CHEME 7770: Problem Set 3

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1. The Urea cycle involves different compounds. For simplicity, all required compounds are summarized in Table 1, which contains all abbreviations used hereafter and the chemical formula of each reagent.

Reagent	**Abbreviation**	**Chemical Composition**
Citruline	C	$C_6H_{13}N_3O_3$
Orthophosphate	Ort	H_3PO_4
Ornithine	O	$C_5H_{12}N_2O_2$
Carbamoyl Phosphate	CP	CH_4NO_5P
Adenosin Triphosphate	ATP	$C_{10}H_{14}N_5O_{13}P_3$
Aspartate	A	$C_4H_7NO_4$
Adenosin Monophosphate	AMP	$C_{10}H_{14}N_5O_7P$
Diphosphate	PPi	$H_4P_2O_7$
Arginino succinate	Arsu	$C_{10}H_{18}N_4O_6$
Fumarate	F	$C_4H_4O_4$
Arginine	Arg	$C_6H_{14}N_4O_2$
Urea	U	CH_4N_2O
Water	H_2O	H_2O
Ammonia	NH_3	NH_3

Table 1: Summary of reagents involve in the Urea Cycle. All abbreviations used in the text are according to the information displayed in this table

According to the information found in KEGG, the following reactions are involved in the Urea cycle. Enzymes involved in the cycle are notated as v_i, whereas enzymes involved in source/sinks are notated as b_i.

$$\mathrm{CP} + \mathrm{O} \xrightarrow{\mathrm{v4}} \mathrm{C} + \mathrm{Ort} \tag{1}$$

$$\mathrm{ATP} + \mathrm{C} + \mathrm{A} \xrightarrow{\mathrm{v1}} \mathrm{AMP} + \mathrm{PPi} + \mathrm{Arsu} \tag{2}$$

$$\mathrm{Arsu} \xrightarrow{\mathrm{v2}} \mathrm{F} + \mathrm{Arg} \tag{3}$$

$$\mathrm{Arg} + \mathrm{H_2O} \xrightarrow{\mathrm{v3}} \mathrm{O} + \mathrm{U} \tag{4}$$

$$\mathrm{C} \xrightleftharpoons{\mathrm{v5}} \mathrm{Arg} \tag{5}$$

$$\mathrm{Source} \xrightarrow{\mathrm{b_1}} \mathrm{CP} \tag{6}$$

$$\text{Source} \xrightarrow{\text{b}_2} \text{A} \tag{7}$$

$$\text{Source} \xrightarrow{\text{b}_5} \text{ATP} \tag{8}$$

$$\text{Source} \xrightarrow{\text{b}_6} \text{H}_2\text{O} \tag{9}$$

$$\text{F} \xrightarrow{\text{b}_3} \text{sink} \tag{10}$$

$$\text{U} \xrightarrow{\text{b}_4} \text{sink} \tag{11}$$

$$\text{Ort} \xrightarrow{\text{b}_7} \text{sink} \tag{12}$$

$$\text{AMP} \xrightarrow{\text{b}_8} \text{sink} \tag{13}$$

$$\text{PPi} \xrightarrow{\text{b}_9} \text{sink} \tag{14}$$

Which gives us a total of 14 reactions with 13 metabolites. Note that equation 5 was not found in KEGG, so we might need to change it a bit after reviewing the balances of species. From this set of equations, the following stochiometric matrix can be derived. In the matrix, columns correspond to v1, v2, v3, v4, v5, b1, b2, b3, b4, b5, b6, b7, b8 and b9, and rows correspond to the metabolites in the same order that are listed in Table 1. Note that NH_3 is not included.

$$\mathbf{S} = \begin{bmatrix} -1 & 0 & 0 & 1 & -1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & -1 & 0 & 0 \\ 0 & 0 & 1 & -1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & -1 & 0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ -1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 & 0 & 0 \\ -1 & 0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & -1 & 0 \\ 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & -1 \\ 1 & -1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 1 & 0 & 0 & 0 & 0 & 0 & -1 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 1 & -1 & 0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 & -1 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & -1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 & 0 \end{bmatrix} \tag{15}$$

2. Before computing the system, it is necessary to check that all the elements are balanced, so that no matter is being created or destroyed. To do so, one can create a matrix with the elements present in each compound, to check which reactions are unbalanced. This matrix would look as follows, where each column corresponds to the metabolites (in the same order as the stochiometric matrix) and each row to C, H, N, O and P.

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

When multiplying **A** with **S**, the only unbalanced reaction is that governed by enzyme v_5, which corresponds to equation 5. Chemically, this reaction is given by:

$$C_6H_{13}N_3O_3 \xrightleftharpoons{\text{v5}} C_6H_{14}N_4O_2 \tag{17}$$

Note that adding a molecule of ammonia to the reagents will produce a molecule of water, which will balance the equation as follows:

$$C_6H_{13}N_3O_3 + NH_3 \xrightleftharpoons{\text{v5}} C_6H_{14}N_4O_2 + H_2O \tag{18}$$

We can now change equation 9 to be a source of ammonia instead of a source of water and add an extra metabolite to our stochiometric array. Adding ammonia as the last row and fixing the water balance yields the following balanced stochiometric array:

$$\mathbf{S} = \begin{bmatrix}
-1 & 0 & 0 & 1 & -1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & -1 & 0 & 0 \\
0 & 0 & 1 & -1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & -1 & 0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
-1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 & 0 & 0 \\
-1 & 0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & -1 & 0 \\
1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & -1 \\
1 & -1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 1 & 0 & 0 & 0 & 0 & 0 & -1 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 1 & -1 & 0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 & -1 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & -1 & 0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & -1 & 0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 & 0
\end{bmatrix} \tag{19}$$

3. The balanced stochiometric matrix can be used to solve a FBA problem as a linear programming problem to find the optimal flux that maximizes the production of Urea. To do so, we need to define the upper and lower boundaries of each flux. This can be obtained by multiplying the concentration of the enzyme that catalyzes the reaction with its k_{cat}. This will provide an upper value for the flux. However, this value can be corrected by multiplying it with a corrector factor (between 0 and 1) given by the following equation:

$$\alpha = \frac{m_i}{K_m + m_i} \tag{20}$$

Where m_i represents the concentration of metabolite i in the flux. K_m and m_i values were obtained from BRENDA. The upper bounds and lower bounds used in this problem set are summarized in Table 2.

