KUMARAGURU college of technology
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Register Number:……..

M.TECH DEGREE EXAMINATIONS: NOV/DEC 2023

(Regulation 2018)

First Semester

BIOTECHNOLOGY

P18BTI1203**:** Bioproduct Recovery and Purification

COURSE OUTCOMES

CO1: Understand the various principles involved in bio separation and cell disruption techniques.

CO2: Explain the different types of filtration and centrifugation techniques used in bioproduct Recovery.

CO3: Understand the various techniques in different unit operations involved for the isolation and extraction of bio-products from biological samples.

CO4: Select and use various methods of chromatography in protein purification.

CO5: Illustrate different methods of final polishing for bio-products produced at lab and industrial level.

CO6: Develop a process design and choose the appropriate purification steps and perform the techno-economical analysis for purification of bioproducts.

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

Answer all the Questions:-

PART A (10 x 1 = 10 Marks)

1. A) Urokinase is an example of Very high value low volume product CO1 [K_2]

   B) Low value high volume product is produced in the range of 100 kg to tons in quantity annually.

   a) A is True but B is False
   b) B is True but A is False
   c) Both A and B are True
   d) Both A and B are False

2. Match the correct pair CO1 [K_2]

Separation factor	Unit operations
A. Particle size	i. Reverse osmosis
B. Hydrophobicity	ii. Electrophoresis
C. Ionic charge	iii. Precipitation
D. Diffusivity	iv. Microfiltration

   a) A-i), B-ii), C-iii), D-iv)
   a) A-ii), B-i), C-iv), D-iii)
   c) A-ii), B-iv), C-i), D-iii)
   d) A-iv), B-iii), C-ii), D-i)

3. Which downstream process is NOT used to remove solids from slurry? CO2 [K_2]

i) Sedimentation
ii) Filtration
iii) Drying
iv) Precipitation

a) i), ii) & iii) b) ii) & iii)
c) i) & iii) d) iii) & iv)

4. Which of the following set of statement are NOT applicable for cross flow filtration? CO2 [K_4]
i) Reverse osmosis is based on the principle of cross-flow filtration
ii) Type of the filtration membrane required is independent of its application
iii) It decreases the operating duration of the filter unit
iv) Not suitable for continuous process of filtration

a) (i), (ii) & (iv) b) (ii), (iii) & (iv)
c) (i), (iii) & (iv) d) (i), (ii) & (iii)

5. If extraction efficiency is 6.5, what will be the percentage extraction in a single stage? CO3 [K_3]

a) 86.7 b) 65.1
c) 79.2 d) 85.0

6. Assertion (A): The proteins are prevented to form hydrogen bonds with water and the salt facilitates their interaction with each other forming aggregates that afterward precipitate out of solution. CO3 [K_4]
Reason (R): The solubility of a nonelectrolyte in water increases with increasing salt concentration.

a) A and R are True and R is the correct reason for A b) A and R are True but R is NOT the correct reason for A
c) A is True but R is False d) Neither A nor R is True

7. If the retention time of a solute is 12 mins and width at the base is 2 mins, how many theoretical plates does the column have? CO4 [K_3]

a) 96 b) 576
c) 8 d) 199

8. The correct order of elution sequence for reverse phase column is CO4 [K_2]

a) Carboxylic acid, alcohols, ethers, alkanes b) Ketones, alcohol, alkane, amines
c) Sulphides, olefins, sulfoxides, alkanes d) Nitro compounds, aromatics, esters, aldehydes

9. A filter costs Rs 10,00,000 and it annual depreciation is 10%. What will be its depreciated value after 5 years? CO6 [K_3]

a) 478658 b) 366779
c) 590473 d) 667538

10. Which of the following is CORRECT about Fractional crystallization? CO5 [K_4]
Statement 1: Repeated crystallization takes place
Statement 2: It increases the purity of the material

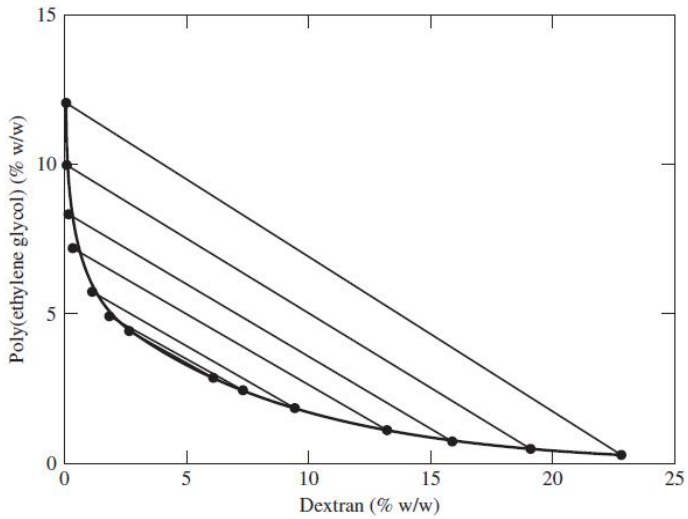
a) Statement 1 and 2 are Correct and 1 is the reason for 2. b) Statement 1 and 2 are Correct but 1 is Not the reason for 2.
c) Statement 1 is Correct but 2 is Incorrect d) Statement 2 is Correct but 1 is Incorrect

