ORDRE DES INGÉNIEURS DU QUÉBEC

NOVEMBER 2012 SESSION

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

04-Chim-B4 Biochemical engineering

QUESTION 1 (5+10+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. The data in the presence and absence of each inhibitor are shown in the table below. Determine the manner of action of each inhibitor and calculate the kinetic constants r_{MAX}, K_M and $K_{S,i}$ (related to a Haldane enzyme kinetics) as well as the inhibition constant K_i for A, B and C, if the total enzyme concentration is 2 µM.

	r (µM/min)			
S (mM)	**Without inhibition**	**With A at 20 mM**	**With B at 25 mM**	**With C at 30 mM**
0,1	47,6	24,4	13,6	45,9
0,2	90,7	47,6	25,9	84,8
0,5	197,5	110,3	56,4	170,7
1	320,0	195,1	91,4	252,0
2	444,4	307,7	127,0	313,7
5	493,8	412,4	141,1	308,3
10	408,2	377,4	116,6	241,7
20	277,8	270,3	79,4	160,6

a) Plot the graphs that are required to identify the enzyme kinetics and the inhibition type; Indicate any useful information on this graph;
b) Determine the type of kinetics in absence of inhibitor and estimate its parameter values;
c) Determine the inhibition type caused by A, B and C (competitive, non-competitive or uncompetitive) and estimate their parameters, K_i;

QUESTION 2 (5+5+5+5+10+5+5 = 40 points): Microbial batch culture

A new bacterium strain, *P. quebecensis*, has been found to very useful for the treatment of soluble wastes from the paint industry. It's kinetics has been characterized. It has been found that this microorganism follows a substrate inhibition rate law (Haldane) of the form:

$$\mu = \frac{\mu_{MAX} \cdot S}{K_M + S + S^2 / K_{S,i}}$$

Where μ is the specific growth rate (h^{-1}), S is the only limiting substrate concentration (g/L), and μ_{MAX}, K_M and $K_{S,i}$ are the three parameters of that kinetic law, of values 1,39 h^{-1}, 5 g/L and 20 g/L respectively.

You plan to grow P. Quebecensis in a closed system (a batch fermenter) with an initial concentration of biomass X_0=0,1 g/L and of substrate, S_0 = 100 g/L. Before doing so, you want to predict mathematically the evolution of the biomass concentration (X, g/L) during this fermentation.

a) Assuming a constant substrate to yield coefficient ($Y_{X/S}$) of 0,3 g/g, how could you relate mathematically X to S during the fermentation?
b) Using the equation developed in (a), calculate the maximum X value the fermentation would attain;
c) Write the differential balance equation for X, using the microorganism rate law;
d) Substitute in the equation developed in (c) the relation for S developed in (a);
e) Solve the differential equation developed in (d) (you might need to used partial fraction decomposition to do so);
f) Using the equation developed in (e), calculate the time required for the fermentation to reach a point where 50% of the substrate would be converted to microorganism.
g) If you were to use an initial substrate concentration of 70 g/L, how long would it take to reach the same value of X used in (f)? How much time would you save compared to the result in (f)? Why is it so?

QUESTION 3 (5+5+10+10+5 = 35 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 chemostat (continuous) cultures for different dilution rates (D), limiting substrate (S) and biomass (X) concentrations:

D (d^{-1})	S (g/L)	X (g/L)
4,6	1	80
7,2	2	79
10,5	5	78
11,3	10	76
10,2	20	72
6,8	50	60
4,2	100	40
2,4	200	0

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

b) Determine the washout dilution rate, the feed substrate concentration and the cell to substrate yield coefficient ($Y_{X/S}$);

c) Determine this microorganism growth kinetic law and estimate the parameters of that relation;

d) Assuming that the production rate of a very interesting protein expressed by this microorganism obey a Luedeking-Piret law:

$$q_P = \alpha \cdot \mu + \beta$$

where q_p is the specific production rate of that protein (g product · g microorganism^{-1} · d^{-1}) and μ is the specific growth rate of the microorganism (d^{-1}), with α = 0,08 (g product / g microorganism) and β = 0,02 g product · g microorganism^{-1} · d^{-1}. Identify the dilution rate at which maximum volumetric productivity will be attain and provide the value of that productivity.

e) What makes the operation of a chemostat particularly difficult with a microorganism with that type of growth kinetics?