KUMARAGURU
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Register Number:……..

M.TECH DEGREE EXAMINATIONS: MAY 2018

(Regulation 2015)

Second Semester

BIOTECHNOLOGY

P15BTT201 : Bioseparation Technology

COURSE OUTCOMES

CO1: Students get skills to understand the various principles involved in protein purification.
CO2: Understand the characterization of various biomolecues.
CO3: Understand the principles involved in various product isolation techniques.
CO4: Apply the principle involved in product purification.
CO5: Analyse purification strategy and process economics of recombinant proteins.

Time: Three Hours **Maximum Marks: 100**

Answer all the Questions:-

PART A (10 x 1 = 10 Marks)

1. Assertion (A):Glucanase is an enzyme that degrades the murein layer in bacterial cell walls. CO1 [K_2]
   Reason (R):Glucanases are enzymes that break down a glucan, a polysaccharide made of several glucose sub-units
   a) Both A and R are Individually true and R is the correct explanation of A
   b) Both A and R are Individually true but R is not the correct explanation of A
   c) A is true but R is false
   d) A is false but R is true
2. The unit operation involved in product isolation is CO1 [K_1]
   a) Filtration and centrifugation
   b) Cell disruption techniques
   c) Chromatography
   d) Lyophilization
3. Filter aids CO3 [K_2]
   a) increase cake compressibility
   b) increases specific cake resistances
   c) does not adsorb colloidal particles
   d) reduces compressibility of biomass
4. Match List I (Unit operation)to List II (Principle) CO3 [K_2]

List I	List II
A. Centrifugation	i. movement of solute
B. Membrane based separation	ii. movement of solvent
C. Dialysis	iii. density
D. Osmosis	iv. Size of solute

	A	B	C	D
a)	iii	iv	i	ii
b)	i	iv	ii	iii
c)	iv	ii	i	iii
d)	iii	i	ii	Iv

5. Assertion (A): A particle's mass, density, and shape will determine its Svedberg value(S) CO3 [K_3]
Reason (R): It is independent on the frictional forces retarding its movement, which, in turn, are related to the average cross-sectional area of the particle.
a) Both A and R are Individually true and R is the correct explanation of A
b) Both A and R are Individually true but R is not the correct explanation of A
c) A is true but R is false
d) A is false but R is true

6. In crystallization, driving force for removal of solutes from the solution is CO3 [K_2]
a) Nucleation
b) Solubility
c) Supersaturation
d) Crystal growth

7. Sulfitolysis is to add –SO3 moieties to all sulfer residues on the cysteines. CO5 [K_2]
1. Sulfitolysis of the denatured pro insulin takes place in a reaction tank under acid conditions.
2. Sulfitolysis of the denatured pro insulin takes place in a reaction tank under akaline conditions.
3. Sulfitolysis of the denatured pro insulin takes place in a reaction tank under natural pH
4. Sulfitolysis of the denatured proinsulin takes place in a reaction tank under mild acidic condition
Which of these statements are correct.
a) 1
b) 3
c) 2
d) 1,3

8. Composition conditions in which the three coexisting phases of partially soluble components of a three-phase liquid system approach each other in composition is called CO3 [K_2]
a) compatibility point
b) Plaint point
c) Coexit point
d) Tie line point

9. Concentration polarization can be reduced further by CO3 [K_2]
a) pre filtering the solution
b) reducing the flow rate per unit membrane surface area
c) back washing periodically
d) all of the above

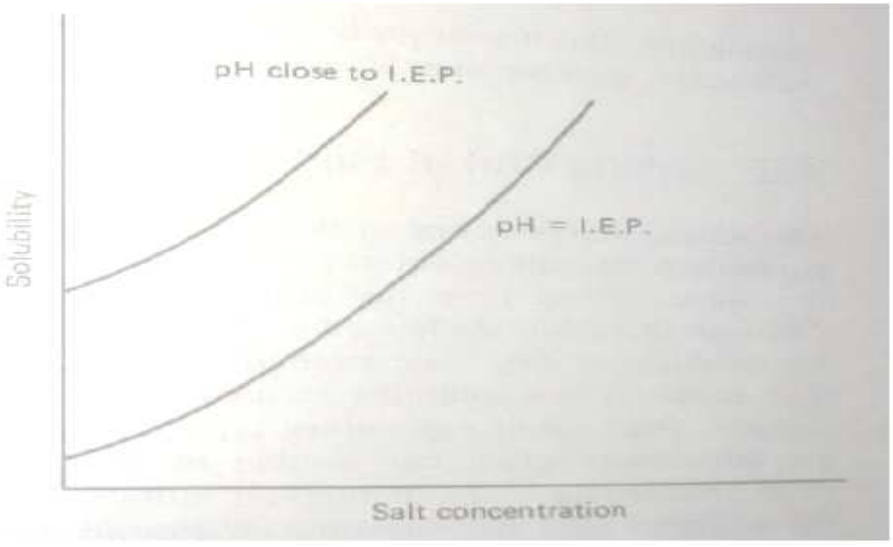
10. Identify the correct sequence of events in bioseparation, CO5 [K_2]

1. Solute-solute separation
2.Liquid-solids separations
3. Solute-liquid separation
4. Pretreatment

a) 2-3-4-1 b) 1-3-2-4
c) 3-4-2-1 d) 4-2-1-3

PART B (10 x 2 = 20 Marks)

