Reg. No.										

Manipal Institute of Technology, Manipal

(A Constituent Institute of Manipal University)

IV SEMESTER B.TECH (BIOTECHNOLOGY)

END SEMESTER EXAMINATIONS, MAY/JUN 2016

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	**2**
1C.	When do you prefer centrifugation over conventional filtration? A tubular bowl centrifuge of length 'l' and rotating at the angular velocity of 'ω'. Obtain an expression for the separating power ∑ of the centrifuge. $$\therefore Q = v_g\left(\frac{2\pi\ell R^2\omega^2}{g}\right) = v_g(\Sigma)$$	**6**
2A.	Why do we need to pretreat a fermentation broth before subjecting it to filtration?	**2**
2B.	Bacterial cells were centrifuged using 5 cm long centrifuge tubes in a laboratory centrifuge having an angled rotor which held the tubes at 60 degree angle with the axis of rotation. The top of the tubes were 5 cm away from the axis and it took 15 minutes at a rotation speed of 10,000 rpm to completely sediment the cells. Calculate the sedimentation coefficient of the cells.	**3**
2C.	Time-pressure drop data for a constant rate cake filtration process is given below:	**5**

Time (s)	0	10	20	30	40	50
ΔP (kPa)	5	10	15	20	25	30

If we are to carry out the filtration at twice the filtration rate, predict the pressure drop required after 2 hours of filtration. Assume that the cake is incompressible.

3A.	Compare and contrast Ultrafiltration and dialysis	**2**

3B.	A protein solution (concentration = 4.4 g/L) is being ultrafiltered using a spiral wound membrane module, which totally retains the protein. At a certain transmembrane pressure the permeate flux is 1.3 x 10^{-5} m/s. The diffusivity of the protein is 9.5 x 10^{-11} m^2 /s while the wall concentration at this operating condition is estimated to be 10 g/L. Predict the thickness of the boundary layer. If the permeate flux is increased to 2.6 x 10^{-5} m/s while maintaining the same hydrodynamic conditions within the membrane module, what is the new wall concentration?	**4**
3C.	A 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?	**4**
4A.	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 (MW: 75). What is the osmotic pressure inside these cells relative to pure water at 37°C?	**5**
4B.	Ammonium sulfate is being used to precipitate a humanized monoclonal antibody from 10 litres of cell culture media, the initial concentration of the antibody in this liquid being 0.5 mg/ml. Solid ammonium sulfate is added to the liquid such that the concentration of the salt is 1.5 kg-moles/m^3. This results in the precipitation of 90% of the antibody. When the ammonium sulfate concentration of the mixture is raised to 1.75 kg-mole/m^3, a further 76.5% of the remaining antibody is precipitated. Predict the ammonium sulfate concentration needed for total antibody precipitation. What is the solubility of the antibody in ammonium sulfate free aqueous medium?	**5**
5A.	What is the significance of nonionic surfactant used in reverse micellar extraction	**2**
5B.	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	**3**
5C	In an experiment, butyl acetate extract which contains 3.13 g/L of the antibiotic is produced at 37 L/hr. We plan to wash this extract with water at pH 6, for which K = 0.11. We will use three stage extractor (counter current) with 95% recovery. What water flow should we use?	**5**