MANIPAL INSTITUTE OF TECHNOLOGY
MANIPAL
(A constituent unit of MAHE, Manipal)

IV SEMESTER B.TECH. EXTERNAL EXAMINATIONS May 2019

SUBJECT: DOWNSTREAM PROCESSING IN BIOTECNOLOGY [BIO2204]

Date of Exam:**04/05/2019** Time of Exam: **2.00 PM – 5.00 PM** Max. Marks: **50**

Instructions to Candidates:

- Answer ALL the questions & missing data may be suitable assumed

<table>
<tr><td>1A.</td><td>Design a downstream process for biological slurry containing volatile impurities mixed with nonvolatile and charged product inside a bacterial cell.</td><td>3</td></tr>
<tr><td>1B.</td><td>A suspension of clay particles for separation in biological process completely clarifies on being left undisturbed for 3 min. The suspension rose to a height of 33 cm in the vessel and the density of clay is known to be 2.9 g/cm³. Estimate the diameter of the clay particles after stating the assumptions.</td><td>2</td></tr>
<tr><td>1C.</td><td>Design a flowchart to show the process design of cost cutting strategies for downstream processing of a high resolution, low throughput biomolecule.</td><td>2</td></tr>
<tr><td>1D</td><td>Determine the partition coefficients for the protein which is extracted using aqueous two phase extraction into top phase using the following data and comment on the results:
<table>
<tr><th rowspan="2">Conc. Of NaCl (M)</th><th colspan="2">Concentration of Protein (mg/ml)</th></tr>
<tr><th>Top phase</th><th>Lower phase</th></tr>
<tr><td>0</td><td>0.49</td><td>0.45</td></tr>
<tr><td>0.1</td><td>0.42</td><td>0.55</td></tr>
<tr><td>1</td><td>0.5</td><td>0.49</td></tr>
</table></td><td>3</td></tr>
<tr><td>2A.</td><td>The following data shows the energy required for the disruption of the same mass of bacterial cells in a bead mill for low volume of sample.
<table>
<tr><th>Average Initial radius (microns)</th><th>Average Final radius (microns)</th><th>Energy Required (J)</th></tr>
<tr><td>7</td><td>6.5</td><td>2</td></tr>
<tr><td>6</td><td>5.5</td><td>1.6</td></tr>
<tr><td>5</td><td>4.5</td><td>3</td></tr>
<tr><td>4</td><td>3.5</td><td>7.5</td></tr>
<tr><td>3</td><td>2.5</td><td>18</td></tr>
</table>
Calculate the amount of energy (in J) required to reduce the average particle radius from 5 microns to 1 micron for the same mass as used in the same bead mill</td><td>5</td></tr>
<tr><td>2B.</td><td>Determine the advantage of using mechanical cell disruption techniques over non mechanical methods while dealing with biological samples. Illustrate and explain the</td><td>5</td></tr>
</table>

	mechanism of operation of a bead mill.	
3A.	Dextran beads are commonly used for the cultivation of different cell types, called as microcarriers. The microcarriers generally have a density of 0.09 g/cm^3 and a radius of 10 μm. A 60 liter vessel is used for the development of the microcarriers and after the completion of growth the microcarrier free fluid is withdrawn. The height to diameter ratio of the tank is 2, the fluid has a density of 0.07 g/cm^3 and the viscosity of fluid is 1.1 cP. Calculate the settling time (in seconds) of these beads if they reach maximum terminal velocity very quickly.	**3**
3B.	A crude laboratory centrifuge, with flinging bucket type set up, is used to collect bacterial cells from aqueous solution post fermentation. The distance between the surface of the liquid and the bottom of the bucket from the axis of rotation, during centrifugation at 500 r/min, is 3 cm and 10 cm, respectively. The equivalent diameter of the cells is 8μm and the density is 1.06 g/cm^3. What is the time needed for complete separation?	**3**
3C.	Derive the following equation for a Disc bowl centrifuge. Several centrifugation processes require the pretreatment of the feed sample. Describe such a pretreatment technique. $$Q = v_g[\frac{2\pi n\omega^2}{3g}(R_0^3 - R_1^3)\cot\theta]$$ Where, Q is volumetric flow rate, n is number of discs, R_0 and R_1 are the external and internal radius, θ is the angle of the discs.	**4**
4A.	Describe the solution diffusion and convection theoretical models for membrane separation.	**3**
4B.	$$t=\frac{1}{AL_p\Delta p}\{(V_o-V)+(\frac{RTn_1}{\Delta p})\ln(\frac{Vo-RTn_1/\Delta p}{V-RTn_1/\Delta p})\}$$ Derive the given transport equation in order to determine the time of filtration. Where, t is time of filtration, A is area of filter, Lp is the permeability, Δp is the pressure drop, V is volume being filtered.	**3**
4C.	The flow channels of an ultrafiltration system has tubes of 0.2 cm diameter and 100 cm long. The protein being filtered to remove low molecular weight species, has a diffusion coefficient of 1.4×10^{-7} cm^2/s. The solution has a viscosity of 2.5 cP and a density of 1.1 g/cc. The unit can operate a bulk stream velocity of 300 cm/s. Determine the polarization modulus for the transmembrane flux of $45 L/m^2h$. (Correlation: $Sh = 0.082\ Re^{0.69}Sc^{0.33}$)	**4**
5A.	Ammonium sulfate precipitation was carried out for protein precipitation. The initial concentration of protein was 15g/L. At ammonium sulfate concentrations of 0.5M and 1 M, the concentration of the protein remaining in the mother liquor at equilibrium were13.5g/L and 5g/L, respectively. From this information, estimate the ammonium sulfate concentration to give 95% recovery of the protein as precipitate.	**3**
5B.	Describe the mechanism of precipitate formation with suitable equations.	**3**
5C.	Explain the theory behind aqueous two phase extraction process.	**4**