11. List the factors that could change the viscosity of a fermentation broth during growth of the process organism? CO1 [K_2]
12. What are all the factors which will influence the efficiency of releasing intracellular components. CO2 [K_2]
13. A settling tank is used to sediment algal pellets with the density of 1.09g/cm^3 and a diameter of 180µm. The particle free molecule has a density of 1.00 g/cm^3 and viscosity is 1.1cP. Calculate the settling time by assuming that the pellets quickly reach their maximum terminal velocity. CO3 [K_3]
14. Classify types of ion –exchangers with examples. CO3 [K_2]
15. Clarified fermentation broth of pH contains 180 mg/litre of penicillin. It is extracted with butyl acetate. The equilibrium consatnt K is 57. The feed solvent planned is 420l/hr and extraction solvent is 40l/hr. Calculate the extraction factor. CO3 [K_3]
16. What do you understand by salting-out? Highlight the factors governing the choice of salt. CO3 [K_2]
17. Define critical temperature and critical pressure of a supercritical fluid. CO5 [K_1]
18. Isoelectric precipitation of low solubility proteins can be improved by inclusion of a divalent metal ions. Please refer the figure below and comment the principle behind. CO4 [K_3]

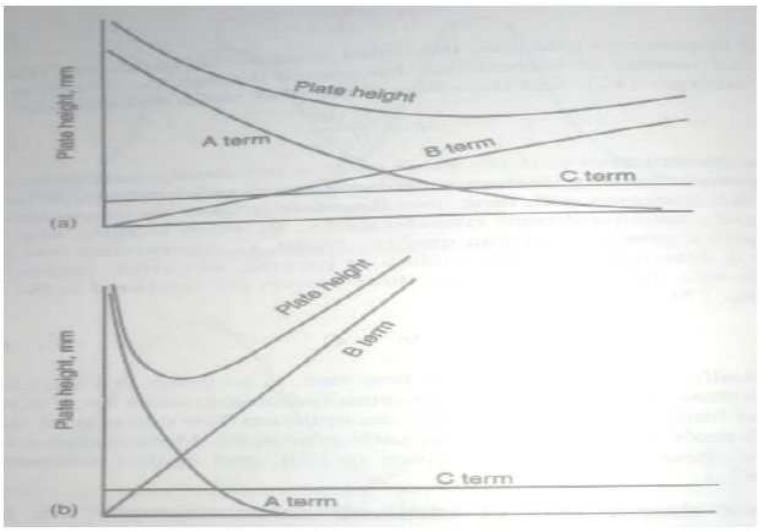


19. A type of animal derived tissue is being ground in a bead mill to recover a pharmaceutical enzyme. It was estimated that 3000 Joules of energy was used to reduce the average particle size of the tissue from 1 mm to 0.5 mm. Predict the amount of energy needed to further reduce the particle size to 0.1 mm. Assume that this grinding process follows Rittinger's law. CO3 [K_2]

20. Give any two actions of synthetic polyelectrolytes. CO2 [K_1]

PART C (6 x 5 = 30 Marks)

21. Compare and contrast how flow rate affect the plate height: a) low-molecular weight solutes and b) high molecular weight solutes in the following figure based on Van Deemter equation factors. CO4 [K_3]



Effect of flow rate on plate height

22. What is the principle of structure determination of proteins by Xray crystallography. CO2 [K_2]
23. Comment the advantages of supercritical fluid extraction for heat labile products. CO4 [K_3]
24. Compare precipitation method with organic solvents and polymers for purification of enzymes. CO5 [K_3]
25. What are different methods to obtain supersaturation. CO5 [K_2]
26. It is customary to prepare hemoglobin by the osmotic lysis of red cells. What is the van't Hoff pressure drop across the membrane of a red blood cell that is isotonic (0.30 osM) on the inside and submerged in a 0.01% NaCl solution? What is the expected outcome of this solution? CO3 [K_4]

Answer any FOUR Questions

PART D (4 x 10 = 40 Marks)

27. Explain the five stages involved in the recovery of bioproduct with an example. CO1 [K_2]

28. 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 m^2. It operates under a vacuum of 20 in Hg. The pretreated broth forms an incompressible cake with the resistance: CO3 [K_3]

$$\frac{\mu\alpha\rho_0}{2\Delta P} = 116 \ s/cm^2$$

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.

29. Gel chromatography scale-up : CO4 [K_5]

Gel chromatography is to be used for commercial-scale purification of a proteinaceous diphtheria toxoid from Corynebacterium diphtheriae supernatant. In the laboratory, a small column of 1.5 cm inner diameter and height 0.4 m is packed with 10 g dry Sephadex gel; the void volume is measured as 23 ml. A sample containing the toxoid and impurities is injected into the column. At a liquid flow rate of 14 ml min-1, the elution volume for the toxoid is 29 ml; the elution volume for the principal impurity is 45 ml.

A column of height 0.6 m and diameter 0.5 m is available for large-scale gel chromatography. The same type of packing is used; the void fraction and ratio of pore volume to total bed volume remain the same as in the bench-scale column. The liquid flow rate in the large column is scaled up in proportion to the column cross-sectional area; the flow patterns in both columns can be assumed identical. The water regain value for the pack ing is given by the manufacturer as 0.0035 m 3 kg /1 dry gel.

(a) Which is the larger molecule, the diphtheria toxoid or the principal impurity?

(b) Determine the partition coefficients for the toxoid and impurity.

(c) Estimate the elution volumes in the commercial-scale column.

(d) What is the volumetric flow rate in the large column?

(e) Estimate the retention time of toxoid in the large column? Compare the retention times in small scale and large scale.

30. With a neat diagram, explain the following crystallizer: CO4 [K_2]

(i) Swenson-walker crystallizer (5)

(ii) Oslo cooling crystallizer (5)

31. Write in detail on different steps used to obtain homogenous recombinant Taq Polymerase. CO5 [K_2]

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