END SEMESTER EXAMINATIONS DEC 2023

FIRST SEMESTER M.TECH. INDUSTRIAL BIOTECHNOLOGY

SUBJECT: ADVANCED BIOSEPARATION PROCESSES [BIO5112]

Date of Exam: 2nd December 2023   Time of Exam: 9:30-12:30   Max. Marks: 50

Instructions to Candidates:

❖ Answer ALL the questions & missing data may be suitable assumed

1a. Complete the following table and decide the best step for protein precipitation by ammonium sulphate

Steps	Total units (U)	Total protein (mg)	Specific activity (U/mg)	Purification fold
Crude	9	0.2		
20 %	7.5	0.12		
40 %	6.5	0.15		
60 %	5.7	0.18		
80 %	5	0.3		

 [2]

1b. Study the process. Complete the material balances around the various separation processes from your knowledge. Tabulate the data. [4]

1c The species *B. Subtilis* and *E.Coli* used in your lab are being tried for lysis by Electroporation, Microwave irradiation and nanostructures. Comment on the feasibility, efficiency, the process parameters. [4]

2a. During the precipitate formation, growth of a particle can occur by Diffusion and by Fluid motion. Explain the phenomena. [4]

2b   In a laboratory test of filtration of a cell culture, the following equation was obtained to describe filtration.

$$\frac{t}{V/A} = K_1 \left(\frac{V}{A}\right) + K_2$$; where K$_1$ =0.18 min/cm^2 and K$_2$=0.017 min/cm. The cell slurry has viscosity of 2cP and the filter cake solids (dry basis) per volume of filtrate was 13g/L. The pressure drop for filtration was 610 mm Hg (0^0 C). Determine the specific cake resistance α

and the medium resistance R_m. Evaluate the relative significance of the medium resistance after 5 minutes. [4]

$$\frac{t}{V/A} = \frac{\mu_o \alpha \rho_c}{2\Delta p}\left(\frac{V}{A}\right) + \frac{\mu_0 R_m}{\Delta p}$$

2c. A system is available for the ultrafiltration of protein solutions. On Monday, you filter a globular protein with 12,000 Da molecular weight, and it is fully retained. On Tuesday, you filter a 120,000 Da molecular weight globular protein at the same molar concentration and using the same membrane. The transmembrane flux was the same on both days. Assuming identical boundary layer thicknesses for both filtrations, in which case will the polarization modulus c_w/c_b be greater? What is the relationship between the polarization moduli for the two cases? [2]

$$J = \frac{\mathscr{D}}{\delta}\ln\frac{c_w}{c_b}$$

3a. Drying rate data for a biological material are known. Determine the drying time for drying material from 0.3333 g-moisture/g-dry solid to 0.0416 g-moisture/g-dry solid. The dry material weighs 112.5 kg and the material has a surface area of 3.75 m^2. [5]

Moisture content g-moisture/g-dry weight	Drying rate, g-moisture/m^2.s
0.02	0.26x10^{-1}
0.06	0.75 x10^{-1}
0.122	1.49 x10^{-1}
0.173	2.17 x10^{-1}
0.222	2.75 x10^{-1}
0.255	3.16 x10^{-1}
0.295	3.50 x10^{-1}
0.345	3.51 x10^{-1}
0.391	3.49 x10^{-1}
0.450	3.51 x10^{-1}

3b. What are the factors that affect protein portioning by ATPS? [2]

3c. Explain the concept of Stern model for electric double layer and the effect of ionic concentration on Potential ψ. [3]

4a. In a counter-current extraction of a pharmaceutical product, partition coefficient $K = 5.0$ and a solvent-to-feed ratio $(S/F) = 0.5$. Determine the number of stages required graphically and analytically, if $x_f = 0.0975$ and the final concentration in the raffinate (feed stream) is 0.003941. Assume fresh solvent. Also determine y_n.

$$x_f = \left(\frac{E^{n+1} - 1}{E - 1}\right)x_n$$

[5]

4b. What are the factors to be considered during the selection of a centrifuge for continuous operation? List the parameters [2]

4c. Determine the maximum time required for sedimentation by centrifugation of tube 10 cm in height, centre of rotation is 4 cm from the liquid surface. The cells have a density of 1.08 g/cm^3, and they are suspended in liquid with a density of 1.00 g/cm^3 and viscosity of 1.0 cp. RPM is 10,000. Cell have diameter of 2 μm. [3]

$$v_g = \frac{2a^2(\rho-\rho_0)g}{9\mu} \qquad\qquad v = \frac{2a^2(\rho-\rho_0)\omega^2 R}{9\mu}$$

5a. What do you understand by "size-exclusion limit" of a gel filtration column"? [2]

5b. Compare and contrast the mechanistic aspects of normal phase and reverse phase forms of chromatography. [3]

5c. Albumin is being separated from an immunoglobulin by isocratic chromatography using a 50 cm long column having a voidage fraction of 0.25 and a diameter of 10 mm at a mobile phase flow rate of 10 mL/min. The distribution coefficients for the immunoglobulin and albumin are 1 and 0.1 respectively. If the albumin peak has a characteristic peak width of 0.52 minutes, predict the selectivity and resolution. *[Hint: Find the volume of the column]* [5]

Penicillin Production

Glucose 2958 kg
Water 2958 kg
Corn Steep Liquor 198 kg
Phenyl acetic acid 9 kg
H₂O 2603 kg
P. chrysogenum 0.01 kg

Off gas

Bioreactor
25⁰C, 1 atm
pH = 5

Air in

8726 kg
PenG 480 kg
Cells/Water

Batch Nutsche Filter
T = 2⁰C

Cells 393 kg

Waste

7631 kg
PenG 466 kg

382 kg H₂SO₄

8013 kg
pH = 2.5

Centrifugal Solvent Extractor

Water

Raffinate

1209 kg
PenG 458 kg
Butyl acetate 726 kg

726 kg Butyl Acetate

134 kg Potassium Acetate

Fresh Butyl Acetate

Butyl Acetate OUT 1306 kg

Butyl Acetate IN 906 kg

1343 kg
PenGK 510 kg
Butyl Acetate 726 kg

Butyl Acetate 326 kg

Waste

25⁰C, pH 7.5
H₂O 139 kg   NH₄OH 20 kg

698 kg
6-APA 255 kg
38⁰C

Semi-batch Reactor 1

Catalyst - PGA 49 kg

PenG 490.25 kg

Penicillin Storage

490.25 kg
PenGK 490 kg
H₂O 0.25 kg

Fluidized Bed Dryer

515 kg
PenGK 490 kg
H₂O 25 kg

828 kg   Butyl Acetate 726 kg
Waste

Solvent Recovery & Recycle - Butyl Acetate

Amoxicillin Production

Raffinate

Butyl Acetate 404 kg

Raffinate

Butyl Acetate 502 kg

25⁰C, pH 7.5

Centrifugal Separator 1

462 kg
6-APA 250 kg

Semi-batch Reactor 2

PHPGME 252 kg

715 kg
AMOX 317 kg

Centrifugal Separator 2

Filter

PGA 49 kg

480 kg
AMOX 285 kg

131 kg

Waste

349 kg
AMOX 271 kg

Crystallizer

302 kg
AMOX 267 kg
H₂O 35 kg

AMOX Storage