BT513 – PRINCIPLES OF BIOCHEMICAL ENGINEERING

MID TERM IMPORTANT QUESTIONS

Prepared by: *Sir Mughal*

Admin: **BS Biological Sciences** (One place to all your solutions)

For timely updates, join our Main Group: https://chat.whatsapp.com/DWXwJpXahbxFVQutey8JXN

Click to join the subject group: https://chat.whatsapp.com/DOV46JIi1THGYi8EytmynT

1. Why bottom entry requires maintenance?

Bottom entry requires maintenance because:

- Impellers, bearings, and seals are located at the tank bottom, submerged in the medium, making them prone to wear, fouling, and corrosion.
- Seal replacement and lubrication are difficult without emptying the vessel.
- Sediment or solids accumulation at the bottom can damage mechanical parts.
- Continuous exposure to high shear or viscous fluids increases maintenance frequency.

2. Write functions of agitation system.

Functions of Agitation System:

1. ***Homogenization:*** Ensures uniform distribution of cells, nutrients, and temperature.
2. ***Mass Transfer Enhancement:*** Improves oxygen transfer in aerobic fermentations.
3. ***Heat Transfer:*** Promotes efficient heating or cooling of the medium.
4. ***Suspension of Solids:*** Keeps cells, particles, or substrates uniformly suspended.
5. ***Reaction Rate Improvement:*** Increases contact between reactants, enhancing biochemical reactions.
6. ***Preventing Sedimentation:*** Avoids settling of biomass or insoluble components.

3. Write role of Turbidostat.

Role of Turbidostat:

- Maintains constant culture turbidity (cell density) by continuously adjusting the flow rate of fresh medium.
- Ensures cells grow at maximum specific growth rate without nutrient limitation.
- Used for continuous cultivation where steady-state high-density growth is required.
- Allows study of microbial physiology under constant environmental conditions.

4. Write method used for measuring cell density.

Methods for Measuring Cell Density:

1. ***Optical Density (OD)/Spectrophotometry:*** Measures turbidity at 600 nm (OD_{600}); quick and non-destructive.
2. ***Dry Weight Measurement:*** Cells are filtered, dried, and weighed; gives absolute biomass.
3. ***Cell Counting:*** **Hemocytometer** – direct microscopic counting and **Flow Cytometry** – counts individual cells rapidly.
4. ***Packed Cell Volume (PCV):*** Measures volume fraction of cells after centrifugation.
5. ***Turbidostat/Continuous Monitoring:*** Uses optical sensors to maintain constant cell density.

5. Differentiate between Chemostat and Quasi Steady State.

A chemostat is a continuous culture system where fresh medium is supplied at a constant dilution rate, maintaining a true steady state with constant cell density, substrate concentration, and product formation. It allows precise control of growth under nutrient-limited conditions and is widely used to study microbial kinetics. In contrast, a quasi-steady state occurs when the culture appears stable for a short period, but parameters like biomass or substrate gradually change over time. It is less controlled, typically seen in batch or semi-continuous cultures, and is useful for short-term studies where maintaining a true steady state is not practical.

6. When agitator is introduced from bottom, then what maintenance is needed?

When a bottom-entry agitator is used, the following maintenance is typically needed:

- ***Seal and Bearing Inspection/Replacement:*** Mechanical seals and bearings at the bottom are prone to wear, corrosion, and leakage due to constant contact with the medium.
- ***Lubrication:*** Bearings and moving parts require regular lubrication to prevent friction and overheating.
- ***Impeller Cleaning:*** Impellers can accumulate solids, biofilm, or sediment, reducing efficiency.
- ***Alignment Check:*** Shaft misalignment can cause vibration and mechanical failure, so periodic alignment is necessary.
- ***Corrosion Monitoring:*** Continuous exposure to medium, especially corrosive or high-shear fluids, requires inspection and preventive treatment.

7. What is meant by fed time and batch time?

Fed Time refers to the period during a fed-batch fermentation when substrate is continuously or intermittently supplied to the bioreactor. The feeding is controlled to maintain optimal nutrient levels, prevent substrate inhibition, and support prolonged microbial growth or product formation. It helps achieve higher cell density or product yield than conventional batch processes.

Batch Time is the total duration of a batch culture, starting from inoculation until the fermentation is terminated or harvested. During this time, the system evolves naturally without any additional substrate, and the growth, substrate consumption, and product formation follow the inherent kinetics of the microorganism.

