DEPARTMENT OF BIOTECHNOLOGY

INDIAN INSTITUTE OF TECHNOLOGY KHARAGPUR

AUTUMN END SEMESTER EXAMINATION — 18-11-2022 (3PM-5PM)

BIOPROCESS TECHNOLOGY (BT30201 AND BT40009) — B.TECH / DUAL

TIME: 3 HOURS — FULL MARKS: 50

NO. OF STUDENTS: 100 — No. of question paper pages: 05

Part-A

Questions 1-12 carry One Marks each. Choose the correct Option.

1. Refolding of proinsulin and removal of SO_3^- moieties is done under the following condition:
   A. Treating with mercapto ethanol at 1.5:1 ratio of SO_3^-
   B. Treating with 4M urea
   C. Treating with sodium sulphite ate alkaline pH
   D. A and B
   E. B and C

2. During preparation of recombinant human serum albumin the following treatment is essential for binding to the cation exchange resin
   A. Raise the conductivity of the solution by heating
   B. Reduce the conductivity of the solution by cooling
   C. Reduce the conductivity of the solution by diluting
   D. Raise the conductivity of the solution by adding extra salt

3. Ultrafiltration techniques can be used for:
   A. Buffer exchange
   B. Protein concentration
   C. Removal of contaminants
   D. All of the above
   E. None of the above

4. Which of the following steps during recombinant Insulin production and purification is required for unfolding of proinsulin?
   A. Enzymatic treatment
   B. Differential centrifugation
   C. Oxidative sulfitolysis
   D. CNBr cleavage

5. CNBr treatment is done during preparation of Insulin from bacterial cells to
   A. Remove the C-peptide
   B. Remove the signal peptide
   C. Separate the A and B chains
   D. None of the above

6. Which of the following component of β-lactamase gene cluster is a metalloprotease?
   A. BlaI
   B. BlaR
   C. BlaZ
   D. All of the above.

7. Addition of which of the following component/components is expected to improve clavulanic acid production?
   A. L-Arginine
   B. Glycerol
   C. Pyruvate
   D. All of the above

8. In proinsulin method of Insulin production, Insulin is produced as
   A. Proinsulin comprising of 82 amino acids
   B. A chimeric peptide containing 121 amino acid linker fused to proinsulin
   C. Separate A and B chains which are reconstituted later
   D. A chimeric peptide containing 121 amino acids fused to insulin

9. Prior to refolding of proinsulin, the proinsulin $(SO_3^-)_6$ is subjected to
   A. Cation exchange chromatography operated at pH4
   B. Anion exchange chromatography operated at pH8
   C. Reversed-phase HPLC
   D. Anion exchange chromatography operated at pH4

10. Chinese Hamster Ovarian (CHO) Cells are used for commercial production of which of the following biologics?
   A. Insulin
   B. Plasminogen Activator
   C. Human Serum Albumin
   D. None of the above

11. Which of the following chromatographic conditions is preferred for purification of Human Serum albumin?

A. Expanded bed Anion exchanger at high pH
B. Packed bed Cation exchanger at acidic pH
C. Expanded bed cation exchanger at acidic pH
D. Packed bed cation exchanger at neutral pH

12. Which of the following in NOT a possible mechanism for beta lactum resistance:

A. Drug modifying enzymes
B. Alteration in Penicillin Binding Proteins
C. Endocytosis
D. Efflux pumps

Questions 13 &14 carry 2 Marks each. Write the detailed answers with justifications.

13. You have 50 mL of sample (MW = 54KD) collected from a membrane chromatography capsule that was eluted in a buffer solution (0.05M Tris, 0.5M NaCl). You need to reduce the salt concentration below 0.05M and then concentrate to 10 mL. Using a Minimate TFF capsule with a 10KD membrane on a Minimate TFF system, how long will it take you if the average filtrate flux rate is 40 LMH and 3 diafiltration volumes (constant volume diafiltration) are required to get the salt concentration below 0.05M. Membrane area is 0.005 sqm

A. 1 hour
B. 2 hours
C. 3 hours
D. 4 hours

14. Recombinant Human Serum albumin is purified from *P. pastoris* cells. The downstream processing steps for product recovery are listed below:

I. Cation Exchange
II. Anion Exchange
III. Ultrafiltration-I
IV. Ultrafiltration-II
V. Hydrophobic Interaction Chromatography

The correct order of the recovery steps is:

A. I, II, V, III, IV
B. II, I, V, III, IV
C. I, V, II, III, IV
D. III, I, V, II, IV

PART B

Answer all questions

[2+2+2+3=9]

Q1.

