BT513 – PRINCIPLES OF BIOCHEMICAL ENGINEERING

FINAL TERM MOST IMPORTANT QUESTIONS

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GROUP LINK: https://chat.whatsapp.com/BjC6W0jZx8o9yaC2ISh1Gu

1. Why we use stainless steel of3-4% Molybdenum in a fermenter for citric acid fermentation?

Purpose of Using Stainless Steel with 3–4% Molybdenum in Citric Acid Fermentation

1. Corrosion Resistance in Acidic Conditions:

Citric acid fermentation creates a highly acidic environment. Molybdenum-containing stainless steel, such as grade 316 or 316L, offers superior resistance to corrosion compared to standard stainless steel (like 304). This is especially important because citric acid can aggressively corrode metal surfaces over time.

2. Resistance to Pitting and Crevice Corrosion

Molybdenum improves resistance to localized corrosion, such as pitting and crevice attack. These types of corrosion are common in fermentation vessels due to the presence of acid, heat, and sometimes chloride ions.

3. Prevention of Metal Contamination

Using corrosion-resistant steel prevents the leaching of metal ions like iron or chromium into the fermentation medium. This is important because such contamination can negatively affect the growth of microorganisms (e.g. *Aspergillus niger*) and reduce citric acid yield.

4. Durability and Hygiene

Fermenters need to be durable and easy to clean to maintain sterile conditions. Molybdenum-enhanced stainless steel maintains its surface quality over time, reducing maintenance needs and supporting clean-in-place (CIP) systems.

2. Write factors that are used to consider for the recovery of the fermented product.

Factors to Consider for the Recovery of the Fermented Product

Recovery of a fermented product involves separating and purifying the desired compound from the fermentation broth. The following factors are important when designing or selecting a recovery process:

1. Nature of the Product:

Whether the product is intracellular (inside the cells) or extracellular (secreted into the medium) greatly influences the recovery steps. Solubility, stability, volatility, and sensitivity to heat or pH are also important.

2. Product Concentration

Low concentrations require more extensive purification and concentration steps, which affect process cost and efficiency.

3. Fermentation Broth Composition

The broth may contain cells, metabolites, nutrients, and by-products. These components must be removed or separated during recovery. The presence of similar compounds can complicate purification.

4. Cell Removal

Methods like centrifugation, filtration, or sedimentation are chosen based on the size and density of microbial cells. Efficient removal of biomass is essential before downstream purification.

5. Purity Requirements

The final application (e.g., pharmaceutical, food, industrial) determines how pure the product needs to be. Higher purity typically requires more steps and higher costs.

6. Product Stability

Some products degrade quickly if not recovered promptly or if exposed to heat, light, or oxygen. Stabilization methods (cooling, pH adjustment) may be needed during processing.

7. Economic Feasibility

The cost of recovery should not exceed the product's market value. Recovery methods must be scalable and cost-effective for industrial production.

8. Environmental and Regulatory Concerns

Use of hazardous solvents or energy-intensive processes must comply with environmental regulations. Waste treatment and disposal are also considered.

9. Downstream Processing Compatibility

Recovery methods should integrate smoothly with purification, concentration, and formulation steps that follow.

3. Give the difference between activation energy for the spore destruction and the activation energy for nutrient degradation.

The activation energy for spore destruction is significantly higher than that for nutrient degradation. Spore destruction typically requires activation energies in the range of 250 to 450 kJ/mol, while nutrient degradation processes, such as the breakdown of vitamins, proteins, or sugars, usually involve activation energies between 40 to 150 kJ/mol. This means that destroying bacterial or fungal spores requires much more energy and higher temperatures compared to degrading nutrients. As a result, spore destruction is highly temperature-sensitive, with the rate of inactivation increasing rapidly at elevated temperatures. In contrast, nutrient degradation is less sensitive to temperature changes and can begin to occur at relatively lower energy levels. This difference is important in food and pharmaceutical processing, where it is necessary to apply enough heat to ensure microbial safety without excessively damaging the nutritional or functional quality of the product.