8. Explain the terms Identification, metabolites and medicine recovery according to Borrow et al and Bu'Lock et al.

In the context of microbial metabolism as discussed by Borrow et al. and Bu'Lock et al., the terms relate to how microbial products are classified and utilized in bioprocesses:

- ***Identification:*** This refers to detecting and characterizing biochemical products formed in a bioreactor or microbial culture, including primary and secondary metabolites, using analytical methods to distinguish compounds by structure and function.

- ***Metabolites:*** According to Bu'Lock's interpretation of secondary metabolism in industrial microbiology, metabolites include both primary metabolic products (essential for growth such as acids, amino acids) and secondary metabolites, which are species-specific products produced in the idiophase and not essential for growth but often of industrial or medicinal value (e.g., antibiotics) and arise from pathways linked to primary metabolism but regulated differently.

- ***Medicine Recovery:*** In fermentation and bioprocess engineering, this term refers to the downstream separation and purification of pharmaceutically active metabolites produced by microorganisms (especially secondary metabolites) into finished drug substances suitable for use, involving extraction, concentration, and purification steps from the culture broth.

9. What is the role of Qag in energy balance equation?

In a bioreactor, Q_ag represents the energy supplied by the agitator. It contributes to the energy balance by providing mechanical energy that helps mix the culture, suspend cells, and enhance mass and heat transfer. Part of this energy is dissipated as heat, which affects the temperature of the medium. Therefore, Q_ag is included as a source term in the energy balance equation to account for the energy input from agitation.

10. Differentiate between stationary phase and exponential phase regarding cell density and growth.

- ***Exponential (Log) Phase:*** Cells grow rapidly and continuously at their maximum specific growth rate. Cell density increases exponentially over time, and nutrients are abundant.

- ***Stationary Phase:*** Growth ceases or slows due to nutrient depletion, waste accumulation, or limited oxygen. Cell density remains nearly constant, as the rate of cell division equals the rate of cell death.

11. Write role of Turbidostat in continuous fermentation. How it maintains the cell concentration.

In continuous fermentation, a turbidostat is used to maintain a constant cell concentration in the bioreactor. Its role is to allow microorganisms to grow at their maximum specific growth rate without nutrient limitation. The system continuously monitors the turbidity (optical density) of the culture, which reflects cell density. When turbidity exceeds the set value, fresh medium is added or culture is withdrawn to adjust the biomass, keeping the cell concentration constant. This ensures steady-state high-density growth, optimal productivity, and uniform environmental conditions for the culture.

12. Write properties of axial flow.

Properties of Axial Flow Agitators:

1. ***Flow Direction:*** Impeller pushes fluid parallel to the shaft, creating top-to-bottom circulation.
2. ***Low Shear:*** Produces gentle mixing, suitable for shear-sensitive cells or proteins.
3. ***High Flow, Low Head:*** Moves a large volume of liquid with low energy dissipation per unit volume.
4. ***Efficient Bulk Mixing:*** Ensures uniform temperature, pH, and nutrient distribution in the reactor.
5. ***Good for Gas Dispersion:*** Effective in sparged bioreactors for oxygen transfer.
6. ***Typical Impellers:*** Pitched-blade, hydrofoil, or propeller type.

13. Explain relationship between qp and u.

In biochemical engineering, the relationship between $\mathbf{q_p}$ (specific product formation rate) and $\boldsymbol{\mu}$ (specific growth rate) describes how product formation depends on microbial growth:

- ***Growth-associated product:*** q_p is directly proportional to μ, meaning product is formed simultaneously with cell growth (e.g., primary metabolites like amino acids).

  $$q_p = Y_{P/X} \cdot \mu$$

  Where $Y_{P/X}$ is the product yield coefficient per biomass.

- ***Non-growth-associated product:*** q_p is independent of μ, produced mainly in stationary phase (e.g., secondary metabolites like antibiotics).
- ***Mixed-growth product:*** q_p has a component dependent on μ and a constant non-growth-associated component.

14. Write limitations and advantages of axial flow.

Advantages:

- Produces top-to-bottom circulation, ensuring uniform bulk mixing.
- Low shear, suitable for shear-sensitive cells and proteins.
- Provides high volumetric flow with low energy consumption per unit volume.
- Effective for gas–liquid mass transfer in sparged bioreactors.
- Suitable for large-scale fermenters requiring gentle mixing.

