Midterm 2

CHE 170A: Biochemical Engineering

November 12, 2010

Name: _____ SID: _____

Problem 1: _____ pts/20 pts

Problem 2: _____ pts/25 pts

Problem 3: _____ pts/20 pts

Problem 4: _____ pts/15 pts

Problem 5: _____ pts/20 pts

Total: _____ pts/100 pts

Problem 1.

Consider the growth of a microorganism in batch culture. When the substrate concentration is high, the cell density doubles every 0.75 h, the observed substrate yield coefficient is 0.3 g DCW/g, and substrate consumption is allocated towards biosynthesis (60%), maintenance (10%), as well as product formation (30%). The product formation is strictly growth-associated.

The batch reactor is inoculated with 0.01 g DCW/L and 10 g/L substrate.

a. Estimate the maximum cell density and the time (after lag phase) required to achieve it. (6 pts)

$$X_{\text{from substrate}} = 10\frac{\text{g substrate}}{\text{L}} \cdot 0.6 \cdot 0.3\frac{\text{g DCW}}{\text{g substrate}} = 1.8\frac{\text{g DCW}}{\text{L}}$$

$$X_{\max} = X_o + X_{\text{from substrate}} (Ok \text{ if they left out } X_o)$$

$$X_{\max} = 0.01\frac{\text{g DCW}}{\text{L}} + 1.8\frac{\text{g DCW}}{\text{L}}$$

$$X_{\max} = 1.81\frac{\text{g DCW}}{\text{L}} \;\; or \;\; 1.80\frac{\text{g DCW}}{\text{L}}$$

$$X = X_o e^{\mu t}$$

$$\mu = \frac{\ln(2)}{t_d} = \frac{\ln(2)}{0.75h} = 0.924\ hr^{-1}$$

$$t = \frac{\ln\left(\frac{X}{X_o}\right)}{\mu} = \frac{\ln\left(\frac{1.81}{0.01}\right)}{0.924\ hr^{-1}} = 5.63\ hr \text{ (t should be about the same if they used } 1.8\frac{\text{g DCW}}{\text{L}})$$

b. Determine the value of the maintenance coefficient (g substrate/g DCW-h). (6 pts)

- 10% of the substrate goes to maintaining the cells.

$$m = \frac{10\frac{\text{g substrate}}{\text{L}} \cdot 0.10}{1.81\frac{\text{g DCW}}{\text{L}} \cdot 5.63hr}$$

$$m = 0.099\frac{\text{g substrate}}{\text{g DCW} \cdot \text{hr}}$$

c. Before inoculation of the batch reactor, you need to sterilize the medium, which contains 10^5 spores L^{-1}. The value of k_d has been determined to be 1 min^{-1} at 121 °C and 61 min^{-1} at 140 °C. For each temperature, determine the required time in the holding section so as to insure that the medium is 95% sterile. The volume of the reactor is 20 L. Neglect heating and cooling. (8 pts)

$$P(t) = \left(1 - p(t)\right)^{N_o} = \left(1 - e^{-kt}\right)^{N_o} \text{ where } N_o = \frac{\#\text{of spores}}{L} \cdot V_{liquid}$$

Solve for t:

$$t = \frac{\ln\left[1 - P(t)^{1/N_o}\right]}{-k}$$

At T = 121 °C

$$t = \frac{\ln\left[1 - 0.95^{1/\left(10^5 \frac{\text{spores}}{L} 20L\right)}\right]}{-1\,\text{min}^{-1}} = 17.5\,\text{min}$$

At T = 140 °C

$$t = \frac{\ln\left[1 - 0.95^{1/\left(10^5 \frac{\text{spores}}{L} 20L\right)}\right]}{-61\,\text{min}^{-1}} = 0.287\,\text{min}$$

Problem 2.

