INDIAN INSTITUTE OF TECHNOLOGY GUWAHATI
Department of Biosciences and Bioengineering

SUBJECT: Bioseparation Engineering **20 February 2023**
COURSE # BT 306 **FULL MARKS: 10**

QUIZ-I

Duration: 3.00 PM-3.45 PM

Answer all questions

In case of Q4 and Q5, 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 and PART MARKING IS NOT APPLICABLE FOR THESE QUESTIONS.

1. Consider that yeast cells are subjected to high pressure homogenization to isolate an intracellular recombinant protein. (a) Briefly explain how the same degree of homogenization can be achieved at low number of passes (*N*). (b) Indicate any two advantages of reducing the number of passes (*N*) while operating the homogenizer. **(1.0 + 1.0 = 2.0)**

2. Consider that two separate bacterial cell suspension samples are subjected to sedimentation by centrifugation. The cells in both the samples are spherical in shape and suspended in sterile water. The concentration of cells in sample 1 and sample 2 is 0.5 mg/mL and 5.0 mg/mL, respectively. If both samples are centrifuged under the same operating conditions, briefly explain how the increase in the cell concentration in sample 2 is likely to impact the sedimentation velocity of the cells in suspension. **(2.0)**

3. As a bioprocess engineer, you are aiming to isolate and purify a recombinant viral core antigen (rVcAg) produced intracellularly in an engineered strain of *E. coli*. In two separate sets, you load equal amounts of *E. coli* cell suspension into a 500 mL glass chamber of a bead mill loaded with 0.2 mm zirconia beads (80% v/v). You perform cell disruption in these sets for 2 h under an impeller tip speed of 18 m/s and 10 m/s, respectively. Following cell disruption, you subject the cell lysates to sucrose density gradient (5% - 30%) ultracentrifugation and the concentration of rVcAg (mg/mL) obtained in various fractions after centrifugation (top to bottom in the centrifuge tube) are indicated in the table shown below. (a) Provide a brief explanation for the obtained data shown in the table. (b) Which of the two bead mill operations you would prefer? Briefly explain with justification. **(1.0 + 1.0 = 2.0)**

Fraction Number	Concentration of rVcAg (mg/mL)	
	Impeller Tip Speed (18 m/s)	Impeller Tip Speed (10 m/s)
2	0.21	0.08
4	0.28	0.12
6	0.32	0.36
8	0.36	0.62
10	0.35	0.41
12	0.24	0.11
14	0.09	0.04

4. In a research project, you are trying to clarify a suspension of ribosomes obtained from yeast cells in order to constitute a cell-free translation system. To accomplish the desired level of clarification, you decide to centrifuge a tube containing the yeast ribosome suspension at an operating speed of 10,000 rpm. Prior to centrifugation, the initial distance from the center of rotation to the ribosomes nearest to the center of rotation is 4.0 cm. Following centrifugation, if the maximum distance travelled radially outward by the ribosomes in the centrifuge tube is 2.0 cm, estimate how long will it take **(in hours)** to completely clarify the suspension of the ribosomes? Given the sedimentation coefficient for the ribosomes is 80S. **(2.0)**

5. You are involved as a bioprocess engineer in a production plant aiming to commercially produce glucose-6-phosphate dehydrogenase enzyme from yeast cells. The enzyme is an intracellular product. Hence to recover the enzyme, you have decided to subject the yeast cell suspension to cell disruption in a bead mill operated in batch mode and monitor the efficiency of cell disruption by measuring the percentage of total protein release from the sample. In a pilot scale operation, 60% of the total protein is released in 9.0 litres of the sample in 20 minutes. How long will it take **(in hours)** to release 90% of total protein from the yeast cells in 200 litres of the sample in a scale-up operation? The bead mill operating conditions are maintained during scale-up (bead mill loading, bead diameter, impeller tip speed is the same) and you may consider that the cell disruption constant is the same for both the scales of operation. **(2.0)**

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