**Homework Assignment on Metabolic Flux Models**

Cells convert nutrients such as glucose and oxygen into stored energy and basic cellular building blocks such as amino and nucleic acids through a complex network of metabolic reactions. The stoichiometry of each reaction is often known from basic biochemistry. Steady-state mass balances on each intracellular species (reactants and products of these reactions) yield a linear

algebraic system **Ax** = **b** where **A** is a known matrix of stoichiometric coefficients, **x** is an unknown vector of reaction rates (also known as fluxes) and membrane transport rates for nutrients and products, and **b** is a known vector of measured transport rates. Each row of the matrix **A** corresponds to an intracellular species and each column corresponds to a particular reaction between these species. Solution of the stoichiometric model yields the unknown fluxes.

The stoichiometric model used in this assignment accounts for primary metabolism in a yeast cell. The model was originally published in a research paper that is available on the course webpage. The dimension of the **A** matrix in the published model is 21x19, but this model assumes some known reaction rates. We have added some additional fluxes to this model to represent unknown rates for O2, CO2 and ethanol transport as well as biomass growth. With only the glucose transport rate specified, the dimension of the **A** matrix becomes 21x 23 with the last four columns corresponding to CO2 transport, biomass growth, O2 transport and ethanol transport. The stoichiometric matrix for this adjusted model is available on the course webpage.

A nonzero element in the **b** vector corresponds to a species with a measured (known) transport rate, while a zero element corresponds to a species that is not transported across the cell membrane or has an unknown transport rate. When a measurement of a previously unknown transport rate becomes available, the corresponding column is removed from the **A** matrix and the known rate is entered into the corresponding element of the **b** vector.

All calculations are based on a normalized glucose transport rate of 1. The **b** vector should be constructed such that **b**(10)=-1 to represent this uptake flux. Because the **A** matrix and **b** vector will be modified for each problem, we suggest that you save different versions denoted {**A**1,..., **A**5} and {**b**1,…, **b**5}. The **A**1 matrix is stored in a file available on the course webpage, A1.dat . After downloading and placing this matrix in the Matlab working directory, the matrix can be loaded into Matlab with the following command: load A1.dat . Make sure that you save all your variables in the Matlab workspace throughout the assignment to perform the analysis of your results for the last part. We recommend that you write Matlab scripts for solving the problems in this assignment.

1. Underdetermined stoichiometric model (21x23): no additional measurements.

* Check the rank of the stoichiometric matrix, **A**1.
* Compute the determinant of **A**1**A**1T and determine if **A**1**A**1T is invertible.
* Form the vector **b**1: b1=zeros(size(A1,1),1); b1(10)=-1.
* Compute **x**1. Are any of the fluxes in the solution negative? A disadvantage of this approach is that constraints such as **x** must be non-negative cannot be imposed. A linear programming formulation would be required to include such constraints on the solution.

2. Underdetermined stoichiometric model (21x22): measurement of CO2 added.

* Reformulate the **A** matrix and the **b** vector to account for the additional measurement, **b**CO2= 1.2. The last column of the **A** matrix needs to removed and the element of the **b** vector corresponding to CO2 needs to be changed from 0 to 12: A2=A1(:,1:end-1); b2=b1; b2(4)=1.2.
* Compute the rank of the new matrix A2.
* Compute the determinant of **A**2**A**2T.
* Compute **x**2.

3. Completely defined stoichiometric model (21x12): CO2 and biomass measured.

* Reformulate the **A** matrix and **b** vector based on the presence of biomass and CO2 measurements. The last two columns of the original **A** matrix should now be removed. The measured rates are bbiomass=b3(3)=0.16 and bCO2=b3(4)=1.2.
* Compute the rank of the **A**3 matrix.
* Compute the determinant of **A**3.
* Compute **x**3.

4. Overdetermined stoichiometric model (21x19): CO2, biomass, O2 and ethanol measured.

* Reformulate the **A** matrix and **b** vector accounting for the measurements. The last four columns of the original **A** matrix should be removed. The measured rates are bbiomass=b4(3)=0.16, bCO2=b4(4)=1.2, bO2=b4(15)=-0.35, and bETOH= b4(5)=0.65.
* Compute the rank of the **A**4 matrix.
* Compute the determinant of **A**4**A**4T.
* Compute **x**4.

5. Uncertainty in the measurements: A measurement covariance matrix **Q** is available on the course website. **Q** is a diagonal matrix of weights indicating the relative uncertainty of the measurements associated with each species. Consider the same **A** matrix and **b** vector from part 4: **A**5=**A**4 and **b**5=**b**4.

* Compute the determinant of **A**5T**Q**-1**A**5.
* Compute **x**5 = (**A**5T**Q**-1**A**5)-1**A**5T**Q**-1**b**5.

6. Analysis of the results.

* Create a bar graph of the first 19 fluxes in **x**2, **x**3 and **x**4:

bar([x2(1:19) x3(1:19) x4(1:19)]), and add a legend and labels for the axes. Identify the fluxes that exhibit significant differences.

* Compute the 2-norm of the difference between the first 19 fluxes for **x**2 and **x**3. **x**2 and **x**4, and **x**3 and **x**4. For example: norm(x2(1:19)-x3(1:19)).

7. Comprehension.

* The method used to resolve the fluxes of the underdetermined systems in parts 1 and 2 calculates a solution that minimizes the norm of the fluxes. Does such a strategy seem biologically plausible?
* Provide insight on the following statement: “The overdetermined system in part 4 was solved by determining fluxes that approximately satisfy the species balances in a least-squares sense. A problem with this method is that reaction stoichiometry may be violated.”
* What does the 2-norm you calculated for the difference in fluxes represent?