National Exams May 2019

04-Bio-A5, Enzyme and Microbial Kinetics

3 hours duration

NOTES:

1. If doubt exists as to the interpretation of any question, the candidate is urged to submit with the answer paper, a clear statement of any assumptions made.

2. This is an OPEN BOOK EXAM.
   Any non-communicating calculator is permitted.

3. SIX (6) questions constitute a complete exam paper.

4. The mark weighting per question is attached.

5. Most questions require a worked answer, and part marks will be awarded for correct equations and process. Clarity and organization of the answer are important.

Marking Scheme

1. 10 marks
2. 15 marks
3. (a) 15 marks; (b) 5 marks
4. (a) 15 marks; (b) 5 marks
5. 15 marks
6. (a) 10 marks; (b) 10 marks

1. An enzyme (E) is used to catalyze the transformation of a substrate (S), to a final product (P). In the reaction vessel, two inhibitors are present. The reaction mechanism can be described by:

$$ESI_1$$

$$k_{-I,1} \downarrow\uparrow k_{I,1}$$

$$I_1$$

$$+$$

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_2} E + P$$

$$+$$

$$I_2$$

$$k_{-I,2} \uparrow\downarrow k_{I,2}$$

$$ESI_2$$

Using the rapid equilibrium approach, please write the equations that would allow you to find an expression that describes how the rate of formation of P depends on S, the initial enzyme concentration (E_0), the concentration of each one of the inhibitors and the various rate constants in Figure above. No need to solve the equations.

2. The following data were obtained for an enzymatic reaction with an inhibitor.

I [mmol/ml]	S [mmol/ml]	V [mmol/ml min]
0	0.1	1.64
0	0.02	0.9
0.6	0.1	1.33
0.6	0.02	0.57

Determine the K_I for the inhibitor and decide what type of inhibitor is being used.

3. A batch reactor, with a volume V_L is used to perform an enzymatic reaction. At time zero, the substrate concentration is S_0. The enzyme is immobilized in vertical plates as a monolayer, with a total area equal to A_T . The kinetics of the enzymatic reaction can be approximated using a first-order rate law:

   $(-r_s) = V_m^{'} \cdot S_W \cdot A_T$ where:

   - $(-r_s)$ is the rate at which the substrate is consumed
   - $V_m^{'}$ is the maximum specific rate of reaction per unit area
   - S_W is the substrate concentration at the surface of the plates.
   - A_T is the total area of the plates.

A stagnant layer is present between the surface of the plates and the bulk liquid leading, to external mass transfer limitation that can be described using a mass transfer coefficient equal to k_L..

a. Please write two differential equation that describe how the rate at which the substrate concentration in the bulk phase is related to the rate of mass transfer and to the rate of enzymatic reaction.

b. Please develop an expression that describes how the concentration in the bulk of the liquid changes as a function of time.

4. The growth of a culture in a closed, rigid bioreactor can be described by:

$$C_6H_{12}O_6 + aO_2 + bNH_3 \longrightarrow cCH_{1.6}N_{0.20}O_{0.55} + dCO_2 + eH_2O$$

Initially 15 mmol of glucose is supplied, and there is excess nitrogen supplied. Part way through the reaction, 0.32 g dry cell weight has been formed (assume a very small inoculum). The molecular weight of the biomass is 25.2 g/mol. Careful measurements find that 16 mmol of O_2 were consumed.

a) Estimate Yx/s (g DCW/mol glucose) and the final amount of glucose in the medium (mmol) after the cells were harvested.

b) Estimate how much CO_2 was produced (mmol).

5. A 100 liter fermenter is operated in batch mode, with an initial substrate concentration equal to 2000 mg/L and an initial biomass concentration of 50 mg/L. The specific growth rate has a first order dependence on the substrate concentration:

$$\mu_g = 0.002 \cdot S \text{ hr}^{-1}$$

The contribution of cell death can be neglected for this culture. The true growth yield is $Y^M_{X/S} = 0.3 g\ dw / g\ substrate$. A growth related product is formed with a product yield equal to $Y_{P/X} = 0.2$ g product/g cells. Please determine the time at which the product concentration is equal to 50% of the maximum product concentration that can be achieved in this reactor.

6. A tubular sterilizer is used to sterilize the feed going into a bioreactor operated in a continuous mode. This is achieved by directly injecting steam into the pipe leading the feed to the reactor (volumetric rate of steam added is negligible compared to the volumetric rate of feed). The pipe (i.e. the tubular sterilizer) has a length L and a cross-sectional area equal to A. The feed is added at a flow rate equal to F and it contains a thermally labile vitamin at a concentration $C_{V,0}$ and spores at an initial concentration equal to N_0. The rates of degradation of vitamin and spores are dependent on temperature according to Arrhenius relationships: $k_{D,\text{vitamin}} = \alpha_{vit} e^{-\frac{E_{a,vit}}{RT}}$ and $k_{D,\text{ spores}} = \alpha_{spores} e^{-\frac{E_{a,spores}}{RT}}$

   b) Please develop an expression that allows you to calculate the vitamin concentration after sterilization (C_V) as a function of the feed flow rate and the temperature at which sterilization takes place.
   c) Please develop an expression that allows you calculate the probability of a successful sterilization as a function of the feed flow rate, the temperature at which sterilization takes place and the total amount of time of operation of the system (t_{oper}).

List of Integrals

$$\int_{x_1}^{x_2} dx = (x_2 - x_1)$$

$$\int_{x_1}^{x_2} x^n dx = \frac{1}{n+1}\left(x_2^{n+1} - x_1^{n+1}\right)$$

$$\int_{x_1}^{x_2} \frac{1}{x} dx = \ln x_2 - \ln x_1$$

$$\int_{x_1}^{x_2} e^{ax} dx = \frac{1}{a}\left(e^{ax_2} - e^{ax_1}\right)$$

$$\int_{x_1}^{x_2} \frac{1}{ax+b} dx = \frac{1}{a}\ln\frac{ax_2+b}{ax_1+b}$$

$$\int_{x_1}^{x_2} \frac{1}{ax^2+bx} dx = \frac{1}{b}\ln\left(\frac{\frac{x_2}{ax_2+b}}{\frac{x_1}{ax_1+b}}\right)$$