SUBJECT: Bioseparation Engineering
COURSE # BT 360

27 February 2023
FULL MARKS: 25

MID-SEMESTER EXAMINATION

Answer all questions

SECTION-A

For this section, the answers should be comprehensive and with proper justification

1. You are trying to estimate the sedimentation velocity for two spherical-shaped bacterial cells B1 and B2 having a radius of 1.0 µm and 0.5 µm, respectively. The density (g cm^{-3}) of the cells is the same. The density and viscosity of the suspending medium is 1.0 g cm^{-1} and 0.01 g cm^{-1} s^{-1}, respectively. Both the cells are subjected to centrifugation under the same conditions (all operating conditions are the same and the same rotor and centrifuge is used for both). Which one of the cells (B1 or B2) is likely to have a higher sedimentation velocity during centrifugation? Provide a brief justification for your answer. **(2.0)**

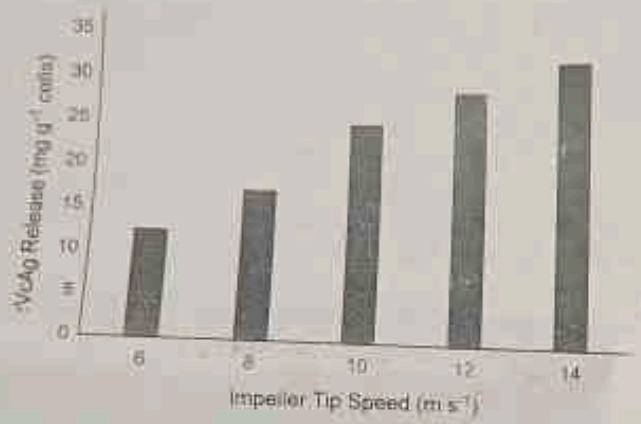
2. You are involved in the recovery of the intracellular enzyme β-galactosidase from a recombinant strain of *E. coli*. To this end, you have initially grown the *E. coli* strain in a 5.0 litre bioreactor in separate sets on (i) a synthetic medium and (ii) a complex medium. Following growth in the respective medium, the cells were harvested and equal concentration of the cells were subjected to disruption in a homogenizer operating at 30 MPa for varying number of passes (1 to 8). Draw a hypothetical plot for release of the enzyme from the *E. coli* strain grown on the respective medium versus number of passes (*N*) and provide a brief explanation for the obtained plots. **(2.0)**

3. A recombinant therapeutic protein (rTPX) expressed in *E. coli* accumulates as inclusion body (IB). The *E. coli* cells were harvested and concentrated by crossflow filtration and homogenized to obtain an inclusion body slurry (IB slurry). The IB slurry was then washed in diafiltration mode with 25 mM phosphate buffer (between 0 - 2.0 diafiltration volumes) and subsequently with a mixture of 25 mM phosphate buffer and 5.0 mM EDTA (from 2.0 - 5.0 diafiltration volumes). The concentration of UV-absorbing soluble contaminants was determined by measuring the absorbance of the filtrate samples at 280 nm (A_{280}) during IB-wash, as indicated in the table shown below. Provide a brief explanation for the obtained data. **(3.0)**

Diafiltration Volumes	Absorbance of filtrate (A_{280})
0	0.22
0.5	0.33
1.5	0.36
2.0	0.37
2.5	0.54
3.0	0.67
3.5	0.75
4.0	0.87
4.5	0.89
5.0	0.88

4. You have grown a recombinant strain of yeast in a bioreactor having a working volume of 300 litres. The yeast cells are engineered to secrete a therapeutic antibody. You are considering using a rotary vacuum drum filtration process for the separation of yeast cells. Briefly explain how (i) the pressure drop (Δp), (ii) sample viscosity (μ_l) and (iii) specific cake resistance (α) can influence the rate of the filtration process? With respect to the mentioned parameters (Δp, μ_l and α), what strategies will you adopt to enhance the filtration rate? **(1.0 + 1.0 + 1.0 = 3.0)**

