Reg. No.										

Manipal Institute of Technology, Manipal

(A Constituent Institute of Manipal University)

V SEMESTER B.TECH (BIOTECHNOLOGY)

END SEMESTER EXAMINATIONS, NOV/DEC 2015

SUBJECT: **FERMENTATION ENGINEERING** [BIO 303]

REVISED CREDIT SYSTEM

Time: 3 Hours MAX. MARKS: 50

Instructions to Candidates:

- Answer **ANY FIVE FULL** the questions.
- Missing data may be suitable assumed.

1A.	Assume that your responsibility is to formulate a media for a high-value protein using recombinant DNA technology. Your senior colleague insists you to add inducers, inhibitors and precursors to the media. What could be the reason? Justify the addition of these compounds with one example each:	**5**
1B.	You are provided with a heat exchanger consisting of 10 m length of pipe, with internal diameter of 0.074 m. Steam is available at 135°C, and you need to ensure sterility of 2,000 L medium in 1 h. Past experience has shown that the complex medium contains 5 x 10^6 spores/mL. The activation energy of spore destruction and frequency factor are 80,000 cal/mole and 2.5 x 10^{44} min^{-1} respectively. At the temperature of sterilization, you may assume that the medium has the same density and viscosity as water (ρ = 927 kg/m^3; μ = 0.21 x 10^{-3} kg/m.s). i. Calculate the number of spores surviving after sterilization, and the probability of contamination. ii. For the problem above, what would be the impact of operating the sterilizer with a lower value of Peclet number?	**2.5 + 2.5**
2A.	Filtration method can be used as a sterilization technique. Justify this statement with two examples:	**2.5+2.5**
2B.	A continuous steriliser is constructed using a 21 m length of pipe of internal diameter 8 cm. Liquid medium in the pipe is maintained at 128°C using saturated steam. At this temperature, the specific death constant of the contaminating organisms is 340 h^{-1} and the density and viscosity of the medium are 1000 kg/m^3 and 0.9 cP, respectively. The concentration of contaminants in the raw medium is 6.5 x 10^5 ml^{-1}. If sterile medium is required on the fermentation floor at a rate of 0.9 m^3 / h, what is the frequency of contamination in the fermentation factory?	**5**
3A.	i. Assume that the concentration of a limiting substrate inside a spherical biocatalyst falls to zero inside the particle for a zero order reaction. Under this condition, what happens to the effectiveness factor? ii. How do you maintain the concentration of limiting substrate greater than zero inside the particle?	**2.5+2.5**
3B.	Invertase 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.	**2.5+2.5**

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<tr><td></td><td>i. Does the availability of the substrate limit the reaction? Justify your answer:
ii. Now instead of immobilized beads, you are asked to try with rectangular shaped strips of immobilized invertase enzyme. The volume of each strip is 1×10^{-9} m^3 and its surface area is 6×10^{-6} m^2. Will this new condition affect the availability of the substrate inside the enzyme? Discuss:</td><td></td></tr>
<tr><td>4A.</td><td>An enzyme (K_M = 1M) is immobilized uniformly in a gelatin slab (thickness L and area A). One side is in contact with a substrate solution (10 M) and the other side is in contact with a glass plate. Derive the equation for the substrate concentration with respect to the distance inside the slab. 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.</td><td>5</td></tr>
<tr><td>4B</td><td>The bioconversion of glucose to ethanol is carried out in a packed-bed, immobilized-cell bioreactor containing yeast cells entrapped in calcium alginate beads. The rate-limiting substrate is glucose and its concentration in the feed bulk liquid phase is 5 g/L. The particle size of beads is 0.5 cm. The rate expression for this bioconversion is given by, $r_s = (r_m\ S) / (K_s + S)$, where r_m = 100 mg/cm^3 h and K_s = 10 mg / cm^3. The effective diffusivity of glucose inside the bead is 10^{-6} cm^2/s. Determine the effectiveness factor and comment on the results:</td><td>5</td></tr>
<tr><td>5A.</td><td>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.
i. Calculate the volumetric rate of oxygen uptake by the culture.
ii. What is the value of k_La?</td><td>2.5+2.5</td></tr>
<tr><td>5B.</td><td>The dynamic pressure method is applied for measurement of k_La in a 3000 L stirred fermenter containing a suspension culture of Micrococcus glutamicus. The stirrer is operated at 60 rpm and the gas flow rate is fixed at 800 L/min. The following dissolved oxygen concentrations are measured using a polarographic dissolved oxygen electrode after a step increase in fermenter pressure.
<table>
<tr><td>Time (s)</td><td>6</td><td>10</td><td>25</td><td>40</td></tr>
<tr><td>C_{AL} (% air saturation)</td><td>50.0</td><td>56.1</td><td>63.0</td><td>64.7</td></tr>
</table>
The steady-state dissolved oxygen tension at the end of the dynamic response is 66% air saturation. Estimate the value of kLa.</td><td>5</td></tr>
<tr><td>6A.</td><td>Develop a model for a filamentous organism which form microbial pellet at high cell densities in suspension cultures.</td><td>5</td></tr>
<tr><td>6B</td><td>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.
i. Determine μ_{max}.
ii. Determine Yxs
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 ($S >> K_s$).</td><td>1+2+2</td></tr>
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