Reg. No.										

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V SEMESTER B.TECH (B. E. BIOTECHNOLOGY)

END SEMESTER EXAMINATIONS, NOV/DEC 2015

SUBJECT: DOWNSTREAM PROCESSING TECHNOLOGY [BIO 305]

REVISED CREDIT SYSTEM

Time: 3 Hours MAX. MARKS: 50

Instructions to Candidates:

- Answer **ANY FIVE FULL** the questions.
- Missing data may be suitable assumed.

1A.	A continuous stirred tank adsorption process is carried out to separate modified vancomycin from the bacterial culture. Using a graphical representation, explain the adsorption process in a stirred tank for rapid adsorption, no adsorption and ideal adsorption.	**2**
1B.	For a continuous stirred tank, derive the following equation: $$q(t) = q(0) + \frac{H y_F t}{(1-\varepsilon)V} - \frac{H}{(1-\varepsilon)V}\int_0^t y\,dt - \left(\frac{\varepsilon}{1-\varepsilon}\right) y(t)$$ Where: V-volume of the tank y and y_F are the solute concentrations in the effluent and the feed H is the feed rate and q is the adsorbed solute concentration (at time 0 and t)	**3**
1C.	Leaching process is utilized for extraction of a soluble constituent from a solid by means of a solvent. However, there are certain stages involved in the leaching process. Illustrate the steps involved in leaching with the help of a sketch, describing the major rate limiting factors involved in the process.	**3**
1D.	An adsorption column has the capacity to adsorb 7.6×10^{-6} mol/cm^3 of a genetically modified protein. The adsorption isotherm follows the following equation: $$q = \frac{q_m y}{K+y}$$; Where K is 2.3×10^{-5} mol/liter. If the operation is to be managed such that 90%, of the column capacity will be exhausted for a 0.085 liter column, what would the feed concentration be, for 2 liters of the feed solution?	**2**
2A.	Derive the equations for yield and purity for a chromatography process operating from time 0 to t.	**2**

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Note: $\operatorname{erf}(x) = \frac{2}{\sqrt{\pi}} \int_0^x \exp(-x^2)dx$; $\int_a^b \exp(-x^2)dx = \frac{\sqrt{\pi}}{2}[\operatorname{erf}(b) - \operatorname{erf}(a)]$

$\operatorname{erf}(-x) = -\operatorname{erf}(x)$; $\operatorname{erf}(0) = 0$; $\operatorname{erf}(\infty) = 1$; $\operatorname{erf}(x > 2.5) = 1$ $\operatorname{erf}\left(\frac{-1}{\sqrt{2}\sigma}\right) = -1$

2B.	An insulin purification plant is set up which uses polyacrylamide beads. The data obtained by the process is such that the maximum concentration (0.021mg/ml) is observed at 185 liter. At 165 liters, the concentration observed is 0.012 mg/ml. If the bed volume is 18 liters, determine the values for yield at 185 and 200 liters.	**3**
2C.	Three proteins, having distribution coefficients as 0, 2 and 6, are being separated by affinity chromatography using a 9 cm long column having 200 theoretical plates. The voidage of the column is 0.46 and t_m is 9 minutes. Using this data, determine the t_R for each protein.	**3**
2D.	Compare and contrast the techniques of ion exchange chromatography and size exclusion chromatography.	**2**
3A.	An aqueous solution containing albumin is maintained at $25^{O}C$ such that the protein in the solution has a charge of +12, and the axial diffusion coefficient of 8.5×10^{-7} cm^2/sec. What is the velocity due to a force of 2.5 volt/cm? The same solution also contains an unknown protein, having the axial diffusion coefficient of 7.6×10^{-7} cm^2/sec. Determine the location and the resolution of the peaks if the following set of conditions are followed for 2 hours: The electric filed strength is set at 130 v/cm for separation and the values of mobility of the proteins is 1×10^{-5} cm^2/volt sec and 0.8×10^{-5} cm^2/volt sec, under the applied electric filed. Note: F= 96500C/mol, R=8.315 J/mol. K and u_{osm}= 0	**5**
3B.	Design a technique for the separation of two proteins that differ in their subunit molecular weights. If the subunits had to be purified further by utilizing their mobility in a pH gradient medium, how would the process be upgraded?	**3**
3C.	Illustrate the principle of adsorption used in the separation of tagged proteins in the process of column chromatography, during the polishing stages.	**2**
4A.	Derive the equation for the crystal growth. $\frac{dm}{dt} = K_G\ (\Delta c)$; where m is the mass deposited in time t	**3**
4B.	Design a separation process which will be most suitable for extraction of a highly temperature sensitive protein product from an aqueous mixture, using the liquid membrane technique.	**3**
4C.	Sketch and compare the advantages that different types of stirred crystallizers have over batch crystallizers.	**2**