Limitations:

- Generates low pressure head, so not ideal for highly viscous fluids.
- Less effective for localized mixing near impeller compared to radial flow.
- May require multiple impellers in tall reactors for uniform circulation.
- Not ideal when high shear or intense mixing is needed.

15. What is the role of baffles in agitation?

Role of Baffles in Agitation:

- ***Prevent Vortex Formation:*** Baffles stop the liquid from spinning with the impeller, ensuring proper mixing.
- ***Enhance Turbulence:*** Increase fluid turbulence, improving mass and heat transfer.
- ***Improve Gas–Liquid Mixing:*** Aid in efficient oxygen dispersion in aerated bioreactors.
- ***Increase Impeller Efficiency:*** Convert rotational energy into effective bulk flow rather than rotational motion.
- ***Promote Uniform Conditions:*** Help maintain homogeneous temperature, pH, and nutrient distribution throughout the reactor.

16. Write importance of exit air sterilization of bioreactor for oxygen demand.

Importance of Exit Air Sterilization for Oxygen Demand:

- ***Prevents Contamination:*** Sterilizing the exhaust air stops microorganisms from entering or leaving the bioreactor, maintaining a sterile environment.
- ***Maintains Accurate Oxygen Supply:*** Ensures that oxygen transfer rates are not affected by microbial contamination in incoming or outgoing air.
- ***Protects Product Quality:*** Avoids unwanted microbial growth that could consume oxygen, altering cell metabolism and reducing product yield.
- ***Regulates Gas Balance:*** Helps maintain controlled oxygen levels for optimal microbial growth and product formation.
- ***Environmental Safety:*** Prevents release of genetically modified or pathogenic microbes into the environment.

17. Elucidate the role of microbial kinetics of the fed batch for variable volume.

In fed-batch fermentation with variable volume, microbial kinetics play a key role in controlling growth and product formation as substrate is added over time. The specific growth rate (μ) and substrate uptake rate determine how biomass accumulates while preventing substrate inhibition. By feeding nutrients gradually, the substrate concentration remains near the optimal level, allowing cells to grow efficiently and produce desired metabolites. The variable volume affects dilution and residence time, so microbial kinetic models (e.g., Monod kinetics) are used to predict biomass, substrate, and product profiles. This approach helps maximize yield, control metabolic shifts, and avoid nutrient limitation or overflow metabolism.

18. Define log phase, lag phase and stationary phase.

Microbial Growth Phases:

- ***Lag Phase:*** Initial period after inoculation where cells adapt to the new environment. Little or no division occurs, but metabolic activity prepares cells for growth.
- ***Log (Exponential) Phase:*** Cells divide at a constant maximum specific growth rate (μ_{max}). Biomass increases exponentially, and nutrients are abundant.
- ***Stationary Phase:*** Growth slows or stops due to nutrient depletion, waste accumulation, or oxygen limitation. Cell density remains nearly constant as cell division equals cell death.

19. Define seals and o rings in fermenter.

Seals in a Fermenter

Seals are mechanical devices used to prevent leakage of liquid, gas, or sterile culture medium along the rotating agitator shaft. They are essential for maintaining sterility of the bioreactor and protecting the culture from contamination while also preventing the escape of potentially hazardous materials. Common types include stuffing box seals, mechanical seals, and lip seals, each designed to handle different pressures, temperatures, and shear conditions in the fermenter. Proper maintenance of seals ensures reliable operation and longevity of the bioreactor.

O-Ring in a Fermenter

An O-ring is a circular elastomeric ring placed in grooves at joints or connections of the fermenter, such as lids, ports, or sampling lines, to create a tight and flexible seal. It helps maintain sterility, pressure, and vacuum conditions within the vessel and is reusable after proper cleaning. O-rings are simple yet critical components for preventing contamination and ensuring the safe and efficient operation of the fermenter.

20. Write steps for growth of organism.

Steps for Growth of an Organism:

1. ***Inoculation:*** Introduce a pure culture of the microorganism into a sterile growth medium.
2. ***Lag Phase:*** Cells adapt to the new environment, synthesizing enzymes and metabolites; little or no division occurs.
3. ***Exponential (Log) Phase:*** Cells divide at a constant maximum specific growth rate (μ_{max}); biomass increases exponentially.
4. ***Nutrient Uptake and Metabolism:*** Cells consume substrates for energy and biosynthesis, producing primary metabolites.
5. ***Stationary Phase:*** Growth slows or stops due to nutrient depletion, waste accumulation, or oxygen limitation; cell density becomes constant.
6. ***Death Phase:*** If conditions worsen, cells begin to die at an exponential rate, and metabolites may be released into the medium.

