PROBLEM SET 3

PART A

Based on the reactions found on KEGG a stoichiometric matrix was formed using all the species contained in the reactions as well as boundary transport species. The fifth reaction EC# 1.14.13.39 was composed of a multi-step reaction with an intermediate that is completely consumed. The step reactions are RN R00558 & RN R00111. Based on what was provided the reaction is not balanced, to balance it we simply need two R00558 reactions for each R00111. Adding the reaction steps together then recovers the accurate RN 00557 described in EC 1.14.13.39. The full STOIC matrix is shown below along with the corrected matrix that does not include the intermediate species.

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  |  | **R01954** | **R01086** | **R00551** | **R01398** | **R00557** | **R00557** | **R00558** | **R00558** | **R00111** | ***Carbamoyl Phosphate*** | ***Aspartate*** | ***Fumarate*** | ***Urea*** | ***ATP*** | ***AMP*** | ***Diphosphate*** | ***Orthophosphate*** | ***O2*** | ***NADPH*** | ***H+*** | ***NO*** | **NADP+** | ***H2O*** | ***H2O*** |
|  |  | **v1** | **v2** | **v3** | **v4** | **v5\_for** | **v5\_rev** | **v5\_1\_for** | **v5\_1\_for\_adj** | **v5\_2\_for** | **b1** | **b2** | **b3** | **b4** | **b5** | **b6** | **b7** | **b8** | **b9** | **b10** | **b11** | **b12** | **b13** | **b14\_for** | **b14\_rev** |
| **1** | **Carbamoyl Phosphate** | 0 | 0 | 0 | -1 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| **2** | **Citrulline** | -1 | 0 | 0 | 1 | 2 | -2 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| **3** | **Aspartate** | -1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| **4** | **Arginosuccinate** | 1 | -1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| **5** | **Fumarate** | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | -1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| **6** | **Arginine** | 0 | 1 | -1 | 0 | -2 | 2 | -1 | -2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| **7** | **Orinithine** | 0 | 0 | 1 | -1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| **8** | **ATP** | -1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| **9** | **Urea** | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | -1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| **10** | **Orthophosphate** | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | -1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| **11** | **AMP** | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | -1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| **12** | **Diphosphate** | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | -1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| **13** | **NO** | 0 | 0 | 0 | 0 | 2 | -2 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | -1 | 0 | 0 | 0 |
| **14** | **O2** | 0 | 0 | 0 | 0 | -4 | 4 | -1 | -2 | -2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| **15** | **H+** | 0 | 0 | 0 | 0 | -3 | 3 | -1 | -2 | -1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 |
| **16** | **NADPH** | 0 | 0 | 0 | 0 | -3 | 3 | -1 | -2 | -1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 |
| **17** | **NADP+** | 0 | 0 | 0 | 0 | 3 | -3 | 1 | 2 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | -1 | 0 | 0 |
| **18** | **H2O** | 0 | 0 | -1 | 0 | 4 | -4 | 1 | 2 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | -1 | 1 |
| **19** | **Hydroxyarginine** | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 2 | -2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  |  | **R01954** | **R01086** | **R00551** | **R01398** | **R00557** | **R00557** | ***Carbamoyl Phosphate*** | ***Aspartate*** | ***Fumarate*** | ***Urea*** | ***ATP*** | ***AMP*** | ***Diphosphate*** | ***Orthophosphate*** | ***O2*** | ***NADPH*** | ***H+*** | ***NO*** | **NADP+** | ***H2O*** | ***H2O*** |
|  |  | **v1** | **v2** | **v3** | **v4** | **v5\_for** | **v5\_rev** | **b1** | **b2** | **b3** | **b4** | **b5** | **b6** | **b7** | **b8** | **b9** | **b10** | **b11** | **b12** | **b13** | **b14\_for** | **b14\_rev** |
| **1** | **Carbamoyl Phosphate** | 0 | 0 | 0 | -1 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| **2** | **Citrulline** | -1 | 0 | 0 | 1 | 2 | -2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| **3** | **Aspartate** | -1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| **4** | **Arginosuccinate** | 1 | -1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| **5** | **Fumarate** | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | -1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| **6** | **Arginine** | 0 | 1 | -1 | 0 | -2 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| **7** | **Orinithine** | 0 | 0 | 1 | -1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| **8** | **ATP** | -1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| **9** | **Urea** | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | -1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| **10** | **Orthophosphate** | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | -1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| **11** | **AMP** | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | -1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| **12** | **Diphosphate** | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | -1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| **13** | **NO** | 0 | 0 | 0 | 0 | 2 | -2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | -1 | 0 | 0 | 0 |
| **14** | **O2** | 0 | 0 | 0 | 0 | -4 | 4 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| **15** | **H+** | 0 | 0 | 0 | 0 | -3 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 |
| **16** | **NADPH** | 0 | 0 | 0 | 0 | -3 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 |
| **17** | **NADP+** | 0 | 0 | 0 | 0 | 3 | -3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | -1 | 0 | 0 |
| **18** | **H2O** | 0 | 0 | -1 | 0 | 4 | -4 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | -1 | 1 |

PART B

To check to see the reactions are balanced we need to perform an atom balance of the metabolites. The table for constructing the ATOM matrix is below.