The Julia code *Flux.jl* was used to solve the FBA problem by indicating it to maximize the flux of Urea, which is given by b_4. The code is attached at the end of this document. The maximum rate of Urea production was found to be $45.65 \mu mol/gDWh$.

Enzyme	Lower Bound [mmol/gDW s]	Upper Bound [mmol/gDW s]
v1	0	0.00203
v2	0	0.000345
v3	0	0.00249
v4	0	0.000881
v5	-0.0000127	0.0000127
b1	0	0.00277
b2	0	0.00166
b3	0	0.000233
b4	0	0.00277
b5	0	0.00276
b6	0	0.00277
b7	0	0.00277
b8	0	0.00109
b9	0	0.00277

Table 2: Lower and upper bounds used during the simulation of the FBA problem. Each enzyme corresponds to the reactions shown in equations 1-14

1. Appendix A: Julia Code

```
        #Script for PS3-S19

#Magic function
include("Flux.jl");

#Plot plot plot
using PyPlot

#Parameters
E = 0.01/1000; #steady-state enzyme concentration [mmol/gDW]
b1 = 10/3600; #Carbanoyl Phosphate input [mmol/gDW s]
b2 = (10/3600)*((1.49*10^(-2))/(1.49*10^(-2)+1*10^(-2))); #Aspartate input [mmol/gDW s]
b3 = (10/3600)*((4.85*10^(-4))/(4.85*10^(-4)+5.3*10^(-3))); #Fumarate output [mmol/gDW s]
b4 = 10/3600; #Urea output [mmol/gDW s]
b5 = (10/3600)*((4.67*10^(-3))/(4.67*10^(-3)+3*10^(-5))); #ATP input [mmol/gDW s]
b6 = 10/3600; #Ammonia input [mmol/gDW s]
b7 = 10/3600; #Orthophosphate output [mmol/gDW s]
b8 = (10/3600)*((4.23*10^(-5))/(4.23*10^(-5)+6.46*10^(-5))); #AMP output [mmol/gDW s]
b9 = 10/3600; #Diphosphate output [mmol/gDW s]
v1 = 203*E; #6.3.4.5 [mmol/gDW s]
v2 = 34.5*E; #4.3.2.1 [mmol/gDW s]
v3 = 249*E; #3.5.3.1 [mmol/gDW s]
v4 = 88.1*E; #2.1.3.3 [mmol/gDW s]
v5 = (13.7*E)*((2.55*10^(-4))/(2.55*10^(-4)+2.5*10^(-3))); #1.14.13.39 [mmol/gDW s]
```

```
td = 20*3600; #Doubling time [mmol/gDW s]

#Stochiometric matrix.
#Columns: v1,v2,v3,v4,v5,b1,b2,b3,b4,b5,b6,b7,b8,b9
#rows: C,Ort,O,CP,ATP,A,AMP,PPi,Arsu,F,Arg,U,H2O,NH3
stoichiometric_matrix = [[-1.0 0 0 1 -1 0 0 0 0 0 0 0 0 0];
                         [0 0 0 1 0 0 0 0 0 0 0 -1 0 0];
                         [0 0 1 -1 0 0 0 0 0 0 0 0 0 0];
                         [0 0 0 -1 0 1 0 0 0 0 0 0 0 0];
                         [-1 0 0 0 0 0 0 0 0 1 0 0 0 0];
                         [-1 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 -1 0];
                         [1 0 0 0 0 0 0 0 0 0 0 0 0 -1];
                         [1 -1 0 0 0 0 0 0 0 0 0 0 0 0];
                         [0 1 0 0 0 0 0 -1 0 0 0 0 0 0];
                         [0 1 -1 0 1 0 0 0 0 0 0 0 0 0];
                         [0 0 1 0 0 0 0 0 -1 0 0 0 0 0];
                         [0 0 -1 0 1 0 0 0 0 0 0 0 0 0];
                         [0 0 0 0 -1 0 0 0 0 0 1 0 0 0]];


Lb = zeros(Int8, 8, 1); #Lower bound array
Ub = [10;10;10;10;10;10;10;10]; #Upper bound array

#Bounds array
default_bounds_array = [[0 v1];
                        [0 v2];
                        [0 v3];
                        [0 v4];
                        [-v5 v5];
                        [0 b1];
                        [0 b2];
                        [0 b3];
                        [0 b4];
                        [0 b5];
                        [0 b6];
                        [0 b7];
                        [0 b8];
                        [0 b9]];
#Species bounds array
species_bounds_array = zeros(Float64, 14, 2);
#Objective array
objective_coefficient_array = [0.0; 0; 0; 0; 0; 0; 0; 0; -1; 0; 0; 0; 0; 0]; #Change -1 to ma

#Flux calculation
flux, n1, n2, n3, n4, n5 = calculate_optimal_flux_distribution(stoichiometric_matrix,default_
flux*3600*1000 #Flux in [umol/gDW h]
```