ORDRE DES INGÉNIEURS DU QUÉBEC

NOVEMBER 2017 SESSION

- All documents allowed
- Calculator: authorized models only
- Examination duration: 3 hours
- One (1) sheet of millimetered paper required

16-CH-A7 Génie biochimique, english version (total: 100 points)

QUESTION 1 (5+5+5+5+10 = 30 points): enzyme kinetics determination (use the millimetered paper to illustrate your graphical calculations)

The kinetic model of a complex irreversible enzymatic reaction involving two substrates, A and B:

$$A + B \xrightarrow{enzyme} C$$

This model has to be determined in order to design a large-scale process. Data at hand involve initial reaction rate measurements for different initial concentration of A and B, at a constant enzyme concentration of 100 µM:

C_A (mM)	C_B(mM)	r (M/h)
400	200	3,2
400	400	6,4
400	600	9,6
400	800	12,8
200	400	1,6
400	400	6,4
600	400	14,4
800	400	25,6

Determine the kinetic model of this enzymatic reaction.

a) Evaluate the partial order of the reaction rate with respect with the concentration of B

b) Evaluate the partial order of the reaction rate with respect with the concentration of A

c) Evaluate the global order of the reaction

d) Evaluate the reaction kinetic constant, k, in $mM^{-2} \cdot h^{-1}$

e) For the same enzyme concentration, and initial concentrations of A and B of 800 mM, evaluate the reaction time required (in h) to reach a substrate conversion of 90%

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

You will perform a batch fermentation for the growth of a micro-organism which has a first-order kinetics with respect to the limiting substrate concentration:

$$\mu = k \cdot S \qquad (1)$$

Where μ is the specific growth rate (s^{-1}), k = 0,01 $L \cdot g^{-1} \cdot h^{-1}$ is the kinetic constant ($L \cdot g^{-1} \cdot s^{-1}$) and S is the limiting substrate concentration (mM). Assuming the yield of micro-organism produced per substrate consumed, $Y_{X/S}$ = 0,4 g/g, is constant, that the micro-organism maintenance energy consumption and mortality rate are negligible, develop the cell concentration (X) growth model and predict the evolution of X with respect to time. The fermentation will take place from an initial concentration of substrate, S_0 = 100 g/L and of micro-organism, X_0 = 0,2 g/L

a) Compute the final and maximum micro-organism concentration that this fermentation will attain

b) Relate by a mathematical expression S to [$Y_{X/S}$, S_0, X_0, X]

c) Substitute the relation developed in (b) into the kinetic expression (eqn 1) and then the micro-organism balance equation:

$$\frac{dX}{dt} = \mu \cdot X \qquad (2)$$

d) Integrate the equation developed in (c) using partial fractions in order to obtain an explicit analytical solution for the determination of t, the culture time, with respect to a given X.

e) Estimate the time required for this batch fermentation for the micro-organism to reach 80% of the maximum cell concentration (identified in (a)).

f) Does this kinetics reminds you of any classical microbial growth kinetics, and if so which one?

QUESTION 3 (5+5+5+5+5 = 25 points): Continuous immobilized enzyme bioreactor

A series of immobilized lactase bioreactors is used to produce lactose-free milk for people suffering from lactose intolerance. The process is treating 10 millions L of milk / week. Raw milk contains 0,146 M lactose, and the process must reduce that level to 0,00292 M (so-called "lactate-free" milk). The enzyme has a Haldane-type kinetics:

$$q_S = \frac{q_{S,\max} S}{K_M + S + \frac{S^2}{K_i}}$$

where q_S is the specific substrate (lactate) consumption rate in (mole lactate·mole $enzyme^{-1} \cdot s^{-1}$, or more simply s^{-1}), S is the lactate concentration (M), $q_{S,max}$, K_M and K_i are the kinetic parameters, with values of 30 s^{-1}, 0,005 M and 0,05 M, respectively. The immobilized enzyme concentration in bioreactors is 10 μM, stays constant and 100% active.

You plan to design a 2-bioreactor system, where the first one will operate at maximum reaction rate and the second will bring lactate concentration to the required level.

a) Given the enzyme kinetic type, calculate the lactate concentration at which the bioreaction rate will be maximum as well as the value of q_S at that S?

b) Calculate the feed rate of milk for this bioreactor system, F, in L/s

c) Develop the lactate mole balance equation for a steady-state continuous stirred-tank reactor (CSTR) with an immobilized enzyme, and isolate the bioreactor volume (V) from this equation

d) Calculate the first bioreactor volume (V1), operating at the rate obtained in (a)

e) Calculate the second bioreactor volume (V2), assuming it is fed from the first bioreactor at the lactate concentration calculated in (a) and deliver milk with the lactate concentration required for it to be considered “lactate free”.

QUESTION 4 (5 points): General questions

a) Describe the general effect that micro-organism maintenance energy consumption would have on a fermentation;

b) Describe what would be the effect of a constant cell death rate on a chemostat fermentation;

c) Describe what diauxic cell growth is;

d) Describe what an autotrophic micro-organism is;

e) Describe what an anaerobic micro-organism is.