BT612 – FERMENTATION TECHNOLOGY

MID TERM IMPORTANT QUESTIONS

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

1. What is differential media? Give on example.

Differential media are culture media designed to distinguish between different types of microorganisms based on their biochemical characteristics. They allow bacteria to grow but produce visible changes (like color changes, gas, or precipitate) that help identify specific species.

***Example*:** MacConkey Agar – Differentiates lactose-fermenting bacteria (pink colonies, e.g., *E. coli*) from non-lactose fermenters (colorless colonies, e.g., *Salmonella*).

2. Differentiate between constitutive and inducible enzymes.

Constitutive vs Inducible Enzymes

Constitutive enzymes are produced continuously by the cell, regardless of the presence of their substrate, because they are required for basic cellular metabolism. Examples include enzymes involved in glycolysis and DNA replication. In contrast, inducible enzymes are synthesized only when their specific substrate is present, allowing the cell to save energy by producing them only when needed. A common example is β-galactosidase, which is produced in the presence of lactose. Thus, constitutive enzymes are always available, while inducible enzymes are substrate-dependent.

3. What is the role of oil in fermenter?

Role of Oil in a Fermenter

Oil in a fermenter plays several important roles, particularly in microbial fermentation:

- ***Foam Control:*** During fermentation, gas bubbles can form foam on the surface. Oil acts as an antifoaming agent, reducing foam formation and preventing overflow.
- ***Oxygen Transfer Regulation:*** A thin layer of oil can help control oxygen transfer in aerobic fermentations, maintaining optimal dissolved oxygen levels.
- ***Protection of Microorganisms:*** Some oils provide a protective layer that reduces shear stress from agitation and protects delicate microorganisms.
- ***Nutrient Source:*** In certain fermentations, oils can also act as a carbon source or energy source for specific microbes.

Example**:** Vegetable oil or mineral oil is commonly used in large-scale fermenters to control excessive foam.

4. Differentiate between batch fermentation and continuous fermentation.

Batch Fermentation vs Continuous Fermentation

In **batch fermentation**, all ingredients are added at the start, and the process runs until the nutrients are exhausted. There is no addition or removal of medium during fermentation, so microbial growth passes through the typical phases of lag, log, and stationary. This method is simple to control and is used in products like yogurt and batch antibiotic production. In contrast, **continuous fermentation** involves the constant addition of fresh medium and simultaneous removal of product and cells. This allows the microbes to be maintained in a steady growth phase, usually the log phase, and produces a more consistent product yield over time. Continuous fermentation requires more complex control systems and is commonly used for industrial enzyme and alcohol production.

5. Write names of 5 recombinant products.

Five Recombinant Products

1. ***Insulin*** – for diabetes treatment
2. ***Human Growth Hormone (hGH)*** – for growth disorders
3. ***Erythropoietin (EPO)*** – to treat anemia
4. ***Interferons*** – for antiviral and anticancer therapy
5. ***Tissue Plasminogen Activator (tPA)*** – for dissolving blood clots

6. Write factors that influence the carbon source.

Factors That Influence the Choice of Carbon Source in Microbial Growth

The carbon source is a critical nutrient for microorganisms, providing energy and building blocks for growth. Several factors influence its selection:

- ***Type of Microorganism:*** Different microbes utilize different carbon sources efficiently. For example, *E. coli* prefers glucose, while some fungi utilize sucrose or maltose.
- ***Metabolic Pathway Efficiency:*** The carbon source should support high growth rate and desired product formation**.**
- ***Cost and Availability:*** Readily available and inexpensive sources (e.g., glucose, molasses, and starch) are preferred for industrial processes.
- ***Solubility and Transport:*** The carbon source should be soluble and easily transported into microbial cells.
- ***Effect on pH and Osmotic Pressure:*** Some carbon sources can alter medium pH or osmotic conditions, affecting microbial growth.
- ***Influence on Product Formation:*** Certain carbon sources may enhance or inhibit specific metabolite production, e.g., lactose for β-galactosidase production.

