National Exams May 2017

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 below.
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. (a) 18 marks (b) 7 marks
3. 15 marks
4. (a) 5 marks; (b) 5 marks; (c) 10 marks
5. (a) 10 marks; (b) 5 marks
6. (a) 10 marks; (b) 5 marks

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

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} ES \underset{k_{-2}}{\overset{k_2}{\rightleftarrows}} E + P$$

$$k_3 \ \downarrow\uparrow \ k_{-3}$$

$$\boldsymbol{ES_2}$$

Using the quasi-steady state 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) and the various rate constants in Figure above. No need to solve the equations.

2. A CSTR with a volume of 1000 L, is fed at a flow rate of 10 L/min and the substrate concentration in the feed is 10 mol/L. The reactor is used to perform an enzymatic reaction where each molecule of substrate is broken into two molecules of product: $S + E \rightarrow E + 2P$

   The enzyme is immobilized in porous spherical agarose beads with a radius of 5 mm. There is external mass transfer resistance, such that the substrate concentration at the surface of the beads is 1 mol/L. The kinetic parameters for the immobilized enzyme are $V_m'' = 10 \dfrac{\text{mol}}{\text{L} \cdot \text{min}}$ and $K_M = 100$ mol/L. The diffusivity of the substrate is $1 \cdot 10^{-4}$ m^2/min.

   a. If the product concentration in the output is to be 5 mol/L, what is the volume of particles required to achieve this target concentration?
   b. How does the rate of reaction in the particles compare to the rate of reaction you would observe if an equivalent mass of enzyme associated with your response in part (a) was present free in solution (not immobilized in the spheres)? Explain your answer and be quantitative.

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 reaction is substrate inhibited. Use the textbook equations developed using rapid equilibrium assumptions. If the initial substrate concentration is 10 mol/L, Km' is 5 mol/L and Ks is 1mol/L, when will the substrate concentration drop to half the initial amount? As the initial substrate concentration approaches zero, comment on how this calculation relates to the time it takes to use half the substrate for a non-substrate inhibited reaction.

4. A 10 L fermenter is operated in batch mode. The initial substrate concentration in the reactor is 20 g/L and the initial biomass concentration in the reactor is 0.1 g/L. A growth associated product (P) is formed during the batch and its concentration affects the specific growth of the cells according to $\mu_g = \frac{0.1}{P+1} h^{-1}$ where P is product concentration (g/L). The initial product concentration in the reactor is equal to zero. The contribution of cell death can be neglected for this culture. The true growth yield is $Y^M_{X/S} = 0.3\ gcells/gsubstrate$ and the yield for the product is $Y_{P/X} = 0.2\ gproduct/gcells$.

   a. What is the biomass concentration in the reactor at the end of the batch (when all the substrate is consumed)?
   b. What is the product concentration in the reactor at the end of the batch?
   c. How long will it take for the substrate to be totally consumed in the reactor?

5. A bioreactor is operated as a chemostat (CSTR without cell recycle) to grow bacterial cells. Cell growth can be described using the Contois equation: $\mu_g = 0.6\frac{S}{0.1 \cdot X \cdot S}h^{-1}$ where S is the glucose concentration (g/L) and X is the biomass concentration (g/L). Cell death follows first order kinetics with respect to the biomass concentration with a rate constant $k_D = 0.1\ h^{-1}$. The true growth yield is $Y^M_{X/S} = 0.4\ gcells/gsubstrate$. The substrate concentration in the feed is 10 g/L.

   a. What is the substrate concentration in the reactor when the dilution rate is equal to 0.2 h^{-1}?

   b. What is the maximum dilution rate at which the reactor can be operated before the cells are washed out from the reactor?

6. A culture is grown in a fed batch reactor and the volume of the reactor at time zero is equal to V_o. The feed flow rate to the reactor is given by $F = F_o + \alpha \cdot t$. The growth kinetics for this culture are given by: $\mu_g = k \cdot S\ (h^{-1})$ where S is the substrate concentration. Cell death is negligible. The true growth yield is given by $Y^M_{X/S}$.

   a. Please develop an expression that describes how the volume of liquid in the reactor depends on time.

   b. Assuming that pseudo-steady state conditions are applicable to this system, please develop an expression that describes how the substrate concentration in the reactor changes with time.

List of Integrals

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

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

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

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

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

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