MICROBIOLOGY

UNIT 5 NOTES

MICROBIAL SPOILAGE

- TYPES OF SPOILAGE
- FACTORS AFFECTING SPOILAGE
- SOURCES AND TYPES
- PRESERVATION OF PHARMACEUTICAL PRODUCTS

ANIMAL CELL CULTURE

- GROWTH
- GENERAL PROCEDURE
- APPLICATION OF CELL CULTURE

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SPOILAGE

- Spoilage refers to the process by which a material, substance or product becomes unusable or unfit for its intended purpose
- Now, Pharmaceutical Spoilage refers to the degradation or deterioration of pharmaceutical product over time, leading to decrease in their effectiveness, safety and quality.
- Spoilage often results in economical loss and poses potential health or safety risks, especially in Food & Pharmaceutical products.

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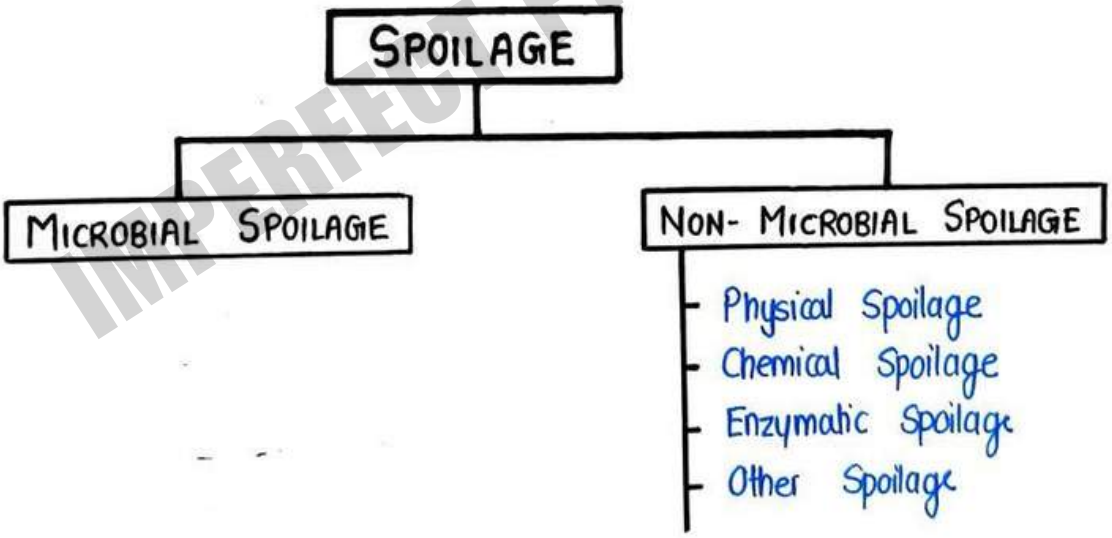
TYPES OF SPOILAGE



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① Microbial Spoilage

- Microbial Spoilage refers to degradation of food, beverages or Pharmaceutical Products caused by growth and metabolic activity of microorganisms such as bacteria, moulds or yeasts.
- These microorganisms break down the components of the product. leading to changes in texture, color, smell, taste and safety. often rendering the item unsuitable for consumption or use.
- Example: Moldy Bread caused by Fungi

② Non - Microbial Spoilage

- Non - Microbial Spoilage refers to degradation of food or products caused by Physical, chemical or environmental factors, rather than by the activity of microorganisms.
- This type of spoilage often results in changes to texture, appearance, flavour or nutritional quality and can render items unsuitable for consumption or use.
- It can be subdivided into following types:

ⓐ Physical Spoilage
ⓑ Chemical Spoilage
ⓒ Enzymatic Spoilage
ⓓ Other Spoilage

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ⓐ Physical Spoilage

- Physical Spoilage refers to degradation of food or pharmaceutical products caused by physical factors such as mechanical damage, improper storage or environmental conditions
- Physical spoilage does not involve microorganisms but can make products more susceptible to microbial contamination.
- Example: Damage to packaging allows exposure to air, moisture or contaminants.

ⓑ Chemical Spoilage

- Chemical Spoilage refers to the deterioration of food, beverages or pharmaceutical products caused by chemical reactions.
- It often includes oxidation, hydrolysis of products.
- Rancidity in oils, discoloration of potatoes or bananas after cutting are some common examples of Chemical Spoilage.
- Chemical Spoilage often lead to undesirable changes in color, often making the product unfit for consumption or use.

