ISSN PRINT 2319 1775 Online 2320 7876

Research paper UGC CARE Listed (Group -I) Journal Volume 11,S Iss 3, Dec 2022

A Summary of The Use of Microbial Cell Culture

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ABSTRACT

Microbial cultures, also known as microbiological cultures, are used to cultivate microorganisms in a controlled laboratory setting. Microbial cultures are essential diagnostic tools in molecular biology. Microbial cultures identify species, abundance, or both. One of the basic microbiological diagnostic methods used to diagnose infectious disease is letting the agent multiply in a specified medium. Microbial medium contains inorganic salts, carbohydrates, amino acids, vitamins, fatty acids and lipids, proteins and peptides, serum, and trace elements. Culture media can also be put into three groups based on how they feel: liquid (broth), solid, and solid. Like synthetic and non-synthetic media, there are three types of microbial cell culture media: general purpose, enrichment, and selective and enrichment. This article is a review of everything there is to know about bacterial cell culture. It shows how to make and store culture media and talks about things like aeration, mixing, and sterilizing that help cultures grow. Microbial fermentor components and operation have also been examined. This contains bacterial culture techniques including batch, feed batch, continuous, and synchronous, as well as relevant figures and diagrams.

Keywords: Microbial culture, Culture medium, Sterilization, Fermentation

Introduction

Microbiological cultures, also known as microbial cultures, enable microscopic organisms to multiply in predefined culture material in a controlled laboratory setting. Microbial cultures are essential diagnostic tools in molecular biology. Microbial cultures identify species, abundance, or both. One of the most basic ways to use microbiology to diagnose an

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infectious disease is to let the agent grow in a certain medium. A pure microorganism culture is essential. A pure (axenic) culture is a group of cells or organisms with more than one cell that grow without any other species. Pure cultures are genetic clones of one cell or organism. Agar gels microbiological cultures. Agar comes from seaweed. Guar gum is cheaper than agar for isolation and maintenance. Culture media contains microbial growth ingredients and conditions. Many germs cannot grow in any culture medium [1].

Basic Constituents of Media

Inorganic Salts

Inorganic salts in medium have several roles. They deliver sodium, potassium, and calcium ions to maintain osmotic balance and membrane potential. The cell matrix needs them for cell adhesion and enzyme cofactors.

Carbohydrates

Carbs mostly sugars provide energy. Some media use maltose or fructose in addition to glucose and galactose. Sugar contents vary from 1g/L to 4.5g/L in advanced medium. Sugar-rich media support more cell kinds. Pyruvate is an energy source for certain media.

Amino Acids

Amino acids make proteins. Culture media needs "essential" amino acids since cells cannot make them. If the culture medium runs out of amino acids, the cells will stop multiplying.

Vitamins

In cell culture, serum is one of the most important ways to get vitamins. However, many media are also full of vitamins, which makes them more suitable for a wider range of cell lines. A number of co-factors are made up of vitamins. Many vitamins, especially those in the B group, are needed for cells to grow and divide, and B12 is especially important for many kinds of cell growth. Vitamins A and E are found in higher amounts in a few different types of media. Some vitamins that are often talked about in the media are riboflavin, thiamine, and biotin.

Proteins and Peptides

Particularly in serum-free medium, they are crucial. The most often employed proteins and peptides, which are added to the medium together with serum, include albumin, transferrin, fibronectin, and fetuin.

Fatty Acids and Lipids

Like proteins and peptides, serum components like cholesterol and steroids are important in serum-free media because they are needed by specialized cells.

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Trace Elements

These contain intermediates of tricarboxylic acid, as well as trace elements including zinc, copper, selenium, and others. As a detoxifier, selenium aids in the elimination of oxygen free radicals [2].

Consistency-based classification of culture medium used in microbiology labs

Solid medium

Solid medium contains 1.5–2.0% agar or similar inert solidifier. Solid media organise germ development and teach or benefit the organism (e.g. as colonies or in streaks). Solid media can isolate bacteria or analyze colony properties.

Solid Media

They are made using 0.5% agars. They can cultivate microaerophilic bacteria or assess their motility due to their custard-like consistency.