4. Explain the Kinetic description of the bacterial death through heat sterilization.

Kinetic Description of Bacterial Death Through Heat Sterilization

The bacterial death during heat sterilization follows first-order reaction kinetics, meaning the rate of microbial death is directly proportional to the number of viable microorganisms present at any given time. This process can be described using several key kinetic parameters:

1. First-Order Kinetics

The rate of bacterial inactivation can be expressed as: $dN/dt = -kN$

Where:

- N = number of surviving microorganisms at time t
- k = death rate constant
- t = time

By integrating this equation, we get: $\log(N/N_0) = -kt / 2.303$

Where N_0 is the initial number of microorganisms. This shows that the number of living cells decreases exponentially over time during heating.

2. Decimal Reduction Time (D-value)

The D-value is the time required at a specific temperature to reduce the microbial population by **90%** (or by one log cycle). It is a practical measure of microbial resistance to heat. A smaller D-value indicates faster death at that temperature.

3. Z-value

The Z-value represents the temperature change needed to reduce the D-value by one log cycle. It shows how sensitive the microorganism is to changes in temperature. A lower Z-value means greater sensitivity.

4. Thermal Death Time (TDT)

Thermal death time is the total time required to kill a specific number of microorganisms at a given temperature. It depends on both the D-value and the number of organisms initially present.

5. Activation Energy (Ea)

The rate constant k increases with temperature according to the Arrhenius equation:

$$k = A \times e^{(-Ea/RT)}$$

Where Ea is the activation energy, R is the gas constant, and T is temperature in Kelvin. A high activation energy indicates that the death rate increases rapidly with temperature.

5. What is Effluent treatment? Give example.

Effluent treatment refers to the process of treating wastewater (effluent) generated from industrial, commercial, or domestic sources before it is released into the environment or reused. The goal is to remove harmful contaminants such as organic matter, chemicals, heavy metals, and pathogens that could damage ecosystems or pose health risks to humans and animals.

Effluent treatment typically involves several stages:

1. ***Preliminary Treatment*** – Removal of large solids or debris (e.g., through screening).
2. ***Primary Treatment*** – Sedimentation of suspended solids.
3. ***Secondary Treatment*** – Biological processes to degrade organic matter using microorganisms.
4. ***Tertiary Treatment*** – Advanced processes such as filtration, chemical treatment, or membrane technologies to remove fine particles, nutrients, or pathogens.
5. ***Disinfection*** – Killing of remaining pathogens, often using chlorine, ozone, or UV light.

Example:

In a sugar mill, large volumes of wastewater are generated containing high organic load, suspended solids, and sometimes toxic substances. Effluent from the mill is treated in an Effluent Treatment Plant (ETP)**,** where it undergoes sedimentation, biological treatment (using activated sludge), and final clarification before being safely discharged or reused for irrigation or cooling purposes.

6. Define interception.

Interception is the process by which precipitation (rain, snow, or dew) is caught and held by plant surfaces such as leaves, branches, and stems before it reaches the ground. The water that is intercepted may evaporate back into the atmosphere or slowly drip down to the soil. Interception plays an important role in the water cycle by temporarily storing rainfall, reducing soil erosion, and influencing the amount of water that actually infiltrates into the soil.

7. What is membrane pore filter and its importance?

A membrane pore filter is a thin, porous material that acts as a physical barrier to separate particles, microorganisms, or contaminants from a fluid (liquid or gas) based on size exclusion. The filter contains pores of a specific size that allow substances smaller than the pore diameter to pass through while retaining larger particles. Membrane filters are commonly made from materials like cellulose acetate, polycarbonate, or nylon and come in different pore sizes, typically ranging from 0.01 to 10 micrometers.

Importance of Membrane Pore Filters

1. ***Sterilization and Microbial Removal***
   Membrane filters with pore sizes of 0.22 micrometers or smaller are widely used to sterilize heat-sensitive liquids by removing bacteria and other microorganisms without using heat.
2. ***Particle Separation***
   They help remove suspended solids, debris, and other particulate matter from liquids in laboratory, pharmaceutical, food, and water treatment processes.
3. ***Sample Clarification***
   Before analysis or further processing, membrane filters clarify samples by removing impurities that might interfere with results or damage equipment.
4. ***Water Purification***
   Used in water treatment to remove pathogens and improve water quality for drinking or industrial use.

