Reg. No.										

MANIPAL INSTITUTE OF TECHNOLOGY

MANIPAL

(A constituent institution of MAHE, Manipal)

I SEMESTER M.TECH. (INDUSTRIAL BIOTECHNOLOGY)
END SEMESTER EXAMINATIONS, NOV/DEC 2018
SUBJECT: BIOPROCESS ENGINEERING [BIO 5121]
REVISED CREDIT SYSTEM
(20/11/2018)

Time: 3 Hours MAX. MARKS: 50

Instructions to Candidates:

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

1A. You Wish to produce a high value protein using recombinant DNA technology. Would you try to develop a chemical defined medium or a complex medium? Why? **2**

1B. Cite five major biological functions of proteins. **2**

1C. Tannin acyl hydrolase (tannase) production by *Lactobacillus plantarum* MTCC1407 was studied in submerged fermentation process. Eight medium components were evaluated initially by Plackett–Burman two level factorial design. Identify the most significant parameters that affect the tannase production, by keeping variable (G) as dummy. A-Tannic acid; B-Glucose; C-NH_4Cl; D-$CaCl_2$; E-K_2HPO_4; F-KH_2PO_4; G-$MgSO_4$. $7H_2O$; H-$MnSO_4$. $7H_2O$. **6**

Run	A	B	C	D	E	F	G	H	Tannase activity (U/mL)
1	+	+	-	+	+	+	-	-	2.36
2	+	-	+	+	+	-	-	-	2.77
3	-	+	+	+	-	-	-	+	0.99
4	+	+	+	-	-	-	+	-	1.23
5	+	+	-	-	-	+	-	+	2.12
6	+	-	-	-	+	-	+	+	3.97
7	-	-	-	+	-	+	+	-	2.02
8	-	-	+	-	+	+	-	+	2.33
9	-	+	-	+	+	-	+	+	2.64
10	+	-	+	+	-	+	+	+	4.59
11	-	+	+	-	+	+	+	-	0.85
12	-	-	-	-	-	-	-	-	1.50

2A. The thermal death kinetics of thermo tolerant spore forming bacterium *Clostridium thermocellum* are shown below. Find the best values of the kinetic coefficients that describe the data. If a sample of *Clostridium thermocellum* were to be replaced in boiling water, how long it takes to reduce the spore population by 50%? **5**

		Temp (^oC)	120	130	140	150	
		k_d (min^{-1})	0.109	1.45	17	177.8	
2B.	A continuous sterilizer with a steam injector and a flash cooler will be employed to sterilize medium continuously with the flow rate of $2m^3/h$. the time for heating and cooling is negligible with this type of sterilizer. The typical bacteria count of the medium is about $5x10^{12}m^{-3}$ which needs to be reduced to such an extent that only one organism can survive during two months of continuous operation. The heat resistant bacterial spores in the medium can be characterized by Ed = $2.834x\ 10^5$kJ/kmol and A= $5.7x10^{39}$ /h. The sterilizer will be constructed with the pipe with an ID of 0.102 m. Steam at 600kPa (gauge pressure) is available to bring the sterilizer to an operating temperature of 125ºC. The physical properties of this medium at 125ºC are Cp= 4.187 kJ/kg K, Density= $1000kg/m^3$; Viscosity=4 kg/m.h. What length should the pipe be in the sterilizer if you consider dispersion?						**5**
3A.	An enzyme with K_M of 0.001M was assayed using an initial substrate concentration of $3x10^{-5}$ M. After 2 min 5% of the initial substrate was converted. How much substrate will be converted after 10, 30 and 60 min?						**4**
3B.	Aspirin inhibits glutamate dehydrogenase. Following Data have been obtained for the enzyme catalysed and inhibition reaction. Determine the kinetic constants, type of inhibition and K_I:						**6**
		Product (mmoles/min)	4.58	6.40	7.72	8.72	9.50
		Substrate (mmoles)	3	5	7	9	11
		Asprin (mmoles/min) I = 5 mmoles	3.66	5.12	6.18	6.98	7.60
4A.	Design the 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.						**6**
4B.	*Pseudomonas methylotrophus* is used to produce single-cell protein from methanol in a l000 m^3 pressure-cycle airlift fermenter. The biomass yield from substrate is 0.41 g/g, Ks is 0.7 mg/l, and the maximum specific growth rate is 0.44 h^{-1}. The medium contains 4% (w/v) methanol. A substrate conversion of 98% is desirable. The reactor operated in batch mode with an inoculum of 0.01 %(w/v) and the downtime between batches is 20 h. Neglecting maintenance requirements, determine the annual biomass production from batch reactor. [Hint: Total time = Batch time + downtime].						**4**
5A.	A strain of *Azotobacter vinelandii* is cultured in a 15 m^3 stirred fermenter for alginate production. Under current operating conditions k_La is 0.17 s^{-1}. Oxygen solubility in the broth is approximately 8 x 10^{-3} $kg.m^{-3}$. a. The specific rate of oxygen uptake is 12.5 $mmol.g^{-1}.h^{-1}$. What is the maximum possible cell concentration? b. The bacteria suffer growth inhibition after copper sulphate is accidently added to the fermentation broth. This causes a reduction in oxygen uptake rate of 3 $mmol.g^{-1}.h^{-1}$. What maximum cell concentration can now be supported by the fermenter?						**2+2**
5B.	Assume that experimental measurements for a certain organism have shown that cells can convert two-thirds (wt/wt) of the substrate carbon (glucose) to biomass. a. Calculate the stoichiometric coefficients for the following biological reaction: $C_6H_{12}O_6 + a\ O_2 + b\ NH_3$ -------→ $c\ (C_{4.4}H_{7.3}N_{0.86}O_{1.2}) + d\ H_2O + e\ CO_2$ b. Calculate the yield coefficients $Y_{x/s}$ (g dw cell/ g substrate), $Y_{x/O2}$ (g dw cell/ g O_2) and degree of reduction of biomass.						**3+3**