UNIVERSITI SAINS MALAYSIA

Second Semester Examination
2011/2012 Academic Session

June 2012

EKC 574 – Downstream Processing of Biochemical and Pharmaceutical Products

Duration : 3 hours

Please ensure that this examination paper contains THREE printed pages and TWO printed pages of Appendix before you begin the examination.

Instruction: Answer **ALL** questions.

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1. [a] What are the fundamental properties of biological material relevant to bioseparation? Give three characteristics and suggest how each can be used to effect separation.

[6 marks]

[b] Monoclonal antibodies produced in serum free conditions were harvested from supernatants of murine hybridomas. The major stages of downstream processing of monoclonal antibodies include clarification, concentration and purification. Give an example of the process for each scheme.

[9 marks]

[c] Chromatography is similar to adsorption because both involve the interaction between solute and solid matrix. State what is the major different between these two processes? For designing and operating a chromatograph, the yield and purity of the separated products are the two important parameters to be controlled. Explain their differences and express their formulation in integral forms.

[10 marks]

2. [a] Explain the similarities and differences of filtration and centrifugation in the first scheme of downstream processing of biochemical and pharmaceutical products. How to increase the efficiency of the centrifugal separations?

[12 marks]

[b] It is desired to achieve complete recovery of bacterial cells from a fermentation broth with a pilot plant scale tubular centrifuge. It has been already determined that the cells are approximately spherical with a radius of 0.5 μm and a density of 1.10 g/cm^3. The speed of the centrifuge is 5000 rpm, the bowl diameter is 10 cm, the bowl length is 100 cm, and the outlet opening of the bowl has a diameter of 4 cm. Estimate the maximum flow rate of the fermentation broth that can be attained. After cell disruption, the cell diameter is reduced by one-third and the viscosity is increased by a factor of 4. Calculate the new feed flow, considering that the centrifuge operates at the same speed and efficiency. Assume that the cell suspension density and viscosity are close to those of water, i.e.: ρ_w = 1.00 g/cm^3, μ = 0.01 g/cm·s.

[13 marks]

3. [a] Based on the structure of the gram-positive bacteria, suggest three (3) disruption techniques that is suitable to disintegrate the cells. Justify your answer.

[6 marks]

[b] An adsorption column with a diameter of 2.0 cm and a bed height of 5.8 cm was used to isolate trypsin. The concentration of trypsin in the feed was 0.190 mg/ml. The flow rate of the feed is 0.109 cm^3/s. The external void fraction in the bed was 0.35. The bulk density of the adsorbent (in its hydrated state in the bed) is 1.03 g/cm^3. The volumes at breakthrough and exhaustion point are 150 and 450 cm^3, respectively. Estimate the loading capacity of the adsorbent for trypsin.

[9 marks]

[c] The data in Table Q.3.[c]. has been measured for the growth of crystals for an antibiotic. From this data set, obtain an expression for the relationship between *dL/dt* and the supersaturation, *c - c**.

Table Q.3.[c].

*c – c** (g/liter)	*dL/dt* (μm/min)
0.20	0.21
0.35	0.45
0.67	0.90
1.25	1.80
2.05	3.30
2.75	5.00

[10 marks]

4. [a] An antibiotic, cycloheximide, is to be extracted from the clarified fermentation beer by using methylene chloride as solvent. The distribution coefficient, K is 23. The initial concentration of cycloheximide in the feed is 150 mg/L. The solvent containing 5 mg/L of cycloheximide is being used with the flow rate of 1 m^3/h. The required recovery of the antibiotic is 98 percent. With equal solvent flow rate in three (3) stages, how much feed can be processed per hour for co-current and counter-current flow.

[15 marks]

[b] The solubility of a certain protein in water is 250 g/L. When 20 ml of ethanol was added to 100 ml of a 80 mg/ml protein solution in water, 40% of the protein was found to precipitate. If the dielectric constant of pure water and ethanol are 78.3 and 24.3, respectively, calculate the volume of ethanol needed to precipitate 95% of the protein in a 100 mL protein aqueous solution. Organic solvents are used for precipitation of some biomaterial. Explain and comment on how precipitation in presence of organic solvents canbe increased.

[10 marks]

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Appendix

$$\upsilon_g = \frac{\rho_p - \rho_f}{18\mu} D_p{}^2 g$$

$$\upsilon_c = \frac{\rho_p - \rho_f}{18\mu} D_p{}^2 \omega^2 r$$

$$Q = \upsilon_g \left[\frac{2\pi l R^2 \omega^2}{g} \right]$$

$$Q = \upsilon_g \left[\frac{2\pi n \omega^2}{3g} \left(R_0^3 - R_1^3 \right) \cot\theta \right]$$

$$Q = \upsilon_g \left[\frac{\pi l (R_0^2 - R_1^2)\omega^2}{g \ln(R_0 / R_1)} \right]$$

g = 980 cm/s^2

$$t_E = \frac{1}{\upsilon\varepsilon}(K + \varepsilon - K\varepsilon)$$

$$\upsilon y_F t^* = L\rho_b q_{sat}$$

$$\theta = 1 - \left(\frac{t_E - t_B}{2t_B} \right)$$

$$q = Ky \qquad q = Ky^n \qquad q = \frac{q_0 y}{K + y}$$

$$B = \frac{dN}{dt} = k_n (c - c^*)^n$$

$$\frac{dM}{dt} = kA(c - c^*)$$

$$G \equiv \frac{dL}{dt} = k_g (c - c^*)^n$$

$$C_{R,n+1} = \left(1 + \lambda + \lambda^2 + .. + \lambda^n\right) C_{R,1} = \left(\frac{\lambda^{n+1} - 1}{\lambda - 1} \right) C_{R,1}$$

$$\ln\left(\frac{S}{S_w} \right) = \frac{A}{RT} \left[\frac{1}{\varepsilon_w} - \frac{1}{\varepsilon} \right]$$

