Reg. No.										

MANIPAL INSTITUTE OF TECHNOLOGY

MANIPAL

(A constituent institution of MAHE, Manipal)

V SEMESTER B.TECH BIOTECHNOLOGY
END SEMESTER EXAMINATIONS, NOV/DEC 2018
SUBJECT: BIOPROCESS ENGINEERING [BIO 3102]
REVISED CREDIT SYSTEM

Time: 3 Hours **(21/11/2018)** MAX. MARKS: 50

Instructions to Candidates:

- Answer **ALL** the questions.
- Missing data may be suitable assumed.

1A. During a test of kinetics of an enzyme-catalyzed reaction, the following data were recorded. Determine the kinetic constants, type of inhibition and K_I: (Use LB-plot) **5**

S (mol/ L)	0.2	0.1	0.06	0.04	0.02
V (mol / L-min) @ I = 0	3.22	2.68	2.02	1.62	1.34
V (mol /L-min) @ I = 1.2 mol/ L	2.68	2	1.34	1	0.8

1B. An enzyme is used to produce a compound used in manufacture of sunscreen lotion. V_{max} for the enzyme is 2.5 mmol /m^3.s; K_M is 8.9 mM. The initial concentration of substrate is 12 mM. Plot the time required for batch reaction as a function of substrate conversion. If the enzyme deactivates with half-life 4.4 h, determine the time required to achieve 90% substrate conversion. **5**

2A. The temperature history of the heating and cooling of a 40,000 L tank during sterilization of medium is: 0 to 15 min, T = 85°C; 15 to 40 min, T = 121°C; 40 to 50 min, T = 85°C; 50 to 60 min, T = 55°C; > 60 min, T = 30°C. The medium contains vitamins, the most fragile of the vitamins has an activation energy for destruction of 10 kcal/mol, and the value of A is $1x10^4$ min^{-1}. Assume vitamin destruction is first order and the initial concentration is 50 mg/L and the medium contains 2.5×10^3 spores/L. The spores have an E_d of 65 kcal/mol and k_d at 121°C is 1.02 min^{-1}. Estimate the probability of unsuccessful sterilization and what fraction of the vitamin remains active? **5**

2B. Fifty thousand liters of a complex medium are to be pumped from a tank held at ambient conditions through an HTST sterilizer consisting of 20 m of 8-inch Schedule 80 pipe. The medium volume is to be sterilized and added to the production vessel within *one* hour, and the acceptable contamination probability is 1 in 350 h. The model spore is *Bacillus thermophillum*, and the coefficients for its sterilization are: Frequency Factor = $4.93x10^{40}$ min^{-1} and Activation Energy = 71,395 cal/mole. **5**

a. Calculate the sterilization temperature, If the initial cell population of *B. thermophillum* is $5.6x10^6$/mL.
b. In part (a) if the temperature were to drop by 1.5°C, what would be the probability of contamination?

Medium Properties: density = 930 kg/m^3; viscosity = $2.36x10^{-4}$ kg/m-s

	For 8 inch Schedule 80 pipe, ID = 7.625 inches.	
3A.	L-Lactate 2-monooxygenase from *Mycobacterium smegmatis* is immobilised in spherical agarose beads. The enzyme catalyses the reaction: $C_3H_6O_3$ (Lactic acid) + O_2 ----------→ $C_2H_4O_2$ (Acetic acid) + CO_2 + H_2O Beads 4 mm in diameter are immersed in a well-mixed solution containing 0.5 mM oxygen. A high lactic acid concentration is provided so that oxygen is the rate-limiting substrate. The effective diffusivity of oxygen in agarose is 2.1 x 10^{-9} m^2/s. K_m for the immobilized enzyme is 0.015 mM; V_{max} is 0.12 mol / s. kg enzyme. The beads contain 0.012 kg enzyme / m^3 gel. External mass-transfer effects are negligible. Determine the largest bead size that allows the maximum conversion rate?	**4**
3B.	Assume that an enzyme is uniformly immobilized in soft gel of spherical shape and is kept in contact with the substrate solution. Derive an expression for concentration profile of substrate when it is catalysed by the immobilized enzyme which follows first order reaction kinetics. Briefly list the assumptions made.	**6**
4A.	Data collected during fermentation of *Pseudomonas ovalis* B 1486 at stirrer speed of 700 rpm are given by Mukhopadhyay and Ghose. The fermenter volume was 3 L. Air flow into the vessel was maintained at 1 vvm. The air pressure was 3 atm. and the temperature 29ºC. The following data were measured: Fermentation Time, h: 0 | 4 | 6 | 7 | 8 | 9 | 10 | 11 C_{AL}, ppm: 5.9 | 5.7 | 5.4 | 5.1 | 4.7 | 4.1 | 4.1 | 4.2 $(Y_{AG})_o$: 0.21 | 0.209 | 0.208 | 0.207 | 0.206 | 0.204 | 0.204 | 0.205 a. Determine k_La. b. What is the solubility of oxygen in the fermentation broth? (Use Oxygen Balance Method)	**6**
4B.	A genetically-engineered strain of yeast is cultured in a bioreactor at 30°C for production of heterologous protein. The oxygen requirement is 80 mmol/L-h; the critical oxygen concentration is 0.004 mM. The solubility of oxygen in the fermentation broth is estimated to be 10% lower than in water due to solute effects. (Given H = 26.1 atm-m^3/kg) a. What is the mass-transfer coefficient necessary to sustain this culture if the reactor is sparged with air at approximately 1 atm pressure? b. What mass-transfer coefficient is required if pure oxygen is used instead of air?	**2+2**
5A.	Define the following: a. Diauxic growth b. Cryptic growth c. Maintenance coefficient d. Y_{xs} e. Endogenous metabolism	**1x5**
5B.	The growth of a microbial population is a function of pH and is given by the following equation: $$\mu_g = \frac{1}{X}\frac{dX}{dt} = \frac{\mu_m S}{K_s\left\{1+\frac{H^+}{k_1}\right\}+S}$$ a. With a given set of experimental data (X and S versus t), describe how you would determine the constants μ_m, K_s, and k_1. b. How would the double reciprocal plot $1/\mu_g$ vs 1/S change with pH (H^+) concentration?	**5**