This is crossed with the STOIC matrix in the equation E = transpose(ATOM) X STOIC. If the values for the E are 0 then they are balanced. If not, they need to be adjusted. When running the matrix through my Julia program the resultant E was:

Reaction Set

[0 0 0 0 0 0; 0 0 0 0 0 0; 0 0 0 0 0 0; 0 0 0 0 0 0; 0 0 0 0 0 0; 0 0 0 0 0 0]

Transport Set

[1 4 -4 -1 10 -10 0 0 0 21 0 0 -21 0 0; 4 7 -4 -4 16 -14 -4 -3 0 30 1 0 -29 -2 2; 1 1 0 -2 5 -5 0 0 0 7 0 -1 -7 0 0; 5 4 -4 -1 13 -7 -7 -4 2 17 0 -1 -17 -1 1; 1 0 0 0 3 -1 -2 -1 0 3 0 0 -3 0 0; 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0]

Stoichiometric Matrix

[0 0 0 0 0 0 1 4 -4 -1 10 -10 0 0 0 21 0 0 -21 0 0; 0 0 0 0 0 0 4 7 -4 -4 16 -14 -4 -3

0 30 1 0 -29 -2 2; 0 0 0 0 0 0 1 1 0 -2 5 -5 0 0 0 7 0 -1 -7 0 0; 0 0 0 0 0 0 5 4 -4 -1 13 -7 -7 -4 2 17 0 -1 -17 -1 1; 0 0 0 0 0 0 1 0 0 0 3 -1 -2 -1 0 3 0 0 -3 0 0; 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0]

The values for the reaction set are 0 indicating that they are balanced. The transport values are no not though.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
|  |  | **C** | **H** | **N** | **O** | **P** | **S** |
| **1** | **Carbamoyl Phosphate** | 1 | 4 | 1 | 5 | 1 | 0 |
| **2** | **Citrulline** | 6 | 13 | 3 | 3 | 0 | 0 |
| **3** | **Aspartate** | 4 | 7 | 1 | 4 | 0 | 0 |
| **4** | **Arginosuccinate** | 10 | 18 | 4 | 6 | 0 | 0 |
| **5** | **Fumarate** | 4 | 4 | 0 | 4 | 0 | 0 |
| **6** | **Arginine** | 6 | 14 | 4 | 2 | 0 | 0 |
| **7** | **Orinithine** | 5 | 12 | 2 | 2 | 0 | 0 |
| **8** | **ATP** | 10 | 16 | 5 | 13 | 3 | 0 |
| **9** | **Urea** | 1 | 4 | 2 | 1 | 0 | 0 |
| **10** | **Orthophosphate** | 0 | 3 | 0 | 4 | 1 | 0 |
| **11** | **AMP** | 10 | 14 | 5 | 7 | 1 | 0 |
| **12** | **Diphosphate** | 0 | 4 | 0 | 7 | 2 | 0 |
| **13** | **NO** | 0 | 0 | 1 | 1 | 0 | 0 |
| **14** | **O2** | 0 | 0 | 0 | 2 | 0 | 0 |
| **15** | **H+** | 0 | 1 | 0 | 0 | 0 | 0 |
| **16** | **NADPH** | 21 | 30 | 7 | 17 | 3 | 0 |
| **17** | **NADP+** | 21 | 29 | 7 | 17 | 3 | 0 |
| **18** | **H2O** | 0 | 2 | 0 | 1 | 0 | 0 |

PART C

The optimization of the Urea flux was completed using a Julia code that references the Flux.jl code provided by the Varner lab which was then modified to solve the problem in our conditions. The output of the Solve.jl script is below. The max flux of Urea was 1.242 mmol/gDW-hr.

(-1.242, [1.242, 1.242, 1.242, 1.242, 0.0, 0.0, 1.242, 1.242, 1.242, 1.242 … 1.242, 1.242, 1.242, 0.0, 0.0, 0.0, 0.0, 0.0, 0.0, 1.242], [0.0, -1.0, 0.0, 0.0, 0.0, 0.0, 0.0, 0.0, 0.0, 0.0 … 0.0, 0.0, 0.0, 0.5, 0.0, 0.0, 0.0, 0.0, 0.0, 0.0], [0.0, 0.0, 0.0, 0.0, 0.0, 0.0, 0.0, 0.0, 0.0, 0.0, 0.0, 0.0, 0.0, 0.0, 0.0, 0.0, 0.0, 0.0], 0, 5)