École Polytechnique Fédérale de Lausanne

ChE-320: Bioreactor modeling and simulation

Project 1: Enzyme and microbial kinetics

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Introduction

In 1913, Michaelis and Menten (MM) refined their professor Henri's original investigation into the kinetics of enzymatic reactions, specifically on the catalysis of glucose and fructose hydrolysis by invertase. They introduced significant simplifications to Henri's approach, disregarding negligible factors such as the loss of catalytic activity and omitting consideration of the reverse reaction, which becomes relevant only as products accumulate. They highlighted the utility of initial rate equations, which offer a substantial simplification in derivation, and capitalized on the fact that conditions are stationary at the beginning of the reaction rendering the equation the standard for steady-state experiments for over a century [1]. Through this report, one explores by a series of exercises enzymatic and microbial kinetics through the lens of Michealis-Menton, depicting a quantitative and qualitative assessment of their outlined methodology for steady-state experiments.

Exercise 1

Question a)

The general mass balance equation for any system is; with F_{A0}, F_A, R_A, and N_A denoting the inflow rate, outflow rate, transformation rate, and molar amount of component A respectively.

$$\text{Accumulation} \quad = \text{In} - \text{Out} + \text{Reaction} \iff \frac{dN_A}{dt} \quad = F_{\mathrm{A0}} - F_A + \int_0^V R_A \, dV \tag{1}$$

Applied to our batch reactor, which has no inflow nor outflow, and assuming it is perfectly mixed:

$$\frac{dN_A}{dt} = \int_0^V R_A \, dV \iff \frac{dN_A}{dt} = R_A \int_0^V dV = R_A \cdot V \tag{2}$$

Given the reaction is taking place in a constant volume reactor, the equation then simplifies to:

$$\frac{dV \cdot C_A}{dt} \quad = V\frac{dC_A}{dt} \quad = R_A \cdot V \iff \frac{dC_A}{dt} \quad = R_A \tag{3}$$

The system's component-wise mass balances are therefore:

$$\begin{cases} \dfrac{d[E]}{dt} = -k_f[E][S] + k_b[ES] + k_{\mathrm{cat}}[ES] & \\ \qquad\quad = (k_b + k_{\mathrm{cat}})[ES] - k_f[E][S] & (i) \\ \dfrac{d[S]}{dt} = -k_f[E][S] + k_b[ES] & (ii) \\ \dfrac{d[ES]}{dt} = -k_b[ES] - k_{\mathrm{cat}}[ES] + k_f[E][S] & \\ \qquad\quad = -(k_b + k_{\mathrm{cat}})[ES] + k_f[E][S] & (iii) \\ \dfrac{d[P]}{dt} = k_{\mathrm{cat}}[ES] & (iv) \\ [E]_0 = [ES] + [E] & (v) \end{cases}$$

Equation (v) comes from making the assumption that the total amount of enzyme is conserved. Thus:

$$\frac{d[E]_0}{dt} = 0 \iff \frac{d[ES]}{dt} + \frac{d[E]}{dt} = 0 \tag{vi}$$

Question b)

Solving the ODE system, using the enclosed Python code, gives the graph presented in **Figure 1**.

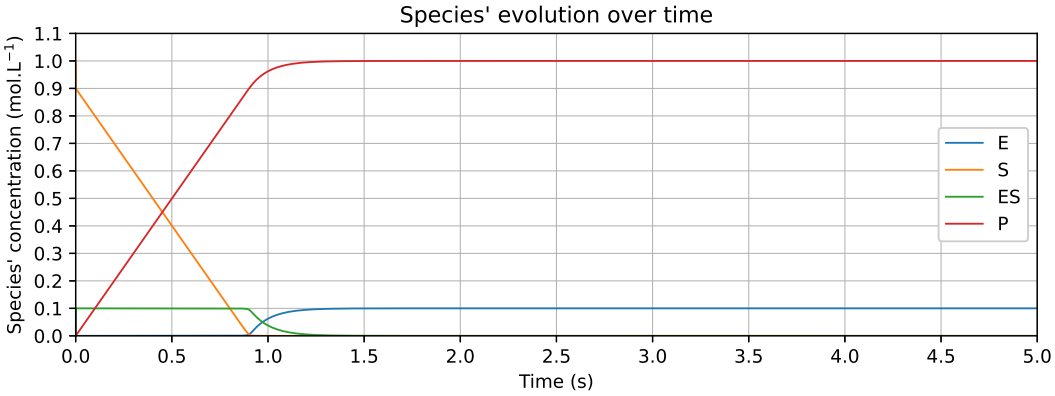

Figure 1: Plot of the temporal evolution of the enzyme (E), substrate (S), enzyme-substrate complex (ES), and the product (P).