Liquid (Broth) medium

These medium contain exact nutritional concentrations but no gelatin or agar. Broth medium is used in fermentation and species growth investigations. Example: Sugar fermentation in MR-VR broth

Classifying cultural media according to their composition

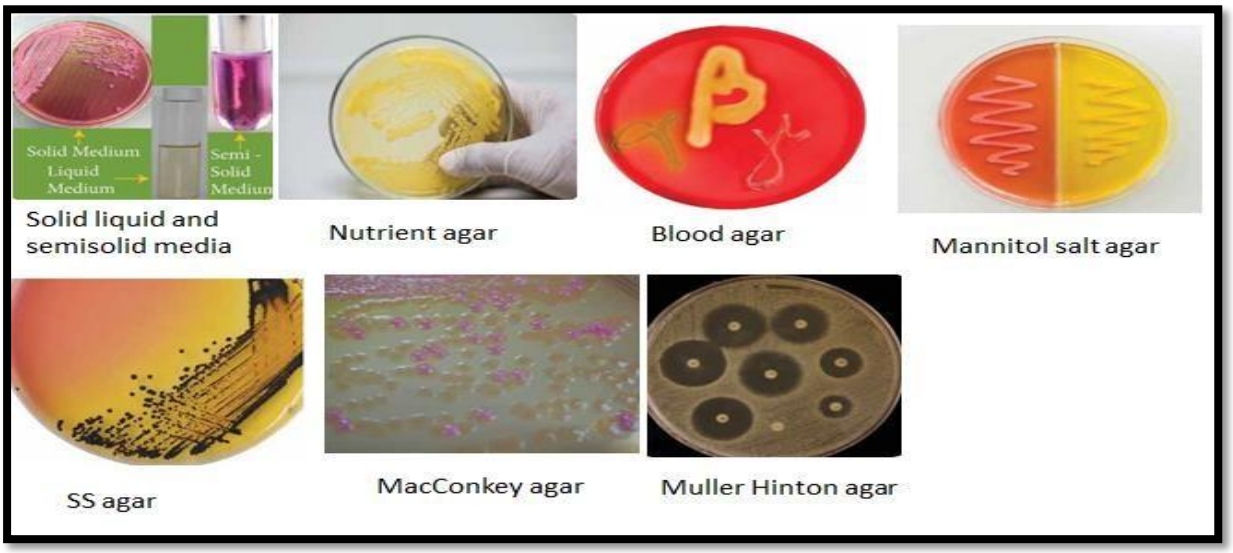

Fig: 1 Classification of culture media

ISSN PRINT 2319 1775 Online 2320 7876

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Chemically defined media

A synthetic medium is made from purified ingredients and has a known composition. Synthetic media's complexity depends on the addition.

Chemically undefined medium

At least one component of non-synthetic media is undefined, refined, or constant from batch to batch. Proteins from several species are partially digested. Nutrient broth is yeast-based. Simple non-synthetic media may nourish organisms with low growth factor demands, whereas more complex media can support more finicky bacteria [3].

A System for Classifying Bacterial Culture Using media according to its intended usage, functionality, or application

It takes a lot of special purpose medium to make it easier to identify, count, and isolate certain bacterial species. There are several media options to satisfy these objectives.

Media for all purposes/Basic media

Basal media supports most non-fastidious microorganisms. Peptone water, nutritional broth, and agar are basal media. These mediums isolate microbes initially.

Additional growth ingredients in the enriched medium

Enriched media include blood, serum, egg yolk, and other nutrients. Enriched media grows fastidious bacteria. Blood, chocolate, Loeffler's serum slope, and other enriched mediums exist. Blood agar is made by adding 5–10% (volume) blood to a base. Chocolate agar is boiled or lysed blood agar [4].

Selective and enrichment media

The goal of selective and enrichment media is to isolate pathogens from a wide range of bacteria by killing off unwanted commensal or contaminating microorganisms. The selective media are made of agar, while the enrichment media are liquid. Both of these methods get the job done. Any agar medium can be made selective by adding certain compounds that stop the growth of pathogens but don't hurt the target pathogen. Some ways to make a medium selective are to add antibiotics, dyes, chemicals, change the pH, or use more than one of these methods.

