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MANIPAL INSTITUTE OF TECHNOLOGY

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I SEMESTER M.TECH (INDUSTRIAL BIOTECHNOLOGY)

END SEMESTER EXAMINATIONS, NOV/DEC 2016

SUBJECT: ADVANCED BIOSEPARATION PROCESSES [BIO 5124]

REVISED CREDIT SYSTEM
(01/12/2016)

Time: 3 Hours MAX. MARKS: 50

Instructions to Candidates:

- Answer **ALL** the questions.
- Missing data may be suitable assumed.

1A.	The purification of a recombinant protein is carried out starting with 100 L of a clarified cell lysate (i.e., the cells have been lysed, and the cell debris has been removed to give a clarified solution), which has a total protein concentration of 0.36 mg/mL and a recombinant protein concentration of 2.2 U/mL, where U denotes units of biological activity of the recombinant protein. It is known that the completely pure recombinant protein has a specific activity of 40.0 U/mg. Purification is continued until a chromatography step that yields 2.0 L of a fraction containing the protein, with a total protein concentration of 1.11 mg/mL and a recombinant protein concentration of 43.2 U/mL. For the recombinant protein, calculate the starting and ending purity, the starting and ending specific activity, and the percentage yield and fold purification through the chromatography step.	**4**
1B.	Two spherical molecules A and B were found to have diffusivities of 4×10^{-10} m^2 /s and 8×10^{-10} m^2 /s respectively in a particular medium. Which molecule has the larger diameter and by what percent is this diameter greater than that of the other.	**2**
1C.	Why do we need bioseparation? Briefly explain challenges and opportunities in bioseparation processes.	**4**
2A.	A rough analysis of cell contents suggests that cytoplasm contains 8% by weight of solutes: 2% proteins of average molecular weight 33,000; 1.5% sugars of molecular weight 180; 1.5% is soluble lipids of molecular weight 340; and 3% is salts like $CaCl_2$. What is the osmotic pressure inside the cells relative to pure water at 25˚C?	**5**
2B.	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 The microcarrier-free fluid is then withdrawn to isolate the vaccine.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**

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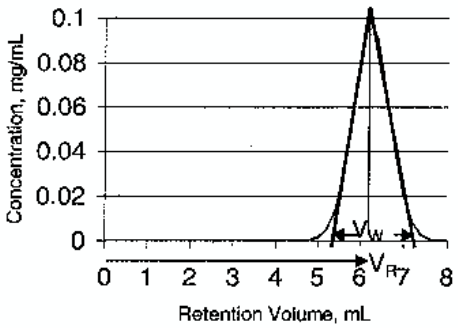
3A.	A broth of 80 L contains the desired protein at 12.8 g/L as well as a contaminant protein at 1.8 g/L. Calculate the ammonium sulphate concentration required to recover 98% of the desired protein if the precipitation constants b and k of the desired protein are 9.33 and 1.1 respectively and that of the contaminant protein are 8.8 and 0.95 respectively. What will be the purity of the desired protein at 98% recovery?	**5**
3B.	A clarified fermentation beer (H) containing 260 mg/liter of this antibiotic is to be extracted using butyl acetate (L). Because the beer's pH is 3.5, the equilibrium constant (K) is 57. You plan to let H equal 450 liters/hr and L equal 37 liters/hr in an counter current mode; you hope to recover 99% of the antibiotic in the feed. How many stages will you need to accomplish this separation?	**5**
4A.	A solution of raffinose containing 100 g/L of NaCl is to be dialysed in a shell-and-tube type of hollow-fiber dialyzer operating countercurrently. With a dialyzer having 1000 cm^2 area of membranes the dialysis coefficient for NaCl was determined to be 0.0415 cm/min, when the feed rate was 200 cm^3/min, and the flow rate of pure water was 500 cm^3/min. If 90% of the salt is to be removed, what area of the hollow-fiber membranes will be needed, if the same flow rates for feed and water are used?	**5**
4B.	We are carrying out the ultrafiltration of chymotrypsin in a spiral wound module at a rate of 1.3×10^{-5} cm/s. The solution concentration is 0.44 wt%, the protein's diffusion coefficient is 9.5×10^{-7} cm^2/s, and the boundary layer is about 0.018 cm thick. How high is the surface concentration?	**5**
5A.	A modified dextran will adsorb up to 8×10^{-8} mol of immunoglobulin G per cm^3 dextran. The adsorption follows a Langmuir isotherm with a constant K equal to 2×10^{-8} mol/liter. How much dextran do you need to adsorb 90% of the protein in 1.2 liters of solution initially containing 4×10^{-6} mol/liter?	**5**
5B.	A 50 μL sample of blue dextran at an initial concentration of 2 mg/mL is injected to a column to measure the plate count at a flowrate of 1 mL/min. The resulting chromatogram is as follows:  If the retention volume V_R = 6.3 mL and peak width V_w = 2 mL, calculate the plate counts.	**5**