The enzyme is initially present in a concentration 10 times smaller than that of the substrate. On top of that, the rate of formation of the ES complex is 500 times larger than its rate of dissociation into E and S. These two arguments thus explain the rapid decrease of the enzyme concentration to 0 M right from 0 s. From around 0.8 s, once the substrate is fully consumed and the product is formed, the enzyme concentration increases with a rate constant k_{cat} before reaching a steady-value of 0.1 M. This is, therefore, consistent with the expectation that enzymes are regenerated.

The substrate curve displays three domains. The first one, at 0 s, is characterized by a sharp decrease due to it forming the complex with the enzyme (ES) with a forward rate constant (k_f) that is 500 times bigger than the rate constant (k_b) for the backward reaction. Once the ES complex is formed, the substrate concentration decreases more slowly, from 0 to about 0.8 s, because the catalytic rate constant (k_{cat}) is 1000 times smaller than k_f. Starting from 1 s, the substrate concentration approaches 0 due to it being depleted.

The concentration of the enzyme-substrate complex initially rapidly increases as the substrate reacts with the enzyme at rate k_f. It remains relatively constant because the enzyme is depleted and both its rate of disappearance (k_b and k_{cat}) are 500 and 1000 times smaller, respectively. It then breaks down into the products and enzyme, at rate k_{cat}, to reach 0 M from 1.2 s.

Initially, the product concentration is zero. As the reaction proceeds, the enzyme-substrate complex breaks down to form product and free enzyme. The product concentration increases over time until it reaches a steady-state concentration, after about 1.25 s, equal to the initial substrate concentration of 1 M.

Question c)

The Quasi Steady-State Approximation (QSSA) on the ES concentration, [ES], assumes the existence of a dynamic equilibrium where the rates of formation and dissociation of the ES complex are equal, which results in [ES] being constant.

$$[ES] = constant \iff \frac{d[ES]}{dt} = 0 \tag{4}$$

Given that $[E]_0$ is constant (cf. Question a)) and that the only other state in which the enzyme exists is the one where it is free, it comes from **Equation (v)** that [E] is also constant.

$$[E] = constant \iff \frac{d[E]}{dt} = 0 \tag{5}$$

From **Equation (5)** and **Equation (v)**, **Equation (iii)** hence becomes:

$$\begin{aligned} -(k_b + k_{\text{cat}})[ES] + k_f[E][S] &= 0 \\ -(k_b + k_{\text{cat}})[ES] + k_f[S]([E]_0 - [ES]) &= 0 \\ [ES](k_b + k_{\text{cat}} + k_f[S]) &= k_f[S][E]_0 \\ [ES] &= \frac{k_f[S][E]_0}{k_b + k_{\text{cat}} + k_f[S]} \end{aligned} \tag{6}$$

Question d)

Using **Equations (v)** and **Equation (7)**, the mass balance for the system can be re-written as:

$$\begin{cases} \frac{d[E]}{dt} = 0 & (i) \\ \frac{d[S]}{dt} = -k_f[S]([E]_0 - [ES]) + k_b[ES] & \\ \quad = -k_f[S]([E]_0 - \frac{k_f[S][E]_0}{k_b + k_{\text{cat}} + k_f[S]}) + k_b(\frac{k_f[S][E]_0}{k_b + k_{\text{cat}} + k_f[S]}) & (ii) \\ \frac{d[ES]}{dt} = 0 & (iii) \\ \frac{d[P]}{dt} = k_{\text{cat}}[ES] = k_{\text{cat}}(\frac{k_f[S][E]_0}{k_b + k_{\text{cat}} + k_f[S]}) & (iv) \\ [E]_0 = [ES] + [E] & (v) \end{cases}$$

Solving this QSSA-ODE system, by the enclosed Python code, yields the graph in **Figure 2**.

