Project 1: Enzyme and microbial kinetics

Batch reactor design and operation

03.03.2025

This project consists of 2 exercises + 1 bonus question, where you will learn how to work with data and how to interpret the enzyme kinetics.

The report has to be uploaded in Moodle by Sunday, the 16th of March at 23:59.

It should be uploaded in 1 pdf file containing max. 9 pages per group.

In this report, we expect that you:

(i) report the numerical answers and plots obtained defining the *correct units*.
(ii) *briefly* explain how you have obtained these answers.
(iii) give an interpretation of the values/plots obtained.

You also need to upload separately all the *code files* that you created and used to produce the results. Try to separate the code in sections, following a detailed structure and adding comments to document the procedure.

Code should run by simply executing the script/main function

No extra arguments. No external data.

EXERCISE 1

We assume an enzymatic reaction in a constant volume reactor. The kinetics for this reaction are given by the Michaelis-Menten equation. The latter is based on the following elementary reaction mechanisms:

Given a substrate S, an enzyme E, a complex ES, and a product P:

$$E + S \underset{k_b}{\overset{k_f}{\leftrightarrow}} ES \xrightarrow{kcat} E + P$$

a) Write the mass balance for this system and simplify it to concentration balance.
b) Solve the ODE system from a) for the time interval [0,5]s. Consider as initial concentrations $[S_0] = 1\ \frac{mol}{L}$, $[E_0] = 0.1\ \frac{mol}{L}$, $[P_0] = [ES_0] = 0\ \frac{mol}{L}$ and the following values for the parameters, $k_f = 10^4\ \frac{L}{mol\ s}$, $k_b = 20\ s^{-1}$, $k_{cat} = 10\ s^{-1}$. Plot and discuss the time-evolution of the concentrations of all the species.

A common approximation is the Quasi-Steady State Approximation (QSSA) of MM where we assume that the concentration of the enzyme complex $[ES]$ is time invariant for a given substrate concentration $[S]$.

c) Use the mass balance with QSSA to find an expression for ES as a function of the parameters k_f , k_b , k_{cat} the substrate concentration $[S]$ and the initial enzyme concentration $[E_0]$.
d) Rewrite the mass balance of the system using the approximation for $[ES]$ derived in c). Solve this ODE system for the time interval [0,5] s. Consider the same values for initial concentrations and parameters as in b). Plot the product concentration and compare it with the plot of the product concentration resulting from the system in b).
e) The original MM equation is:

$$\nu = \frac{\nu_{max}\, S}{K_M + S}$$

Perform simulations, for $[S_0] = 1\ \frac{mol}{L}$, $[E_0] = 0.1\ \frac{mol}{L}$, $[P_0] = [ES_0] = 0\ \frac{mol}{L}$, under the QSSA and plot the initial ν as a function of the initial enzyme concentration $[E_0]$ and the parameters k_f, k_b, k_{cat}. How does each one of these parameters affect the initial reaction rate? *Hint: use the MM equation to calculate v(t=0). Consider the following ranges for the parameters:* $k_f = 1 - 10^8\ \frac{L}{mol\ s}$, $k_b = 1 - 10^4\ s^{-1}$, $k_{cat} = 1 - 10^4\ s^{-1}$. *For* $[E_0]$, *think about the validity of the QSSA and determine a range that is in accordance with this.*
f) Identify the most important parameter(s) that determine ν using scaled sensitivity. To do so, plot $ln\nu$ vs logarithm of each of the parameters and comment on the slope.

Assume now that the enzyme is not specific, but it can also produce P'.

$$E + S \underset{k_b}{\overset{k_f}{\leftrightarrow}} ES \xrightarrow{kcat_1} E + P$$
$$\downarrow \text{kcat}_2$$
$$E + P'$$

g) Write the mass balance for this system and simplify it to concentration balance.
*Hint: **DO NOT** consider the QSSA.*

h) Solve the ODE system from a) for the time interval [0,5] s. Consider as initial concentrations $[S_0] = 1\ \frac{mol}{L}$, $[E_0] = 0.1\ \frac{mol}{L}$, $[P_0] = [ES_0] = [P'_0] = 0\ \frac{mol}{L}$ and the following values for the parameters, $k_f = 10^4\ \frac{L}{mol\ s}$, $k_b = 20\ s^{-1}$, $k_{cat1} = 10\ s^{-1}$, $k_{cat2} = 5\ s^{-1}$.

i) Which parameter(s) would you change to increase the selectivity?

EXERCISE 2

In his thesis, which was published as a book in 1948, Monod first proposed the celebrated equation that bears his name. As experimental support for this equation he presented results from four batch reactor runs on the growth of a pure bacterial culture in a lactose solution (see Monod, 1958, p3 74). Here are a set of simplified data for one of his runs.

Time Interval Number	t(h)	Cell concentration (g/l)	Lactose concentration (g/l)
1	0-0.54	15.5 to 23	137
2	0.54-0.9	23 to 30	114
3	0.9-1.23	30 to 38.8	90
4	1.23-1.58	38.8 to 48.5	43
5	1.58-1.95	48.5 to 58.3	29
6	1.95-2.33	58.3 to 61.3	9
7	2.33-2.7	61.3 to 62.5	2

Table 1: concentration data obtained

Analyze this data following the suggestions below:

A. Plot the cell concentration (X) and lactose concentration (S) as a function of time. Cell concentration changes with a constant slope in each time interval, however, Lactose concentration profile is constant in each time inetrval. Comment on the X vs. t curve with respect to the expected phases of batch growth, i.e., what phases do you see, what is their duration, etc.
   - Plot 1/μ vs. 1/S using a Lineweaver-Burke type plot. Find K_s and μ_{max}. In order to find corresponding μ in each time interval, use the following formula:

$$\mu = \frac{1}{X_{min}}\left(\frac{dX}{dt}\right)$$

   - Work only with the exponential growth phase. The data from lag phase and stationary phase should be neglected. Try to find the points by qualitatively looking to the plot.

B. What is the mass doubling time t_d (The time require to double the mass of the cell)? Explain what value you assumed for μ and why.

BONUS QUESTION

At room temperature sucrose is hydrolyzed by the enzyme sucrase as follows:

$$\text{sucrose} \xrightarrow{\text{sucrase}} \text{products}$$

A young scientist measured following sucrose concentrations in different time points in a batch reactor (concentrations are calculated from optical rotation measurements). Initial sucrase concentration is C_{E0} = 0.01 mmol/L.

$C_{SUCROSE,}$ mmol/L	1	0.68	0.16	0.006
t, hr	0	2	6	10

Help her find Michaelis-Menten kinetic parameters in this process.