5. ***Precision and Consistency***
   Membrane filters provide a reliable and reproducible method of filtration with defined pore sizes, making them essential for quality control in many industries.

8. What are the requirements for designing fermenter?

Requirements for Designing a Fermenter

Designing a fermenter involves considering several important factors to ensure optimal microbial growth and product formation. The key requirements include:

1. ***Sterility:*** The fermenter must be designed to maintain sterile conditions throughout the fermentation process to prevent contamination by unwanted microorganisms. This involves sealed vessels, sterilizable components, and sterile inlet/outlet systems.

2. ***Material of Construction:*** The materials should be corrosion-resistant, durable, and non-toxic. Stainless steel (often with molybdenum) is commonly used because it withstands acidic conditions, is easy to clean, and resists corrosion.

3. ***Aeration and Oxygen Transfer:*** For aerobic fermentations, the fermenter must provide adequate aeration and oxygen transfer to meet microbial demands. This includes spargers or diffusers and agitation systems to disperse oxygen efficiently.

4. ***Agitation and Mixing:*** Proper mixing ensures uniform distribution of nutrients, temperature, and dissolved gases, and prevents sedimentation of cells. Agitators must be designed to provide sufficient mixing without damaging microorganisms.

5. ***Temperature Control:*** Microbial growth is sensitive to temperature; therefore, fermenters must have systems for heating and cooling to maintain optimal temperature throughout the process.

6. ***pH Control:*** Fermenters should allow for monitoring and controlling pH by adding acid or base to maintain conditions favorable for microbial activity.

7. ***Foam Control:*** Fermentation often produces foam; the fermenter must have mechanisms like antifoam addition or foam breakers to prevent overflow or operational issues.

8. ***Sampling and Monitoring Ports:*** Ports are necessary for aseptic sampling, addition of nutrients or inoculum, and insertion of sensors to monitor parameters like temperature, pH, dissolved oxygen, and pressure.

9. ***Volume and Scale:*** The size and shape of the fermenter should suit the scale of production, balancing space, cost, and operational efficiency.

10. ***Efficient Drainage and Cleaning:*** Design should allow for easy drainage, cleaning-in-place (CIP), and sterilization-in-place (SIP) to maintain hygiene and reduce downtime.

9. Write role of spiral heat exchanger.

Role of Spiral Heat Exchanger

A spiral heat exchanger is used to transfer heat efficiently between two fluids while handling fluids with suspended solids or viscous properties. Its key roles include:

1. ***Efficient Heat Transfer***
   The spiral design creates a high heat transfer area within a compact space, allowing effective heating or cooling of process fluids.
2. ***Handling Fouling Fluids***
   Because of its smooth, continuous spiral channels, it resists clogging and fouling better than conventional heat exchangers, making it ideal for fluids containing solids or sludge.
3. ***Self-Cleaning Effect***
   The spiral flow path induces turbulence, which helps minimize the buildup of deposits on the heat transfer surfaces.
4. ***Compact Design***
   Spiral heat exchangers occupy less space and require less maintenance, making them suitable for industrial processes with space constraints.
5. ***Application in Fermentation and Waste Treatment***
   They are commonly used to control temperature in fermentation processes and to treat effluents where fluids are often thick or contain particulate matter.

10. Give reason for the use of double heat exchanger in cooling periods?

Reason for the Use of Double Heat Exchanger in Cooling Periods

Double heat exchangers are used during cooling periods to improve the efficiency and control of the cooling process. The main reasons include:

1. ***Better Temperature Control:*** Using two heat exchangers in series or parallel allows more precise control of the cooling rate, preventing sudden temperature drops that could harm the product or process.

2. ***Improved Heat Transfer Efficiency:*** Double exchangers increase the total surface area for heat exchange, enabling faster and more effective cooling compared to a single exchanger.

3. ***Handling Variable Loads:*** During cooling, the load on the system can vary. Two heat exchangers provide flexibility to adjust cooling capacity according to changing process demands.

4. ***Redundancy and Reliability:*** If one exchanger requires cleaning or maintenance, the other can continue operating, reducing downtime and ensuring continuous cooling.

5. ***Stepwise Cooling:*** Some products or processes require gradual cooling to prevent thermal shock or maintain quality. Double heat exchangers allow staged cooling to meet these needs.

