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Manipal Institute of Technology, Manipal

(A Constituent Institute of Manipal University)

I SEMESTER M.TECH (INDUSTRIAL BIOTECHNOLOGY)

END SEMESTER EXAMINATIONS, NOV/DEC 2015

SUBJECT: ADVANCED BIOSEPARATION PROCESSES [BIO 507]

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. Many animal cells can be cultivated on the external surface of dextran beads. These cell-laden beads or "microcarriers" have a density of 1.02 g/cm^3 and a diameter of 150 *m*m. A 50-liter stirred tank is used to cultivate cells grown on microcarriers to produce a viral vaccine. After growth, the stirring is stopped and the microcarriers are allowed to settle. The microcarrier-free fluid is then withdrawn to isolate the vaccine. The tank has a liquid height to diameter ratio of 1.5; the carrier-free fluid has a density of 1.00 g/cm^3 and a viscosity of 1.1 cP. Estimate the settling time by assuming that these beads quickly reach their maximum terminal velocity. **5**

1B. A biochemist discovers and purifies a new enzyme, generating the purification table below **5**

Procedure	Total Protein (mg)	Activity (units)
Crude Extract	25000	4000000
Precipitation (Salt)	5000	3000000
Precipitation (pH)	4000	1000000
Ion exchange Cellulose Chromotography	200	800000
Affinity Chromotography	50	750000
Size exclusion Chromotography	45	675000

Calculate the yield and specific activity at each fraction and pick out the most efficient and the least efficient purification steps

2A. Calculate specific resistance of cake and filter medium resistance on basis of the following data for a constant pressure filtration. The mass of solid deposited per unit volume of filtrate was 24.6 g/L on a filter medium of area 5 cm^2. The pressure drop was 500 psi and viscosity of filtrate was 1.1 × 10^{-3} kg/m s **5**

Time (s)	6	10	16	23	31
V (cm^3)	30	40	50	60	70

2B. A suspension of kaolin (a type of clay used as adsorbent for biological material) in water became clear upon being allowed to stand undisturbed for 3 min at 20ºC. **5**

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	The height of the suspension in the vessel was 30 cm and the density of kaolin is known to be 2.6 g/cc. Estimate the diameter of the kaolin particles.	
3A.	A bead mill was used to grind *Penicillium* filaments and the energy required for different size reductions for the same mass of material was determined (see Table below): **Average Initial radius (microns)** / **Average Final radius (microns)** / **Energy Required (J)** 6 / 5.5 / 1.8 5 / 4.5 / 2.7 4 / 3.5 / 4.3 3 / 2.5 / 8.0 2 / 1.5 / 20 Calculate the amount of energy required to reduce the average filament radius from 5 microns to 1 micron for the same mass of *Penicillium* as used in the same bead mill.	**5**
3B.	For a nonessential amino acid, the equilibrium relation between toluene and pure water is $x^2 = (0.001\ \text{mol/L})\ y$ We plan to contact 4.7 L of toluene containing 0.006 M amino acid with 1 L of water. What fraction of the amino acid can we extracted?	**5**
4A.	The solubility of ovalbumin in water is 390 kg/m^3. When 30 mL of ethanol was added to 100 mL of a 50 mg/mL ovalbumin solution in water, 33% of the protein was found to precipitate. How much protein would precipitate if 100 mL of ethanol were added to 100 mL of a similar protein solution at the same temperature? Assume that the dielectric constant of the medium varies linearly with volumetric composition of the two solvents. Dielectric constant for water and ethanol are 80 and 24 respectively	**5**
4B.	Leucine dehydrogenase was recovered from a homogenate of disrupted Bacillus cereus cells using an aqueous two phase polyethylene glycol-salt system. 100 L of homogenate initially containing 5.44 U/mg protein were processed. A polyethylene glycol-salt mixture was added and two phases formed. The phase volume ratio was 1.3. Leucine dehydrogenase activity in the top and bottom phase were found to be 5.29 U/mL and 1.61 U/mL. The concentration of protein in the top and bottom phase were 0.389 mg/mL and 1.06 mg/mL respectively. Calculate the selectivity, purification fold and & % yield.	**5**
5A.	Two solutes have linear equilibrium constants of K_1 = 7.5 and K_2 = 7.8, respectively. For a flow rate of 1.5 L/min, in a column 63 cm in diameter with a void fraction of 0.33, what column length is required to separate the two solutes by 5 min?	**5**

