Register Number: ……..

M.TECH DEGREE EXAMINATIONS: JUNE/JULY 2013

Second Semester

BIOTECHNOLOGY

BTY506: Bioseparation Technology

Time: Three Hours **Maximum Marks: 100**

Answer all the Questions:-

PART A (10 x 2 = 20 Marks)

1. Why do fermentation yield low concentration of products?
2. What problems can be caused by microbial contamination of the product stream in downstream processing?
3. Write the short notes on coagulation and flocculation
4. How do you calculate the Gt values in centrifugation during scale-up studies?
5. What properties of a supercritical fluid are very much important in separation process?
6. Why the salts are likely to dissolve in water?
7. Define isocratic elution
8. What are the advantages of dye-affinity chromatography?
9. What is prepro insulin?
10. Why the eutectic point is give much importance in lyophilization?

PART B (5 x 16 = 80 Marks)

11. a) (i) Discuss in detail about the physio-chemical characterization of various biomolecules (12)

(ii) List out the guidelines to recombinant protein purification. Categories of bioproducts (4)

(OR)

b) (i) Explain the five stages involved in the recovery of bioproduct. (4)

(ii) Discuss in detail about the purification of various biomolecules using HPLC (12)

12. a) (i) The production of a recombinant protein involves fermentation of *E.Coli* , with the production of protein being intracellular. The protein forms precipitates in the form of submicron size inclusion bodies inside the cell. The protein in the inclusion bodies is not active. It is desired to recover inclusion bodies so that the protein can later be recovered in an active form. Give a conceptual process diagram that indicates the keys steps in recovery of the protein in an active form. (8)

(ii) We want to filter 25,000 L/h of a beer containing erythromycin using a rotary vacuum filter originally purchased for another product. Our filter has a cycle time of 50 s and an area of 32.7 m^2. It operates under a vacuum of 20 in Hg. The pretreated broth forms an incompressible cake with the resistance: (8)

$$\frac{\mu\alpha\rho_0}{2\Delta P} = 58 \text{ s/cm}^2$$

We want to wash the cake until only 1% of the retained soluble is left, and we expect that the washing efficiency will be 72 % and that 1% of the filtrate is retained.
(a) Calculate the filtration time per cycle.
(b) Find the washing time.

(OR)

b) (i) A batch of yeast cells was disrupted using ultrasonic vibrations to release an intracellular product. The concentration of released product in the solution was measured during the process (see table below): If the ultrasonic cell disruption were carried out for 240 seconds, predict the product concentration. (4)

Time (second)	**Concentration of protein (mg/L)**
60	3.49
120	4.56

(ii) What are the different mechanical methods available for cell disruption? Explain in detail with diagram. (12)

13. a) (i) What are aqueous biphasic systems? Explain the steps involved in the aqueous two phase extraction of an enzyme. (8)

(ii) Streptomycin is extracted from the fermentation broth using an organic solvent in a counter current staged extraction unit. The distribution coefficient of streptomycin at pH=4 is K_d=Yi/Xi=40,and the flow rate of the aqueous(H) phase is H=150 l/min. If only five extraction units are available to reduce the streptomycin concentration from 10 g/l in the aqueous phase to 0.2 g/l, determine the required flow rate of the organic phase (L) in the extraction unit. (8)

(OR)

b) (i) Water containing 6.8 mg/L of a steroid is extracted with initially pure methylene dichloride. The equilibrium constant for the steroid is 170 and the ratio of water to solvent is 82. What is the concentration in the organic phase after the extraction? What fraction of the steroid has been removed? (12)

(ii) Why ammonium sulfate is preferred over other salts for the precipitation of proteins? Also add a note on salting-out. (4)

14. a) (i) With a neat diagram explain the working principles of gel filtration chromatography and reverse phase chromatography. (12)

(ii) A myoglobin which has molecular mass 17 KDa and a stroke radius of 2.06 nm. If the viscosity of water at 25 ºC in mPas, what is the diffusion coefficient of myoglibin in water at 25 ºC (4)

(OR)

b) (i) What are the advantages of bioaffinity chromatographic techniques? (4)

(ii) Consider the use of gel chromatography to separate two proteins A and B. The partition coefficient (KD) for A is 0.5 and for B is 0.15 Vo, the void volume in the column, is 20 cm3. Vi, the void volume within the gel particles, is 30 cm3. The total volume of the column is 60 cm3. The flow rate of elutant is 100cm3/h. Ignoring dispersion and other effects, how long will it take for A to exit the column? How long for B? (12)

15. a) (i) Discuss in detail about the theory of crystallization (12)

(ii) Write a note on various applications of spray drying. (4)

(OR)

b) (i) Write in detail on different steps used to obtain homogeneous Taq polymerase (12)

(ii) List any four typical charactertics of modern biotechnology versus classical biotechnology in terms recovery of bioproducts (4)

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