ORDRE DES INGÉNIEURS DU QUÉBEC

MAY 2010 SESSION

- All documents allowed
- Calculator: authorized models only
- Examination duration: 3 hours
- Two (2) sheets of millimetered paper required

**04-Chem-B4 Biochemical Engineering**

**QUESTION 1 (10+5+10 = 25 points): enzyme inhibition kinetics**

The initial rate of reaction of an enzyme catalyzed reaction was determined in the presence of several inhibitors, A, B and C, at various concentrations of substrate S ([S]). The rate data, either in the absence, or in the presence of each inhibitor are shown in the table below. Determine the manner of action of each inhibitor and calculate the enzyme kinetic constants $r_{max}$ and $K_m$, and the inhibition constant $K_i$ for A, B and C, if the total enzyme concentration is 10 μM.

| | Initial reaction rate (mM/min) | | | |
|---|---|---|---|---|
| **[S] (mM)** | **without inhibitor** | **A at 20 mM** | **B at 15 mM** | **C at 5 mM** |
| **1** | 3,3 | 1,4 | 1,7 | 2,0 |
| **2** | 5,0 | 2,5 | 2,5 | 2,5 |
| **5** | 7,1 | 4,5 | 3,6 | 2,9 |
| **10** | 8,3 | 6,3 | 4,2 | 3,1 |
| **20** | 9,1 | 7,7 | 4,5 | 3,2 |

a) Plot the graph that is required to identify the inhibition type. Indicate any useful information on this graph.

b) Determine the inhibition type caused by A, B and C

c) Calculate the kinetic parameters $v_{max}$, $K_s$ and $K_i$.

**QUESTION 2 (5+5+5+5 = 20 points): Microbial kinetics**

Initial rate measurements were performed to evaluate the growth kinetics of a new bacterium strain, P. Quebecensis in terms of a limiting substrate concentration (S). All other factors being equal, the increase in concentration of the microorganism, X, is measured, for a very short period, for different initial substrate concentrations, $S_o$. Results are presented on the Table below.

| | Microorganism concentration (g/L) | | | | |
|---|---|---|---|---|---|
| t (min) | So=2 g/L | So=6 g/L | So=10 g/L | So=20 g/L | So=30 g/L |
| 0 | 1,00 | 1,00 | 1,00 | 1,00 | 1,00 |
| 2,5 | 1,05 | 1,08 | 1,09 | 1,08 | 1,06 |
| 5 | 1,10 | 1,17 | 1,18 | 1,16 | 1,13 |
| 7,5 | 1,15 | 1,26 | 1,28 | 1,25 | 1,21 |
| 10 | 1,20 | 1,36 | 1,39 | 1,35 | 1,29 |

a) Calculate the specific growth rate ($\mu$) for each initial concentration of S, using the slope of the data and the initial microorganism concentration as a basis for your calculations;

b) Transform data according to the Lineweaver-Burke relation, and plot the results on a $1/\mu$ vs $1/S$ graph;

c) Identify the kinetic type of the microorganism and calculate the kinetic parameters;

d) Calculate the time that would be required by this microorganism to use 60% of the substrate in a batch culture if the initial substrate concentration was $S_0$ = 100 g/L, the initial biomass concentration was $X_0$ = 0,7 g/L, and the cell per substrate yield coefficient was Yx/s = 0,2 g/g.

**QUESTION 3 (5+5+15 = 25 points): Batch culture**

The research and development laboratory of your company has developed a microorganism that produces a protein against cancer, recombinant P-53. This microorganism has been characterized: the growth kinetic obey a Monod law, with kinetic parameter values of $\mu_{max}$ = 2 $h^{-1}$, $K_s$ = 5 g/L, the yield of biomass growth with respect to substrate consumption, Yx/s = 0,4 g/g, and the production kinetic is growth associated with a yield coefficient value of Yp/x = 0,2 g/g. You are asked to produce P53 in a 100L bioreactor, starting the culture with an initial limiting substrate concentration of 40 g/L, and an initial microorganism concentration of 0,1 g/L. Calculate:

a) The maximum cell concentration you will obtain under these conditions (state clearly your hypothesis if required);

b) The maximum product concentration that will be reached;

c) Fermentation times and product concentrations attained when the microorganism concentration will reach values of 20, 30 and 36 g/L.

**QUESTION 4 (5+5+5+5+5+5 = 30 points): Chemostat fermentation**

You are provided with these data about the fermentation of a very interesting microorganism that can attain very high cell density. From these data, obtained in steady state, perfectly mixed cultures (chemostat) for different dilution rates (D), microorganism (X), limiting substrate (S) and product (P) concentrations:

| D (h-1) | X (g/L) | S (g/L) | P (g/L) |
|---|---|---|---|
| 0,77 | 45 | 10 | 0,23 |
| 1,16 | 40 | 20 | 0,35 |
| 1,54 | 30 | 40 | 0,46 |
| 1,73 | 20 | 60 | 0,52 |
| 1,85 | 10 | 80 | 0,55 |
| 1,93 | 0 | 100 | 0,58 |

a) Plot the operating graph of this chemostat process (X,S and P vs D);

b) Identify the growth kinetic type of this microorganism and calculate the kinetic constants of that kinetic model;

c) Determine the cell from substrate yield coefficient, $Y_{x/s}$;

d) Determine the coefficient for the specific productivity relation;

e) Determine the value of the wash-out dilution rate, $D_{wash\text{-}out}$;

f) Determine the value of D for which the process maximum volumetric productivity will be reached ($D_{opt}$).