Consider a culture of bacteria that secrete a product in a chemostat operated at steady state. The specific growth rate of biomass is adequately described by the Monod equation, and the rate of product formation is described by Leudeking-Piret kinetics: $r_P = (\alpha\mu + \beta)X$

This system is well characterized, such that the following constants are known:

$Y_{X/S}$ = 0.4 g/g	α = 0.2 g/g	S_o = 10 g/L
μ_{max} = 0.7 h^{-1}	β = 0.3 g/g-h	F = 15 L hr^{-1}
K_S = 0.2 g/L	$Y_{P/S}$ = 0.8 g/g	V = 500 L

The liquid feed to the chemostat is sterile, and the flow rates entering and exiting the chemostat are equal.

a. Write steady state mass balances for S, X, and P. (5 pts)

$$X \text{ balance: } 0 = -FX + \mu XV \text{ or } DX = \mu X \text{ or } 0 = -FX + \frac{\mu_{max} S}{S + K_s} XV$$

$$\text{S balance: } 0 = F(S_o - S) + r_S V \quad \text{or} \quad 0 = F(S_o - S) - V\left(\frac{1}{Y_{X/S}} \frac{\mu_{max} S}{S + K_s} X + \frac{\alpha\left(\frac{\mu_{max} S}{S + K_s}\right)X + \beta X}{Y_{P/S}}\right)$$

$$P \text{ balance: } 0 = -FP + r_p V \quad \text{or} \quad 0 = -FP + (\alpha\mu X + \beta X)V \quad \text{or} \quad 0 = -FP + \left(\alpha \frac{\mu_{max} S}{S + K_s} X + \beta X\right)V$$

$$\text{or } DP = (\alpha\mu + \beta)X$$

b. What is the steady state concentration of S? (5 pts)

Using the X balance:

$$0 = -FX + \frac{\mu_{max} S}{S + K_s} XV$$

$$\frac{F}{V} = \frac{\mu_{max} S}{S + K_s}$$

$$\frac{F}{V}(S + K_s) = \mu_{max} S$$

$$\frac{F}{V} S + \frac{F}{V} K_s = \mu_{max} S$$

$$\frac{F}{V} K_s = S\left(\mu_{max} - \frac{F}{V}\right)$$

$$S = \frac{\frac{F}{V} K_s}{\left(\mu_{max} - \frac{F}{V}\right)} = \frac{\frac{15\frac{L}{hr}}{500L} 0.2\frac{g}{L}}{\left(0.7hr^{-1} - \frac{15\frac{L}{hr}}{500L}\right)} = 9x10^{-3}\frac{g}{L}$$

c. What is the steady state concentration of X? If you were unable to obtain an answer for S in part b, use S = 0.2 g/L. (5 pts)

Use the S balance:

$$0 = \mathrm{F}\left(\mathrm{S_o} - S\right) - \mathrm{V}\left(\frac{1}{Y_{X/S}}\frac{\mu_{\max} S}{S + K_s}\mathrm{X} + \frac{\alpha\left(\frac{\mu_{\max} S}{S + K_s}\right)\mathrm{X} + \beta \mathrm{X}}{Y_{P/S}}\right)$$

$$\left(\frac{1}{Y_{X/S}}\frac{\mu_{\max} S}{S + K_s} + \frac{\alpha\left(\frac{\mu_{\max} S}{S + K_s}\right) + \beta}{Y_{P/S}}\right) X = \frac{\mathrm{F}\left(\mathrm{S_o} - S\right)}{V}$$

$$X = \frac{\mathrm{F}\left(\mathrm{S_o} - S\right)}{V\left(\frac{1}{Y_{X/S}}\frac{\mu_{\max} S}{S + K_s} + \frac{\alpha\left(\frac{\mu_{\max} S}{S + K_s}\right) + \beta}{Y_{P/S}}\right)} = \frac{15\frac{\mathrm{L}}{\mathrm{hr}}\left(10 - 9x10^{-3}\frac{g}{L}\right)}{500L\left(\frac{1}{0.4}\frac{0.7hr^{-1}9x10^{-3}\frac{g}{L}}{9x10^{-3}\frac{g}{L} + 0.2\frac{g}{L}} + \frac{0.2\left(\frac{0.7hr^{-1}9x10^{-3}\frac{g}{L}}{9x10^{-3}\frac{g}{L} + 0.2\frac{g}{L}}\right) + 0.3}{0.8}\right)}$$

$$X = 0.655\frac{g}{L}$$

d. What is the productivity (g product per g substrate per time) of this process? If you were unable to answer parts b or c, use S = 0.2 g/L and X = 0.2 g/L. (5 pts)

