | $b_6$ | $b_7$ | $b_8$ | $b_9$ | $b_{10}$ | $b_{11}$ | $b_{12}$ | $b_{13}$ | $b_{14}$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **M.1** | 0 | 1 | -1 | 0 | -2 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| **M.2** | 0 | 0 | 1 | -1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| **M.3** | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | -1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| **M.4** | 0 | 0 | 0 | -1 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| **M.5** | -1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| **M.6** | -1 | 0 | 0 | 1 | 2 | -2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| **M.7** | 1 | -1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| **M.8** | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | -1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| **M.9** | 0 | 0 | -1 | 0 | 4 | -4 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 |
| **M.10** | -1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| **M.11** | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | -1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| **M.12** | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | -1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| **M.13** | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | -1 | 0 | 0 | 0 | 0 | 0 | 0 |
| **M.14** | 0 | 0 | 0 | 0 | -3 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | -1 | 0 | 0 | 0 | 0 |
| **M.15** | 0 | 0 | 0 | 0 | 3 | -3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 |
| **M.16** | 0 | 0 | 0 | 0 | -4 | 4 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | -1 |
| **M.17** | 0 | 0 | 0 | 0 | 2 | -2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 |
| **M.18** | 0 | 0 | 0 | 0 | -3 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | -1 | 0 | 0 | 0 |

(Convention for $v_5$ reversibility has been assumed to be: citrulline ===> arginine)

Solution 1 (b)

**(answer)** Table 4 **Atom Matrix, A**

| | M.1 | M.2 | M.3 | M.4 | M.5 | M.6 | M.7 | M.8 | M.9 | M.10 | M.11 | M.12 | M.13 | M.14 | M.15 | M.16 | M.17 | M.18 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **C** | 6 | 5 | 1 | 1 | 4 | 6 | 10 | 4 | 0 | 10 | 10 | 0 | 0 | 21 | 21 | 0 | 0 | 0 |
| **H** | 14 | 12 | 4 | 4 | 7 | 13 | 18 | 4 | 2 | 16 | 14 | 4 | 3 | 30 | 29 | 0 | 0 | 1 |
| **N** | 4 | 2 | 2 | 1 | 1 | 3 | 4 | 0 | 0 | 5 | 5 | 0 | 0 | 7 | 7 | 0 | 1 | 0 |
| **O** | 2 | 2 | 1 | 5 | 4 | 3 | 6 | 4 | 1 | 13 | 7 | 7 | 4 | 17 | 17 | 2 | 1 | 0 |
| **P** | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 3 | 1 | 2 | 1 | 3 | 3 | 0 | 0 | 0 |

I have used the atom matrix for this part and the **epsilon = A*S** matrix can be generated by running the ("**S_and_epsillon_generator.jl**") file. Here, S is the stoichiometric matrix for reactions in the urea cycle only ($v_1 - v_6$)

- in julia console run include("a,b S_and_epsillon_generator.jl")

This will generate “atom_matrix.csv” and “epsilon.txt” files.

If the urea cycle reconstruction is elementally balanced, all components of the epsilon matrix should be zero, which is true in this case. (view “epsilon.txt”)

If all elemental matrix components were not zero, then we would have to check the stoichiometry for the reactions corresponding to the non-zero columns in the matrix.

Solution 1 (c)

The code for solving the FBA problem is given in “c run.jl”

- Upper bounds for the fluxes were calculated using:

$$v_{max,i} = k_{cat,i} \times E \times \prod_{i=1}^{n} \frac{C_i}{K_m + C_i}$$

- $K_m$ values and metabolite concentration , $C_i$ were obtained from Park et al Nat Chem Biol 12:482-9, 2016 and used to calculate:

For these values, the following order of preference was followed:

$$Homo\ sapiens > Mus\ musculus > other\ organism$$

Data for S. cerevisiae was used for the enzyme E.C.2.1.3.3

In the absence of enzyme and substrate specific data, it was assumed $C_i >> K_m$, which effectively gives $\frac{C_i}{K_m + C_i} = 1$

- **in julia console run** include("c run.jl")

*Note*: I have assumed the metabolites involved in reaction $v_5$ can be exchanged through the boundary (so there is a net positive flux in arg ==> cit direction [Table5]).

Deactivate lines 25, 26 and activate lines 29 and 30 in the "c run.jl" file to see optimum fluxes with **no boundary exchange of reaction $v_5$ metabolites** (in this case flux $v_5 = v_6 = 0.0$ mmol/gDW.hr to maximize urea)

**(answer)** Table 5 Optimum fluxes for maximum urea production

| Flux ID | Reaction | LB | UB | Optimum value (mmol/gdW.hr) |
|---|---|---|---|---|
| $v_1$ | *e.c.6.3.4.5* asp-->arg_s | 0 | $v_{max1}$ | 1.242 |
| $v_2$ | *e.c.4.3.2.1* arg_s-->arg | 0 | $v_{max2}$ | 1.242 |
| $v_3$ | *e.c.3.5.3.1* arg-->orn | 0 | $v_{max3}$ | 1.271095 |
| $v_4$ | *e.c.2.1.3.3* orn-->cit | 0 | $v_{max4}$ | 1.271095 |
| $v_5$ | *e.c.1.14.13.39* arg-->cit | 0 | $v_{max5}$ | 0.478652 |
| $v_6$ | rev. $v_5$ *e.c.1.14.13.39* cit-->arg | 0 | $v_{max6}$ | 0.4932 |
| $b_1$ | [ ]-->camp | 0 | 10 | 1.271095 |
| $b_2$ | [ ]-->asp | 0 | 10 | 1.242 |
| $b_3$ | fum-->[ ] | 0 | 10 | 1.242 |
| **$b_4$** | **u--> [ ]** | **0** | **10** | **1.271095** |
| $b_5$ | [ ]-->ATP | 0 | 10 | 1.242 |
| $b_6$ | AMP-->[ ] | 0 | 10 | 1.242 |
| $b_7$ | PPi-->[ ] | 0 | 10 | 1.242 |
| $b_8$ | $H_3PO_4$-->[ ] | 0 | 10 | 1.271095 |
| $b_9$ | [ ]<-->$H_2O$ | -10 | 10 | 1.329286 |
| $b_{10}$ | NADPH<-->[ ] | -10 | 10 | 0.043643 |
| $b_{11}$ | H<-->[ ] | -10 | 10 | 0.043643 |
| $b_{12}$ | NADP<-->[ ] | -10 | 10 | 0.043643 |
| $b_{13}$ | NO<-->[ ] | -10 | 10 | 0.029095 |
| $b_{14}$ | $O_2$<-->[ ] | -10 | 10 | 0.05819 |