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ⓒ Enzymatic Spoilage

- Enzymatic Spoilage refers to the degradation of food caused by the natural activity of enzymes present in food itself.
- Enzymes are biological catalysts that regulate various biochemical reactions, but their activity can lead to undesirable changes in food quality, such as texture, flavour, color or nutritional value.
- Example: Breakdown of Protein by Protease enzyme.

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④ Others Spoilage

- Spoilage by other factors involves external or environmental agents that indirectly affect the quality, safety or usability of a product.
- It often occurs due to insects, rodents, birds and other animals resulting change in color, odour & chemical nature.

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FACTORS AFFECTING MICROBIAL SPOILAGE

There are various factors which can affect Microbial Spoilage of Pharmaceutical Products such as :

- Types & size of Microbe.
- Nutritional Factors
- Water
- Storage Temperature
- pH
- Package Designing
- Storage Conditions

① Types & Size Of Microbe

- Types & size of microbe significantly affect the microbial spoilage.
- Different types of microorganisms such as bacteria, mold, yeast have different mechanism of action in spoilage.
- Molds such as Aspergilus & Penicillium can grow on surfaces & produce mycotoxins, which can be toxic to humans and animals.
- Smaller microbes, like bacteria and viruses, can penetrate deeper into food tissues, causing more extensive spoilage.
- Larger microbes like molds & yeasts, tend to grow on surfaces, causing visible spoilage.

② Nutritional Factors

- Microorganisms requires nutrition for energy & proliferation.
- The presence of nutrients like carbohydrates, proteins and vitamins in the formulation can support microbial growth.

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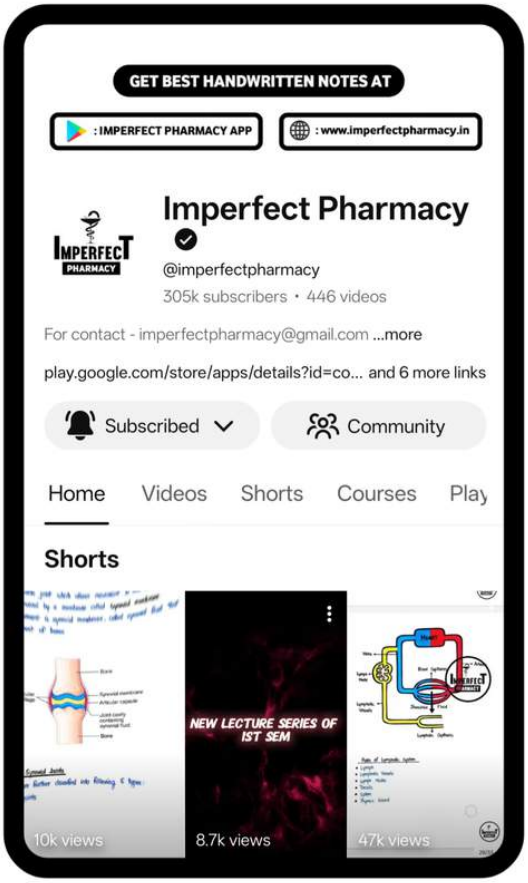

