BIOPROCESS ENGINEERING
(CHEN 3141)

Time Allotted : 3 hrs **Full Marks : 70**

Figures out of the right margin indicate full marks.

Candidates are required to answer Group A and any 5 (five) from Group B to E, taking at least one from each group.

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

Group – A
(Multiple Choice Type Questions)

1. Choose the correct alternative for the following: **10 × 1 = 10**

(i) Competitive inhibition of an enzyme can be reduced by
(a) Reducing the amount of substrate (b) Increasing the amount of substrate
(c) Decreasing the amount of the enzyme (d) Diluting the reaction mixture.

(ii) When the rate of product formation is half the maximum forward velocity, the value of Michaelis-Menten constant Km equals
(a) half the substrate concentration (b) substrate concentration
(c) zero (d) square of substrate concentration.

(iii) The end product of glycolysis is
(a) Oxaloacetic acid (b) Pyruvic acid (c) Succinic acid (d) Fumaric acid.

(iv) In a chemostat under steady state, the specific growth rate of microorganisms is equal to the
(a) feed flowrate (b) dilution rate
(c) culture volume (d) substrate concentration.

(v) In a bioprocess, assume that only cell mass is formed. Due to a variation in process conditions, if the microbial cell yield is halved, what would be the rate of substrate consumption to maintain the same rate of cell mass production?
(a) It would be doubled (b) It would be halved
(c) It would be unchanged (d) It would increase four fold.

(vi) A microorganism following Monod kinetics is grown in a chemostat with working volume 5 *l* and inlet substrate concentration 1g/l. If the μmax and Ks of the organism are 0.5 h^{-1} and 0.25 g/*l* respectively, washout occurs when the flowrate(*l* h^{-1}) exceeds
(a) 0.5 (b) 1 (c) 2 (d) 2.5.

(vii) Observed retention by a membrane is given by
(a) Ratio of permeate concentration to feed concentration of solute
(b) (1- Ratio of permeate concentration to feed concentration of solute)
(c) Ratio of retentate concentration to feed concentration of solute
(d) Ratio of feed concentration to permeate concentration of solute.

(viii) The property involved in the separation of a mixture of analytes using gas chromatography is
(a) Conductivity (b) Partitioning (c) Polarity (d) Viscosity.

(ix) In a batch bioreactor, the specific growth rate
(a) Continuously increases with time (b) Reaches a maximum in the exponential phase
(c) Continuously decreases with time (d) Does not change with time.

(x) Scale up of a fermenter is based on constant impeller tip speed. If the diameter of the impeller increases by 5 fold, the agitator speed will
(a) Increase by 5 fold (b) Decrease by 5 fold (c) Remain constant (d) Increase by 25 fold.

Group- B

2. (a) Derive the Michaelis-Menten equation for enzyme catalyzed reaction stating the necessary assumptions. [(CO1)(Remember/LOCQ)]
(b) Discuss the significance of enzyme inhibition. [(CO1)(Understand/LOCQ)]
(c) Sucrose is hydrolyzed in a batch bioreactor in presence of the enzyme sucrase (E) as follows

Sucrose (A) $\longrightarrow$ 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)(Evaluate/HOCQ)]

4 + 2 + 6 = 12

3. (a) An enzymatic reaction takes place in a mixed flow reactor (V=6 litre). From the entering and leaving concentration and flow rate, find a rate equation to represent the action of enzyme on substrate. [(CO1,CO2)(Evaluate/HOCQ)]

C_{E0}, mol/lit	C_{A0}, mol/lit	C_A, mol/lit	V_0, lit/h
0.01	1.0	0.1	0.3
0.01	1.5	0.5	1.0
0.01	2.5	2.0	4.0

(b) Enzyme urease is immobilized in calcium alginate beads 2 mm in diameter. When urea concentration in bulk liquid is 0.5 mM, the rate of urea hydrolysis is 10 mmol/l.h. Diffusivity of urea in calcium alginate is 1.5×10^{-5} cm^2/s and Michaelis-Menten constant is 0.2 mM. Assuming the urea concentration on the surface of beads is same as the bulk concentration, compute the maximum reaction velocity, Thiele modulus and effectiveness factor. [(CO1)(Evaluate/HOCQ)]
(c) Enumerate the different techniques of enzyme immobilization. [(CO1)(Understand/LOCQ)]

5 + 5 + 2 = 12

Group – C

4. (a) A fermentation broth with viscosity 10^{-2} Pa s and density 1000 kg/m^3 is agitated in a 50 m^3 baffled tank using a marine propeller 1.3 m diameter. Calculate the power requirement for a stirrer speed of 4 s^{-1}. The ratio of tank diameter to impeller diameter and the ratio of liquid height to tank diameter are 3. In the laminar regime, power number is inversely proportional to Reynolds number and in turbulent regime, power number is 0.35. [(CO2) (Evaluate/HOCQ)]

(b) *Saccharomyces cerevisiae* is grown anaerobically in continuous culture at 30°C. Glucose is used as carbon source, ammonia is the nitrogen source. A mixture of glycerol and ethanol is produced. At steady state, mass flows to and from the reactor are as follows:

Glucose in(kg/h)	NH_3 in (kg/h)	Cells out (kg/h)	Glycerol out (kg/h)	Ethanol out (kg/h)	CO_2 out (kg/h)	H_2O out (kg/h)
36	0.4	2.81	7.94	11.9	13.6	0.15

Estimate the cooling requirements. [(CO1,CO2) (Evaluate/HOCQ)]

(c) With the aid of diagram, discuss the types of baffle arrangements in fermentation broths of different viscosities. [(CO2) (Understand/LOCQ)]

4 + 6 + 2 = 12

5. (a) A strain of *Azetobactervinelandii* is cultured in a 15 m^3 fermenter for alginate production. Under current operating conditions, $k_La=0.17\ s^{-1}$. Oxygen solubility in the broth is 0.008 kg/m^3. Specific rate of oxygen uptake is 12.5 mmol/g/h.

