# PS 3 Solution

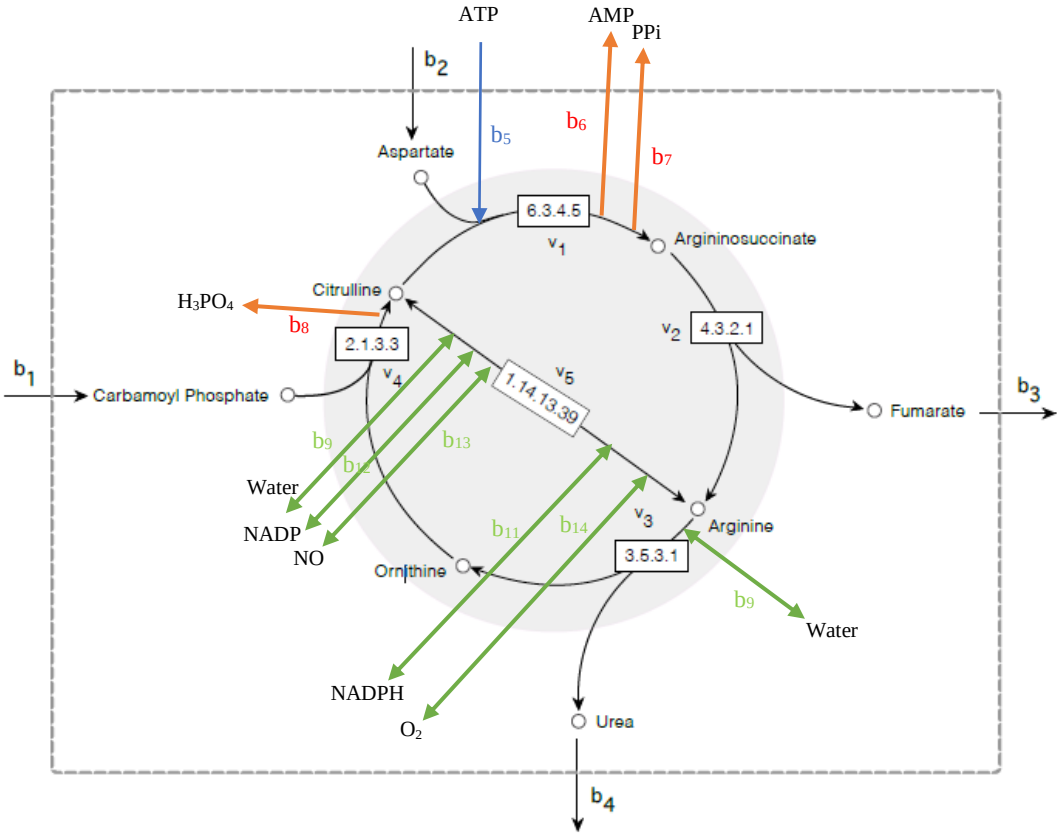


**Figure 1:** Schematic of the Urea cycle.

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)

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.

- 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”)

Solution 1 (c)

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

- Upper bounds for the fluxes were calculated using:

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

- $K_m$ 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 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 |