7. Enlist the name of fermentation scales.

Fermentation Scales

Fermentation is carried out at different scales depending on the purpose, ranging from research to industrial production. The main scales are:

1. ***Laboratory Scale*** – Small-scale fermenters used for research and experimentation.
2. ***Pilot Scale*** – Intermediate scale used to test processes before full industrial production.
3. ***Industrial Scale (Commercial Scale)*** – Large-scale fermenters for mass production of products like antibiotics, enzymes, and alcohol.
4. ***Bench Scale*** – Very small-scale setups used for initial trials and optimization of conditions.

8. What is selective media? Give one example.

Selective media are culture media designed to allow the growth of specific microorganisms while inhibiting the growth of others. This helps isolate a particular species from a mixed culture.

Example**:** MacConkey Agar – Inhibits the growth of Gram-positive bacteria while allowing Gram-negative bacteria like *E. coli* to grow.

9. What is importance of pH in fermentation?

Importance of pH in Fermentation

pH is a critical factor in fermentation because it directly affects microbial growth, enzyme activity, and product formation**.**

1. ***Microbial Growth*** – Most microorganisms have an optimal pH range; deviation can slow growth or kill cells.
2. ***Enzyme Activity*** – Enzymes involved in fermentation are sensitive to pH; wrong pH can reduce their efficiency.
3. ***Product Formation*** – The yield and quality of the product (like acids, alcohol, or enzymes) depend on maintaining the right pH.
4. ***Contamination Control*** – Maintaining a suitable pH can inhibit unwanted microbes that might spoil the fermentation.

Example: Yogurt fermentation requires a pH around 4.5 to 4.8 for proper coagulation and flavor development.

10. Write 5 important groups of fermentation.

Fermentation can be classified based on the type of end products or the microorganisms involved. The five important groups are:

1. ***Alcoholic Fermentation*** – Produces ethanol and carbon dioxide (e.g., *Saccharomyces cerevisiae* in beer and wine).
2. ***Lactic Acid Fermentation*** – Produces lactic acid (e.g., *Lactobacillus* in yogurt and cheese).
3. ***Acetic Acid Fermentation*** – Produces acetic acid (e.g., *Acetobacter* in vinegar).
4. ***Butyric Acid Fermentation*** – Produces butyric acid, hydrogen, and carbon dioxide (e.g., *Clostridium butyricum*).
5. ***Propionic Acid Fermentation*** – Produces propionic acid and carbon dioxide (e.g., *Propionibacterium* in Swiss cheese).

11. What is efficiency treaty of carbon sources?

Efficiency of Carbon Sources

The efficiency of a carbon source refers to how effectively a microorganism can utilize a particular carbon source for growth and product formation during fermentation. It is a measure of the conversion of the carbon source into biomass or desired metabolites**.**

1. High-efficiency carbon sources are easily metabolized, leading to rapid growth and high product yield (e.g., glucose).
2. Low-efficiency carbon sources are metabolized slowly or incompletely, producing less biomass or product (e.g., complex polysaccharides).
3. Efficiency depends on the microorganism's metabolic pathways, enzyme availability, and environmental conditions**.**
4. Choosing an efficient carbon source is important for cost-effective and productive fermentation processes**.**

12. Write types of fermentation on the base of state of mixing.

Types of Fermentation Based on State of Mixing

Fermentation can be classified according to the physical state and mixing of the substrate as follows:

1. ***Submerged Fermentation (SmF)***
   - Microorganisms grow in a liquid medium with continuous mixing and aeration.
   - Used for industrial production of enzymes, antibiotics, and alcohol.

2. ***Solid-State Fermentation (SSF)***
   - Microorganisms grow on a solid substrate without free-flowing water**.**
   - Used for production of enzymes, citric acid, and fermented foods like tempeh.

13. What are the micro and macro minerals that are used in fermentation process?

Minerals Used in Fermentation

Minerals are essential in fermentation because they act as cofactors for enzymes and support microbial growth and metabolism**.** They are classified as macrominerals and microminerals based on the quantity required.

