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# MANIPAL INSTITUTE OF TECHNOLOGY

MANIPAL

*A Constituent Institution of Manipal University*

**V SEMESTER B.TECH. (BIOTECHNOLOGY)**
**END SEMESTER EXAMINATIONS, NOV/DEC 2017**
**SUBJECT: BIOPROCESS ENGINEERING [BIO 3102]**
**REVISED CREDIT SYSTEM**
**(17/11/2017)**

Time: 3 Hours MAX. MARKS: 50

**Instructions to Candidates:**

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

**1A.** In the mashing step in beer and whisky brewing, ground partially sprouted barley grain is suspended in liquid (called a wort) to allow the enzyme amylase to break down starch contained in the grain into sugars. The mashing step is carried out at warm temperature to speed up the sugar conversion process, but the enzyme also deactivates at high temperature. As a biochemical engineer operating this brewing process, you are asked to find an optimal temperature and time. How will you model this process? What are the parameters in your model? What do you vary and what do you measure experimentally to estimate these parameters? **5**

**1B.** During a test of kinetics of an enzyme-catalyzed reaction, the following data were recorded. Determine the kinetic constants, type of inhibition and KI: **5**

| **S (mol/ L)** | 0.1 | 0.05 | 0.03 | 0.02 | 0.01 |
|---|---|---|---|---|---|
| **V (mol / L-min) @ I = 0** | 1.61 | 1.34 | 1.01 | 0.81 | 0.67 |
| **V (mol /L-min) @ I = 0.6 mol/ L** | 1.34 | 1 | 0.67 | 0.5 | 0.4 |

**2A.** Consider the data given in the table on the temperature changes in a 10,000 L fermenter. The values of the Arrhenius constant and $E_d$ for the spores are $1x10^{36}$ $min^{-1}$ and 65 kcal/mol respectively. For the inactivation of the vitamin, the values are $1x10^{4}$ $min^{-1}$ and 10 kcal/mol respectively. Assume an initial spore concentration of $10^5$ / L and a vitamin concentration of 30 mg/l. **5**

a. What is the probability of unsuccessful sterilization?
b. What fraction of the vitamin remains undegraded?

| **Time (min)** | 0 | 10 | 20 | 30 | 40 | 50 | 55 | 60 | 65 | 70 | 90 | 100 | 120 | 140 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Temp (C°)** | 30 | 40 | 54 | 70 | 95 | 121 | 121 | 121 | 106 | 98 | 75 | 64 | 46 | 32 |

| | | |
|---|---|---|
| **2B.** | A continuous culture system is being constructed. The fermentation tank is to be 50, 000 L in size and the residence time to be 2 h. A continuous sterilizer is to be used. The un-sterilized medium contains $10^4$ spores/L. the value of $k_d$ has been determined to be 1 $min^{-1}$ at 121°C and 61 $min^{-1}$ at 140°C. For each temperature, determine the required residence time in the holding section so as to ensure 99% of the time four weeks of continuous operation can be obtained without contamination. | **5** |
| **3A.** | Amylase enzyme is immobilized in the form of spherical beads. The reaction follows a first order kinetics with a reaction rate constant of 3.6 $h^{-1}$. The size of the beads is 12 mm and the effective diffusivity of substrate is 1 x $10^{-6}$ $m^2$/sec.<br>a. Does the availability of the substrate limit the reaction? Justify your answer:<br>b. Now instead of immobilized beads, you are asked to try with rectangular shaped strips of immobilized amylase enzyme. The volume of each strip is 1 x $10^{-9}$ $m^3$ and its surface area is 6 x $10^{-6}$ $m^2$. Will this new condition affect the availability of the substrate inside the enzyme? Discuss: | **3+2** |
| **3B.** | An enzyme is immobilized uniformly in a gelatin slab (thickness L and area A). One side is in contact with substrate solution and the other side is in contact with a glass plate. Derive the equation for the substrate concentration with respect to x when the substrate is catalyzed by zero order reaction. Assume that the substrate is transferred by molecular diffusion in the x direction only and the gelatin slab is thick enough to catalyze all the substrate while it diffuses into the slab. What is the critical thickness at which all the substrate is consumed? | **5** |
| **4A.** | A 20 L stirred fermenter containing *Bacillus thuringiensis* is used to produce an insecticide. The oxygen balance method is applied to determine $k_La$. The fermenter operating pressure is 150 kPa and the culture temperature is 30°C. The oxygen tension in the broth is measured as 82% using a probe calibrated to 100% in situ using water and air at 30°C and 150 kPa. The solubility of oxygen in the culture fluid is the same as in water. Air is sparged into the vessel; the inlet gas flow rate measured outside the fermenter at 1 atm pressure and 22°C is 0.23 L/s. The exit gas from the fermenter contains 20.1% oxygen and has a flow rate of 8.9 L/ min.<br>i. Calculate the volumetric rate of oxygen uptake by the culture.<br>ii. What is the value of $k_La$? | **3+3** |
| **4B.** | *E.coli* have a maximum respiration rate, $qO_{2\ max}$ of 0.24 $gO_2$/g dry wt-hr. It is desired to achieve a cell mass of 20g dry wt/L. The $k_La$ is 120$h^{-1}$ in a 1000L reactor. A gas stream enriched in oxygen is used (i.e., 80% $O_2$) which gives a value of C* = 28 ppm. Under these conditions the respiration rate $qO_2$ is given by, $qO_2 = (qO_{2\ max}\ C_L) / (0.2\ ppm + C_L)$, where $C_L$ is the DO concentration in the fermentor. What is the value of $C_L$? | **4** |
| **5A.** | How do the Logistic equations fit in a better way for the growth of microorganism? Explain with an example: | **5** |
| **5B.** | Consider the growth of a microorganism in batch culture, inoculated at a concentration of 0.1 g/L, growing on fructose as the limiting substrate with an initial concentration of 10 g/L. After a lag time of 3 h, the culture grows exponentially with a doubling time of 2 h. Stationary phase is reached after a total time of 14 h. Assume that there was no decline phase.<br>i. Determine $\mu_{max}$.<br>ii. Determine Yxs<br>iii. The total time of culture to reach stationary phase if the initial substrate concentration were 2 g/L by assuming that this concentration is also sufficient to support maximal growth ($S >> K_s$). | **5** |