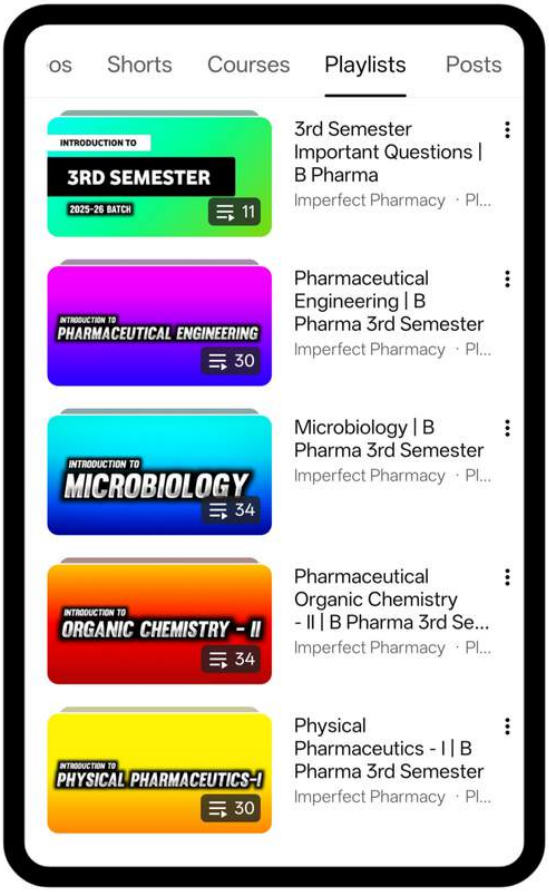

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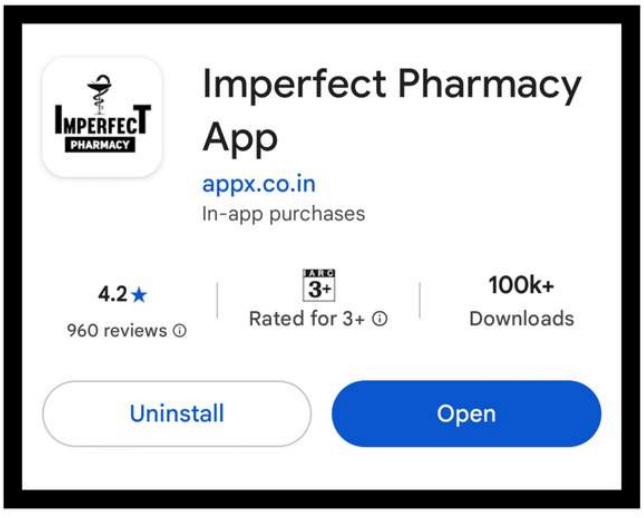

③ Water

- Microbial growth, especially bacteria and fungi, is favored in products with higher water content.
- Creams, ointments and liquid formulations are more prone to microbial contamination than dry powders or tablets.

④ Storage Temperature

- The formulations can be affected by microorganisms b/w the temperature range of 20°C to 60°C
- Storage in deep freeze at -20°C is preferred to preserve the preparation from Microorganisms.
- Water for Injection is stores at 80°C before preparation to minimize bacterial growth and to prevent bacterial activity.

⑤ pH

- Microorganisms have specific pH ranges in which they grow best.
- Many bacteria grow at neutral pH, while fungi may grow in more acidic solutions.
- Extremes of pH generally prevent microbial attack.

⑥ Packaging Design

- Improper packaging, such as cracks or leaks in packaging, can expose pharmaceutical products to environmental contaminants, leading to microbial spoilage.
- Multi dose containers are generally more susceptible for microbial contamination.

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⑦ Storage Conditions

- Improper storage conditions such as high humidity or temperature fluctuations, can promote microbial growth.
- Oxygen exposure can contribute to both chemical degradation and microbial contamination, especially in products stored without proper seals.

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SOURCES OF MICROBIAL CONTAMINATION

There can be various sources of Microbial Contamination in pharmaceutical products as follows :

- Raw Material
- Airborne Particles
- Personnel
- Equipment
- Water Systems
- Aseptic Processing
- Storage Conditions

① Raw Material

- Raw Materials like active pharmaceutical ingredients, excipients can attract microorganisms if not properly sourced, stored or handled.
- Contamination can occur during the transfer, weighing and mixing of raw materials.

② Airborne Particles

- Dust, spores and other microorganisms can be present in the air and settle on exposed surfaces or products.

③ Personnel

- Human skin, hair and respiratory tract can act as a source of microorganisms, especially in non-sterile environments.

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④ Equipment

- Manufacturing Equipments, if not properly cleaned and sanitized, can play a major role in contamination of Pharmaceuticals.

⑤ Water Systems

- Contaminated water used for cleaning, rinsing or product formulation can introduce microorganisms.

⑥ Aseptic Processing

- Improper aseptic techniques during the filling & sealing of sterile products can introduce microorganisms.

⑦ Storage Conditions

- Improper storage conditions, such as high humidity or temperature fluctuations, can promote microbial growth.

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TYPES OF MICROBIAL CONTAMINANTS

Microbial Spoilage in pharmaceuticals, food and other products is caused by various types of microorganisms, each with different characteristics and effects.

① BACTERIA

- They are single-celled microorganisms that reproduce quickly under favorable conditions.
- Some most common bacterial contaminants are as follows:
- Pseudomonas spp: Frequently found in water and aqueous pharmaceutical formulations, causing spoilage.
- Klebsiella spp: They can grow in oral and topical products, leading to spoilage and contamination.
- Staphylococcus spp: They can contaminate products through human handling and poor hygiene.

