Project 2: Enzyme kinetics and microbial growth

22.03.2024

Submission Deadline: Thursday, 11th of April, at 23:59.

Submission Format: Single PDF file.

Page Limit: Maximum of 9 pages per group.

Submission Platform: Upload to Moodle.

This project consists of 4 exercises.

In this report, we expect that you:

(i) Report the numerical answers and plots obtained to define the correct units!
(ii) Briefly explain how you obtained these answers.
(iii) Give an interpretation of the values/plots obtained.

You can use the template file found in Moodle.

Alongside your report, you must upload all related code files. This includes MATLAB files (either .m files or MATLAB notebook .mlx files) or Python files (.py files or Jupyter notebook files). The code should run by executing the script/main function without extra arguments or external data.

Organise your code files logically, dividing them into sections. Each section should be well-documented with comments to explain the code and the steps involved in your procedure.

EXERCISE 1

The kinetics of an enzyme were analyzed in both the absence and presence of Inhibitor A and Inhibitor B. Given the following data (substrate concentration [S], and reaction rate v), calculate or construct the following for inhibitor A and B on separate graphs:

a) Plot the data in the Lineweaver-Burk format (*Make sure to label both the inhibitor line and the no inhibitor line)
b) Determine the K_m and V_{max} values from Lineweaver-Burk plot.
c) What types of inhibitors are A and B? How can you tell? (*Use the equations in slide 6 of Lecture 1 and compare them to your results.)

	v (mM/min)		
[S] (mM)	No Inhibitor	Inhibitor A [A]=C_A	Inhibitor B [B]=C_B
0.2	5.0	3.0	2.0
0.4	7.5	5.0	3.0
0.8	10.0	7.5	4.0
1.0	10.7	8.3	4.3
2.0	12.5	10.7	5.0
4.0	13.6	12.5	5.5

This part of the question is independent of the previous parts.

d) The kinetics of an enzyme was analyzed in the presence of an Inhibitor I at different concentrations (I given in mM) and following data (substrate concentration [S], and reaction rate v) are recorded:

	v (mM/min)			
[S] (mM)	[I]= 1mM	[I]= 2mM	[I]= 3mM	[I]= 4mM
0.1	1.09	0.74	0.52	0.42
0.3	2.89	1.96	1.47	1.17
0.7	5.24	3.89	3.01	2.51
2.0	8.85	7.40	6.31	5.43
4.0	10.89	9.82	8.76	8.07
6.0	12.11	11.36	10.02	9.37

Plot the data in the Lineweaver-Burk format (indicate in the legend different I concentrations). From visual inspection of the reciprocal plot determine the type of the inhibitor I.

Given the inhibitor concentrations estimate the Inhibition constant K_I. (**Hint:** Think about plotting slope and/or intercept of the Lineweaver-Burk plots vs inhibitor concentration [I], what does the slope and the intercept of this new plot stand for?)

EXERCISE 2

Bibal et al. (1988, 1989) studied the inhibition of lactic acid on *Streptococcus cremoris*, and in this exercise we will analyse their data:

a) The influence of lactic acid on the growth of *S. cremoris* was examined by measuring the specific growth rate μ **under substrate saturation** during batch growth of the bacterium in media containing various concentrations of lactic acid (*p*):

$\boldsymbol{p(g\ L^{-1})}$	$\boldsymbol{\mu\ (h^{-1})}$
0.0	0.9
12.0	0.68
39.0	0.52
55.0	0.13

We assume that only the **un-dissociated acid (P)** can pass through the cell membrane and induce a toxic effect that affects the growth rate.

Plot the **relative specific growth rate, i.e.** $\boldsymbol{\mu(P)/\mu(P=0)}$**,** versus the concentration of the **un-dissociated acid (P)** concentration (in mM) assuming that lactate is a weak acid and the volume is constant. In the experiment pH = 6.3 was used, and the pK_a and molar mass for lactic acid are and 3.88 and 90.08 g/mol, respectively.

b) Assume that the kinetics follow the following equation:

$$\mu = \mu_{max}\frac{S}{K_M + S}\frac{1}{1+\frac{P}{K_I}}$$

Given the substrate saturation and the data provided explain why $\mu(P=0) = \mu_{max}$. Find the inhibition constant K_I . (Use the function polyfit). Plot the model together with the experimental data.

c) Calculate the parameter of the inhibition model given below (what is the model parameter?). Plot both models and the experimental data in one plot, then decide which inhibition model best describes the kinetics and explain why (Use a quantitative measure to justify your findings).

$$\mu = \mu_{max}\frac{S}{K_M + S}\left(1-\frac{P}{P_{max}}\right)$$

d) Plot the **relative specific growth rate** as a function of the pH in a medium containing 1 g L^{-1} and 10 g L^{-1} of lactic acid (total concentration), using the model you decided in c). For the two respective lactic acid concentrations what is the pH at which growth stops?

