Reg. No.										

MANIPAL INSTITUTE OF TECHNOLOGY
MANIPAL

A Constituent Institution of Manipal University

I SEMESTER M.TECH. (INDUSTRIAL BIOTECHNOLOGY)

END SEMESTER EXAMINATIONS, NOV/DEC 2016

SUBJECT: BIOPROCESS ENGINEERING [BIO 5121]

REVISED CREDIT SYSTEM
(24/11/2016)

Time: 3 Hours MAX. MARKS: 50

Instructions to Candidates:

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

<table>
<tr><td>1A.</td><td>Briefly describe the primary, secondary, tertiary, and quaternary structure of proteins. What could happen if you substituted a tyrosine for a cysteine in the active site? What might happen if the substitution occurred elsewhere?</td><td>4</td></tr>
<tr><td>1B.</td><td>What are major sources of carbon, nitrogen, and phosphorus in industrial fermentations?</td><td>3</td></tr>
<tr><td>1C.</td><td>You wish to produce a high-value protein using recombinant DNA technology. Would you try to develop a chemical define medium or a complex medium? Why?</td><td>3</td></tr>
<tr><td>2A.</td><td>A fermentation medium contains an initial spore’s concentration of 8.5 x 10^{10}. The medium is sterilized thermally at 120^{o}C, and the spore density was noted with the progress of time as given below
<table>
<tr><td>Time (min)</td><td>0</td><td>5</td><td>10</td><td>15</td><td>20</td><td>30</td></tr>
<tr><td>Spore density (m^{-3})</td><td>8.5 x 10^{10}</td><td>4.23 x 10^{9}</td><td>6.2 x 10^{7}</td><td>1.8 x 10^{6}</td><td>4.5 x 10^{4}</td><td>32.5</td></tr>
</table>
a) Find the thermal specific death rate, and b) Calculate the survival factor at 40 min.</td><td>4</td></tr>
<tr><td>2B.</td><td>The first step of an industrial fermentation is identification and isolation of the microorganism which brings out the desired bioconversion. To enhance the bioproduct, what are the strategies for isolation of a suitable industrial microorganism from the environment</td><td>3</td></tr>
<tr><td>2C.</td><td>Describe the Lederberg method of preserving bacterial strains.</td><td>3</td></tr>
<tr><td>3A.</td><td>You are working for company A and you join a research group working on immobilized enzymes. Harry, the head of the lab, claims that immobilization improves the stability of the enzyme. His proof is that the enzyme has a half-life of 10 days in free solution, but under identical conditions of temperature, pH, and medium composition, the measured half-life of a packed column is 30 days. The enzyme is immobilized in a porous sphere 5mm in diameter. Is Harry’s reasoning, right? Do you agree with him? Why or why not?</td><td>3</td></tr>
</table>

3B. During a test of kinetics of an enzyme catalyzed reaction, the following data were recorded,

E0 (g/L)	T (ºC)	I (m.mol/mL)	S (m.mol/mL)	V (m.mol/mL.min)
1.6	30	0	0.1	2.63
1.6	30	0	0.03	1.92
1.6	30	0	0.02	1.47
1.6	30	0	0.01	0.96
1.6	30	0	0.005	0.56
1.6	49.6	0	0.1	5.13
1.6	49.6	0	0.033	3.70
1.6	49.6	0	0.01	1.89
1.6	49.6	0	0.0067	1.43
1.6	49.6	0	0.005	1.11
0.92	30	0	0.1	1.64
0.92	30	0	0.02	0.90
0.92	30	0	0.01	0.58
0.92	30	0.6	0.1	1.33
0.92	30	0.6	0.033	0.80
0.92	30	0.6	0.02	0.57

i). Determine the Michalis-Menten constant for the reaction with no inhibitor present at 30 and 49.6ºC
ii). Determine the maximum velocity of the uninhibited reaction at 30ºC and an enzyme concentration of 1.6 g/L.
iii). Determine the KI for the inhibitor at 30ºC and decide what type of inhibitor being used.

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4A. Ethanol formation from glucose is accomplished in a batch culture of *S. cerevisiae*, and the following data were obtained,

Time (h)	0	2	5	10	15	20	25	30
Glucose (S0, (g/L)	100	95	85	58	30	12	5	2
Biomass (X), (g/L)	0.5	1.0	2.1	4.8	7.7	9.6	10.4	10.7
Ethanol (P), (g/L)	0.0	2.5	7.5	20.0	34.0	43.0	47.5	49.0

i). By fitting the biomass data to the logistic equation, determine the carrying-capacity coefficient, k.
ii). Determine yield coefficients, $Y_{P/S}$ and $Y_{X/S}$.

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4B. A researcher is working on optimizing the growth pattern of *E. coli*. He conducted shake flask experiment with the inoculum size of 5% v/v, incubation at room temperature for 36 hr. Aliquots of samples is withdrawn from culture media in every 1 hr to construct microbial growth curve.
i). Illustrate and explain the growth pattern and kinetics in batch culture of *E. coli*.
ii). Explain the methods used to quantify the cell mass.

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5A. The growth of *S. cerevisiae* on glucose may be simply described as,

$$C_6H_{12}O_6+3O_2+0.48NH_3 \longrightarrow 0.48C_6H_{10}NO_3+4.32H_2O+3.12CO_2$$

In a batch reactor of volume 10^5L. The final desired *S. cerevisiae* concentration is 50g/L. Using the above equation find,
i). Concentration and total amount of glucose and $(NH_4)_2SO_4$ in nutrient medium.
ii). Yield coefficients, $Y_{X/S}$ and $Y_{X/O2}$.
iii). Determine the total amount of oxygen required.

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5B. Discuss in brief the production of lactic acid with a process flow sheet. **4**