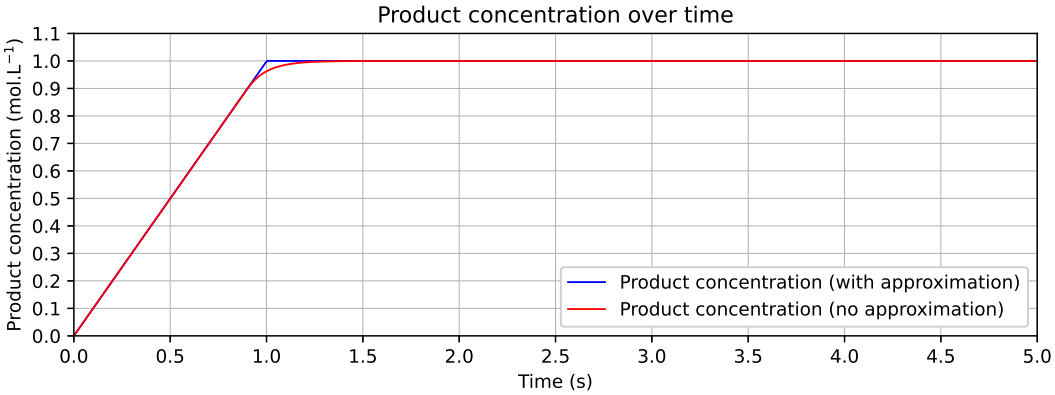

Figure 2: Plot of the temporal evolution of the product (P) with and without making the Quasi-Steady-State Approximation.

Figure 2 shows a similar temporal evolution of the product for both with and without QSSA for most time points. However, right before reaching steady-state, the blue plot shows a small overshoot which is likely due to the assumption made.

Question e)

The original Michealis-Menten (MM) equation is as follows.

$$\nu = \frac{\nu_{max} S}{K_M + S} = \frac{k_{cat} \cdot E_0 \cdot S}{\frac{k_b + k_{cat}}{k_f} + S} \tag{7}$$

To understand how k_f, k_b, k_{cat} and E_0 influence the initial reaction rate (ie when S = $[S_0]$), one plots ν_0 with respect to the respective parameter ranges: $k_f = 1 - 10^8 [\frac{L}{mols}]$, $k_b = 1 - 10^4 [\frac{1}{s}]$, $k_{cat} = 1 - 10^4 [\frac{1}{s}]$ and $E_0 = 0 - [S_0][\frac{mol}{l}]$. To select the range for E_0, one looks at the validity domain for the Quasi-State Approximation. This approximation assumes that the concentration of the enzyme complex remains constant through time, implying its swift formation and subsequent breakdown. Consequently, during the initial transient phase, it is presumed that the substrate concentration remains at the approximately initial substrate concentration, while the concentration of enzyme-substrate complexes increases. Therefore, the enzyme concentration must be kept lower than the substrate concentration.