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② FUNGI

- They are multicellular molds and unicellular yeasts that grow in warm, moist and nutrient rich environments.
- They are capable of growing in low pH & low water activity.

③ SPORE FORMING MICROORGANISMS

- They produce spores that are highly resistant to heat and disinfectants.
- They can survive in adverse conditions and germinate when favorable conditions return.

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④ ALGAE

- They are photosynthetic microorganisms found in water systems used during manufacturing.
- They are rare but possible contaminants in Pharmaceutical water systems or liquid formulations.

⑤ PROTOZOA

- They are single-celled organisms that are less common in spoilage but can contaminate water systems.

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Assessment Of Microbial Contamination & Spoilage

- Assessing microbial contamination & spoilage in Pharmaceutical products is crucial to ensure their safety, efficacy & shelf life.
- Various methods are employed to detect and quantify microbial presence as follows :

① Physical Or Chemical Indicators

- Changes in product's physical or chemical properties can signal microbial spoilage.
- Indicators include alterations in viscosity, pH, emulsion stability and surface activity.

② Microbial Limit Test

- They are designed to enumerate and identify microorganisms in non-sterile products, ensuring they meet acceptable microbiological standards.
- These tests typically involve :

ⓐ Total Aerobic Microbial Count
ⓑ Total Yeast & Mold Count
ⓒ Specified Microorganisms Testing

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③ STERILITY TESTING

- For products required to be sterile, such as Injectables and Ophthalmic solutions, sterility test confirms the absence of viable microorganisms.
- It mainly involve two methods :

ⓐ Direct Inoculation
ⓑ Membrane Filtration.

④ ENVIRONMENTAL MONITORING

- Regular monitoring of the manufacturing environment, including air, surfaces and personnel helps identifying potential sources of contamination.

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PRESERVATIVES

- Preservatives are chemical agents incorporated into Pharmaceutical formulations to prevent microbial contamination and proliferation during manufacturing, storage and use.
- Their primary role is to maintain the sterility, safety, efficacy and stability of the product by inhibiting or destroying microorganisms such as Bacteria, Fungi & Molds.
- Preservatives are especially critical in multi-dose formulations, aqueous preparations and products that are exposed to environment during use.

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IDEAL CHARACTERISTICS OF PRESERVATIVES

- It should have a broad spectrum Antimicrobial Activity.
- It should be Non-toxic to humans.
- It should be physically & chemically stable.
- It should not reacts with API, excipients or packaging material.
- It should be effective at low concentrations.
- It should be Non-volatile.
- It should have rapid action.
- It should be cost-effective & readily available.

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Types Of Preservatives

On the basis of chemical nature & mechanism of action, Preservatives can be classified as follows:

① Antimicrobial Preservatives

These are the preservatives that prevent the growth of bacteria, fungi and molds in pharmaceutical products by:

- Cell membrane disruption
- Protein denaturation
- Inhibition of nucleic acid synthesis

They can be further subdivided into following types:

ⓐ **Parabens:**
- Examples: Methylparaben, Propylparaben.
- They are effective against fungi & some bacteria
- They are commonly used in syrups, creams & ointments.

ⓑ **Organic Acids:**
- Examples: Benzoic Acid, Sorbic Acid etc.
- They are primarily effective in acidic formulations.

ⓒ **Alcohols:**
- Examples: Ethanol, Benzyl Alcohol etc.
- They have broad spectrum activity.
- They are common in oral, injectable & topical formulations.

ⓓ **Quaternary Ammonium Compounds:**
- Examples: Benzalkonium Chloride.
- They are effective against Gram-positive bacteria & Fungi.
- Common in Ophthalmic and nasal preparations.

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⑥ Phenolic Compounds :

- Examples : Phenol, Chlorocresol etc.
- They have broad spectrum antimicrobial action.
- They are used in creams, ointments and vaccines.

② ANTIOXIDANT PRESERVATIVES

- They prevent the pharmaceutical products from oxidative degradation, which can ultimately lead to microbial growth.
- Examples : Butylated Hydroxytoluene, Butylated Hydroxyanisole, Ascorbic Acid etc.
- They are commonly used in lipid- based formulations.

③ CHELATING AGENTS

- They bind with metal ions in formulation, preventing their use by microorganisms for growth and metabolism.
- Examples : Disodium Edetate (EDTA).
- They are often used in combination with other preservatives to enhance their efficacy.