Common Engineering Conversion Factors

Length	Volume
1 ft = 12 in = 0.3048 m, 1 yard = 3 ft 1 mi = 5280 ft = 1609.344 m 1 nautical mile (nmi) = 6076 ft	1 ft^3 = 0.028317 m^3 = 7.481 gal , 1 bbl = 42 U.S. gal 1 U.S. gal = 231 in^3 = 3.7853 L = 4qt = 0.833 lmp.gal. 1 L = 0.001 m^3 = 0.035315 ft^3 = 0.2642 U.S. gal
Mass	**Density**
1 slug = 32.174 lb$_m$ = 14.594 kg 1 lb$_m$ = 0.4536 kg = 7000 grains	1 slug/ft^3 = 515.38 kg/m^3 , 1 g/cm^3 = 1000 kg/m^3 1 lb$_m$/ft^3 = 16.0185 kg/m^3 , 1 lb$_m$/in^3 = 27.68 g/cm^3
Acceleration & Area	**Velocity**
1 ft/s^2 = 0.3048 m/s^2 1 ft^2 = 0.092903 m^2	1 ft/s = 0.3048 m/s , 1 knot = 1 min/h = 1.6878 ft/s 1 min/h = 1.4666666 ft/s (fps) = 0.44704 m/s
Mass Flow & Mass Flux	**Volume Flow**
1 slug/s = 14.594 kg/s. 1 lb$_m$/s = 0.4536 kg/s 1 kg/m^{2}s = 0.2046 lb$_m$/ft^{2}s = 0.00636 slug/ft^{2}s	1 gal/min = 0.00228 ft^3/s = 0.06309 L/s 1 million gal/day = 1.5472 ft^3/s = 0.04381 m^3/s
Pressure	**Force and Surface Tension**
1 lb$_f$/ft^3 = 47.88 Pa, 1 torr = 1 mm Hg 1 psi = 144 psf, 1 bar = 10^5 Pa 1 atm = 2116.2 psf = 14696 psi = 101,325 Pa = 29.9 in Hg = 33.9 ft H_2O	1 lb$_f$ = 4.448222 N = 16 oz, 1 dyne = 1 g cm/s^2 = 10^{-5}N 1 kg$_f$ = 2.2046 lb$_f$ = 9.80665 N 1 U.S. (short) ton = 2000 lb$_f$, 1 N = 0.2248 lb$_f$ 1 N/m = 0.0685 lb$_f$/ft
Power	**Energy and Specific Energy**
1 hp = 550 (ft.lb$_f$)/s = 745.7 W 1 (ft.lb$_f$)/s = 1.3558 W 1 Watt = 3.4123 Btu/h = 0.00134 hp	1 ft lb$_f$ = 1.35582 J, 1 hp·h = 2544.5 Btu 1 Btu = 252 cal = 1055.056 J = 778.17 ft lb$_f$ 1 cal = 4.1855 J, 1 ft.lb$_f$/lb$_m$ = 2.9890 J/kg
Specific Weight	**Heat Flux**
1 lb$_f$/ft^3 = 157.09 N/m^3	1 W/m^2 = 0.3171 Btu/(h ft^2)
Viscosity	**Kinematic Viscosity**
1 slug/(ft.s) = 47.88 kg/(m.s) = 478.8 poise (p) 1 p=1 g/(cm.s) 0.1 kg/(m.s) = 0.002088 slug/(ft s)	1 ft^2/h = 2.506 .10^{-5} m^2/s, 1 ft^2/s = 0.092903 m^2/s 1 stoke (st) = 1 cm^2/s = 0.0001 m^2/s = 0.001076 ft^2/s
Temperature Scale Readings	
^{o}F = (9/5)^{o}C + 32 ^{o}C = (5/9) (^{o}F – 32)	^{o}R = ^{o}F + 459.69 ^{o}K = ^{o}C + 273.16
Thermal Conductivity*	**Gas Constant***
1 cal/(s.cm.^{o}C) = 242 Btu/(h.ft.^{o}R) 1 Btu/(h.ft.^{o}R) = 1.7307 W/(m.K)	R = 82.057 atm.cm^3/(gmol.K) = 62.361 mm Hg.L/(gmol.K) = 1.134 atm.ft^3/(lbmol.K) = 0.083144 bar.L/(gmol.K) = 10.73 psi. ft^3/(lbmol. ^{o}R) = 555.0 mm Hg.ft^3/(lbmol. ^{o}R)

- Note that the intervals in absolute (Kelvin) and ^{o}C are equal. Also, 1 ^{o}R = 1 ^{o}F.

Latent heat: 1 J/kg = 4.2995 × 10^{-4} Btu/lb$_m$ = 10.76 lb$_f$.ft/slug = 0.3345 lb$_f$.ft/lb$_m$, 1 Btu/lb$_m$ = 2325.9 J/kg

Heat transfer coefficient: 1 Btu/(h.ft^2.^{o}F) = 5.6782 W/(m^2.^{o}C).

Heat generation rate: 1 W/m^3 = 0.09665 Btu/(h ft^3)

Heat transfer per unit length: 1 W/m = 1.0403 Btu/(h ft)

Mass transfer coefficient: 1 m/s = 11.811 ft/h, 1 lb$_{mol}$/(h.ft^2) = 0.013562 kgmol/(s.m^2)