ORDRE DES INGÉNIEURS DU QUÉBEC

NOVEMBER 2011 SESSION

- Open-book examination
- Calculator: only authorized models
- Duration: 3 hours
- Three (3) sheets of millimetered paper required

04-Chim-B4 Génie biochimique en anglais

QUESTION 1 (5+5+5+5+3+2 = 25points): enzyme kinetics (millimetered sheet #1)

The volumetric reaction rate (r) of an enzyme with respect to a limiting substrate concentration (S) has been measured by an initial rate method. Results of this experiment are shown in the table below

S (mM)	r (mM/min)
4100	177
950	173
520	125
103	106
49	80
10,6	67
5,1	43

Assuming a Michaelis-Menten type kinetics, evaluate as precisely as possible both parameters of that kinetic by:

a) The Lineweaver-Burke method.
b) The Eadie-Hofstee method.
c) The Hanes-Woolf method.

d) Which of the plot above do you think gives the best results? Discuss.

e) If you could suggest a better method, what would it be? Discuss.

f) If the enzyme concentration in all of these tests was 1 µM, what would be the turnover rate (specific activity) of this enzymatic reaction?

QUESTION 2 (2+3+5+10 = 20 points): Microbial kinetics (millimetered sheet #2)

The following data were collected during the course of a batch culture of a very interesting ethanol producing microorganism.

t (min)	S (g/L)	X (g/L)	P (g/L)
0	30	0,1	0
20	30	0,1	0
174	20,5	2	0,95
214	10,5	4	1,95
230	5,5	5	2,45
256	0,5	6	2,95

Where t is time, S is the limiting substrate concentration, X is the microorganism concentration and P is the product concentration. You want to evaluate the parameters of that microorganism kinetics.

a) What is the lag-phase duration of this fermentation?
b) What is the microorganism to substrate yield coefficient ($Y_{x/s}$) of this fermentation? Is it constant?
c) What is the product to substrate yield coefficient ($Y_{p/s}$) of this fermentation? Is it constant?
d) If this microorganism growth is govern by a Monod kinetics, what would be the maximum specific growth rate (μ_{max}) and saturation constant (Ks)?

QUESTION 3 (3+2+5+5+5+5+5+5 = 35 points): Fed-batch culture design

Your project consists in optimizing the course of a fed-batch fermentation for a bacterium whose kinetics follow a Haldane (i.e. substrate inhibition) law, with:

$\mu_{max} = 0{,}012\ min^{-1}$

$K_s = 2\ g/L$

$K_i = 10\ g/L$

$Y_{x/s} = 0{,}4\ g/g$

Your fermentation will start as a fully active 100 L culture of microorganism at a concentration of 0,2 g/L that you will feed with a concentrated limiting-substrate solution of S_{in} = 260 g/L. The final volume (V_{final}) of the fermentation will be 200 L. You will assume that the culture will not be submitted to oxygen transfer limitation nor product inhibition and that it will have no lag phase. Your objective is to maintain the optimal substrate concentration at all time during the course of the culture.

a) Identify the optimal substrate concentration at which the specific growth rate will be the highest.
b) How will you program the feed rate of the concentrated solution to maintain a constant substrate concentration and specific growth rate at the optimal value throughout the fermentation? Do you think the feed rate could be maintained constant to reach that objective? What trend should follow the feed rate?
c) Do a differential volume balance on the bioreactor and solve it as much as possible.

d) Do a balance on the total quantity of microorganism in the bioreactor ($X \cdot V$) and solve the differential equation considering that quantity as a variable.

e) Do a substrate balance on the bioreactor. With the adequate hypothesis, solve the differential equation in order to propose a function for the feed rate (F) with respect to time.

f) What will be the initial feed rate to the bioreactor?

g) What will be the total duration of the fed-batch?

h) What quantity of microorganism will be produced during this fed-batch?

QUESTION 4 (5+5+5+5 = 20 points): Perfectly mixed continuous fermentor (CSTR) operation design (millimetered sheet #3)

Your company is setting up a new fermentation project for polyhydroxybutyrate (PHB, a biopolymer) production with bacterial specie Azotobacter vinelandii. This microorganism follows Monod law kinetics:

$\mu_{max} = 0{,}017\ min^{-1}$

$K_s = 20\ g/L$

$Y_{p/s} = 0{,}05\ g/g$

But with a cell to substrate yield ($Y_{x/s}$) that is not constant, as it tends towards a value of $Y_{x/s,\ ideal} = 0{,}3\ g/g$ at very high growth rate, while substrate specific consumption rate always maintains a basal maintenance value of m=0,02 g S/(g $X \cdot$min).

You are in charge of this project, and decide the best PHB productivity will be obtained in a CSTR. To evaluate the performance of the process:

a) Draw a CSTR operation diagram, starting with S as a function of the dilution rate, D. Identify the washout dilution rate.

b) Draw the X and P curve on the same operation diagram.

c) Identify the dilution rate at which you will obtain a maximum product concentration (with a dilution rate precision of $0{,}002\ min^{-1}$).

d) How many kilograms of PHB will be produced per year in a 1000L bioreactor at the dilution rate identified in (c), assuming a 24h/day, 365 day/year operation? Is this the maximum productivity obtainable with this process?