11. Why axial impeller are used?

Axial impellers are used in fermenters and mixing tanks because they provide efficient axial flow of the fluid, which means the liquid is pushed along the shaft axis, creating a top-to-bottom circulation. The main reasons for using axial impellers are:

1. ***Efficient Mixing and Circulation:*** Axial impellers generate strong flow along the vertical axis, ensuring good circulation of the broth and uniform distribution of nutrients, gases, and temperature throughout the fermenter.

2. ***Low Shear Stress:*** They produce relatively low shear forces compared to radial impellers, which is important when handling shear-sensitive microorganisms or cells to avoid damage.

3. ***Energy Efficiency:*** Axial impellers require less power for mixing large volumes of low-viscosity fluids, making them energy-efficient in many fermentation processes.

4. ***Good Gas Dispersion:*** They promote effective gas-liquid mass transfer, which is critical in aerobic fermentations where oxygen needs to be dispersed evenly.

12. Write effectiveness of heat sterilization.

Effectiveness of Heat Sterilization

The effectiveness of heat sterilization depends on several factors that determine how well microorganisms, including spores, are destroyed during the process. Key aspects influencing effectiveness include:

1. ***Temperature:*** Higher temperatures generally increase the rate of microbial death. There is a threshold temperature below which sterilization is ineffective.

2. ***Time:*** The duration of exposure to heat is critical. Sufficient time at the target temperature is needed to ensure complete destruction of all microorganisms.

3. ***Microbial Load and Type:*** The initial number and type of microorganisms affect sterilization. Spores and heat-resistant bacteria require higher temperatures or longer times compared to vegetative cells.

4. ***Moisture Content:*** Moist heat (steam) is more effective than dry heat because it penetrates cells better and denatures proteins more efficiently.

5. ***Heat Penetration and Distribution:*** Uniform and adequate heat distribution throughout the material or medium is necessary. Incomplete heating results in survival of microbes.

6. ***Physical and Chemical Environment:*** pH, presence of organic matter, and protective substances can affect heat penetration and microbial resistance.

13. Write environmental and economic advantages of by-products of sterilization.

Environmental Advantages:

1. ***Waste Reduction:*** By-products from sterilization processes can often be recycled or reused, reducing the amount of waste sent to landfills or discharged into the environment.

2. ***Resource Conservation:*** Using by-products minimizes the need for raw materials, conserving natural resources and reducing environmental impact.

3. ***Lower Pollution:*** Proper utilization of sterilization by-products can reduce harmful emissions and effluents, leading to cleaner air and water.

4. ***Energy Savings:*** Some by-products, like heat or steam recovered during sterilization, can be reused, reducing overall energy consumption.

Economic Advantages:

1. ***Cost Savings:*** Recycling or reusing by-products reduces disposal costs and the need to purchase new raw materials.
2. ***Additional Revenue:*** By-products can sometimes be sold or used as inputs for other processes, generating extra income.
3. ***Improved Process Efficiency:*** Using sterilization by-products effectively can optimize production cycles and reduce operational costs.
4. ***Compliance and Avoidance of Penalties:*** Proper management of by-products helps meet environmental regulations, avoiding fines and legal costs.

14. Explain Del factor.

The Del factor (also called the decimal reduction factor) is a term used in sterilization and microbial inactivation processes. It represents the time required at a specific temperature to reduce the microbial population by 90%**,** or by one logarithmic cycle (i.e., to kill 90% of the organisms present). In other words, the Del factor is the time needed to achieve a one-log reduction in the number of viable microorganisms under certain conditions.

Importance of Del Factor

- It helps quantify the efficiency of a sterilization or pasteurization process**.**
- It allows comparison of resistance among different microorganisms.
- It is used to design and validate heat treatment processes ensuring safety and product quality.

15. What agents are used in fermenter to control the pH?

In a fermenter, pH control is crucial for maintaining an optimal environment for microbial growth and product formation. The pH is typically managed by adding acidic or basic agents through automated systems. Here are the most commonly used agents:

1. Acidic Agents (to lower pH):

- ***Hydrochloric acid (HCl):*** Common, strong acid, fast-acting, inexpensive.
- ***Sulfuric acid (H_2SO_4):*** Also widely used, more cost-effective in bulk, but can lead to sulfate accumulation.
- ***Phosphoric acid (H_3PO_4):*** Less common; can be used when phosphate buffering is desired.