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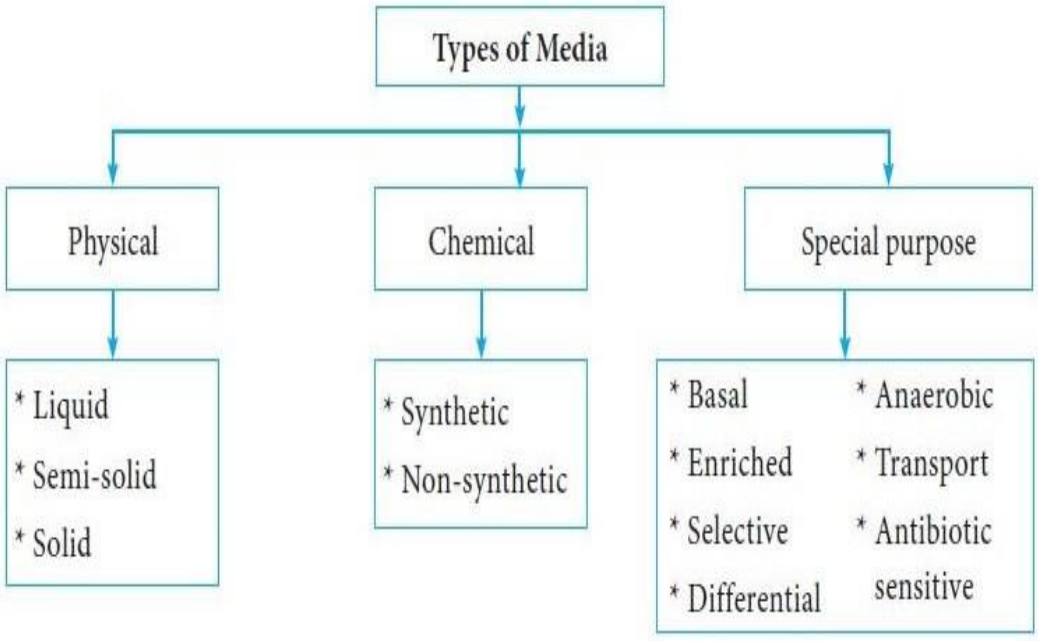

Fig:2 Types of media

Creation and Preservation of Cultural Media

Adjust medium pH before autoclaving. pH indicators include phenol red, neutral red, bromothymol blue, and bromocresol purple. Reconstitute commercial dehydrated medium according to the manufacturer's recommendations. Autoclaving sterilizes most cultural items. Glucose, urea, serum, and blood media cannot be autoclaved. Filtered pieces may be introduced after autoclaving the medium. Wilson and Blair's medium and trypticase soy broth (TSB) agar are extremely selective and do not need sterilization. Before using, examine a sample from each batch for performance and contamination. Media may be refrigerated for one to two weeks at 4 to 50 C. Some liquid media held at room temperature in screw-capped bottles, tubes, or cotton plugs may last weeks [5].

Sterilization

Disinfecting the media and culture vessel prevents bacteria growth. If laboratory-scale experiments are done in 100 to 1000 ml flasks or 50 or 10 ml vials, the media and culture flasks or vials may be autoclaved. Depending on how many items are autoclaved, a practical pressure cooker may sterilize them. Steam sterilization at 120 °C under 15 psi in an autoclave or pressure cooker takes 15–20 minutes. When bacteria are cultured in a fermentor for large-scale operation, it is practicable to sterilize the fermentor without media. Media may be sterilized individually or in the fermentor. Steam sterilizes the media and fermentor through the sterilizing jacket or coil. After disinfecting the fermentor without media, steam may be sparged into all ports to gently leave. A sparging mechanism at the bottom of the fermentor

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circulates sterile air or steam through the medium. Steam circulates within the vessel or jacket at 1.5 psi for 20–30 minutes [6].

The conditions for the development of microbes

The medium's nutrients, salt ionic concentration, pH, and temperature affect microbial growth and metabolism. Most bacteria grow at neutral pH, unlike yeast and fungus. Many species have different active development and reproduction temperatures. In the case of small-scale cultures, an incubator must be used to assist maintain the culture's ideal temperature, and the fermentor's jacket must be circulated with water that is the right temperature.

Mixing and aeration

The broth's mixing distributes nutrients and microbes evenly. Aeration is needed for simple gas exchange. Medium oxygen will be high. A shaker can aerate and mix tiny cultures (shake flasks cultures). Large-scale bioreactor oxygen transmission is difficult because it requires appropriate mixing. To mix cells, media, and oxygen, fermentors employ mechanical stirrers with baffles. Baffles maintain turbulence. Forced aeration with microbial-free air mixes media, cells, and oxygen.