(i) What is the maximum possible cell concentration?

(ii) The bacteria suffers growth inhibition after $CuSO_4$ is added to the fermentation broth accidentally. This causes a three-fold reduction in oxygen uptake rate. What maximum cell concentration can be supported by the fermenter? [(CO2)(Evaluate/HOCQ)]

(b) A strain of mold is grown in a batch culture on glucose and the following data were obtained

Time(h)	0	7	16	24	30	35	37	40
Cell concentration (g/l)	1.25	2.45	5.1	10.5	22	34	37.5	41
Substrate concentration(g/l)	100	96	90	77	48	21	9	0.6

(i) Calculate the maximum net specific growth rate

(ii) Calculate the apparent growth yield

(iii) What maximum cell concentration would one expect if 100 g glucose were used with the same size inoculum? (mm graph required). [(CO2)(Evaluate/HOCQ)]

5 + 7 = 12

Group - D

6. (a) *Zymomonasmobilis* is used to convert glucose to ethanol in a batch fermenter under anaerobic conditions. Yield of biomass from the substrate is 0.06 g/g, $Y_{P/X}$ is 7.7 g/g, the maintenance coefficient is 2.2 g/g/h, specific rate of product formation due to maintenance is 1.1 h^{-1}. Maximum specific growth rate of *Zymomonasmobilis* is 0.3 h^{-1}. 5 g bacteria are inoculated into 50 litre medium containing 12 g/l glucose. Determine batch culture times required to

(i) produce 20 g biomass

(ii) achieve 90% substrate conversion

(iii) produce 100 g ethanol. [(CO3)(Evaluate/HOCQ)]

(b) A 5 m^3 fermenter is operated continuously with feed substrate concentration 20 kg/m^3. The microorganism cultivated in the reactor has following characteristics:
$\mu_{max}=0.45\ h^{-1}$, $Ks=0.8\ kg/m^3$, $Y_{X/S}=0.55\ kg/kg$

(i) What feed flowrate is required to achieve 90% substrate conversion?

(ii) How does the biomass productivity at 90% substrate conversion compare with the maximum possible? [(CO2,CO3)(Evaluate/HOCQ)]

(c) Show that the biomass concentration obtained from a chemostat with product formation is less than that obtained in absence of product formation. [(CO2)(Understand/LOCQ)]

5 + 4 + 3 = 12

7. (a) Justify the following statements:

(i) A chemostat can be operated at dilution rates less than or equal to the specific growth rate

(ii) In a fed-batch reactor, specific growth rate of a microorganism decreases with time
(iii) Time and temperature of sterilization are two essential factors affecting the degree of sterilization. [(CO3)(Analyze/IOCQ)]

(b) In a chemostat with recycle, the feed flowrate and culture volumes are F=100 ml/h and V=1000 ml. The system is operated under glucose limitation and the yield coefficient $Y_{X/S}^{M}$ is 0.5 g dw cells/g substrate. Glucose concentration in the feed is S_0=10 g glucose/l. The kinetic constants of the organism are μm=0.2 $h^{-1}$and Ks=1 g glucose/l. Value of cell concentration factor is 1.5 and recycle ratio is α=0.7. The system is at steady state.
(i) Determine the substrate concentration in recycle stream
(ii) Calculate the specific growth rate(μnet) of the organisms
(iii) Compute the cell concentration in recycle stream
(iv) Compute the cell concentration in centrifuge effluent. [(CO3)(Evaluate/HOCQ)]

(2 + 2 + 2) + 6 = 12

Group – E

8. (a) Justify the following statements:
(i) Real retention indicates the true separation efficiency of solute by a membrane.
(ii) Chromatography involves partitioning of solutes between two phases.
[(CO4)(Analyze/IOCQ)]

(b) Explain the basic principle of osmotic lysis of microbial cells with an illustration of the process of osmosis. [(CO4)(Understand/LOCQ)]

(c) Derive the relation between concentration polarization modulus and solvent flux in cross-flow filtration. [(CO4)(Remember/LOCQ)]

(2 + 2) + 3 + 5 = 12

9. (a) With the aid of a flowsheet, explain the process of production of acetone-butanol. [(CO4)(Understand/LOCQ)]

(b) Enumerate the criteria of selection of a solvent for extraction. [(CO4)(Remember/LOCQ)]

(c) Solvent extraction using ethanol is used to purify a target metabolite from an aqueous broth. The partitioning coefficient is 3. Calculate the ratio of solvent to broth required to extract 80% metabolite in a single step. [(CO4)(Evaluate/HOCQ)]

6 + 3 + 3 = 12

Cognition Level	LOCQ	IOCQ	HOCQ
Percentage distribution	31.25	10.42	58.33

Course Outcome (CO):

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.