MANIPAL INSTITUTE OF TECHNOLOGY
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SEMESTER M.TECH END SEMESTER EXAMINATIONS NOVEMBER/DECEMBER 2019

SUBJECT: ADVANCED BIOPROCESS ENGINEERING [BIO 5151]

Date of Exam: **15-11-2019** Time of Exam: 2-5 PM Max. Marks: **50**

Instructions to Candidates:

- Answer ALL the questions & missing data may be suitable assumed

1A.	C, H, and O account for 95% of the elements in living organisms. These atoms are present in the ratio C:2H:O, which is equivalent to the general formula for carbohydrates (CH2O). Does this mean living organisms are mostly sugar? Why or why not?	**2**
1B.	Why does boiling an egg white cause it to harden? Why does it require both a detergent and a reducing agent to dissolve the hard-boiled egg white?	**3**
1C	Why do humans see so poorly under water? And why do goggles help?	**2**
1D.	Explain the light microscope with a neat flow diagram. At what points the image of the specimen magnified? Explain the difference between resolution and magnification.	**3**
2A.	A total of seven (n) variables (A, B, C, D, E, F and G) with four dummy variables (H, J, K and L) were selected. The glucose oxidase activity of the 12 trials are listed, perform the ANOVA and identify the significant parameters.	**5**

					Coded variable level						
Run	**x1**	**x2**	**x3**	**x4**	**x5**	**x6**	**x7**	**D1**	**D2**	**D3**	**D4**	**Y(gL^{-1})**
1	H	L	L	L	L	H	H	L	H	H	L	2.823
2	L	L	L	L	L	L	L	L	L	L	L	0.289
3	H	H	H	L	H	H	L	H	L	L	L	0.57
4	L	H	L	L	L	H	H	H	L	H	H	0.95
5	L	L	L	H	H	H	L	H	H	L	H	0.459
6	L	H	H	L	H	L	L	L	H	H	H	0.07
7	L	H	H	H	L	H	H	L	H	L	L	0.508
8	H	H	L	H	H	L	H	L	L	L	H	1.013
9	L	L	H	H	H	L	H	H	L	H	L	0.715
10	H	H	L	H	H	L	L	H	H	H	L	0.285
11	H	L	H	H	L	H	L	L	L	H	H	0.814
12	H	L	H	L	L	L	H	H	H	L	H	1.31

2B.	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 1×10^4 min^{-1}. Assume vitamin destruction is first order and the initial concentration is 50 mg/L. the medium contains 2.5×10^3 spores/L. The spores have an Ed 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**
3A.	For an enzyme that follows Michaelis–Menten kinetics, by what factor does the substrate concentration have to increase to change the rate of the reaction from 20% to 80% *V*max?	**2**
3B.		**4**

	E_0 (g/l)	**T (^{0}C)**	**I (mmol/ml)**	**S (mmol/ml)**	**V (mmol/ 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	0.01	0.56
1.6	49.6	0	0.1	5.13
1.6	49.6	0	0.03	3.7
1.6	49.6	0	0.01	1.89
1.6	49.6	0	0.01	1.43
1.6	49.6	0	0.01	1.11
0.92	30	0	0.1	1.64
0.92	30	0	0.02	0.9
0.92	30	0	0.01	0.58
0.92	30	0.6	0.1	1.33
0.92	30	0.6	0.03	0.8
0.92	30	0.6	0.02	0.57

I. Determine the MM constant for the reaction with no inhibitor present at 30^{0}C and at 49.6 ^{0}C and an enzyme concentration of 1.6 g/L.

II. Determine the maximum velocity of the uninhibited reaction at 30^{0}C and an enzyme concentration of 1.6 g/L.

III. Determine the Ki for the inhibitor at 30^{0}C and decide what type of inhibitor is being used.

3C. (4)

Alpha amylase from malt is used to hydrolyze starch. The dependence of initial reaction rate on temperature is determined experimentally. Results measured at fixed starch and enzyme concentrations are listed below.

Temperature (˚C)	20	30	40	60
Rate of glucose production (mmol/m^3 s)	0.31	0.66	1.2	6.33

I. Determine the activation energy for this reaction.

II. Alpha amylase is used to break down starch. It is proposed to carry out the reaction at a relatively high temperature so that the viscosity is reduced. What is the reaction rate at 55˚C compared with 25˚C?

III. Thermal deactivation of this enzyme is described by the equation:

kd= 2.25 x 10^{27} EXP$^{(-41\,630/RT)}$

where kd is the deactivation rate constant in h^{-1}, R is the ideal gas constant in cal/gmol K, and T is the temperature in K. What is the half life of the enzyme at 55˚C compared with 25˚C?

4A. (3)

Determine the time response of a culture to diauxic growth on glucose and lactose based on the following: $\mu_{glucose}$ = 1.0 h^{-1}; $\mu_{lactose}$ = 0.6 h^{-1}; $Y_{glucose}$ = $Y_{lactose}$ = 0.5; enzyme induction requires 30 min to complete. Plot cell mass, glucose and lactose concentrations, assuming initial values of 2 g/L glucose, 3 g/L lactose, and 0.10 g/L cells.

4B.	The maximum growth yield coefficient for *Bacillus subtilis* growing on methanol is 0.4 g X/g S. The heat of combustion of cells is 21 kJ/g cells and for substrate it is 7.3 kcal/g. Determine the metabolic heat generated by the cells per unit mass of methanol consumption.	**2**
4C.	Ethanol formation from glucose is accomplished in a batch culture of *Saccharomyces cerevisiae* and the following data were obtained were determined. (table below) **I.** Determine μ_{max}, Ks, $Y_{X/S}$ and t_d **II.** By fitting the biomass data to the logistic equation determine the carrying capacity coefficient k.	**5**

Time(d)	0	2	5	10	15	20	25	30
Biomass (g/l)	0.5	1	2.1	4.8	7.7	9.6	10.4	10.7
Glucose (g/l)	100	95	85	58	30	12	5	2
Ethanol g/L	0	2.5	7.5	20	34	43	47	49

5A.	Describe the process of ethanol production explaining the stoichiometry, names of organisms, substrates and process conditions.	**3**
5B.	The growth of baker's yeast (*S. cerevisiae*) on glucose may be simply described by the following equation: $$C_6H_{12}O_6 + a\,O_2 + b\,NH_3 \longrightarrow c\,C_6H_{10}NO_3 + d\,H_20 + e\,CO_2$$ In a batch reactor of volume 10^5L, the final desired yeast concentration is 50 gdw/L. Respiratory coefficient (RQ) =1.04 Using the given data: **I.** Determine the stoichiometric coefficients. **II.** Determine the concentration and total amount of glucose and in the nutrient medium. **III.** Determine the yield coefficients $Y_{X/S}$, $Y_{X/NH3}$ and $Y_{X/O2}$ **IV.** Determine the total amount of oxygen required.	**5**
5C.	The equation for aerobic production of acetic acid from ethanol is: $$C_2H_5OH + O_2 \longrightarrow CH_3CO_2H + H_2O$$ *Acetobacter aceti* bacteria are added to aerated medium 10 gL^{-1} ethanol. After some time the ethanol concentration is 2 gL^{-1} and 7.5 gL^{-1} acetic acid is produced. How does the overall yield of acetic acid from ethanol compare with theoretical yield?	**2**