Dr. Saurabh Bansal

JAYPEE UNIVERSITY OF INFORMATION TECHNOLOGY, WAKNAGHAT

TEST -3 EXAMINATION- May 2019

B.Tech 6th Semester

COURSE CODE: 16B11BT611 MAX. MARKS: 35

COURSE NAME: Downstream Processing

COURSE CREDITS: 04 MAX. TIME: 2 Hrs

Note: All questions are compulsory. Carrying of mobile phone during examinations will be treated as case of unfair means.

[CO3]

1. **a)** Why salt induced precipitation during protein purification is preferred over the organic acid induced precipitation? **[1]**

   b) How does the centrifugal speed and exposure time affect the settling of particles during centrifugation? **[2]**

   c) How the filter aid usage affects the rate of filtration? **[1]**

[CO1, 2]

2. Differentiate between following: **[4.5]**

   a) Upstream processing and Downstream Processing

   b) Extracellular and Intracellular protein purification

   c) HPLC and FPLC

[CO4]

3. A proteinaceous diphtheria toxoid from *Corynebacterium diphtheriae* supernatant was purified using Gel Filtration Chromatography. In the laboratory, a small column of 1.5 cm inner diameter and height 0.4 m is packed with 10 g dry Sephadex gel; the void volume is measured as 23 ml. A sample containing the toxoid and impurities is injected into the column. At a liquid flow rate of 14 ml min^{-1}, the elution volume for the toxoid is 29 ml; the elution volume for the principal impurity is 45 ml. A column of height 0.6 m and diameter 0.5 m is available for large-scale gel chromatography. The same type of packing is used; the void fraction and ratio of pore volume to total bed volume remain the same as in the bench-scale column. The liquid flow rate in the large column is scaled up in proportion to the column cross-sectional area; the flow patterns in both columns can be assumed identical. The water regain value for the packing is given by the manufacturer as 0.0035 m^3 kg^{-1} dry gel.

   a) Determine the partition coefficients for the toxoid and impurity. **[2]**

   b) Estimate the retention time of toxoid in the large column. **[3]**

P.T.O

4. Leucine dehydrogenase is recovered from a homogenate of disrupted *Bacillus cereus* cells using an aqueous two-phase polyethylene glycol-salt system. 150 litres of homogenate initially containing 3.2 units enzyme ml^{-1} are processed; a polyethylene glycol-salt mixture is added and two phases form. The enzyme partition coefficient is 3.5. Determine the volume ratio of upper and lower phases required to achieve 80% recovery of enzyme in a single extraction step? **[2]**

[CO4, 5]

5. How will you purify two different proteins A and B from a mixture if the pI of protein A and B have pI 7.0 and 6.0 respectively and the protein B found to be unstable at acidic conditions. How will you purify both the proteins? Also draw the chromatogram. **[3]**

[CO5, 6]

6. Citric acid is an important component of TCA cycle. However, in the case of iron limitation, citric acid is excreted out by the organism in the production medium.
   - **a)** Why citric acid is excreted out by the organism? **[1.5]**
   - **b)** Why the presence of iron in medium reduces the production of citric acid? **[1.5]**
   - **c)** How can the cycle continue when citric acid is excreted? **[2]**

7. **a)** What are the advantages claimed of using *Zymomonas mobilis* over the yeasts for the alcohol production? **[2]**
   b) What do you understand by azeotropic distillation? How does it help in recovery of highly purified alcohol? **[2]**

8. **a)** Why Rhizopus fermentation is preferred over *Lactobacillus delbruckii* fermentation for lactic acid production? **[1]**
   b) What are the limitations of natural penicillin? **[1.5]**
   c) Draw a self-explanatory flow chart for penicillin recovery from a fungal culture. **[2]**

[CO6]

9. **a)** How will you minimize the loss of a bioproduct (a protein) while its recovery? **[1.5]**
   b) Why the formulation of a bioproduct is necessary before its packaging? **[1.5]**