UNIVERSITI SAINS MALAYSIA

Second Semester Examination
2010/2011 Academic Session

April/May 2011

EKC 376 – Downstream Processing Of Biochemical and Pharmaceutical Products
[Proses Hiliran Untuk Produk Biokimia Dan Farmaseutikal]

Duration : 3 hours
[Masa : 3 jam]

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

[Sila pastikan bahawa kertas peperiksaan ini mengandungi ENAM muka surat yang bercetak dan DUA muka surat Lampiran sebelum anda memulakan peperiksaan ini.]

Instructions: Answer **ALL** questions.

*[**Arahan:** Jawab **SEMUA** soalan.]*

In the event of any discrepancies, the English version shall be used.

[Sekiranya terdapat sebarang percanggahan pada soalan peperiksaan, versi Bahasa Inggeris hendaklah digunapakai].

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1. [a] Based on the information below, suggest and briefly discuss the most likely unit operations that should be used for the isolation and purification of L-Lactic acid:

Berdasarkan maklumat di bawah, cadang dan bincangkan secara ringkas unit operasi yang harus digunakan untuk pemencilan dan penulenan asid L-laktik:

- *Prepared from glucose through fermentation by Lactobacillus delbrueckii.*

  Melalui penapaian disediakan daripada glukosa dengan menggunakan Lactobacillus delbrueckii.

- *Crystal from acetic acid or chloroform, melting point 53^{o}C.*
  Hablur daripada asid asetik atau kloroform, suhu lebur pada 53^{o}C.

- *Formation of salt with many metals.*
  Pembentukan garam dengan pelbagai logam.

- *The salts are more soluble in water than the salts of the racemic acid.*
  Garam lebih melarut dalam air berbanding dengan garam asid rasemik.

[6 marks/markah]

[b] Ultrafiltration process to concentrate protease is to be carried out under continuous operation using modules that consists of tubes of 0.075 cm in diameter and 50 cm long. Given that protease has a diffusion coefficient of 8.8 x 10^{-7} cm^2/s. The solution has a viscosity of 0.012 g/(cm.s) and a density of 1.1 g/cm^3. The bulk stream velocity is 300 cm/s. Based on these information, determine

Proses penurasan ultra untuk memekatkan protease dijalankan di bawah operasi berterusan menggunakan modul yang terdiri daripada tiub berdiameter 0.075 sm dan panjang 50 sm. Diketahui bahawa protease mempunyai pekali resapan 8.8 x *10^{-7} sm^2/s. Larutan ini mempunyai kelikatan 0.012 g/cm.s dan ketumpatan 1.1 g/sm^3. Halaju aliran pukal adalah 300 sm/s. Berdasarkan maklumat di atas, tentukan*

[i] the thickness of concentration polarization layer
ketebalan lapisan pengutuban kepekatan

[ii] the polarization modulus of the fluid for membrane flux of 50 $l/(m^2.hr)$.
Modulus pengutuban bendalir untuk membran dengan fluks 50 $l/(m^2.j)$.

[iii] the intrinsic rejection coefficient of the protease for the same membrane flux if the observed rejection coefficient is 95%.
Pekali pembuangan hakiki untuk protease dengan fluks membran yang sama sekiranya pekali pembuangan tinjauan adalah 95%.

[19 marks/markah]

2. [a] A fermentation of the bacterium *Streptomyces* has been done to recover the cells from the fermentation broth. Which methods would one normally choose? Explain your answer.

Suatu penapaian bakteria Streptomyces *telah dilakukan untuk mendapatkan sel-sel daripada kaldu penapaian. Manakah kaedah yang kebiasaannya dipilih? Jelaskan jawapan anda.*

[7 marks/markah]

[b] In a laboratory centrifuge, it takes a minimum of 17 min to clarify the cell lysate at 12000 rpm. After 17 min, the top of the culture being centrifuged was 32 mm and the top of the packed solids was 79 mm from the center of rotation, respectively.

Dalam pengempar makmal, masa minimum 17 minit diperlukan untuk menjernihkan sel terhancur pada 12000 rpm. Selepas 17 minit, didapati bahagian atas kultur dan pepejal terpadat yang diemparkan masing-masing adalah 32 mm dan bahagian atas pepejal terpadat adalah 79 mm dari pusat putaran.