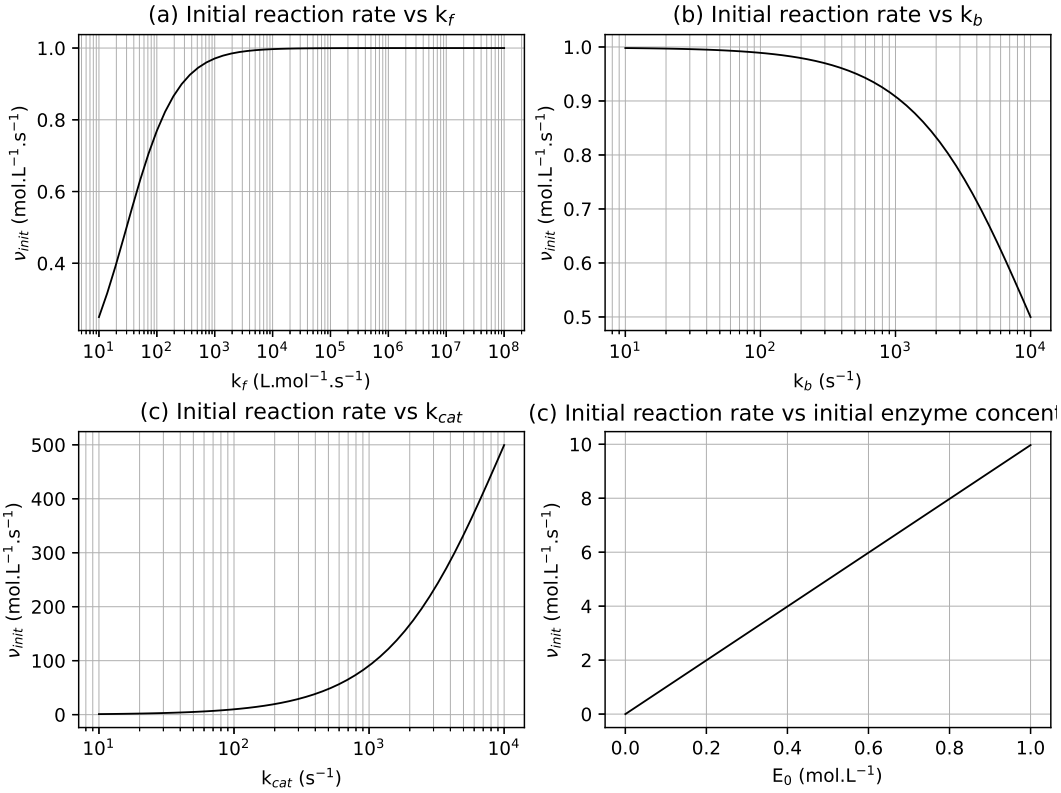

Figure 3: Plot of initial reaction rate calculated using the MM equation and its variation with respect to parameter ranges. Plot a, b and c have been plotted on semi-logarithmic plot due to the large span of the parameters tested.

In **Figure 3 (a)**, one observes a steep increase of the initial reaction rate as k_f ranges from 1 to approximately 1000 L/mol/s. After that threshold, large variations in this parameter yield negligible changes in the initial rate. In **Figure 3 (b)**, small variations of the k_b parameter yields a relatively small variation (in the order of 0.1 mol/L/s), while a sharp decrease is seen from 1000 1/s onwards. When one considers k_{cat} (**Figure 3 (c)**), one sees that the initial reaction rate exhibits an exponential increase as the parameter k_{cat} is increased between 1000 and 10000 1/s. When k_{cat} values are smaller, the resulting changes range from 0 to 100 mol/L/s. This variation is larger compared to the changes

observed in the stationary parts of the k_b and k_f behaviours. Finally, when varying E_0 as shown in **Figure 3 (d)**, a linear variation in the initial reaction rate is observed, with a a change of 1 mol/L of initial enzyme concentration yielding 1 mol/L/s variation of the initial reaction rate.

Question f)