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CLASSIFICATION BASED ON SOURCE

① **Natural Preservatives** : These preservatives are obtained by natural sources that are plant, mineral & animal sources etc. ex : Neem Oil, Lemon etc.

② **Artificial Preservatives** : They are prepare by chemical synthesis & active against various microorganisms at small concentration. ex : Benzoates, Nitrites.

FACTORS AFFECTING EFFICACY OF PRESERVATIVES

There are various factors which can affect the efficacy of Preservatives as follows :

- Type of Microorganism : Different microorganisms have varying levels of susceptibility to different preservatives.
- Concentration : Too low can be ineffective while too high could be toxic.
- pH : The pH of the product can significantly impact preservative efficacy, some preservatives are more effective at certain pH ranges.
- Incompatability : Certain excipients in the formulation can interact with preservatives, reducing their effectiveness.

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EVALUATION OF MICROBIAL STABILITY OF FORMULATIONS

- Ensuring the microbial stability of Pharmaceutical formulations is crucial to maintain their safety, efficacy and shelf life
- This process involves evaluating the formulation's ability to resist microbial contamination and proliferation over time.
- A key method for this assessment is the Preservative Efficacy Test (PET), also known as Antimicrobial Effectiveness Test

PRESERVATIVE EFFICACY TEST

A Preservative Efficacy Test, also known as an Antimicrobial Effectiveness Test, is a critical procedure used to evaluate the effectiveness of Preservatives in Pharmaceuticals, cosmetics and personal care products.

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Procedure

- Inoculation : The product is inoculated with specified concentrations of test microorganisms, including bacteria (Staphylococcus aureus, Escherichia Coli, Pseudomonas aeruginosa), yeast (Candida albicans) & mold (Aspergillus brasiliensis)
- Incubation : The inoculated product is stored under controlled conditions for a predetermined period, typically 28 days.

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- **Sampling** : At specific intervals (e.g., 7, 14 & 28 days) samples are taken to determine the number of viable microorganisms present.

- **Evaluation** : The reduction in microbial counts over time is analyzed to assess the effectiveness of preservative system.

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ANIMAL CELL CULTURE

- Animal Cell Culture is a technique where animal cells are grown and maintained under controlled laboratory conditions, allowing researcher to study cellular behaviours, functions and responses in a controlled environment.
- It is a fundamental technique in biological & medical research. involving the maintainance and growth of animal cells outside their natural environment under controlled conditions.

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KEY PRINCIPLES OF ANIMAL CELL CULTURE

① Asceptic Techniques

- Maintaining a sterile environment is crucial to prevent contamination from bacteria, fungi or other microorganisms that can compromise the culture.

② Culture Medium

- The culture medium provides the essential nutrients, growth factors and physical support for cell growth
- It typically includes :
  - Basal Medium
  - Serum
  - Antibiotics

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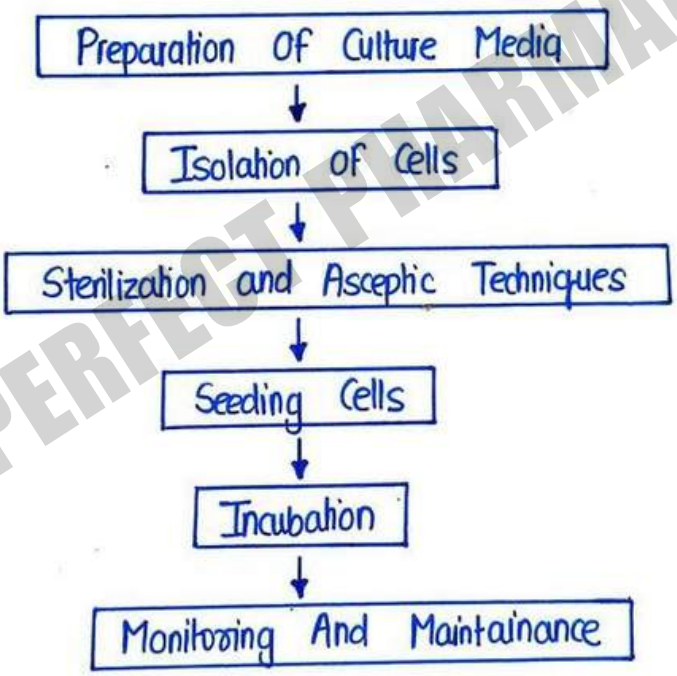
③ Growth Factors