Macrominerals (Required in Larger Amounts)

- ***Calcium (Ca^{2+})*** – Stabilizes cell walls and enzymes
- ***Magnesium (Mg^{2+})*** – Cofactor for many enzymes, important for ATP metabolism
- ***Potassium (K^{+})*** – Maintains osmotic balance and enzyme activity
- ***Phosphorus (P)*** – Component of nucleic acids, ATP, and phospholipids
- ***Sodium (Na^{+})*** – Osmotic balance and nutrient transport

Microminerals / Trace Elements (Required in Small Amounts)

- ***Iron (Fe^{2+}/Fe^{3+})*** – Electron transport and enzyme cofactor
- ***Zinc (Zn^{2+})*** – Cofactor for dehydrogenases and other enzymes
- ***Manganese (Mn^{2+})*** – Enzyme activation and antioxidant defense
- ***Copper (Cu^{2+})*** – Cofactor for oxidases
- ***Cobalt (Co^{2+})*** – Component of vitamin B12 and certain enzymes

14. What is bio fertilizer?

A biofertilizer is a substance containing living microorganisms that, when applied to seeds, soil, or plants, enhances nutrient availability and promotes plant growth. Unlike chemical fertilizers, biofertilizers improve soil fertility naturally by fixing atmospheric nitrogen, solubilizing phosphorus, or decomposing organic matter.

Examples are: ***Rhizobium*** – Nitrogen-fixing bacteria for legumes, ***Azotobacter*** – Free-living nitrogen-fixing bacteria, ***Azospirillum*** – Promotes root growth in cereals, ***Mycorrhizal fungi*** – Enhance nutrient and water uptake, ***Phosphate-solubilizing bacteria*** – Convert insoluble phosphorus into a form usable by plants.

15. Enlist the types of fermentation on the basis of process.

Types of Fermentation Based on Process

Fermentation can be classified according to the nature of the process and conditions used**:**

1. ***Batch Fermentation*** – All nutrients are added at the beginning, and the process runs until substrates are exhausted.
2. ***Fed-Batch Fermentation*** – Nutrients are added gradually during fermentation without removing the culture.
3. ***Continuous Fermentation*** – Fresh medium is continuously added, and product along with cells is continuously removed to maintain steady-state operation.
4. ***Semi-Continuous Fermentation*** – Part of the culture is periodically removed and replaced with fresh medium.
5. ***Solid-State Fermentation (SSF)*** – Microorganisms grow on solid substrates without free-flowing water.
6. ***Submerged Fermentation (SmF)*** – Microorganisms grow in a liquid medium with continuous mixing and aeration.

16. Write advantages of oil and fats in fermentation.

Advantages of Oil and Fats in Fermentation

Oils and fats play important roles in fermentation processes, particularly in controlling the environment and supporting microbial growth. Key advantages include:

1. ***Foam Control*** – Oils act as antifoaming agents, reducing excessive foam formation during fermentation and preventing overflow.
2. ***Protection of Microorganisms*** – Fats provide a protective layer that reduces shear stress from agitation, helping delicate microbes survive.
3. ***Nutrient Source*** – Certain microbes can use oils and fats as carbon or energy sources for growth and metabolite production.
4. ***Improved Oxygen Transfer*** – A thin oil layer can help regulate oxygen levels in aerobic fermentations.
5. ***Enhanced Product Yield*** – Oils can stabilize the medium and enhance microbial activity, contributing to better fermentation efficiency.

17. Why we do mixing?

Mixing in fermentation is essential to ensure uniform conditions throughout the fermenter. The main reasons are:

1. ***Uniform Distribution of Nutrients*** – Ensures all microorganisms have equal access to carbon, nitrogen, and minerals.
2. ***Even Temperature*** – Prevents hot or cold spots, maintaining optimal microbial growth conditions.
3. ***Proper Oxygen Supply*** – In aerobic fermentation, mixing improves dissolved oxygen transfer to microorganisms.
4. ***Prevention of Sedimentation*** – Keeps cells and solids suspended, avoiding clumping or settling.
5. ***Enhanced Product Formation*** – Uniform conditions optimize enzyme activity and metabolite production**.**

18. Name types of fermentation on the basis of moisture/water.

Types of Fermentation Based on Moisture/Water Content

Fermentation can be classified according to the moisture content of the substrate:

1. **Submerged Fermentation (SmF)**
   - Uses a liquid medium with high water content.
   - Microorganisms grow while fully immersed in the liquid.
   - Common for industrial enzymes, antibiotics, and alcohol production.