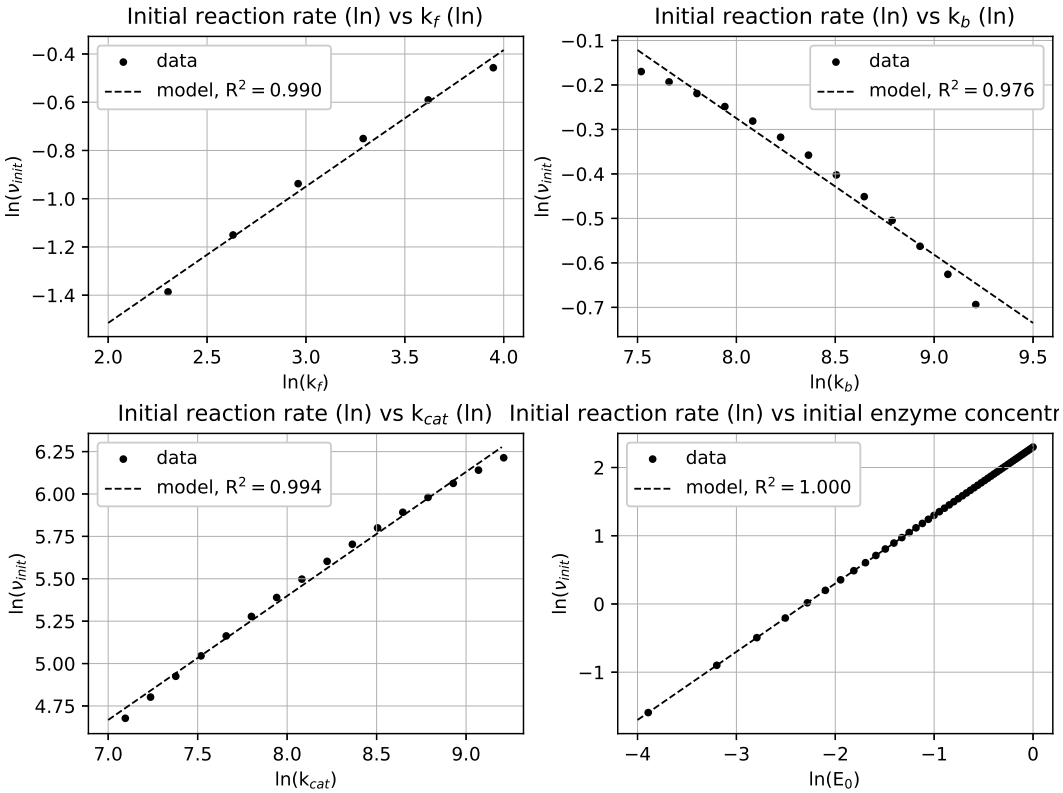

Figure 4: First degree polynomial fit of the maximum slope in 3.

To identify the most important parameters that determine the initial reaction rate via the MM equation, we look at the steepest slope achieved during the parameter variation. The rationale is that a steepest slopes correlates to faster change of the initial reaction rate. As the curves in **Figure 3** are not linear, one fits a first-degree polynomial to the exponential phase of reaction rate with respect to parameter change and then assesses the absolute values of their slopes. The most significant fluctuations arise primarily from variations in the parameters k_{cat} (slope = 0.73) and E0 (slope = 1). Conversely, modifications to k_f or k_b are anticipated to induce comparatively minor alterations in initial velocity.

Question g)

Given the non specific enzymatic process, one writes the general mass balance of the constant volume batch reactor system as **Equation 1**. Our batch system is considered to be isolated, exempted from inflows and outflows and perfectly homogeneously mixed. Thus for a reactant A (such as the substrate) one writes.

$$\frac{dN_A}{dt} = \int_0^V R_A \, dV \iff \frac{dN_A}{dt} = R_A \int_0^V dV = R_A \cdot V \tag{8}$$

One assumes the reaction to be taking place in a volume constant in time, allowing the equation to simplify to:

$$\frac{dV \cdot C_A}{dt} = V\frac{dC_A}{dt} = R_A \cdot V \iff \frac{dC_A}{dt} = R_A \tag{9}$$

For products (such as P and and P'), one expects the transformation rate to evolve oppositely to the reactants. The system's component-wise mass balance is written as follows.

$$\begin{cases} \dfrac{d[E]}{dt} = -k_f[E][S] + k_{cat_1}[ES] + k_{cat_2}[ES] + k_b[ES] \\ \dfrac{d[S]}{dt} = -k_f[E][S] + k_b[ES] \\ \dfrac{d[ES]}{dt} = k_f[E][S] - k_{cat1}[ES] - k_{cat_2}[ES] - k_b[ES] \\ \dfrac{d[P]}{dt} = k_{cat_1}[ES] \\ \dfrac{d[P']}{dt} = k_{cat_2}[ES] \\ [E]_0 = [ES] + [E] \end{cases} \tag{10}$$

Question h)