- Since the organism has to grow at the a rate equal to the dilution rate, the productivity is a function of how quickly the organism grows, i.e. makes product, even though the product is both growth and non-growth associated.

$$\text{Productivity} = \mathrm{Y_{P/S}}\mu = Y_{P/S}\frac{\mu_{\max} S}{S + K_s}$$

$$\text{Productivity} = 0.8\frac{g\ \text{product}}{g\ \text{substrate}}\frac{0.7hr^{-1}9x10^{-3}\frac{g}{L}}{9x10^{-3}\frac{g}{L} + 0.2\frac{g}{L}}$$

$$\text{Productivity} = 0.024\ \frac{g\ \text{product}}{g\ \text{substrate}\cdot\ \text{hr}}$$

e. If the volume of the reactor is kept constant, what value of the flow rate would cause "washout" of the reactor? (5 pts)

$$\text{Wash-out: } X \to 0,\ S \to S_o$$

From biomass S.S. mass balance:

$$\frac{F}{V} = \frac{\mu_{max} S}{S + K_s}$$

$$F = \frac{0.7 hr^{-1} \cdot 10 \frac{g}{L}}{10 \frac{g}{L} + 0.2 \frac{g}{L}} 500 L$$

$$F = 343 \frac{L}{hr}$$

Problem 3.

Rhodobacter sphaeroides is a purple bacterium that can produce hydrogen gas from organic acids, such as acetic acid, in a continuous culture. The substrate is provided continuously in the entering liquid stream.

a) Write the unsteady state mass balances for hydrogen in both the gas and the liquid. Define the variables in your equations. (8 pts)

$$\frac{d\left(\frac{PV_g y_{H_2}}{RT}\right)}{dt} = \frac{P}{RT}\left(Q_1 y_{H_2,in} - Q y_{H_2,out}\right) - K_g a P V_L\left(y_{H_2} - \frac{H}{P} C_{H_2}\right)$$

Liquid:

$$\frac{d\left(C_{H_2} V_L\right)}{dt} = K_g a P V_L\left(y_{H_2} - \frac{H}{P} C_{H_2}\right) + r_{H_2} V_L - F C_{H_2}$$

P: pressure T: temp y_{H_2}: mole frac. of H2 in the gas Q: volumetric gas flow rate

V_g: gas volume V_L: gas volume K_g: mass transfer const. a: gas/liquid interfacial area/volume

C_{H_2}: concentration of H_2 in liquid H: Henry's constant F: liquid flow rate

r_{H_2}: H_2 reaction rate, $r_{H_2} = q_{H_2} X$

b) Derive a steady-state equation for the concentration of hydrogen in the gas given that there is no hydrogen in the entering liquid stream and the hydrogen only leaves in the exiting gas stream. (6 pts)

Assume C_{H_2} in exit stream is zero.

Add liquid and gas H_2 mass balances:

$$Q_2 y_{H_2} \frac{P}{RT} = r_{H_2} V_L$$

Assume $Q_1 = Q_2 = Q$ at steady state.

$$y_{H_2} = \frac{r_{H_2} V_L RT}{Q \cdot P} = \frac{q_{H_2} X V_L RT}{Q \cdot P} \quad \text{or} \quad p_{H_2} = \frac{q_{H_2} X V_L RT}{Q}$$

c) During the process, you monitor the partial pressure of hydrogen (p_{H_2}) in exiting gas stream. What is the biomass concentration $\left(\frac{g\ \text{DCW}}{\text{L}}\right)$ in the reactor if p_{H_2}=30 kPa? (6 pts)