2. **Solid-State Fermentation (SSF)**
   - Uses a solid substrate with low moisture content.
   - Microorganisms grow on the surface or within the solid material.
   - Used for enzymes, citric acid, tempeh, and other fermented foods.

19. What is influence source of carbon source in fermentation?

Influence of Carbon Source in Fermentation

The carbon source in fermentation provides energy and building blocks for microbial growth and product formation. Its nature and quality strongly influence the fermentation process in several ways:

1. ***Microbial Growth*** – Efficient carbon sources support rapid growth of microorganisms, while less favorable sources may slow growth.
2. ***Product Yield*** – The type of carbon source affects the quantity and quality of the desired product (e.g., glucose favors alcohol production in yeast).
3. ***Metabolic Pathways*** – Different carbon sources can activate or repress specific metabolic pathways, influencing enzyme production and metabolite formation.
4. ***pH and Medium Conditions*** – Carbon metabolism can alter pH, osmotic pressure, and nutrient balance, affecting microbial activity.
5. ***Energy Efficiency*** – Easily metabolizable sources save microbial energy, while complex sources may require more energy to break down.

20. What are components of fermenter?

Components of a Fermenter

A fermenter is a specialized vessel used for microbial fermentation, and it includes several key components to control the growth environment:

1. ***Vessel/Body*** – The main container where the fermentation takes place, usually made of stainless steel or glass.
2. ***Agitator/Mixer*** – Ensures uniform distribution of nutrients, oxygen, and temperature by stirring the culture.
3. ***Aeration System*** – Supplies oxygen in aerobic fermentations, often through spargers or air diffusers.
4. ***Temperature Control System*** – Maintains optimal temperature using jackets, coils, or external heaters/coolers.
5. ***pH and Foam Control*** – Probes and additives (like antifoam agents) maintain proper pH and reduce foam.
6. ***Sampling Port*** – Allows collection of samples during fermentation for monitoring.
7. ***Nutrient Addition System*** – Enables addition of substrates or nutrients during fed-batch or continuous fermentation.
8. ***Pressure and Safety Valves*** – Prevent overpressure and ensure safe operation.
9. ***Sensors and Control Systems*** – Monitor pH, temperature, dissolved oxygen, and agitation speed for automated control.

21. What are inorganic nitrogen? What are their uses?

Inorganic nitrogen refers to nitrogen compounds that do not contain carbon, which are directly available for microbial or plant use. They are commonly used as nutrient sources in fermentation and agriculture**.**

Common Inorganic Nitrogen Sources

- ***Ammonium salts:*** Ammonium sulfate ($(NH^4)_2SO^4$), Ammonium chloride (NH_4Cl)
- ***Nitrates:*** Sodium nitrate ($NaNO_3$), Potassium nitrate (KNO_3)
- ***Urea:*** $CO(NH_2)_2$

Uses of Inorganic Nitrogen

1. ***Microbial Growth in Fermentation*** – Provides nitrogen for protein and nucleic acid synthesis in bacteria, yeast, and fungi.
2. ***Plant Nutrition*** – Used in fertilizers to enhance plant growth and crop yield.
3. ***Industrial Fermentation*** – Supports production of enzymes, antibiotics, amino acids, and organic acids**.**
4. ***pH Buffering*** – Some inorganic nitrogen salts help maintain stable pH in fermentation media.

22. Write name of 3 chelating agents.

Three Common Chelating Agents

1. ***EDTA (Ethylenediaminetetraacetic acid)*** – Binds metal ions and prevents them from precipitating.
2. ***Citric Acid*** – Acts as a natural chelator for calcium, magnesium, and iron ions.
3. ***Oxalic Acid*** – Forms complexes with metal ions to keep them soluble in solution.