2. Alkaline Agents (to raise pH):

- ***Sodium hydroxide (NaOH):*** Most common base; strong, effective, and inexpensive.
- ***Potassium hydroxide (KOH):*** Alternative to NaOH, especially in potassium-based media.
- ***Ammonia (NH_3) or Ammonium hydroxide (NH_4OH):*** Used both as a pH control agent and a nitrogen source.

3. Buffer Systems (to stabilize pH):

While not actively controlling pH in the same way as acids/bases, buffers help resist pH fluctuations**:**

- Phosphate buffer (e.g., KH_2PO_4 / K_2HPO_4)
- Citrate buffer
- Tris buffer (less common in industrial fermentation)

Automated pH Control:

- Most fermenters use automated control systems with a pH probe and peristaltic pumps to add acid/base as needed.
- The system continuously monitors pH and adjusts it in real time.

16. What is agitator and how it is classified?

An agitator is a mechanical device used in tanks or fermenters to stir fluids and mixtures. In fermentation processes, its role is to maintain a uniform environment by mixing the contents. This ensures even distribution of nutrients, temperature, and gases like oxygen.

Why is an Agitator Important?

- Keeps cells or solids suspended
- Enhances oxygen transfer (especially in aerobic fermentation)
- Maintains uniform temperature
- Prevents formation of dead zones or gradients
- Helps in foam control by dispersing gases

How Are Agitators Classified?

Agitators can be classified in a few different ways:

1. By Impeller Design

- ***Rushton turbine:*** Has flat blades; produces strong mixing and gas dispersion, but high shear.
- ***Pitched blade turbine:*** Blades are angled; creates axial flow, used for moderate mixing.
- ***Propeller:*** Looks like a boat propeller; good for fast mixing in low-viscosity fluids.
- ***Anchor agitator:*** Blade touches the tank wall; used for viscous fluids to scrape and mix.
- ***Helical ribbon:*** A spiral-shaped agitator for very thick or paste-like materials.
- ***Paddle agitator:*** Flat paddles used for gentle mixing and heat transfer in thick fluids.

2. By Flow Pattern

- ***Axial flow:*** Moves fluid up and down along the shaft. Propellers and pitched blades fall into this category.
- ***Radial flow:*** Moves fluid outward from the shaft, like in Rushton turbines. Better for gas dispersion.
- ***Mixed flow:*** Combines axial and radial movement, offering a balance between mixing and dispersion.

3. By Application

- ***Low-viscosity liquids:*** Use propellers or pitched blades.
- ***High-viscosity fluids or slurries:*** Use anchor, ribbon, or paddle types.
- ***Gas–liquid systems (like aerobic fermentation):*** Use turbines like the Rushton impeller for efficient gas transfer.

17. What is the role of anti-foaming agents in foam formation?

Role of Anti-Foaming Agents in Foam Formation:

Anti-foaming agents do not contribute to foam formation—instead, they prevent or reduce foam in liquid systems like fermentation broths. Here's the direct role they play:

1. Prevent foam formation by reducing surface tension and interfering with the formation of stable bubbles.
2. Break down existing foam by penetrating and collapsing the foam film (bubble walls), causing bubbles to merge and burst.

3. Reduce foam stability by disrupting the stabilizing agents (like proteins or surfactants) present in the liquid.

18. What is the purpose of Freeze Drying?

Purpose of Freeze Drying (Lyophilization):

Freeze drying is a preservation method used to remove water from a product by freezing it and then reducing the surrounding pressure to allow the frozen water to sublimate (turn directly from ice to vapor).