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4D.	1000 kg of a 30% protein solution is cooled to 30^{O}C. During cooling, 10% of water originally present evaporates. The crystals obtained are consisting of bound moisture. The solubility of anhydrous protein at 30^{O}C is 21.5kg/100 kg water. What weight of protein crystallizes out? (Molecular weight of protein with bound water: 286 and anhydrous protein: 106)	**2**
5A.	Propose the technique that can be applied to overcome the feedback inhibition caused by a biocatalyst during biotransformation process.	**3**
5B.	A batch of fine particulate wet solids containing initial moisture content of 40% moisture is dried continuously in a tower drier to 20% of moisture. The drying process is assisted by a counter-current flow of air at 375K. The critical moisture content is at 15% and the constant rate of drying is 0.7 g/s m^2. For using such a process, calculate the approximate drying time, if the drying surface is assumed to be 0.03 m^2/kg dry mass for 100kg feed of wet solids.	**4**
5C.	Derive the equation for denaturation during drying and solve the following problem: Drying operation is utilized during the final processing stages of protein crystals. At 40^{O}C, drying operation for 10 min causes 60% denaturation. When dried at 30˚C for 30 min, protein denatures by only 25%. Estimate the denaturation for 80 min at 2˚C. Note: κ=3.5×10^{15} min^{-1} ; E=23.4×10^3 cal/gmol; R= 1.99 cal/mol. K	**3**
6A.	At the end of a fermentation process, the broth containing 20g/l of solute of interest is sent into a continuous mixer settler extraction system at the flow rate of 100L/min. The extracting solvent enters at the rate of 10 L/min. The equilibrium line for this process is given as: C_E=40C_R. For the first stage, calculate the (a) solute concentration in the extract and raffinate (b) fraction of solute extracted. As the organic extract enters into the second stage at 5 L/min and a varied set of pH conditions, the equilibrium line changes form to: C_E=37C_R. For the second stage, calculate (c) Concentration of solute in extract and raffinate from second extraction (d) Overall fraction of solute extracted.	**4**
6B.	Illustrate and design a drying operation for a wet slurry that has to be converted to dry granular end product by mixing with a friable mass of previously dried mixture.	**2**
6C.	Proteins are extracted from cheese whey using the aqueous two phase extraction system. For the salt concentration of 0.5M and 1 M the protein partitions into the two phases as follows: Determine the % yield for the two salt concentrations.	**2**

Salt conc. (M)	Volume		Concentration	
	Top phase	Bottom phase	Top phase	Bottom phase
0.5	2.4	2.88	1.2	6.05
1	1.8	2.5	0.79	6.6

6D.	The membrane chromatography process is utilized to purify DNA (diffusivity= 9.5 $\times 10^{-12}$ m^2/s) from a DNA protein mixture. If the dimensions of the membrane are as follows: Thickness-3mm, area of membrane-1.89 $\times 10^{-3}$ m^2, Porosity- 0.65 and tortuosity-1.5 and pore diameter-2×10^{-6} m. Determine the diffusion time and convection time for this membrane operating at a flow rate of $1 m^3/sec$.	**2**