



B.TECH DEGREE EXAMINATIONS: NOV/DEC 2023

(Regulation 2018)

Fifth Semester

BIOTECHNOLOGY

U18BTI5202: Bioprocess Engineering

COURSE OUTCOMES

- CO1:** Apply the knowledge of various optimization methods to design the media for fermentation broth
- CO2:** Evaluate the sterilization kinetics of media and able to design the holding time for batch. sterilization
- CO3:** Develop a suitable mathematical model for batch, fed-batch and continuous fermentation and able to simulate and evaluate the constants for microbial growth
- CO4:** Understand and analyse the application of various bioreactors and importance of mass transfer. effect in bioprocess engineering
- CO5:** Apply the various scale-up criteria to design the bioreactors
- CO6:** Identify and provide the solution for non- ideal performance of bioreactor

Time: Three Hours

Maximum Marks: 100

Answer all the Questions:-

PART A (10 x 2 = 20 Marks)

(Answer not more than 40 words)

- In what scenarios or under what conditions, is it more advantageous to use a defined growth medium instead of a complex growth medium for microbial fermentation? CO1 [K₃]
- How do you measure the biomass concentration using ATP in a fermentation broth ? CO1 [K₂]
- An industrial fermenter containing 10,000 L of the medium needs to be sterilized. The initial spore concentration in the medium is 10⁶ spores per mL. The desired probability of contamination after sterilization is 10⁻³. The death rate of spores at 121°C is 4 min⁻¹. Assume that there is no cell death during heating and cooling phases. Calculate the holding time of the sterilization process. CO2 [K₄]
- Illustrate the setup of batch and continuous sterilization process. According to you, which one is superior? Substantiate your opinion. CO2 [K₂]
- 15g of *Bacillus subtilis* biomass is produced from 40 grams of glucose. No by-product is formed was observed, calculate the yield coefficient. CO3 [K₃]
- When does cell washout occur in a continuous fermentation? CO3 [K₂]
- Oxygen transfer was measured in a stirred tank bioreactor using dynamic gas method. The CO4 [K₃]

dissolved oxygen tension was found to be 80% air saturation under steady-state conditions. The measured oxygen tension at 7 sec and 17 sec were 55 and 68 % air saturation, respectively. Calculate the volumetric mass transfer coefficient $K_{L}a$ in seconds.

8. Enlist the assumptions of O_2 -balance method in measurement of $K_{L}a$. CO4 [K₂]
9. The dimensions and operating condition of a lab-scale fermenter are as follows: Volume = 1L, Diameter = 20cm, Agitator speed = 600rpm, Ratio of the impeller diameter to fermenter diameter = 0.3. This fermenter needs to be scaled up to 8000L for a large-scale industrial application. If the scale-up is based on constant impeller tip speed, Calculate the speed of the agitator in the larger reactor in rpm. Assume that the scale-up factor is cube-root of the ratio of fermenter volumes. CO5 [K₃]
10. Enlist any two reasons of existing of non-ideality in bioreactors. CO6 [K₂]

Answer any FIVE Questions:-
PART B (5 x 16 = 80 Marks)
(Answer not more than 400 words)

11. a) Describe the concept of medium optimization using simplex search method 6 CO1 [K₂]
- b) Given the Plackett-Burmann Design for media containing seven variables. 10 CO1 [K₄]
 Identify the key variable affecting the optimization of media. Consider the dummy variable at least two as per your choice. Comment on your prediction.

| Trial | Variables | | | | | | | Yield |
|-------|-----------|---|---|---|---|---|---|-------|
| | A | B | C | D | E | F | G | |
| 1 | H | H | H | L | H | L | H | 1.1 |
| 2 | L | H | H | H | L | H | L | 6.3 |
| 3 | L | L | H | H | H | L | H | 1.2 |
| 4 | H | L | L | H | H | H | L | 0.8 |
| 5 | L | H | L | L | H | H | H | 6.0 |
| 6 | H | L | H | L | L | H | H | 0.9 |
| 7 | H | H | L | H | L | L | H | 1.1 |
| 8 | L | L | L | L | L | L | L | 1.4 |

H, denotes a high level value; L, denotes a low level value.

12. a) Steam sterilizer is used to sterilize liquid medium for fermentation. The initial concentration of contaminating organisms is 10^8 per litre. For design purposes, the final acceptable level of contamination is usually taken to be 10^{-3} cells; this corresponds to a risk that one batch is a thousand will remain contaminated even after the sterilization process is complete. For how long should 1 m^3 be treated if 16 CO2 [K₄]

the temperature is A) 80°C B) 121°C and C) 140°C.

(Use R as $8.3144 \times 10^{-3} \text{ kJ K}^{-1} \text{ gmol}^{-1}$, Activation energy as 283 kJ gmol^{-1} and Arrhenius constant as $10^{36.2} \text{ s}^{-1}$)

13. a) A 200-litre stirred fermenter contains a batch culture of *Bacillus subtilis* bacteria at 28°C. Air at 20°C is pumped into the vessel at the rate of 1vvm. The average pressure in the fermenter is 1atm. The volumetric flow rate of off-gas from the fermenter is measured as 189 L.min^{-1} . The exit gas stream is analyzed for oxygen and is found to contain 20.1 % O₂. The dissolved oxygen concentration in the broth is measured using an oxygen electrode as 52% air saturation. The solubility of oxygen in the fermentation broth at 28°C and 1 atm air pressure is $7.8 \times 10^{-3} \text{ kg m}^{-3}$. (Use gas constant R as $8.205 \times 10^{-5} \text{ m}^3 \cdot \text{atm} \cdot \text{K}^{-1} \cdot \text{gmol}^{-1}$)
- A) Calculate the oxygen transfer rate.
- B) Determine the K_La for the system.
- b) Explain the various resistances involved in the transfer of oxygen from gas bubble to microbial cell with a neat diagram.
14. a) With a neat sketch, explain the production of single-cell production using photobioreactor? Emphasis on the cell harvest process.
15. a) Discuss – “Why would the performance of the biomass differs at 10, 000L and at 10L ?”.
- b) Explain how heterogeneity is attained in a small fermenter. Emphasis on the scale-down approaches in debottlenecking a fermenter operation using a case study.
16. a) Discuss in detail the various methods employed in determining the RTD in a non-ideal fermenter.