(a) State two ways through which downstream processing be improved?

(b) State two ways how you can bring automation into the scale up process?

(c) What is meant by 'Quality by Design' and Process Analytical Technology?

(d) With the help of a flow diagram explain a typical scale up process?

Q.2 [2+3+4=9]

(a) State with example what is a classical subunit vaccine?

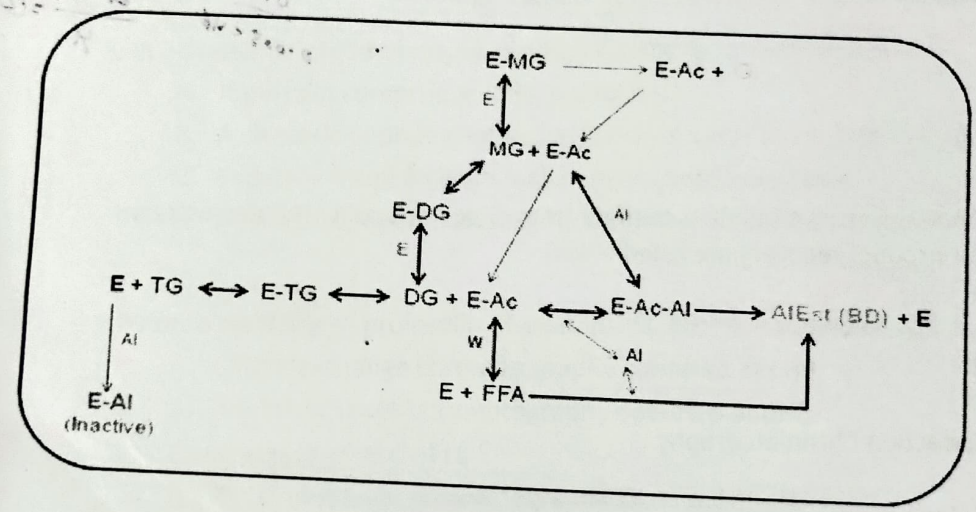
(b) What are serum derived vaccines and state two limitations of serum derived vaccines?

(c) What are the different expression systems used for obtaining recombinant vaccines and state the pros and cons of each system?

PART C

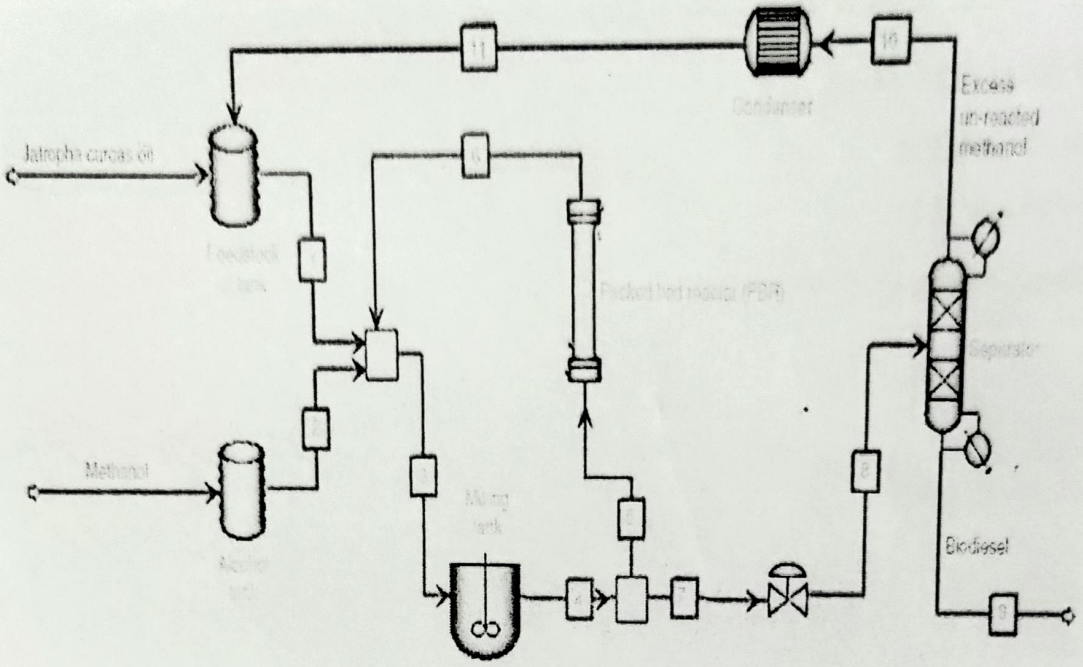
Q.1. (a) Define biodiesel as per an International Standard like ASTM D6751 [1]