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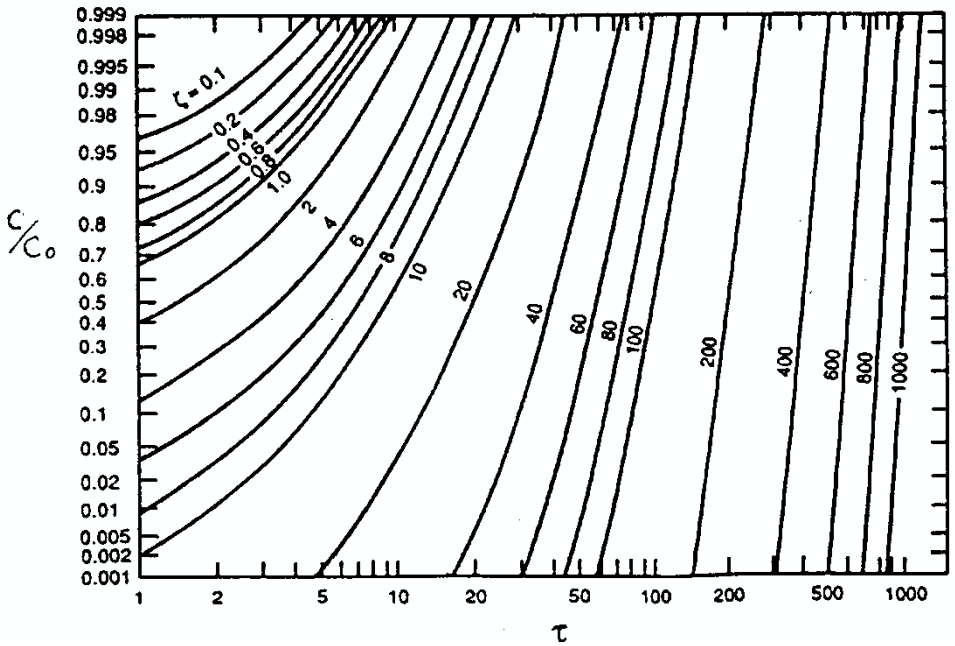
5B.	Bacterial cells having 0.8 micron average diameter are being microfiltered in the cross-flow mode using a membrane having an area of 100 cm^2. The steady state cake layer formed on the membrane has a thickness of 10 microns and a porosity of 0.35. If the viscosity of the filtrate obtained is 1.4 centipoise, predict the volumetric permeate flux at a transmembrane pressure of 50 kPa. When pure water (viscosity = 1 centipoise) was filtered through the same membrane at the same transmembrane pressure, the permeate flux obtained was 10^{-4} m/s	**5**
6A.	An adsorption column with a diameter of 2.0 cm and a bed height of 5.8 cm was used to isolate trypsin. The concentration of trypsin in the feed was 0.19 mg/mL. The external void fraction in the bed was 0.35. The bulk density of the adsorbent is 1.03 g/cm^3. The volumes at breakthrough and exhaustion are 150 and 450 cm^3, respectively. Estimate the loading capacity of the adsorbent for trypsin.	**5**
6B.	You have isolated a protein to be used as a vaccine by adsorption from a buffer on a packed bed of an ion exchange resin. The resin consists of 0.011-cm spheres, with an external void fraction of 0.37. It is packed in a 100-cm column, 83 cm in diameter, fed at a velocity of 0.052 cm/s. Under these conditions, the protein is adsorbed with a mass transfer coefficient k of 9×10^{-6} cm/s and an adsorption equilibrium constant of 27. How long should you run the bed if you choose a 10% breakthrough concentration?	**5**



Where $\zeta = \frac{zka}{v}$, a dimensionless position

$\tau = \frac{ka}{K(1-\varepsilon)}\left(t - \frac{z}{v}\right)$, a dimensionless time