Assignment 1

Question 1: Experimental data from an enzyme kinetics experiment is provided in Kinetics.csv file. The file contains two substrate (S1 and S2) concentrations (in mM) and corresponding rates (in mM/s). (5 points)

1. Identify the underlying enzyme kinetics mechanism
   Hint: Use Chi-squared/R-squared measures
2. Make (a) Eadie-Hofstee and (b) Lineweaver-Burk plots for different S2 values of 1.5, 2.5 and 5 mM
3. Extract Michaelis constants (Km1, Km2) and Vmax from the Eadie-Hofstee plot

Question 2:

The Tricarboxylic Acid (TCA) cycle, also known as the Krebs cycle or citric acid cycle, is a central metabolic pathway that occurs in the mitochondria of cells. It plays a crucial role in cellular respiration by oxidizing acetyl-CoA derived from carbohydrates, fats, and proteins to produce energy in the form of ATP, as well as reducing agents like NADH and FADH2. The cycle involves a series of enzymatic reactions that convert acetyl-CoA into carbon dioxide and high-energy molecules, which are then used in the electron transport chain to generate ATP.

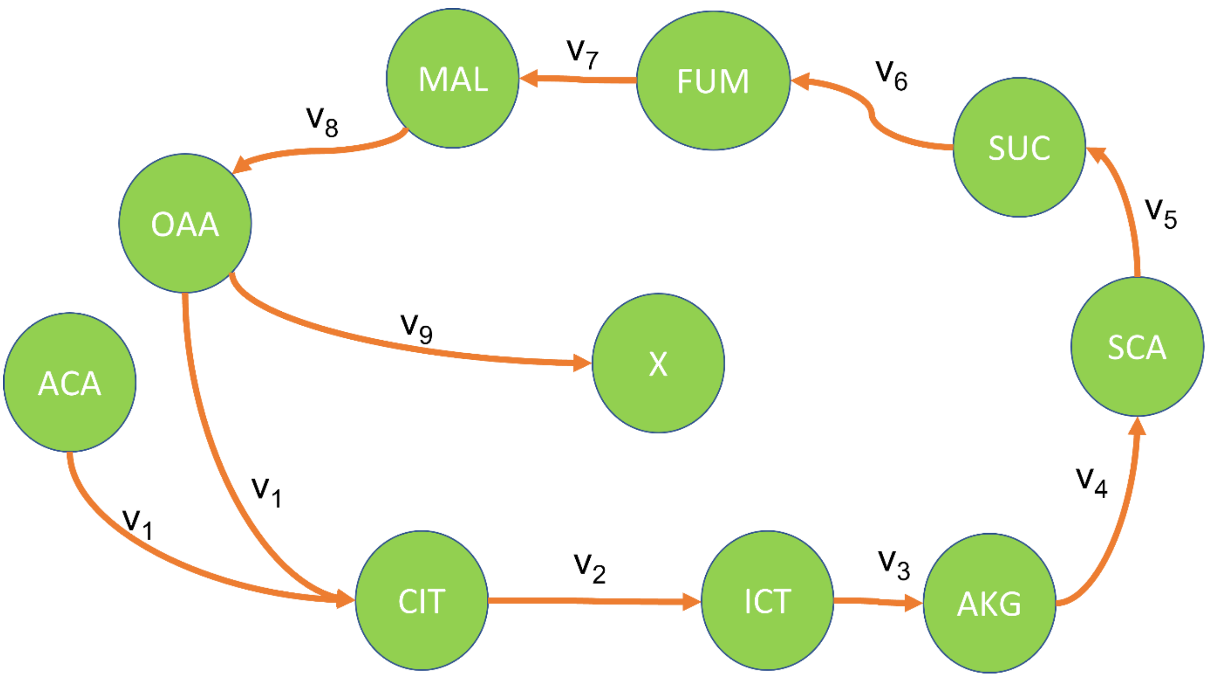

Figure 1: A simplified TCA reaction network

The TCA cycle involves several substrates, intracellular metabolites, and products. For simplicity, consider the following metabolites: Acetyl-CoA (ACA), Citrate (CIT), Isocitrate (ICT), Alpha-ketoglutarate (AKG), Succinyl-CoA (SCA), Succinate (SUC), Fumarate (FUM), Malate (MAL), Oxaloacetate (OAA), and biomass (X). The reaction network is shown in figure 1.

v_6 is reversible with a maximal enzyme capacity constraint $v_6 \leq v_{6,max}$.

Due to the experimental conditions, biomass conversion X, v_9 is fixed to $v_9 = D$.

Now assume that at least one of these reactions—say, the reversible interconversion v_6 between Succ and Fum—is catalyzed by an enzyme that follows Michaelis–Menten kinetics. Specifically, you are to model the rate v_6 with the following MM expression:

$$v_6 = (V_{max} \cdot [Succ]) / (K_m + [Succ]) - (V_{max} \cdot [Fum]) / (K_m + [Fum]),$$

where V_{max} and K_m are the enzyme's characteristic kinetic constants, and [Succ] and [Fum] indicate the concentrations of succinate and fumarate, respectively.

Answer the following (5 points):

a) Write down the stoichiometric matrix and steady-state mass balance equations for the intracellular metabolites Cit, IsoCit, aKG, SuccCoA, Succ, Fum, Mal, OAA, and X.

b) Replace $v_9 = D$ in the corresponding equation and, retaining v_1 and v_6 as independent variables, solve for the other fluxes in terms of v_1, v_6. Identify conditions for which biomass conversion X is possible

c) Incorporate the irreversibility constraints and the MM constraints (the rate equation for v_6 along with its maximal capacity $v_6 \leq v_{6,max}$), and derive the resulting inequality constraints.

Sketch the solution space by plotting v_6 versus v_1 using the derived inequalities and answer the questions (d) and (e) below,

d)Distinguish the two cases below on how they affect the feasible region in solution space:

(1) $v_{6,max} \geq D$

(2) $v_{6,max} < D$

e) Discuss how changes in V_{max} and K_m (from the MM kinetics of v_6) affect the feasible region.