21. Write role of Jacket or Coil for heat in fermenter.

Role of Jacket or Coil for Heat in a Fermenter:

- ***Temperature Control:*** Jackets or coils circulate heating or cooling fluid to maintain the optimal temperature for microbial growth or product formation.
- ***Heat Removal:*** During exothermic microbial metabolism, they remove excess heat to prevent thermal stress or cell damage.
- ***Heat Supply:*** In cold environments or for thermophilic cultures, they provide heat to reach desired fermentation temperature.
- ***Uniform Temperature Distribution:*** Promotes homogeneous conditions throughout the medium, preventing hot or cold spots.
- ***Process Optimization:*** Maintaining precise temperature enhances growth rate, enzyme activity, and product yield in the fermenter.

22. Give methods to determine microbial growth.

Methods to Determine Microbial Growth:

1. ***Direct Microscopic Count:*** Cells are counted under a microscope using a hemocytometer; provides total cell number including live and dead cells.
2. ***Viable (Plate) Count:*** Serial dilution and plating on agar to count colony-forming units (CFU); measures only living cells.
3. ***Turbidity / Optical Density (OD):*** Measures light scattering by cells using a spectrophotometer (OD_{600}); fast and non-destructive, proportional to biomass.
4. ***Dry Weight Measurement:*** Cells are filtered, dried, and weighed; gives absolute biomass concentration.
5. ***Packed Cell Volume (PCV):*** Volume fraction of cells after centrifugation; simple estimate of biomass.
6. ***Indirect Methods:*** Metabolite production rate (e.g., CO_2, O_2 consumption, acid production) & Electrical conductivity or impedance of the culture medium.

23. How axial impeller design helps in aeration and agitation?

Axial Impeller Design in Aeration and Agitation:

- ***Promotes Top-to-Bottom Flow:*** Axial impellers push liquid parallel to the shaft, creating vertical circulation, which distributes oxygen and nutrients uniformly.
- ***Improves Gas–Liquid Contact:*** The flow pattern disperses sparged air bubbles efficiently, enhancing oxygen transfer to the culture.
- ***Low Shear Mixing:*** Gentle agitation protects shear-sensitive cells while maintaining homogeneous conditions.
- ***High Volumetric Flow:*** Moves a large volume of liquid with minimal energy, ensuring efficient bulk mixing and preventing stagnation zones.
- ***Supports Scale-Up:*** Axial impellers maintain uniform aeration and agitation in tall or large bioreactors, critical for industrial fermentation.

24. How process kinetics of microbial growth can be assessed?

Assessment of Process Kinetics of Microbial Growth:

- ***Batch Culture Experiments:*** Monitor biomass, substrate, and product concentrations over time to calculate specific growth rate (μ) and yield coefficients.
- ***Continuous Culture (Chemostat/Turbidostat):*** Maintain steady-state conditions to determine growth parameters under constant nutrient and environmental conditions.
- ***Mathematical Modeling:*** Apply Monod or other kinetic models relating substrate concentration to specific growth rate.
- ***Measurement Techniques:*** Use optical density, dry weight, viable counts, or metabolite analysis to quantify growth.
- ***Determination of Kinetic Constants:*** Calculate μ_{max}, K_s (substrate saturation constant), Y_x/s (biomass yield), and product formation rates.
- ***Data Fitting and Simulation:*** Compare experimental data with models to predict growth, substrate consumption, and product formation under various process conditions.

25. Explain role of dx/dt in the fed batch culture.

In fed-batch culture, dx/dt represents the rate of change of biomass concentration with time. Its role is to quantify microbial growth as cells consume the added substrate. By monitoring dx/dt, engineers can:

- Determine how feeding rates affect biomass accumulation.
- Predict the growth phase and metabolic activity of the culture.
- Optimize substrate addition to maximize cell density or product formation while avoiding substrate inhibition.
- Integrate into kinetic models to control volume changes, nutrient supply, and product yield in fed-batch systems.

LMS Handling

by Sir Mughal

Reliable • Professional • Result-Oriented

Our Key Features

- Timely Activities
- Expert Team Members
- Maximum Quiz Scores
- Assignment Solutions by *Subject-Oriented Experts*
- Paid Project Handling
- Guaranteed Results
- Market Competitive Rates

Contact Us

0313-2310940

shahzeb.zoologist04@gmail.com