T=30 °C Q = 1.75 $\frac{mL}{\text{min}}$ $q_{H_2} = 35\frac{mL\ \text{H}_2}{\text{g DCW}\cdot\ \text{hr}}$ $\rho_{H_2} = 0.08\frac{g\ \text{H}_2}{\text{mL}}$ V=20 L

$$p_{\text{H}_2} = \frac{q_{\text{H}_2} X V_L RT}{\text{Q}}$$

$$X = \frac{Q\cdot p_{H_2}}{q_{H_2} V_L RT} = \frac{1.75\frac{mL}{\text{min}}\cdot 60\frac{\text{min}}{hr}\cdot 30kPa}{35\frac{mL\ \text{H}_2}{\text{g DCW}\cdot\ \text{hr}}\,0.08\frac{g\ \text{H}_2}{mL}\,\frac{mol}{2g\text{H}_2}\,20L\cdot 8.314\frac{kPa\cdot L}{mol\cdot K}\,1000\frac{mL}{L}\left(30+273K\right)}$$

$$X = 4.5x10^{-5}\,\frac{DCW}{L}$$

Problem 4. (15 pts)

Estimate the stirrer power requirement (P) for a tank fermenter, 1.8 m in diameter, stirred by a pitched-blade, turbine-type impeller of diameter D_i = 0.5 m with a rotational speed N of 1 s^{-1}. The tank contains a viscous non-Newtonian broth: κ = 124, and ρ = 1050 kg m^{-3}.

$$\mu_{app} = \kappa\left(11N\right)^{n-1}\left(\frac{3n+1}{4n}\right)^{n} = 124\left(11\cdot 1s^{-1}\right)^{0.75-1}\left(\frac{3(0.75)+1}{4(0.75)}\right)^{0.75}$$

$$\mu_{app} = 72.3\frac{kg}{s\cdot m}$$

or

$$\mu_{app} = \kappa\left(kN\right)^{n-1} = 124\left(10\cdot 1s^{-1}\right)^{0.75-1}$$

$$\mu_{app} = 69.7\frac{kg}{s\cdot m} \text{ or } 68\frac{kg}{s\cdot m} \text{ if used k} = 11$$

$$\text{Re} = \frac{D_i^2 N\rho_L}{\mu_{app}} = \frac{\left(0.5m\right)^2 1s^{-1}\cdot 1050\frac{kg}{m^3}}{72.3\frac{kg}{s\cdot m}} \quad \text{or} \quad \text{Re} = \frac{D_i^2 N\rho_L}{\mu_{app}} = \frac{\left(0.5m\right)^2 1s^{-1}\cdot 1050\frac{kg}{m^3}}{6.75\frac{kg}{s\cdot m}}$$

$$\text{Re} = 3.63 \quad \text{or} \quad 3.86$$

$N_P \approx 14$ from figure on last page of exam

$$N_P = \frac{P}{N^3 D_i^5 \rho_L}$$

$$P = N_P N^3 D_i^5 \rho_L = 14\cdot\left(1s^{-1}\right)^3\left(0.5m\right)^5 1050\frac{kg}{m^3} \quad \text{or} \quad 3\cdot\left(1s^{-1}\right)^3\left(0.5m\right)^5 1050\frac{kg}{m^3}$$

$$P = 459W$$

Problem 5.

