



ENTER B.E/B.TECH DEGREE EXAMINATIONS: NOV/DEC 2022

(Regulation 2018)

Seventh Semester

BIOTECHNOLOGY

U18BTI7204: DOWNSTREAM PROCESSING

COURSE OUTCOMES

- CO1:** Apply the various unit operation principles and engineering fundamentals to design the separation processes specific to biologically derived products.
- CO2:** Analyze and design the various solid-liquid unit operations and different cell-disruption techniques used in downstream processing.
- CO3:** Apply and analyse the various principles underlying the different unit operations used for the isolation and extraction of bio-products.
- CO4:** Select and design the various methods of chromatography used in protein purification.
- CO5:** Apply the different unit operations for polishing and packing the final bioproducts .
- CO6:** Evaluate the techno-economical analysis for purification of bioproducts

Time: Three Hours

Maximum Marks: 100

Answer all the Questions:-

PART A (10 x 2 = 20 Marks)

(Answer not more than 40 words)

- | | | |
|--|-----|-------------------|
| 1. Using graphical representation, correlate between the market volume or concentration of desired bio-products with selling price. | CO1 | [K3] |
| 2. How do the products are released from the cells by osmotic shock? | CO2 | [K2] |
| 3. Write the relation between g-force and RPM in centrifugation. | CO2 | [K ₂] |
| 4. List any two application of ATPS extraction | CO3 | [K2] |
| 5. What are the factors that affects the performance of membrane in filtration? | CO3 | [K2] |
| 6. A chromatographic separation of a two components samples on a 50 cm column gave the retention times for the solutes A and B as 2.5 and 3.1 minutes with base width of the two chromatographic peaks being 0.25 and 0.3 minutes, respectively. Calculate the theoretical plate | CO4 | [K4] |
| 7. How do inner and void volume of gel filtration column is determined ? | CO4 | [K3] |
| 8. Draw the rate of drying curve and mention various zone present on it. | CO5 | [K ₂] |
| 9. Spell out the significance of eutectic point in lyophilization? | CO5 | [K2] |
| 10. What do you mean by direct and indirect cost estimation ? | CO6 | [K2] |

Answer any FIVE Questions:-
PART B (5 x 16 = 80 Marks)
(Answer not more than 400 words)

11. a) The production of a recombinant protein involves fermentation of *E.coli*, with the production of protein being intracellular. The protein forms precipitates in the form of submicron size inclusion bodies inside the cell. The protein in the inclusion bodies is not active. It is desired to recover inclusion bodies so that the protein can later be recovered in an active form. Give a conceptual process diagram that indicates the keys steps in recovery of the protein in an active form. 8 CO1 [K3]
- b) Based on the purification table, calculate the specific activity , fold purification and % Yield. 8 CO1 [K4]

Fraction	Volume (ml)	Total protein (mg)	Activity (units)	Total activity (Units* ml)	Specific activity (units/mg)	Fold purification	% Yield
Lysate	10	56.3	65897	658970			
DEAE	12.25	25.35	42845	524851			
Affinity chromatography	8.25	18.25	51481	424718.3			
Gel filtration Chromatography	4.3	3.75	48239	207427			
Sparry drying	3.5	1.28	46578	163023			

12. a) We want to filter 15,000 L/h of a beer containing erythromycin using a rotary vacuum filter originally purchased for another product. Our filter has a cycle time of 50 s and an area of 37.2 m². It operates under a vacuum of 20 in Hg. The pretreated broth forms an incompressible cake with the resistance: 8 CO2 [K5]

$$\frac{\mu\alpha\rho_0}{2\Delta P} = 29 \text{ s/cm}^2$$

We want to wash the cake until only 1% of the retained soluble is left, and we expect that the washing efficiency will be 70% and that 1% of the filtrate is retained.

- (a) Calculate the filtration time per cycle
 (b) Find the washing time
- b) Write notes on (i) tubular bowl centrifugation and (ii) disc stack centrifugation 8 CO2 [K2]

- | | | | | | |
|-----|----|--|----|-----|------|
| 13. | a) | Cell-free fermentation liquor contains $8 \times 10^{-5} \text{ mol l}^{-1}$ immunoglobulin G. It is proposed to recover at least 90% of this antibody by adsorption on synthetic, non-polar resin. Experimental equilibrium data are correlated as follows: $q = 5.5 \times 10^{-5} y^{0.35}$ where q is mol solute adsorbed per cm^3 adsorbent and y is liquid-phase solute concentration in mol l^{-1} . What minimum quantity of resin is required to treat 2 m^3 fermentation liquor in a single-stage mixed ta | 8 | CO3 | [K5] |
| | b) | With an example, explain the process of blood protein fractionation using Cohn's method for blood protein fractionation | 8 | CO3 | [KL] |
| 14. | a) | Give detailed account of the principle and practice of Ion exchange chromatography | 10 | CO4 | [K3] |
| | b) | Write a note on application of affinity chromatography | 6 | CO4 | [K3] |
| 15. | a) | Draw a neat sketch of spray dryer and explain its process layout and its application | 8 | CO5 | [KL] |
| | b) | With a neat diagram, explain the equipment's used for crystallization | 8 | CO5 | [K2] |
| 16. | a) | L-Lysine is an amino acid that is produced in large quantities ($>100,000$ metric tons/year) via fermentation. It is used as an animal feed supplement mainly for poultry and pigs. Based on information which you studied, develop and evaluate a process for producing $15,000,000 \text{ kg}$ of L-lysine per year via fermentation. Your analysis should include flow sheets, no of unit operations, estimation of capital and operating cost for industrial application. | 16 | CO6 | [K5] |