- These proteins stimulate cell proliferation and differentiation
- Common growth factors include :
  - Temperature : Typically 37°C
  - pH : Around 7.4
  - Humidity : Should be high

GENERAL PROCEDURE FOR CELL CULTURE



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Types Of Cell Culture

Cell Cultures can be classified as follows :

① Primary Cell Culture
- Adherent Cell Culture
- Suspension Cell Culture

② Secondary Cell Culture

③ Cell Lines
- Finite Cell Lines
- Continuous Cell Lines

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① Primary Cell Culture

- Primary Cell Culture refers to the process of isolating cells directly from living tissues (e.g. organ, blood etc) and growing them in a controlled artificial environment, such as culture disk.
- These cells retains many of the characteristics and functions of their tissues of origin, making them highly valuable for biological & medical research.
- It is further subdivided into two types :

ⓐ Adherent Cell Culture

ⓑ Suspension Cell Culture

ⓐ **Adherent Cell Culture** :
- These cells attached to the surface of the culture flask.
- They require regular removal of old media and replacement with fresh media to maintain proper growth.

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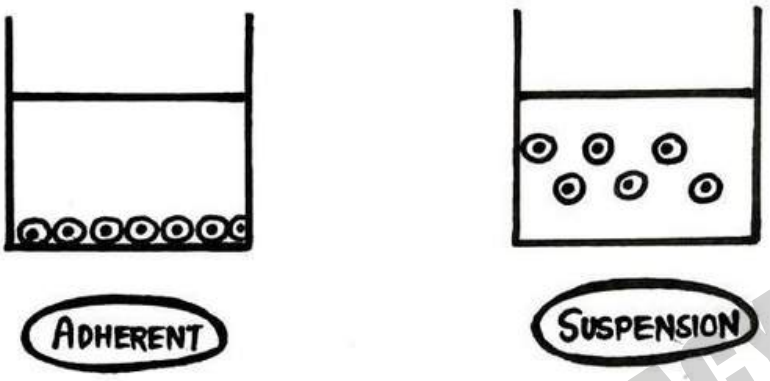
ⓑ Suspension Cell Culture :
- These cells does not get attached to the surface of culture flask.
- They are freely suspended in culture medium.



② SECONDARY CELL CULTURE

- Secondary cell culture happens when cells from a primary culture are moved to a new container with fresh nutrients to keep growing.
- This is done after the cells have grown enough in the first dish and need more space or better conditions.
- It helps remove unwanted cells, making the culture more uniform and easier to work for experiments or studies.

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③ CELL LINES

- Cell lines are animal cultures that can be propagated & maintained in vitro under controlled conditions.
- These cells are derived from primary cell cultures and can either have a finite or continuous lifespan depending on their origin & genetic stability.
- They are further subdivided into two types.

ⓐ Finite Cell lines
ⓑ Continuous Cell lines.

ⓐ **Finite Cell Lines** :
- Cell lines that have a limited lifespan and can divide only a finite number of times.
- They are generally used for studies requiring cells with normal characteristics and limited genetic alterations.

ⓑ **Continuous Cell Lines** :
- Cell lines that have undergo transformation (either spontaneous or induced) and can divide indefinitely
- They are used extensively in cancer research, recombinant protein production and vaccine development.

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APPLICATION OF CELL CULTURE

- Cell culture involves growing cells in a controlled environment outside their natural setting.
- This technique is essential in the pharmaceutical industry and research for several reasons.

① Drug Testing

- Before new drugs are given to humans, scientist test them on cultured cells to see if they work & to check for harmful effects.
- This helps to ensure safety and efficacy of the products / drugs.

② Vaccine Development

- To make vaccines, certain viruses need to be grown in large amounts.
- Cultured cells provide a place for these viruses to multiply, which is crucial for producing vaccines against disease like flu & polio.

③ Studying Disease

- Researchers use cell cultures to understand how disease affect cells.
- By observing infected cells, they can learn how disease develop and find ways to treat them.

④ Producing Import Proteins

- Some medical treatments require specific proteins.
- Scientist can utilize cultured cells to produce these proteins in large quantities, which is important for creating therapies like Insulin for diabetes.

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⑤ Reducing Animal Testing

- By using cell cultures, researchers can conduct many experiments without involving animals.
- This approach is more ethical and often provides quicker results.

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