Main Purposes:

1. ***Preservation of Biological Activity:***
    - Maintains the structure and function of sensitive biological materials like proteins, enzymes, vaccines, and probiotics.
    - Used in pharmaceuticals and biotechnology to preserve drugs, cultures, or biologics.
2. ***Long Shelf Life:***
    - Removal of moisture prevents microbial growth and chemical degradation, allowing products to be stored for long periods without refrigeration.
3. ***Stability and Storage:***
    - Products become stable at room temperature, making storage and transport easier and less expensive.
4. ***Ease of Rehydration:***
    - Freeze-dried materials can be quickly and easily rehydrated (e.g., powdered antibiotics or instant coffee), often returning close to their original state.
5. ***Weight Reduction:***
    - By removing water, freeze-dried products are much lighter, which is useful for shipping or space-saving.

Common Applications:

- Pharmaceuticals (e.g., vaccines, injectables)
- Food industry (e.g., instant meals, coffee, fruit snacks)
- Biotechnology (e.g., microbial cultures, enzymes)
- Cosmetics and nutraceuticals

19. What is impact of effluent composition and rate of flow in sewage works?

Impact of Effluent Composition and Flow Rate in Sewage Works:

The composition and rate of flow of effluent (wastewater) have a major influence on the performance and stability of sewage treatment plants.

1. Impact of Effluent Composition:

- ***Organic Load (BOD/COD):***
    - High biochemical oxygen demand (BOD) or chemical oxygen demand (COD) means more organic matter.
    - Overloads can overwhelm microbes, reduce treatment efficiency, and lead to foul odors or effluent quality failure**.**
- ***Toxic Substances:***
    - Heavy metals, chemicals, or detergents can inhibit microbial activity, causing process upset or even bioreactor collapse.

- ***Nutrient Balance (C:N:P ratio):***
    - Microorganisms require a balanced carbon:nitrogen:phosphorus ratio.
    - Imbalance can lead to poor biomass growth or excessive sludge production.
- ***pH and Temperature:***
    - Extremes in pH or sudden temperature changes can stress or kill microorganisms vital for biological treatment.

2. Impact of Flow Rate:

- ***Hydraulic Overloading:***
    - Too much flow can short-circuit treatment processes, reduce retention time, and bypass biological treatment, leading to poor effluent quality.
- ***Underloading:***
    - Too little flow may lead to dead zones, poor mixing, and inefficient microbial activity.
- ***Shock Loads:***
    - Sudden surges in flow or composition can destabilize biological systems, especially in activated sludge or biofilm reactors.
- ***Sludge Handling:***
    - Changes in flow affect sludge production rates, dewatering, and digestion efficiency.

20. What is CIP? Explain its role in sterilization and filtration system.

CIP stands for Clean-in-Place. It is an automated cleaning system used to clean the internal surfaces of equipment (like fermenters, pipelines, filters, and tanks) without disassembling them.

Purpose of CIP:

- To remove residual product, microbes, and contaminants from process equipment.
- To ensure hygienic conditions and prevent cross-contamination.
- To support compliance with regulatory standards in industries like pharmaceuticals, food, and biotech.

Steps in a Typical CIP Cycle:

1. ***Pre-rinse:*** Flushes out loose debris with water.
2. ***Detergent wash:*** Removes organic/inorganic residues using alkaline or acidic solutions.
3. ***Intermediate rinse:*** Clears out cleaning agents.
4. ***Disinfection or sterilization rinse (optional):*** Uses hot water, steam, or chemicals.
5. ***Final rinse:*** Uses purified water to remove all traces of chemicals.

Role of CIP in Sterilization Systems:

- Prepares equipment for sterilization by removing organic/inorganic material that could shield microbes.
- Ensures that sterilization methods (e.g., SIP – Steam-in-Place) are effective by providing a clean surface.
- Reduces bioburden (initial microbial load) before sterilization.
- Helps prevent contamination during sterile manufacturing (e.g., in aseptic pharma processes).

Role of CIP in Filtration Systems:

- Cleans filter housings and piping used in processes like water purification, media filtration, or sterile air supply.
- Removes fouling materials (proteins, particulates, biofilm) from membrane or cartridge filters.
- Maintains filter efficiency and prolongs filter life.
- Prevents microbial growth and product carryover between batches.

Without CIP:

- Manual cleaning would be required—time-consuming, labor-intensive, and riskier.
- Higher chance of product contamination and equipment downtime.

Disclaimer:

For best results, it is advised NOT to rely on this file only. Go through video lectures, handouts and other sources as well.

Regards,
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