Reg. No.										

MANIPAL INSTITUTE OF TECHNOLOGY

MANIPAL

A Constituent Institution of Manipal University

IV SEMESTER B.TECH. (BIOTECHNOLOGY)

END SEMESTER EXAMINATIONS, APRIL/MAY 2018

SUBJECT: DOWNSTREAM PROCESSING IN BIOTECHNOLOGY [BIO 2204]

REVISED CREDIT SYSTEM

Time: 3 Hours MAX. MARKS: 50

Instructions to Candidates:

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

1A. What are the major problems faced in purification of an extracellular enzyme? **2**

1B. Explain the common stages of downstream processing. Highlight the typical bioseparation techniques employed in each stage. **3**

1C A rough analysis of cell contents suggests their cytoplasm contains 5% by weight of solutes: 1% is proteins of average molecular weight 45,000; 1% is soluble lipids of molecular weight 400; 1% is sugars of molecular weight 170; and 2% is salts like KCl. What is the osmotic pressure inside these cells relative to pure water at 37°C? **5**

2A. We have filtered slurry of sitosterol at constant pressure through a filtration medium consisting of a screen support mounted across the end of a Pyrex pipe. We find that the resistance of this new medium is negligible. We also find the following data in a laboratory test: **5**

Weight of crystals	62 g	Pressure of filtration	15 psi
Filter diameter	5.08 cm	Cake depth	12.5 cm
Cake volume	253.3 cm3	Filtration time	163 min

The cake is essentially incompressible.

On the basis of this laboratory test, predict the number of frames (30 in × 30 in × 1 in thick) needed for a plate-and-frame press & estimate the time required for filtering a 63 kg batch of steroid. In the plate and frame press, pressure of filtration is 3.5 psi.

2B A continuous disc-stack centrifuge is operated at 5000 rpm for separation of bakers' yeast. At a feed rate of 60 L/min, 50% of the cells are recovered. At constant speed, solids recovery is inversely proportional to flow rate. **5**

(i) What flow rate is required to achieve 90% cell recovery if the centrifuge speed is maintained at 5000 rpm?

What operating speed is required to achieve 90% recovery at a feed rare of 60 L/min?

3A. A bowl centrifuge is used to concentrate a suspension of *Escherichia coli* prior to cell disruption. The bowl of this unit has an inside radius of 12.7 cm **5**

and a length of 73.0 cm. The speed of the bowl is 16,000 rpm and the volumetric capacity is 200 L/h. Under these conditions, this centrifuge works well.

(i) Calculate the settling velocity v_g for the cells.

(ii) After disruption, the diameter of debris is about one-half of that of cell and the viscosity is increased four times. Estimate the volumetric capacity of this same centrifuge operating under these new conditions.

$$Q = v_g\left(\frac{2\pi\ell R^2\omega^2}{g}\right)$$

3B. Two chemically modified Cephalosporins A and B dissolved in a clarified beer at pH 3.8 have activities of 1.0 and 2.0 (in arbitrary units). We want to extract these antibiotics into amyl acetate, for which the partition coefficient K_A and K_B are 31 and 11 respectively. For initial experiments, we plan to use an end feed extraction process with H = 9.6 and L = 0.51 L/h. We want to get 90% recovery of Cephalosporin A. What is the required number of stages? What are the exit concentrations of both antibiotics? 5

4A. 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 β 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

4B. A solution containing 0.62 mg/mL of a polypeptide of molecular weight 2360 Da is to be concentrated by ultrafiltration. The ultrafiltration membrane is nearly ideal, passing solvent but completely retaining polypeptide; low molecular weight species have no significant effect. Concentration polarization also has a minor effect. The value of RT is 24 liters atm /mol, the initial solution volume is 64 liters, the final concentration should be 10 mg/mL, the spiral wound module has a total area of 2.6 m^2, and the permeability of the membrane is 0.15 liter/m^2 atm hr with pressure drop of 235psi, how long will the filtration take place? 5

$$t = \frac{1}{AL_p\Delta P}\left[(V_0 - V) + \left(\frac{n_1 RT}{\Delta P}\right)\ln\left(\frac{V_0 - n_1 RT/\Delta P}{V - n_1 RT/\Delta P}\right)\right]$$

5A. Reverse osmosis process is used for desalination of seawater. The volumetric flux of water through the membrane is 3×10^{-5} m/s (or $m^3\ s^{-1}\ m^{-2}$), and the applied feed pressure is 8.0 MPa greater than the product-water pressure. For seawater, the osmotic pressure is 2.5 MPa. What is the water velocity through the membrane if the polarization modulus (c_W/c_B) rises to 1.2-fold of the original? 5

5B. We want to use a four stage countercurrent extractor to separate growth hormones present as part of an aqueous protein concentrate. The activity of each hormone in the feed solution is a nominal value of 1. We add phosphates to this concentrate, and extract with a polyethylene glycol solution which forms a second immiscible aqueous phase. The hormones partition between these phases with values of K equal to 6.0 and 8.0, respectively. In one series of experiments, we use a phosphate feed of 50 kg/h and a polyethylene glycol feed of 10 kg/hr. What is the percent recovery of both hormones in this case? 5