23. Write names of 5 industrial fermentations.

Five Examples of Industrial Fermentation

1. ***Alcohol Fermentation*** – Production of ethanol using *Saccharomyces cerevisiae* (beer, wine, biofuel).
2. ***Lactic Acid Fermentation*** – Production of lactic acid using *Lactobacillus* (yogurt, cheese, sourdough).
3. ***Acetic Acid Fermentation*** – Production of acetic acid using *Acetobacter* (vinegar).
4. ***Antibiotic Production*** – Production of antibiotics like penicillin using *Penicillium* species.
5. ***Enzyme Production*** – Industrial enzymes like amylases, proteases, and cellulases using bacteria or fungi.

24. Write uses of water in industrial scale.

Uses of Water in Industrial-Scale Fermentation

Water plays a crucial role in industrial fermentation for various purposes:

1. ***Solvent for Media Components*** – Dissolves nutrients, salts, and carbon/nitrogen sources to prepare the fermentation medium.
2. ***Temperature Control*** – Used in cooling jackets or coils to maintain optimal fermentation temperature.
3. ***Cleaning and Sterilization*** – Steam or hot water is used to sterilize fermenters, pipelines, and equipment.
4. ***Dissolved Oxygen Supply*** – In aerobic fermentation, water acts as a medium for oxygen transfer to microorganisms.
5. ***Dilution and pH Control*** – Water helps in adjusting concentration of substrates and maintaining proper pH.

25. What is shake flask fermentation?

Shake Flask Fermentation – Definition

Shake flask fermentation is a small-scale, laboratory method of cultivating microorganisms in a liquid medium using flasks placed on a shaking platform. The shaking provides aeration and mixing, allowing microorganisms to grow efficiently under controlled conditions.

Key Features:

1. ***Small-scale cultivation*** – Used for research, strain screening, and media optimization.
2. ***Aeration by shaking*** – Ensures proper oxygen transfer for aerobic microbes.
3. ***Monitoring growth*** – Easy to sample and measure microbial growth, metabolite production, or enzyme activity.
4. ***Cost-effective*** – Simple setup without complex fermenter equipment.

Applications:

- Screening microbial strains
- Preliminary testing of fermentation conditions
- Production of small amounts of enzymes or metabolites for lab studies

26. What are 3 examples of bioweapons?

Bioweapons use disease-causing microbes (bacteria, viruses, fungi) or toxins (like ricin, botulinum) to harm humans, animals, or plants, with historical examples including anthrax, plague, smallpox, and tularemia (bacteria/viruses) and toxins like ricin, often spread via aerosols or contaminated food/water. Key examples of agents weaponized include *Bacillus anthracis* (anthrax), *Yersinia pestis* (plague), *Variola major* (smallpox), *Francisella tularensis* (tularemia), botulinum toxin, and hemorrhagic fever viruses (Ebola, Marburg).

27. Write importance of enrichment culture.

Importance of Enrichment Culture

Enrichment culture is a technique used to favor the growth of specific microorganisms from a mixed population while inhibiting others. Its importance includes:

1. ***Isolation of Specific Microbes*** – Helps in selectively growing microorganisms with desired metabolic traits.
2. ***Studying Rare or Slow-Growing Organisms*** – Allows detection of microbes present in low numbers in natural samples.
3. ***Industrial Applications*** – Useful for obtaining strains that produce enzymes, antibiotics, or metabolites.
4. ***Environmental and Research Studies*** – Helps in studying microbial diversity and ecological functions.
5. ***Improved Yield*** – Increases the proportion of the desired microorganism for further experimentation or production.

28. What is submerged fermentation?

Submerged Fermentation (SmF) – Definition

Submerged fermentation is a type of fermentation in which microorganisms grow in a liquid medium with sufficient water content. The liquid allows for uniform nutrient distribution, aeration, and temperature control, making it suitable for large-scale industrial processes.

Key Features:

1. ***Liquid medium*** – Microbes are fully immersed in the nutrient solution.
2. ***Aeration and Mixing*** – Agitation ensures proper oxygen supply for aerobic microorganisms.
3. ***Industrial Use*** – Commonly used for producing enzymes, antibiotics, organic acids, and alcohol.
4. ***Control*** – Easy to monitor and control pH, temperature, and dissolved oxygen.