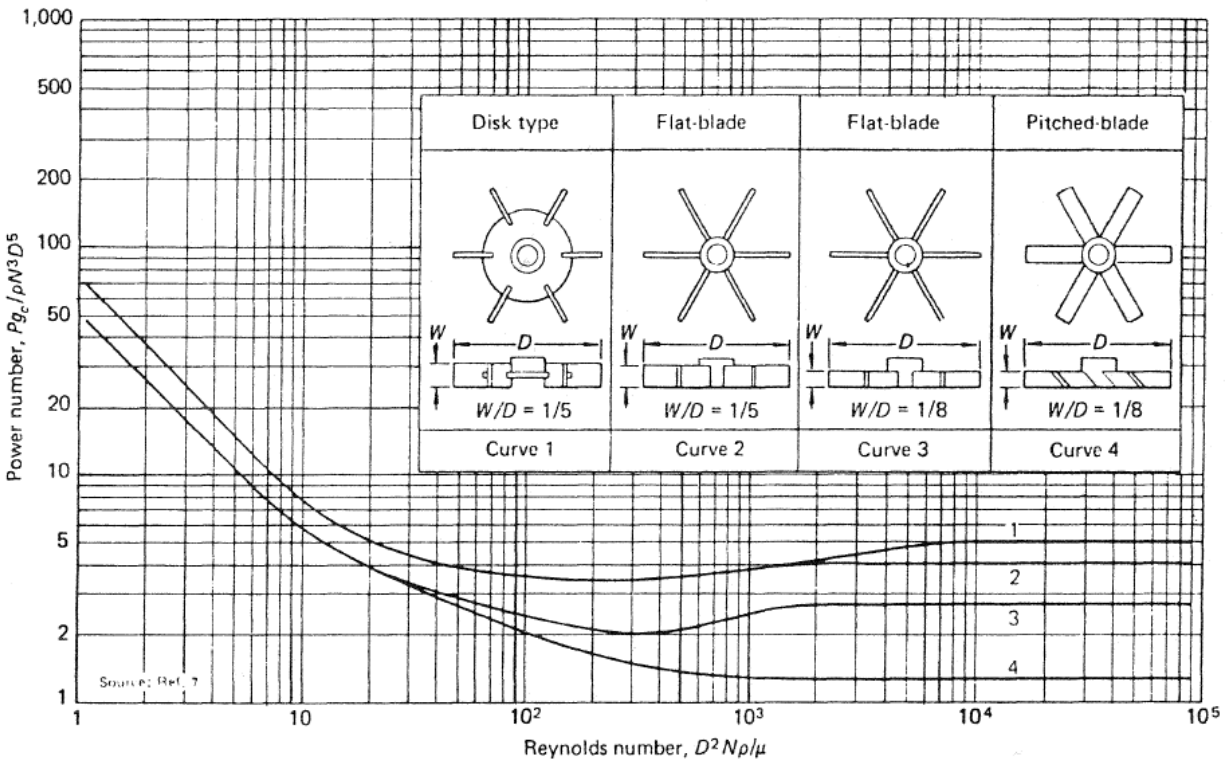
Genzyme has hired you to work at their production facility for Myozyme, an α-glucosidase used in the treatment of Pompe disease. Myozyme is produced in mammalian cells and currently the cell density and product titer are very low. Your collegues, who are recent graduates of Stanford, cannot figure out how to develop a serum-free medium that will improve the production of Myozyme.

a. Discuss your strategy for developing a medium that meets the nutrient requirements of the cell. A bulleted list is acceptable.

1) Identify depleted nutrients by analysis of spent medium.
2) Identify further candidate nutrients by consideration of metabolic pathways.
3) Add each component and determine its maximum non-inhibitory concentration.
4) Keep adding additional candidates until the combination results in a synergistic increase in growth and product expression.

b. You discover that the addition of ammonium chloride induces the production of Myozyme at a certain cell density. Unfortunately, this production facility has also suffered from contamination issues because the Stanford graduates take samples directly from the reactor. How would you measure the cell density in the medium without taking a sample from the reactor?

- Before culture:
    - Measure k_La of bioreactor.
    - Measure q_{O2} using Warburg respirometer.
- During culture:
    - Measure PO_2^G using flow meters on inlet gas stream.
    - Measure PO_2^L using dissolved oxygen probe.



Gas Constant: R = 8.314 $\frac{kPa \cdot L}{mol \cdot K}$

$$k = k_o e^{-E_a/RT}$$

$$N_P = \frac{P}{N^3 D_i^5 \rho_L}$$