(b) Mention various feedstock and methods of biodiesel production. Explain the following mechanism of enzymatic synthesis of biodiesel. [2+2 = 4]



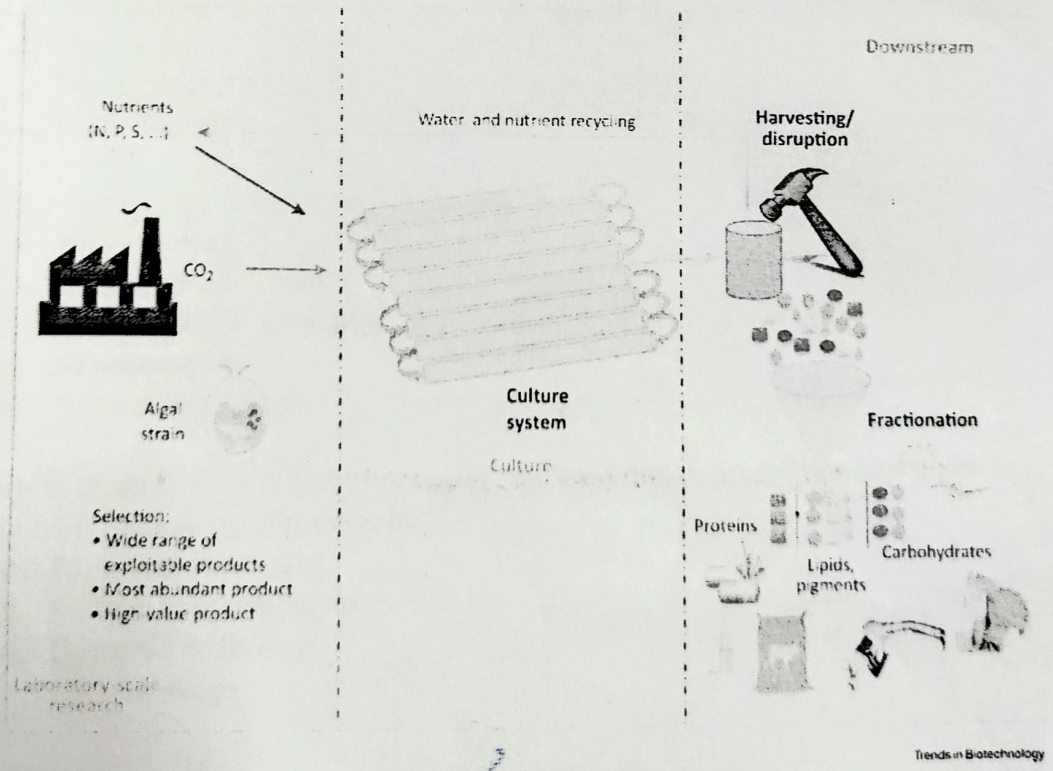
(c) Determine the amount of oil, methanol, glycerol and catalyst required to produce 35x106 lb/yr (5 million gallons per year) of biodiesel. Molecular weight of FAMEs = 292; Molecular weight of methanol = 32; Molecular weight of glycerol = 92; Molecular weight of soybean oil = 885. Assume methanol/oil molar ratio as 6:1. [3]

(d) The following schematic represents a process flow diagram for enzymatic / biocatalytic production of biodiesel. Explain the processing steps as per the following diagram. [3]



Q.2. (a) What is your understanding of an algal biorefinery? Name one algal feedstock. [2]

(b) Explain the steps involved in establishing an algal biorefinery as per the schematic below. [3]



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