BIOPROCESS ENGINEERING
(CHEN 3141)

Time Allotted : 2½ hrs **Full Marks : 60**

Figures out of the right margin indicate full marks.

Candidates are required to answer Group A and any 4 (four) from Group B to E, taking one from each group.

Candidates are required to give answer in their own words as far as practicable.

Group – A

1. Answer any twelve: **12 × 1 = 12**

Choose the correct alternative for the following

(i) We have competitive inhibition when
(a) A (substrate) and B (inhibitor) attack on the same site on the enzyme
(b) when B attacks a different site on the enzyme, but in doing so stops the action of A
(c) both (a) and (b)
(d) none of the above.

(ii) Enzymatic reactions following M. M. Kinetics is a
(a) zero order reaction
(b) first order reaction
(c) shifting order reaction, i.e., initially zero order and then shifting to first order
(d) none of the above.

(iii) A plot of: $\frac{C_A}{-r_A} Vs C_A$ for an enzymatic reaction is known as
(a) Lineweaver and Burk plot (b) Eadie Hopstee plot
(c) Hanse Woolf plot (d) none of the above.

(iv) We have non-competitive inhibition when
(a) A (substrate) and B (inhibitor) attack on the same site on the enzyme
(b) when B attacks a different site on the enzyme, but in doing so stops the action of A
(c) both (a) and (b)
(d) none of the above.

(v) A plot of rate versus substrate concentration of an enzymatic reaction following Michaelis Menten equation gives
(a) a section of rectangular hyperbola (b) a linear plot
(c) an irregular plot (d) none of the above.

(vi) In Monod kinetics, if K_s is the Monod constant, μ_{max} is the maximum specific growth rate and S the substrate concentration, for K_s=S, specific growth rate μ equals
(a) $\mu_{max}/2$ (b) μ_{max} (c) $2\ \mu_{max}$ (d) μ_{max}^2.

(vii) The yield coefficient of biomass(X) with respect to substrate(S) is defined as

(a) $\frac{\Delta X}{\Delta S}$ (b) $\frac{\Delta S}{\Delta X}$ (c) $-\frac{\Delta X}{\Delta S}$ (d) $\Delta X \Delta S$

(viii) During microbial growth, the time taken by the microbial species to adjust to the new environment is known as

(a) Exponential phase (b) Lag phase (c) Stationary phase (d) Death phase.

(ix) The dimensionless group $\frac{P}{\rho N^3 D^5}$ is known as

(a) Reynolds number (b) Froude number
(c) Aeration number (d) Power number.

(x) The culture volume in a fed-batch reactor at a time t is

(a) directly proportional to flowrate (b) directly proportional to time
(c) both (a) and (b) (d) inversely proportional to flowrate.

Fill in the blanks with the correct word

(xi) A common cross-linking agent used for enzyme immobilization is_____.

(xii) The dimensionless group governing the relative rates of reaction and diffusion in immobilized systems is _____.

(xiii) At high substrate concentration, the order of Monod equation is_____.

(xiv) The unit of volumetric oxygen transfer coefficient is_____.

(xv) Example of a non-growth associated product is_____.

Group - B

2. (a) Derive Michaelis Menten equation for free enzyme catalyzed reaction. *[(CO1)(Remember/LOCQ)]*

(b) State the importance of enzyme inhibition study in pharmacology study. *[(CO1)(Understand/LOCQ)]*

(c) Sucrose is hydrolyzed in a batch bioreactor in presence of the enzyme sucrase (E) as follows:
Sucrose (A) → product
With initial concentrations of 1 mM sucrose and 0.01 mM enzyme, the following data are obtained.

C_A, mM	0.84	0.68	0.53	0.38	0.27	0.16	0.09	0.04
$(-r_A)$,mM/hr	0.160	0.154	0.152	0.127	0.110	0.0882	0.0625	0.0400

Using Lineweaver and Burk plot, find the intrinsic kinetic parameters of Michaelis-Menten equation. *[(CO1)(Analyze/IOCQ)]*

2 + 4 + 6 = 12

3. (a) Deduce the performance equation of a batch bioreactor used for carrying out an enzymatic reaction. *[(CO1)(Remember/HOCQ)]*

(b) Substrate A decomposes in the presence of enzyme E (A->R). It is desired to design a batch fermenter for producing 2000 kg R / day from a feed containing C_{A0} = 1000 mol / m^3. The conversion of A is 90 %. The plant should operate day and night and times for filling, cleaning and draining may be taken as 0.5 hrs. The molecular weight of A is 179 and the initial enzyme concentration is 10 mol / m^3. Find the length and diameter of the reactor if the aspect ratio is 2 and the actual

reactor volume is 25% excess of the theoretically calculated volume. The reactor is cylindrical in shape. The system follows Michaelis-Menten equation having k_3 and K_m values 48 hr^{-1} and 336 mol/m^3 respectively. *[(CO2)(Evaluate/HOCQ)]*

(c) Cellulose can be converted to sugar by the following enzymatic attack Cellulose → sugar (in presence of enzyme cellulose) and cellubiose acts to inhibit the breakdown. To study the kinetics of this reaction a number of runs are made in an MFR kept at 50^0C and using a feed of finely shredded cellulose (C_{A0} = 25 kg / m^3), enzyme (C_{E0}, same for all runs). The results are as follows:

Run	Exit stream, C_A, kg / m^3	Residence time with no inhibitor, min	Residence time with cellubiose, C_{B0} = 5 kg / m^3, min
1	1.5	587	691
2	4.5	279	306
3	9.0	171	182
4	21.0	36	38