Determine the maximum flow rate for the clarification of a suspension of lysed *Escherichia coli* cells in a plant scale disk centrifuge. Given that the plant centrifuge has a bowl diameter of 25.4 cm, the maximum dimensionless acceleration, G of 14200, $\theta = 42°$, $R_1 = 8$ cm, $R_0 = 20$ cm and number of disks = 100.

Tentukan kadar aliran maksimum untuk penjernihan ampaian sel-sel Escherichia coli *yang telah hancur dengan menggunakan pengempar cakera berskala loji. Diberikan bahawa pengempar loji ini mempunyai garis pusat mangkuk 25.4 sm dan pecutan maksimum tak berdimensi, G ialah 14200, $\theta = 42°$, $R_1 = 8$ sm, $R_0 = 20$ sm dan bilangan cakera = 100.*

[18 marks/markah]

Information:
Maklumat:

$$G = \frac{\omega^2 R}{g} \qquad \frac{dr}{dt} = \upsilon_g \left(\frac{r\omega^2}{g} \right)$$

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

$$Q = \upsilon_g \left[\frac{2\pi n \omega^2}{3g} \left(R_0^3 - R_1^3 \right) \cot\theta \right] \qquad \upsilon_g = \frac{\rho_p - \rho_f}{18\mu} D_p^{\,2} g$$

$g = 9.81\ ms^{-2}$

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3. [a] A 0.6 m length and 7.5 x 10^{-3} m inner diameter high performance liquid chromatography (HPLC) column is used for the separation of two proteins [bovine serum albumin (BSA) and myoglobin (Mb)] in a 0.1 x 10^{-6} m^3 solution. Pilot runs of HPLC at flow velocity of 2.17 x 10^{-4} m/s indicate that the capacity factors K_1 and K_2 of two protein are 0.8 and 1.1, respectively. The relationship between the heights equivalent to a theoretical plate (HETP) and the flow velocity is obtained as follows,

Turus kromatografi cecair berprestasi tinggi (HPLC) yang panjangnya 0.6 m dan 7.5 x 10^{-3} m diameter dalaman digunakan untuk memisahkan dua protein [albumin serum bovine (BSA) dan myoglobin (Mb)] dalam larutan 0.1 x 10^{-6} m^3. Ujikaji berpandu HPLC pada kelajuan aliran 2.17 x 10^{-4} m/s menunjukkan bahawa faktor kapasiti K_1 dan K_2 daripada dua protein masing-masing adalah 0.8 dan 1.1. Hubungan antara ketinggian setara kepada plat teori (HETP) dan kelajuan aliran diperolehi seperti berikut,

$$\text{HETP (m)} = 6.2 \times 10^{-5}\text{ (m)} + 1.13\text{ (s)}u \qquad (3.1)$$

where u = the flow velocity (m/s)
di mana u = kelajuan aliran (ms^{-1})

[i] Based on the above information, calculate the number of theoretical plates of the HPLC column.
Berdasarkan maklumat di atas, kirakan bilangan plat teori yang diperlukan oleh turus HPLC.

[ii] What resolution R_N of two enzymes may be expected? Comments on the result obtained.
Apakah peleraian R_N antara dua enzim tersebut? Ulaskan keputusan yang diperolehi.

[10 marks/markah]

[b] The equilibrium partitioning of a protein between an organic solvent extract phase and aqueous feed phase has been found to be nonlinear and can be represented by the following equation:

Keseimbangan pemetakan protein antara fasa ekstrak pelarut organik dan fasa suapan akues adalah tidak lelurus dan boleh diwakili oleh persamaan berikut:

$$y = 1.50 \ln x + 6.0 \qquad (3.2)$$

where y and x are concentrations of the protein in the extract and aqueous feed phases, respectively in g/l. It is desired to extract 96% of the protein from a feed stream having a protein concentration of 4.0 g/l. For a feed stream at a flow rate of 5.5 l/min and an extract stream at a flow rate of 3.0 l/min

di mana y dan x adalah kepekatan protein dalam fasa ekstrak dan fasa suapan akues, masing-masing dalam g/l. Ia bertujuan untuk mengekstrak sebanyak 96% protein daripada aliran suapan yang berkepekatan 4.0 g/l protein. Untuk aliran suapan pada kadar aliran 5.5 l/minit dan aliran ekstrak dengan kadar aliran 3.0 l/minit,

[i] What is the concentration of the peptide in the exit extract stream?
Apakah kepekatan peptida dalam aliran keluaran ekstrak?