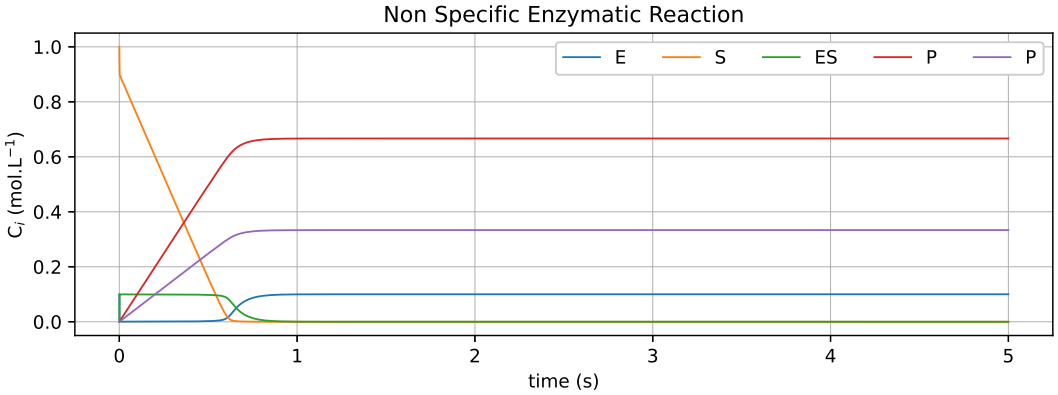

Figure 5: Solution to the system of equations 10. This plot can be achieved by using python solver *odeint* from the *scipy.integrate* library.

The two phases of the process are shown in **Figure 5**. The exponential (transition) phase spans from the initiation of the reaction 0 s to 0.77 s and marks the dynamic evolution of the reaction. As the reaction occurs, one sees that the substrate concentration (yellow, S) strongly declines, concomitant with the formation of reaction intermediates (green, ES). Simultaneously, these intermediates are rapidly consumed to yield products (red and purple, respectively P and P'). This leads to the release of the catalyst (E) back to its initial concentration.

Subsequent to the exponential phase, the system enters a stationary state, where (due to a lack of substrate) no new products are formed from 0.77 s onwards. As expected, less P is formed than in **Question 1 (b)** since the ES complex is involved in two parallel reactions leading to different products, P and P'. Notably, the product distribution demonstrates a selectivity ratio of 2:1, with the primary product (P) prevailing at 0.67 mol/L compared to the secondary product (P') at 0.33 mol/L.

Question i)

Considering the reactions given in the system of **Equations 10**, one understands that increasing the parameter k_{cat_1} allows to increase the selectivity of the reaction. k_{cat_1} considers the catalytic process of transforming the intermediate (ES) to the product (P).

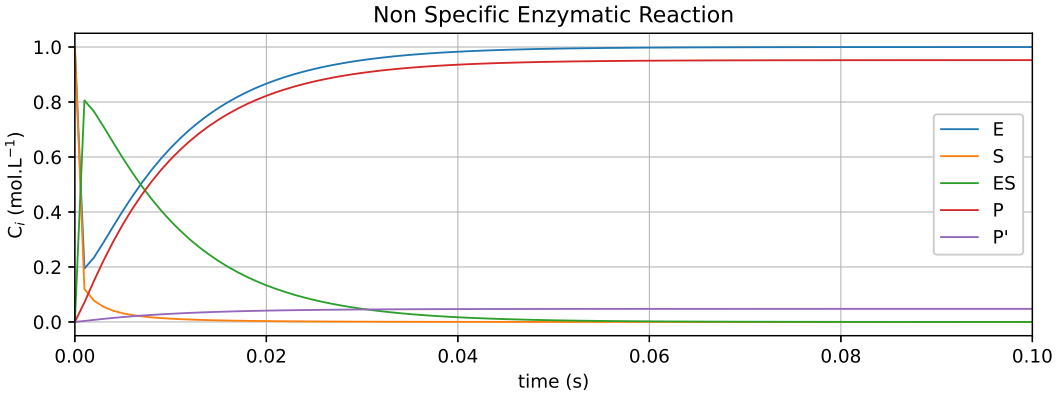

Figure 6: Solution to the system of **Equations 10** with k_{cat_1} increased by 10 folds (100 1/s instead of 10 1/s). This plot can be achieved by using python solver *odeint* from the *scipy.integrate* library.

This handling would allow for the steady state to be reached at 0.142 s, ie nearly 82% faster than the previous case. Moreover, the selectivity increased allowing for 0.95 mol/L of primary product and only 0.05 mol/L of secondary product.