5. As a bioprocess engineer, you are aiming to purify a recombinant viral core antigen (rVcAg) produced intracellularly in *E. coli*. In separate sets, you load equal amounts of *E. coli* cell suspension (10% w/v) into a 1.0 litre glass chamber of a bead mill loaded with 0.2 mm zirconia beads (80% v/v). You perform cell disruption in these sets for 3 hours under various impeller tip speed. Following cell disruption, you measure (a) the amount of rVcAg released (mg g^{-1} cells) by ELISA, (b) purity of rVcAg (%) and (c) temperature of the various cell lysate samples as indicated in the figure and table shown below. Provide an explanation for the obtained data. **Note:** rVcAg is a heat-stable protein, which can retain its native conformation and biological activity till 70 °C. **(3.0)**



Impeller Tip Speed (m/s)	rVcAg Purity (%)	Temperature (°C)
6.0	14.2	22
8.0	18.9	22
10	22.5	25
12	27.8	48
14	32.5	62

SECTION-B

In this section, all the assumptions, hypothesis or steps required for solution of the numerical problems and the final answer along with the required units should be clearly indicated. Incomplete steps/answers or answers shown as fraction will not be considered. PART MARKING IS NOT APPLICABLE FOR THESE QUESTIONS.

1. In a bioprocess plant, you are aiming to achieve complete recovery of *E. coli* cells grown in a bioreactor by using a pilot scale tubular bowl centrifuge. The cells have a radius of 0.5 μm and a density of 1.1 g cm^{-3}. The centrifuge is operated at 10,000 rpm, the bowl diameter is 10 cm, the bowl length is 100 cm and the diameter of the outlet opening is 4.0 cm. Estimate the maximum flow rate **(in litres per minute)** of the cell broth that can be attained in the centrifuge? The density and viscosity of the suspending medium is 1.0 g cm^{-3} and 0.01 g cm^{-1} s^{-1}, respectively. Consider g = 9.81 m s^{-2}. **(3.0)**

2. In a bioprocess plant, you are involved in the isolation and purification of a recombinant therapeutic protein. The protein solution contains 2.0 M NaCl. You have decided to desalt 5000 litres of the protein solution by using a constant volume diafiltration process with a membrane having a retention coefficient R = 0 for NaCl and R = 1.0 for the protein. The filter is operated with a permeate flux of 40 l m^{-2} h^{-1}. The total membrane area is 100 m^2. During diafiltration, water is added till the concentration of NaCl is 0.01 M. Calculate the time required (**in hours**) for the diafiltration process? **(3.0)**

3. Batch cell disruption data for Baker's yeast slurry (50% wet wt. volume^{-1} containing 0.5 kg protein.kg^{-1} yeast cell) in a 50×10^{-4} m^3 mill at 5 °C with a stainless-steel impeller is provided in the table below. (a) Calculate the disruption constant ***k* (in s^{-1})** by using a graphical method. (b) If the same mill is used in continuous mode for the same cell suspension, calculate the volumetric feed rate **(in m^3 s^{-1})** to achieve 90% release of the protein. Given impeller speed = 12 rpm; impeller diameter = 0.05 m; Apparent viscosity and density of yeast cell suspension is 0.80 kg.m^{-1}.s^{-1} and 1100 kg.m^{-3}, respectively.

(2.0 + 1.0 = 3.0)

Time (s)	Wt. of protein released per unit wt. of packed yeast (kg protein.kg^{-1} yeast cell)
10	0.05
20	0.20
40	0.41
60	0.48

4. In a bioprocess plant, you are operating a rotary drum vacuum filter at a constant pressure drop and trying to study the effect of the addition of filter aid on the specific cake resistance during separation of *Streptomyces griseus* biomass. Based on the experiments, the specific cake resistance was 4×10^{14} m kg^{-1} and 5×10^{12} m kg^{-1} in presence of 1% and 2% filter aid, respectively. For the same filtration time, estimate the fold increase in the volume of the filtrate in presence of 2% filter aid as compared to 1% filter aid. The resistance due to the medium is negligible. The filtration parameters (including the area of the filter used and pressure drop) as well as the concentration of the biomass used in presence of 1% and 2% filter aid is the same. **(3.0)**

********** END OF QUESTION PAPER **********