PART B (10 x 2 = 20 Marks)

11. If the separation process involves 3 steps with a yield of 85%, what would be the overall yield of the desired product? CO1 [K_3]

12.	In a bead mill 50% of the intracellular enzymes are released in 5 hours. How long will it take to release 75% of them (assume first order release process).	CO1	[K_2]
13.	Suggest some of the few methods to minimize membrane fouling.	CO2	[K_4]
14.	Relate the working principle of Diafiltration with Dialysis.	CO2	[K_2]
15.	Activated carbon is used as an adsorbent to remove Methylene blue dye in a simulated wastewater. Comment on the kinetics of this adsorption, if it follows Pseudo second order kinetics.	CO3	[K_2]
16.	A researcher is working with a protein that contains four subunits of differing molecular weights. If the researcher performs SDS-PAGE, how many distinct bands should he see on the gel? Justify your answer with proper explanation.	CO3	[K_5]
17.	Two peaks have retention time of 7.5 and 9.5 mins and their corresponding width at the base is 1.45 and 3.55 mins respectively. What is the resolution of the column?	CO4	[K_3]
18.	Indicate the significance of FPLC in the purification of biomolecules.	CO4	[K_2]
19.	If cost of 50 L vessel is Rs 5,00,000, what will be the cost of a 200 L vessel?	CO6	[K_3]
20.	Identify the key variable in the design of a spray dryer and brief on it.	CO5	[K_4]

PART C (6 x 5 = 30 Marks)

21.	Examine the type of kinetics in cell disruption using a bead mill and brief on it.	5	CO1	[K_4]
22.	You are given with an unknown protein sample. Suggest a suitable method to determine the molecular weight of the given protein other than Chromatography or Electrophoresis.	5	CO4	[K_5]
23.	 Comment on the above diagram.	5	CO3	[K_4]
24.	We wish to precipitate the protein α_2-macroglobulin contained in 100 liters of aqueous solution at 20°C in a tank at a concentration of 0.2 g/liter. α_2-Macroglobulin is a globular protein with a molecular weight of 820,000 and a diffusion coefficient of 2.41×10^{-7} cm^2/s at 20◦C. The precipitate particles have a density of 1.3 g/cm^3. The solution is stirred with a 75 W (0.1 hp) motor. Calculate the time for the particles to reach a size of 1.0 μ m, assuming that growth is governed by diffusion only up to this particle size. Given $K_A = 1.08 \times 10^{-11}$cm^{3}s^{-1}; $N_0 = 1.08 \times 10^{-11}$ cm^3 s^{-1}	5	CO3	[K_3]
25.	Compare and contrast the principle and applications of Metal affinity chromatography and dye affinity chromatography.	5	CO4	[K_2]

26.	You are given with a highly purified diagnostic enzyme. Suggest a suitable downstream processing step to convert the above product into powder form and schematically explain its principle.	5	CO6	[K_5]

Answer any FOUR Questions

PART D (4 x 10 = 40 Marks)

27.	An intracellular eukaryotic protein is expressed in *E.coli* through recombinant DNA technology which results in the aggregation of the expressed proteins and the formation of insoluble inclusion bodies. Discuss the cell wall structure of *E.coli* and suggest a suitable cell disruption technique to disrupt its cell wall. Also as a downstream process engineer how would you tackle the problem of recovering protein in active form?	10	CO1	[K_4]
28.	Chlorella cells are often cultivated in an open pond. Upon harvest, the dilute stream of cell mass is first concentrated with disc bowl centrifuges. The resulting concentrated effluent stream is then fed into a tubular bowl type centrifuge to give a cell paste. The centrifuge is 1.2m high, the outer radius is 15 cm, and the distance to the liquid interface is 11 cm. This feed stream has a density of 1.01 g/cm^3 and a viscosity of 1.02 cP. The density of chlorella cells is 1.03 g/cm^3. The rotation speed is 6000 r/min. Assume chlorella cells are spherical. What flow rate should you use to recover all chlorella cells with a diameter larger than 10 μm?	10	CO2	[K_3]
29.	1000 kg/hr of an acetone water mixture containing 20% by weight of acetone is to be counter currently extracted with trichloroethane. The recovered solvent to be used is free from acetone. The water and trichloroethane are immiscible. If 90% recovery of acetone is desired, estimate the number of stages required if 1.5 times the minimum solvent is used. The equilibrium relationship is given by y=1.65x where x and y are the weight fractions of Water and Trichloroethane respectively.	10	CO3	[K_3]
30.	i) With an example case study, depict the practice of Ion exchange chromatography in the separation of a bioproduct.	8	CO4	[K_6]
	ii) You are given with a sample solution containing different proteins with different molecular weight such as Protein A: 12,500 Da, Protein B: 74,500 Da and Protein C: 2,44,000 Da. By using Gel permeation chromatography, identify the order of protein elution.	2	CO4	[K_4]
31.	Illustrate the use of SuperPro Designer to analyze and evaluate the bacterial production of recombinant human insulin.	10	CO6	[K_4]

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