EXERCISE 3

Phosphoglycerate mutase (PGM, enzyme) is part of the lower glycolysis pathway and functions by reversibly transforming 3-phospho-D-glycerate (g3p) into 2-phospho-D-glycerate (g2p). Enzyme displays reversible Michaelis-Menten kinetics. Overall reaction scheme can be given as follows:

$$E + S \underset{k_{1,b}}{\overset{k_{1,f}}{\Leftrightarrow}} ES \underset{k_{2,b}}{\overset{k_{2,f}}{\Leftrightarrow}} E + P$$

a) Write the mass balance of the system, and simplify it to concentration balance (Reaction takes place in a constant volume reactor)

b) As we have already investigated in the first project, QSSA (Quasi Steady State Assumption) is a common approximation where we assume that the concentration of the enzyme complex (here ES) is time invariant for a given substrate concentration [S]. Use the mass balance with QSSA to find an expression for ES as a function of the elementary rate constants, the substrate concentration $[S]$, the initial enzyme concentration $[E_0]$. and the product concentration [P]. Using this expression to write the equation for dP/dt.
Hint: this is a reversible Michaelis-Menten, it is therefore different than the one you derived in the first project.

c) Typical form of the reaction rate v is derived from the QSSA assumption as a function of substrate [S] and product [P] concentration as follows:

$$v([S],[P]) = \frac{\frac{V_{max,f}[S]}{K_{m,S}} - \frac{V_{max,b}[P]}{K_{m,P}}}{1 + \frac{[S]}{K_{m,S}} + \frac{[P]}{K_{m,P}}}$$

Given the parameters $V_{max,f}$, $V_{max,b}$, $K_{m,S}$ and $K_{m,P}$, would you be able to determine all elementary rate constants $k_{1,f}$, $k_{1,b}$, $k_{2,f}$, $k_{2,b}$? If not, what kind of data would you need? Write the expressions for these parameters as a function of elementary rate constants using your answer from part b.
Hint: Try to find expressions for various functions of elementary rate constants (e.g. think of the units of $K_{m,S}$ and $K_{m,P}$ and try to formulate your result in part b according to this such as $\frac{k_{1,b}+k_{2,f}}{k_{2,b}}$ etc.)

d) Kinetic constants for this reaction were calculated in an enzyme assay by Frater *et al.* in 1999, and following constants are noted:

$$k_{1,b} = 10\ s^{-1}$$

$$k_{2,f} = 22\ s^{-1}$$

$$K_{m,S} = 210\mu M$$

$$K_{m,P} = 97\mu M$$

Using these constants, find all elementary rate constants and rewrite the mass balance of the system using the approximation for $[ES]$ derived in b). Solve this ODE system for the time interval [0,5] s. Consider as initial concentrations $[S_0] = 1\ \frac{mol}{L}$, $[E_0] = 0.1\ \frac{mol}{L}$, $[P] = 0\ \frac{mol}{L}$. Plot the product and substrate concentration and comment on it.

e) Now consider as initial concentrations, $[S_0] = 1\ \frac{mol}{L}$, $[E_0] = 0.1\ \frac{mol}{L}$, $[P] = 1\frac{mol}{L}$. Solve the ODE System and comment on the plot. What is the difference of this rate equation compared to the one you had in the 1st Project Exercise 1? What is the effect of the product concentration on the rate equation? Which parameter should be changed in order to make this mechanism similar to the one you have in Project 1- Exercise 1?

f) **BONUS:** Try also for higher initial product concentrations and comment on it (e.g . $[S_0] = 1\ \frac{mol}{L}$, $[E_0] = 0.1\ \frac{mol}{L}$, $[P] = 3\frac{mol}{L}$. Does the product concentration increase? Explain the observed behavior based on the mass-action ratio and the overall equilibrium constant.

EXERCISE 4

The following data (at steady-state) were obtained in a chemostat for the growth of *Yeast* for biomass production:

Dilution rate (h^{-1})	Cell concentration (g/l)	Carbon substrate concentration (g/l)
0.05	3.2	0.012
0.1	3.7	0.028
0.2	4	0.05
0.4	4.4	0.1
0.6	4.75	0.15
0.7	4.9	0.176
0.8	4.5	0.8
0.84	0.5	9

Note: Substrate concentration in the feed is 10 g/l and an excess of oxygen was used.

a) Estimate (assuming Monod kinetics) Maximum growth rate (μ_{max}) and Saturation/Monod constant (K_s).
b) Estimate the Yield on substrate ($Y_{X/S}$) and Maintenance energy (m).
c) You will now examine the effect of the dilution rate on the system. **Ignoring** the maintenance energy, derive **theoretical** formulas for the maximum, D_{max}, and optimal, D_{opt}, values of the dilution rate and calculate them using the parameters found in part a).
Hint: for D_{max} : under which condition does dilution rate reach its maximum? (See Slide 8 of Lecture 2 to remember)
Hint: for D_{opt}:If cells are our product of interest, find the best operating condition that maximizes the productivity of the cells ($D \cdot X$)
d) Based on the values in question c) define a range for D. Use the estimated values in part a and b to compute the substrate and cell concentrations as a function of dilution rate (Do not neglect maintenance energy).
e) Use the estimated values in part a and b to calculate the productivity of the cells ($D \cdot X$) as a function of dilution rate. Repeat this productivity computation for different values of K_s. What happens when the K_s is increased? (Do not neglect maintenance energy)
f) Find the maximum productivity and the corresponding dilution rate with the constant K_s found in a).
g) Compare the D_{opt} from question c) with the one obtained in question f).