Exercise 2

The cell and lactose concentration of the experiment is plotted below:

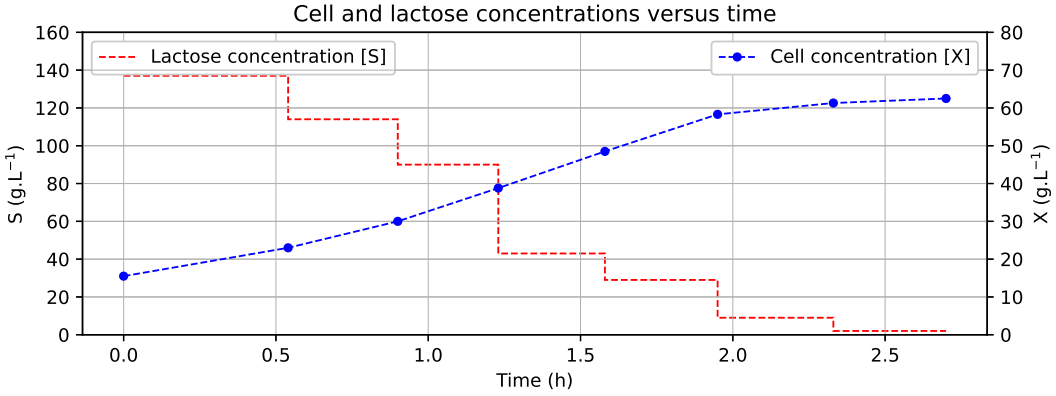

Figure 7: Plot of the evolution of cell and lactose concentrations.

No lag phase seems to be present in this experiment, as the cell concentration immediately starts to increase. Stationary phase seems to start after approximately 2 hours of growth phase, as shown by the stagnation of cell concentration around 120 g.L^{-1}. This is consistent with the lactose concentration approaching 0 g.L^{-1} at the end of the experiment.

Excluding the last 2 measurements that have been taken after 2 h to only keep data from the exponential growth, a Lineweaver-Burke plot can be constructed to obtain the Monod constants to model the kinetics of this culture.

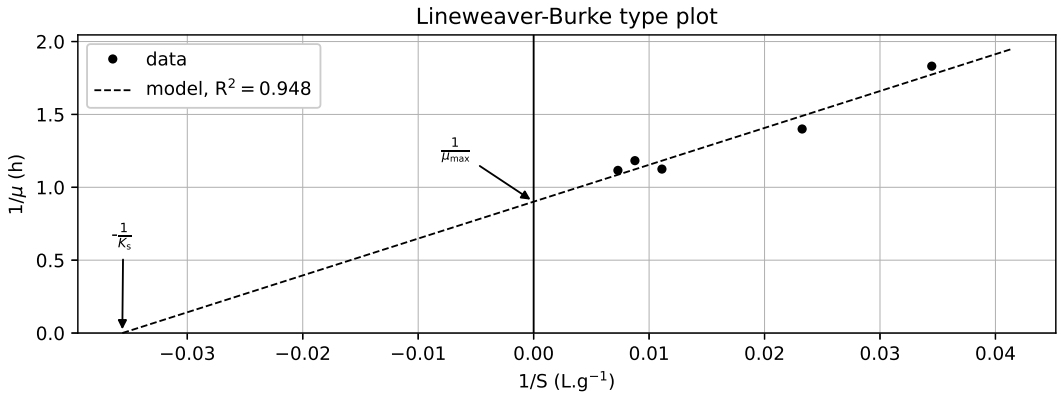

Figure 8: Lineweaver-Burke type plot.

As shown in the graph, the Monod constants K_s and μ_{max} can be easily obtained by looking at the values of the intercepts with the x and y axes, which occur when $x = -0.04$ and $y = 0.90$. That gives a K_s value of 28.07 $g.L^{-1}$ and a μ_{max} value of 1.11 h^{-1}.

Alternatively, the slope of the model (25.30) can also be used to determine the Monod constants using the equation $y = \frac{K_s}{\mu_{max}}x + \frac{1}{\mu_{max}}$.

To obtain the doubling time (t_d) of the culture, only the first 3 growth rates were used, as they were established before signs of stagnation occurred. An average between those 3 has then been performed to reduce possible errors caused by noise during measurements, which gave a $\mu_{average}$ of 0.88 h^{-1}. Knowing that $X = X_0 e^{\mu t}$ and $X(t_d) = 2X_0$, the doubling time can be calculated with:

$$t_d = \frac{ln(2)}{\mu_{average}} = \frac{ln(2)}{0.88} = 0.79\ h^{-1} \tag{11}$$

Using μ_{max} to calculate the doubling time would give $t_d = 0.62\ h^{-1}$. However, this value is purely theoretical and in practice, using the maximal empirical μ gives more information about the real performance of the process.