[ii] Graphically estimate how many equilibrium stages will be required for countercurrent flow of the phases.

Anggarkan secara grafik bilangan tahap keseimbangan yang diperlukan untuk fasa berlawanan aliran

[iii] As the concentration of the peptide in the raffinate decreases, does the partitioning of the protein become more or less favourable?

Apabila kepekatan peptida dalam rafinat berkurangan, adakah pemetakan protein cenderung kepada penambahan atau pengurangan?

[15 marks/markah]

4. [a] List down the methods that can be used to crystallize proteins. What are the key strategies for effective crystallization process?

Senaraikan kaedah-kaedah yang boleh digunakan untuk menghablurkan protein. Apakah strategi-strategi utama untuk proses penghabluran yang berkesan?

[6 marks/markah]

[b] As part of a study of a nystatin analogue, a new ion exchange resin has been used for adsorption process. At a high antibiotic concentration, the resin adsorbs 0.062 g antibiotic per 1 g dry resin. At a solution concentration of 82 mg/l, it adsorbs half of this amount. Estimate the amount adsorbed at a solution concentration of 200 mg/l by assuming that the adsorption follows a Langmuir isotherm.

Sebagai sebahagian daripada kajian analog nistatin, resin penukaran ion yang baru telah digunakan dalam proses penjerapan. Pada kepekatan antibiotik yang tinggi, resin telah menjerap 0.062 g antibiotik bagi 1 g resin kering. Pada kepekatan larutan 82 mg/l, resin telah menjerap separuh daripada jumlah ini. Anggarkan jumlah yang dijerap pada kepekatan larutan 200 mg/l dengan mengandaikan bahawa penjerapan mengikuti garis sesuhu Langmuir.

[7 marks/markah]

[c] A small test filtration of auromycin crystals in an acetone suspension uses a filter medium of negligible resistance. It gives the following data:

Satu ujian kecil penurasan hablur auromisin di dalam ampaian aseton dilakukan menggunakan media penuras dengan rintangan boleh abai. Proses penurasan menghasilkan data berikut:

t (sec) t *(saat)*	*V* (liters) V *(liter)*
10	0.500
20	0.707
30	0.866

In finding these data, a filter with 89 cm^2 area has been used with a pressure drop of 0.25 atm. A much larger crop of crystals containing 7300 liters of solvent is then need to be filtered by using a filter of 1.3 m^2. However, this larger crop has a concentration of 0.28 g/100 cm^3 solvent, while in the test filtration is 0.34 g/100 cm^3 of solvent. How long will it take to filter this new crop at the same pressure drop?

Dalam mencari data-data ini, penuras dengan keluasan 89 sm^2 telah digunakan dengan susutan tekanan 0.25 atm. Kemudian, pertumbuhan hablur yang lebih banyak yang mengandungi 7300 liter larutan perlu dituras menggunakan penuras berkeluasan 1.3 m^2. Walau bagaimanapun, pertumbuhan hablur yang besar ini mempunyai kepekatan 0.28 g/100 sm^3 larutan dan dalam penurasan ujian ialah 0.34 g/100 sm^3 larutan. Berapa lamakah yang diperlukan untuk menuras hasil baru ini pada susutan tekanan yang sama?

[12 marks/markah]

Information:
Maklumat:

$$\frac{At}{V} = \frac{\mu\alpha\rho_0}{2\Delta P}\left(\frac{V}{A}\right) + \frac{\mu R_M}{\Delta P}$$

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Appendix

Graetz-Leveque correlation

$$Sh = 1.62\, Re^{0.33} Sc^{0.33} \left(\frac{d}{l}\right)^{0.33}$$

Dittus-Boelter correlation

$$Sh = 0.023\, Re^{0.8} Sc^{0.33}$$

Column Resolution

$$R_N = \frac{1}{4}\sqrt{N_P}\left(\frac{\delta - 1}{\delta}\right)\left(\frac{k_2}{k_2 + 1}\right)$$

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^2s = 0.2046 lb_m/ft^2s = 0.00636 slug/ft^2s	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	
^oF = (9/5)^oC + 32	^oC = (5/9) (^oF – 32)
^oR = ^oF + 459.69	^oK = ^oC + 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)