ORDRE DES INGÉNIEURS DU QUÉBEC

MAY 2018 SESSION

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

16-CH-A7 BIOCHEMICAL ENGINEERING (total: 100 points)

QUESTION 1 (5+5+5+5+5+5 = 30 points): Comparison of different methods to assess kinetic parameter values (millimetered paper required)

Enzymatic reaction rates (r, in mM/min) have been measured for different limiting substrate concentrations (S, in mM) by performing initial rate experiments. The results obtained are shown below.

S (mM)	r (mM/min)
2,6	86
5,3	134
24,5	160
51,5	212
260	250
475	346
2050	354

Assuming this enzyme is govern by a Michaelis-Menten type kinetics, evaluate as precisely as possible the two parameters of that kinetic relation by three different methods:

a) Lineweaver-Burke.

b) Eadie-Hofstee.

c) Hanes-Woolf.

d) According to these results and your general knowledge, which one of these methods gives the most accurate estimation of parameter values and why?

e) Could you suggest another, better method and if so, which one and why would it be better?

f) If the enzyme concentration during the experiment above was 1 μM, what would be the turnover rate of this enzyme?

QUESTION 2 (5+10+10+5 = 30 points): Microbial batch growth with decay

A batch fermentation has to be performed with a very peculiar microorganism: i) for the conditions under study, its growth can be described as exponential, but 2) it is severely affected by a strong decay (mortality) rate. This mortality is dependant on the accumulation of a toxic by-product, but can be represented by a mortality rate constant, k_d (min^{-1}), that varies exponentially with respect to time:

$$k_d = k_{d0} e^{k \cdot t}$$

where k_{d0} is the initial mortality rate constant and k is the exponential rate constant at which the mortality rate increases.

Given the batch fermentation mass balance on biomass:

$$\frac{dX}{dt} = \left(\mu - k_d\right) X$$

Where X is the biomass concentration (in g/L), μ is the specific growth rate (min^{-1}) and t is the time (min). Given also that: X_0 = 0,1 g/L (initial biomass concentration), μ = 0,046 min^{-1}, k_{d0} = 0,01 min^{-1} and k = 0,01 min^{-1}:

a) Fully develop the differential mass balance for the biomass in this batch fermenter to obtain an explicit differential expression for dX/dt = fcn(t,X)

b) Integrate the relation obtained in (a) to obtain an explicit expression for X = fcn(t)

c) Calculate the value of X for t = [0-250 h] with 50 h intervals. Compare to the X that would be obtained without any mortality for the same time intervals

d) In order to obtain the precise time at which the biomass yield will be maximized, solve the equation developed in (a) to calculate exactly that time and calculate it.

QUESTION 3 (3+2+5+5+5+5+5 = 30 points): Fed-batch fermentation simulation

You have been given a project that aims at simulating the course of a fed-batch fermentation for a bacterium producing acetone following a Haldane-type (substrate-inhibited) growth kinetics.

The kinetic parameters are:

μ_{max} = 0,005 min^{-1}	$Y_{x/s}$ = 0,4 g/g	K_i = 15 g/L
K_s = 1 g/L	$Y_{p/x}$ = 0,15 g/g	

The lag phase, maintenance substrate consumption, cell mortality, mass transfer limitations and byproduct inhibition are all considered negligible. Acetone production is known to be strictly growth-associated.

The fermentation will start at a volume of 100 L, a microorganism concentration of 0,3 g/L and will be fed by a concentrated limiting-substrate solution ($[S]_{in}$ = 300 g/L). The fermentation will stop when the working volume will reach 200 L. Your objective consist in simulating the dynamics of that fermentation in order to identify conditions that will allow a maximum product yield.

a) Identify the substrate concentration that would provide the largest possible specific production rate;

b) What would be the best feeding program strategy to maintain this optimal substrate concentration (identified in (a)) in the fermentation at all time? Do you think that a constant-flowrate strategy would allow you to reach that objective? Else, what trend should follow this feedind strategy?

c) Make a differential volume balance over the fermenter and solve that differential equation as much as possible;

d) Make a differential mass balance on the total microorganism content of the fermenter (X*V), considering this product of variables as a variable as such;

e) Make a differential mass balance on the limiting substrate in the fermenter. State the required hypotheses and solve (integrate) that equation in order to arrive at a feeding program equation, F = fcn(t), where F is the volumetric flowrate of the feeding solution;

f) Following this approach, what would be the initial feed flowrate?

g) What would be the total quantity of acetone produced during this fed-batch fermentation?

QUESTION 4 (2 + 2 + 2 + 2 + 2 = 10 points): Fermentation process development strategy

Assume you are to lead a team in charge of developing a fermentation process. For each of the element below, describe in a few sentences how you would handle each of these issues in the general strategy of this process development project:

a) The dynamic aspects of a continuous culture in a CSTR

b) Including maintenance effects on limiting-substrate consumption as well as cell mortality

c) Including mass transfer limitations

d) Including byproduct toxicity effects

e) Including the effect of multiple substrates, and change in limiting-substrate between these substrates as well as C/N sources