Reg. No.										

INTERNATIONAL CENTRE FOR APPLIED SCIENCES

(Manipal University)

II SEMESTER B.S. DEGREE EXAMINATION

SUBJECT: INTRODUCTION TO BIOPROCESS ENGINEERING (BT 122)

(BRANCH: INDUSTRIAL BIOTECHNOLOGY)

Wednesday, 3 May 2017

Time: 3 Hours **Max. Marks: 100**

✓ **Answer ANY FIVE FULL Questions.**

1. **(A)** Explain the mechanism of action an enzyme. Compare the progress of a reaction in the presence and absence of an enzyme, with respect to free energy. Add a short note on proximity effect and orientation effect. **[10m]**

   (B) What are the approaches devised to derive the velocity of an enzyme-catalyzed reaction? Derive the velocity using Briggs-Haldane approach, mentioning clearly the assumptions made. **[10m]**

2. **(A)** Derive the Eadie-Hofstee Equation and Hanes-Woolf Equation. Show the plot graphically, labelling the slope and intercept. **[10m]**

   (B) What is the accepted international method of enzyme classification? List the subtypes under each of them, as applicable. Name any two biologically-derived products, each: (a) requiring high purity (b) requiring minimum purity. **[10m]**

3. **(A)** What is uncompetitive inhibition? If an uncompetitive inhibitor is added to an enzyme-catalyzed reaction, how does it influence the maximum reaction velocity and Michaelis constant? Derive the expression for velocity for such an inhibited reaction. **[10m]**

   (B) Describe the main features of the air-lift reactor. Present a labelled sketch of three configurations of this type of reactor. State two applications of this bioreactor. **[10m]**

4. **(A)** What is the physical significance of cooperativity coefficient? How is it determined graphically? Explain with a diagram. **[8m]**

   (B) Lipase is being investigated as an additive to laundry detergent for removal of stains from fabric. The general reaction is: Fats → Fatty acids + Glycerol

   The Michaelis constant for pancreatic lipase is 5 mM. At 60°C, lipase is subject to deactivation with a half-life of 8 min. Fat hydrolysis is carried out in a well-mixed

batch reactor which simulates a top loading washing machine. The initial fat concentration is 45 gmol /m^3. At the beginning of the reaction the rate of hydrolysis is 0.07 mmol/ L.s. How long does it take for the enzyme to hydrolyze 80% of the fat present? **[12m]**

5. **(A)** Give four examples of support materials used for immobilizing enzymes by the physical adsorption method. **[4m]**

   (B) Write short notes on direct and indirect methods of determining cell mass concentration in batch growth of a bacterial specimen. **[8m]**

   (C) In a competitive inhibition experiment, a structural analog was used along with the substrate and the following kinetics was observed: At 10μM substrate, the velocity was 25 μM/min. With 2mM of the analog, the velocity dropped to 50%. Calculate the K_i of the inhibitor. Given that the substrate concentration used gives half-maximal velocity, calculate how much inhibitor should be used for increasing the K_m to 10 times the uninhibited value? **[8m]**

6. **(A)** The equation for aerobic production of acetic acid from ethanol is:

   $$C_2H_5OH \text{ (ethanol)} + O_2 \rightarrow CH_3COOH \text{ (acetic acid)} + H_2O$$

   Acetobacter aceti bacteria are added to vigorously-aerated medium containing 10 g/L ethanol. After some time, the ethanol concentration is 2 g/L and 7.5 g/L acetic acid is produced. How does the overall yield of acetic acid from ethanol compare with the theoretical yield? **[4m]**

   (B) The following data have been obtained for two different initial enzyme concentrations for an enzyme-catalyzed reaction.

V at $[E_0]$ = 0.015 g/L (g/L-min)	**[S] (g/L)**	**V at $[E_0]$ = 0.00875 g/L (g/L-min)**
1.14	20.0	0.67
0.87	10.0	0.51
0.70	6.7	0.41
0.59	5.0	0.34
0.50	4.0	0.29
0.44	3.3	
0.39	2.9	
0.35	2.5	

   i. Find K_m **[4m]**

   ii. Find V_m for $[E_0]$ = 0.015 g/L **[4m]**

   iii. Find V_m for $[E_0]$ = 0.00875 g/L **[4m]**

   iv. Find k_2 for both values of $[E_0]$. **[4m]**

7. **(A)** Show graphically, the variation of biomass concentration and product concentration with time in (i) growth-associated product formation (ii) mixed-growth-associated product formation (iii) non-growth-associated product formation. **[6m]**

(B) If you need to produce 10 g of cells using glucose as a carbon source, what is the minimum amount of glucose that would be needed? Assume cell composition as $CH_{1.8}O_{0.5}N_{0.2}$. **[6m]**

(C) A strain of mold was grown in a batch culture on glucose and the following data were obtained.

(i) Calculate the net specific growth rate **[6m]**

(ii) Calculate the growth yield **[2m]**

Time (h)	Cell concentration (g/L)	Glucose concentration (g/L)
0	1.25	100
9	2.45	97
16	5.1	90.4
23	10.5	76.9
30	22	48.1
34	33	20.6
36	37.5	9.38
40	41	0.63

8. **(A)** State any three applications of enzymes used for medical diagnosis and/or therapeutical purposes. **[6m]**

(B) To measure the amount of glucoamylase in a crude enzyme preparation, 10 mL of the crude enzyme preparation containing 8 mg protein is added to 9 mL of 4.44% starch solution. One unit of activity of glucoamylase is defined as the amount of enzyme which produces 1 µmol of glucose per min in a 4% solution of Lintner starch at pH 4.5 and 60°C. Initial rate experiments show that the reaction produces 0.6 µmol of glucose per mL per min. What is the specific activity of the crude enzyme preparation? If V_m = 1 µmol per mL per min, find k_2. **[6m]**

(C) Write a brief note on the immobilizing enzymes using ionic and covalent methods. **[8m]**