National Exams December 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.
   The first six questions as they appear in the answer book will be marked.

4. The mark weighting per question:

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

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.

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

$$E + S + S \overset{k1,k-1}{\longleftrightarrow} ES + S \overset{k2,k-2}{\longleftrightarrow} ES_2 \overset{kcat}{\longrightarrow} E + P$$

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 mechanism above. Do not 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	1.0	16.4
0	0.20	9.00
1.2	1.0	13.30
1.2	0.20	5.70

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

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

   $-r_S = V_m' \cdot S_W \cdot A_T / K_m$

   Where $(-r_S)$ = the rate at which the substrate is consumed

   V_m' = the maximum specific rate of reaction per unit area

   A_T = the total surface area of the plates

   K_m = the Michaelis Menten constant

   S_W = the substrate concentration at the surface of the plates.

   The reaction is well mixed, so you can assume that the wall substrate concentration equals the bulk substrate concentration ($S_W = S_b$).

The enzyme denatures (loses activity) with time according to:

$$\frac{dV'_m}{dt} = -k_1.V'_m$$

Please show that the minimum substrate concentration that can be achieved in this reactor is:

$$S_{min} = S_0 \cdot e^{\left(\frac{-A_T \cdot V_{m,0}}{k_1 \cdot K_m}\right)}$$

Where $V_{m,0}$ is the maximum specific reaction rate per unit area at time zero.

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, 0.3 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 15 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. In a continuous ethanol fermentation system, an enzymatic hydrolysate of corn powder is the substrate, with 10 g/L sugar. The yeast strain that converts the substrate to ethanol is self-flocculating, and can be easily separated by sedimentation. Taking advantage of this, you hypothesize that a recycle system would maximize productivity.

The feed flow rate is 400 ml/h into a 1000 mL bioreactor. The cell separator concentrates the cells to twice the concentration exiting the bioreactor and returns 200 ml/h to the bioreactor. In these conditions, μ_m=0.5 h^{-1} and K_s = 0.2 g/L and $Y_{x/s}^m$ = 0.4. Assume Monod kinetics and no cell death. Calculate the substrate concentration exiting the bioreactor, the cell concentration within the reactor, and the cell concentrations exiting the cell separator.

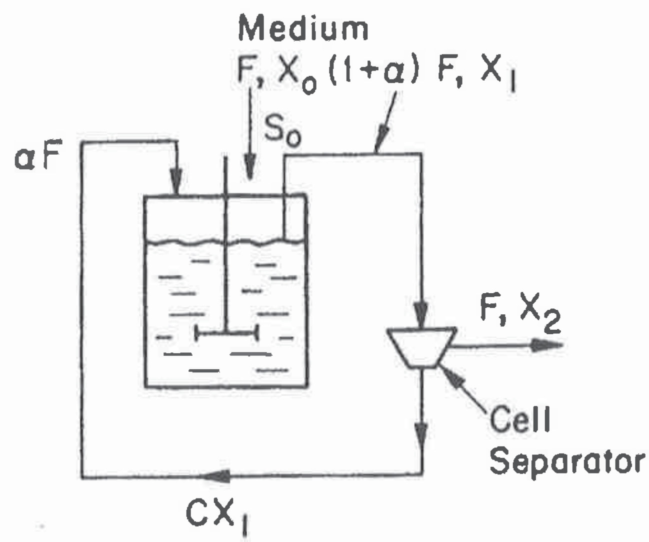

Figure 1. Bioreactor with cell recycle

6. a) In a batch reactor filled to 100 L, *E. coli* are growing and cell death is negligible. The bioreactor is initially inoculated with 1 g DCW of biomass. Glucose is the substrate that is used and the initial glucose concentration in the medium is 10g/L. The yield coefficient is 0.4 g DCW / g glucose, $\mu_m = 0.7\ h^{-1}$ and $K_s = 0.1 g/L$. Assume Monod kinetics. What is the maximum cell density that can occur with a minimal remaining substrate concentration (0.01 g/L), and how long will it take after the end of the lag phase?

   b) If, instead, this culture were grown in a chemostat (CSTR) at steady state, what dilution rate would give maximum productivity?

   c) How does the biomass production rate (g DCW / L / h) compare between batch and continuous systems?

d) Now you decide to split the difference and try a fed batch system. You grow 100 L in the batch system described in part a), but use a 1000 L reactor. You decide to use the initial dilution rate calculated in part b), and then maintain a constant feed rate. Over the fed batch portion (after the initial batch growth), what is the biomass production rate?

<u>List of Integrals</u>

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

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

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

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

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

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