Bonus Exercise

The reaction of sucrose hydrolized by sucrase into products can be described using the Michaelis-Mentend model. First, the total product concentration is deduced from the sucrose concentration (as all sucrose turns into products, one mole of sucrose S gives one mole of products P).

In addition, the reaction rate r_s is approximated by doing a local derivation on each point as so:

$$r_{s1\ \text{local}} = \frac{P_0 - P_1}{t_0 - t_1}$$

Following Michealis Menten model, the reaction rate r_s is given by :

$$r_\text{s} = v_{max} * \frac{S}{K_M + S}$$

To find the most suitable parameters K_M and v_max to fit the observed data, the Lineweaver-Burke representation can bu used. By plotting $1/r_s$ as a function of $1/S$ (with S being the average sucrose concentration between 2 samples) and by doing a linear regression on these points.

Time (h)	$C_{sucrose}$ (mmol.L^{-1})	$C_{products}$ (mmol.L^{-1})	$r_{s_{local}}$
0	0	1	0
2	0.68	0.32	0.16
6	0.16	0.84	0.13
10	0.006	0.994	0.0385

Table 1: Concentrations and estimation of the reaction rate

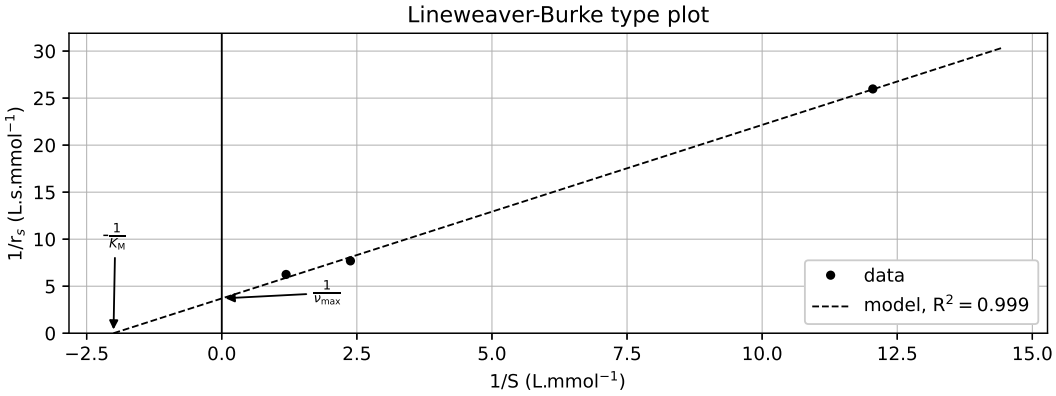

Figure 9: Lineweaver-Burke plot

The value at y = 0 is defined as $-1/K_{\mathrm{M}}$ while the point where x = 0 is defined as $1/v_{\max}$. This gives $K_{\mathrm{M}} = 0.50$ mmol.L^{-1} and $v_{\max} = 0.27$ mmol.L^{-1}.s^{-1}.

1 Conclusion

These three exercises gave an overview on the different models describing enzymatic and microbial kinetics. Exercises focused on two different approaches: Michealis-Menten model and Monod equation. Mass balances along with approximations were settled to evaluate the evolution of the process with time. The influence of reaction constants over the global rate of the process was investigated; k_{cat} and E_0 were shown to significantly drive the reaction rate. However Michaelis Menten parameters can also be extracted from experimental data, using the slope between two measured points as a local rate, and creating a Lineweaver Burk plot, as shown in the bonus exercise. Lastly, experimental data were also used in exercise 2 to model cell growth. The exponential and stationnary phase (once all the lactose was consumed) of the growth can be well described by Monod equation. The use of a Lineweaver-Burk plot enables to estimate the maximum growth rate μ_{max} and the constant K_s, respectively 1.11 h^{-1} and 28.07 $g.L^{-1}$. These parameters are powerful tools to characterize the kinetic of biological reactions.

References

[1] Cornish-Bowden A., One hundred years of Michaelis–Menten kinetics. Consulted on March 18, 2024 https://www.sciencedirect.com/science/article/pii/S2213020914000627