Find a rate equation to represent this reaction. *[(CO2)(Analyze/IOCQ)]*

2 + 4 + 6 = 12

Group - C

4. (a) Xanthan gum is produced in batch culture. For each gram glucose utilized by the bacteria, 0.23 g O_2 and 0.01 g NH_3 are consumed, 0.75 g gum, 0.09 g cells, 0.27 g CO_2 and 0.13 g H_2O are produced. Medium containing glucose and NH_3 dissolved in 20000 litre water is pumped into a fermenter and inoculated. Air is sparged and total amount of off-gas recovered is 1250 kg. Final gum concentration is less than 3.5 weight%. Calculate the amount of glucose and NH_3 required. *[(CO2)(Evaluate/HOCQ)]*

(b) Explain why Monod model is an unstructured non-seggregated kinetic model for microbial growth. *[(CO2)(Analyse/IOCQ)]*

(c) "Increase in impeller diameter and speed of agitation causes proportionally greater increase in power transmission to liquid in case of turbulent flow compared to laminar flow". Comment on the validity of the statement. *[(CO2)(Analyse/IOCQ)]*

6 + 3 + 3 = 12

5. (a) With the aid of a diagram, discuss the steps of glycolysis and calculate the ATP balance of each step. *[(CO2)(Understand/LOCQ)]*

(b) A bacterial strain is cultured in a 12 m^3 fermenter. If the volumetric oxygen transfer coefficient is 0.18 s^{-1} and O_2 solubility is 0.008 kg/m^3, compute the maximum cell concentration if the specific O_2 uptake rate is 12.5 mmol/g/h. *[(CO2)(Evaluate/HOCQ)]*

(c) Discuss the types of spargers used for aeration in a fermentation medium. *[(CO2)(Remember/LOCQ)]*

5 + 4 + 3 = 12

Group - D

6. (a) In a fed-batch culture operating with intermittent glucose addition, following parameter values are given at 2 h when the system is at quasi steady state: volume of the culture 1000 mL, flowrate 200 mL/h, initial substrate concentration 100 g glucose/litre, μ_{max}=0.3 h^{-1}, K_s=0.1 g glucose/litre, $Y_{X/S}$ = 0.5 g dry weight cells/g glucose, and total number of cells at the start is 30 g.
(i) Calculate the initial volume of the culture

(ii) Determine the concentration of growth limiting substrate at steady state
(iii) Determine the concentration and total amount of biomass at 2 h.
[(CO3)(Apply/HOCQ)]

(b) "Smaller the value of Thiele modulus, larger is the diffusional resistance in immobilized cell systems" Justify the statement with the aid of effectiveness factor versus Thiele modulus diagram. *[(CO3)(Analyse/IOCQ)]*

(c) Explain with a diagram the working principle of a continuous sterilization unit. *[(CO3)(Remember/LOCQ)]*

6 + 3 + 3 = 12

7. (a) A bacterial strain converts glucose to ethanol in a batch fermenter under anaerobic conditions. Yield of biomass from the substrate is 0.05 g/g, yield of product from biomass is 7 g/g, and maintenance coefficient is 2.5 g/g/h, and specific rate of product formation due to maintenance is 1.1 h^{-1}. Maximum specific growth rate of the bacteria is 0.4 h^{-1}. 5 g bacteria is inoculated in 50 litre medium containing 12 g/l glucose. Assume inlet air and off-gas is free from moisture, and 100% glucose conversion, determine batch culture times required to
(i) produce 25 g bacteria, (ii) achieve 95% conversion. *[(CO3)(Apply/HOCQ)]*

(b) Discuss the mechanism of formation of biofilms. Perform the differential mass balance for substrate consumption in biofilms, write the appropriate boundary conditions and obtain the expression for Thiele modulus. *[(CO3)(Analyse/IOCQ)]*

(3 + 3) + (3 + 3) = 12

Group - E

8. (a) With the aid of flowsheet, explain the process of production of penicillin. *[(CO4)(Remember/LOCQ)]*

(b) "Aqueous two phase extraction is essential for purification of enzyme" Justify the statement *[(CO4)(Analyse/IOCQ)]*

(c) "Permeate flux during ultrafiltration increases monotonically with transmembrane pressure" Justify. *[(CO4)(Analyse/IOCQ)]*

5 + 4 + 3 = 12

9. (a) Derive the expression for concentration polarization modulus in ultrafiltration. *[(CO4)(Analyse/IOCQ)]*

(b) Explain the basic principle of chromatographic separation. *[(CO4)(Remember/LOCQ)]*

(c) A pilot scale gel chromatography packed column with Sephacryl resin is used to separate two hormones A and B. The column is 5 cm in diameter and 0.3 m high and the void volume is 1.9×10^{-4} m^3. The water regain value of the gel is 3×10^{-3} m^3/kg dry Sephacryl; density of wet gel is 1.25×10^3 kg/m^3. The partition coefficient for hormone A is 0.38 and for B is 0.15. If the eluant flowrate is 0.7 litre/ h, what is the retention time of each hormone? *[(CO4)(Evaluate/HOCQ)]*

4 + 4 + 4 = 12

Cognition Level	LOCQ	IOCQ	HOCQ
Percentage distribution	27.1	39.6	33.3

Course Outcome:
After the completion of the course students will be able to
CO1: Solve biochemical reaction engineering problems for predicting rate equation for both enzymatic and live cell fermentation process
CO2: Design bioreactors for free enzymatic reaction under enzyme uninhibited/inhibited conditions
CO3: Select suitable bioreactor and its design and scale-up for whole cell catalysed reactions
CO4: Suitable modern separation techniques for isolation, purification, and quantitative separation of target biomolecule from live cells.

**LOCQ: Lower Order Cognitive Question; IOCQ: Intermediate Order Cognitive Question; HOCQ: Higher Order Cognitive Question.*