Fermentors

These bioreactors produce microorganisms industrially. This closed metallic or glass vessel provides aeration, agitation-based media mixing, temperature control, pH management, anti-foaming, overflow control, media and vessel disinfection, chilling, and sampling (removal of sample, while the fermentor is on). Stirring, aeration, or both may agitate bioreactor media [7]. This gear may be used for days. The following (Figure 3) details each laboratory fermentor component:

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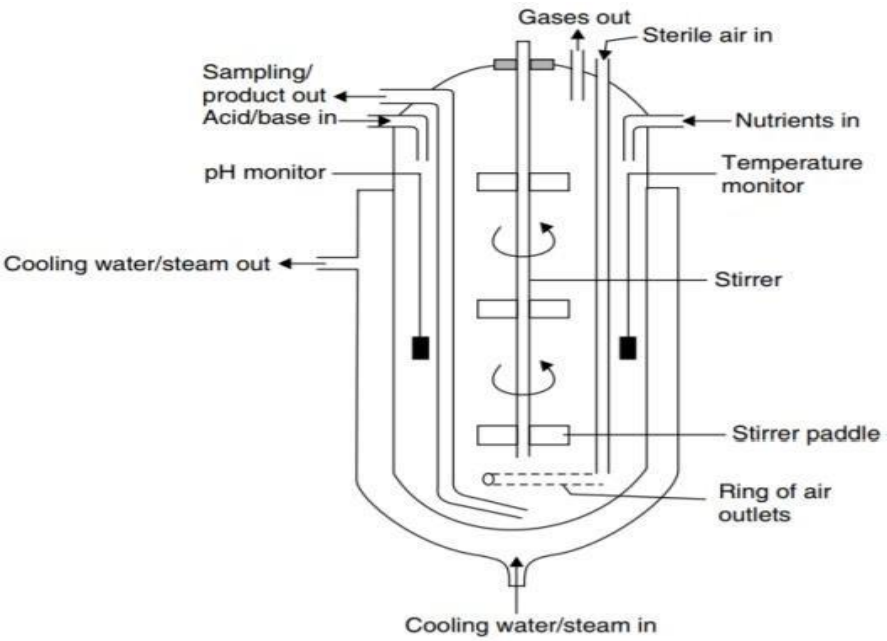

Fig:3 Depicts a laboratory ferment and its constituent parts

The bioreactors have controls for monitoring and changing many physical and chemical parameters, such as temperature, pH, nutrition composition, foaming, etc. Controlling these factors will maximize cell growth and product synthesis. Stirred tank bioreactors with impellers are the most common for microbial culture. A high density of metabolically active cells can quickly deplete dissolved oxygen, creating anaerobic conditions. This could affect product quality or fermentation type. Similar to how cell growth and product production can change the medium's pH, this can also affect cell culture development and metabolism. Rapid growth depletes nutrients needed for product creation and metabolism. These changes are automatically notified by fermentor or bioreactor accessories. When pH deviates from optimal, the medium automatically adds acid or alkali to maintain it. The sensor will also detect foaming and inject an antifoam chemical to stop it. Laboratory fermentors are smaller than industrial bioreactors or fermentors. These lab fermentors vary in size from 10 to 100 litres and are used to adjust nutritional and culture conditions for increased cell growth and metabolite production during laboratory research projects. Microorganisms several techniques may cultivate the microbial system. The culture process depends on the microbial system or result we expect. By changing nutritional and other circumstances or even the culture containers, the same organism may create two separate products [8].

Batch culture

A microbial colony is growing in a tiny flask in this lab experiment. It grows in a flask with a tiny broth culture contaminated with bacteria or microbes. The system is closed because growing bacteria consume the medium's limited resources to grow and excrete byproducts. Batch cultures cannot replenish nutrients, therefore cells only grow exponentially for a few

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generations. The culture's growth phase includes an early lag phase, an exponential log phase, and a stationary period. The log phase consumes the most nutrients and produces the most biomass and product excretion. The stationary phase stops growing. The stationary phase exposes cells to a new environment with fewer nutrients, more cells, and more metabolites, which may limit cell growth.

Fed-batch culture

By feeding the batch culture with new medium successively at the conclusion of the log phase or at the start of the stationary phase without removing cells, the batch culture may be converted into a semi-continuous culture or fed-batch culture. Because of this, the culture will continue to grow when new media is introduced. This approach is particularly appropriate for cultures where the creation of biomass and cell growth is inhibited by high substrate concentrations. In these circumstances, feeding the substrate at low concentrations may promote cell development. A batch fermentor or shake flask culture may not be able to create a high cell density in the culture media as readily as this approach. To maximize product production per biomass, this is crucial when product creation occurs intracellular [9].

Continuous culture

A continuous culture system, created to eliminate the circumstances that prevent exponential development in batch cultures, may be used to keep bacterial cultures in an exponential growth state for extended periods of time. Continuous cultivation in a chemo-stat may maintain bacterial density similar in nature. This method can readily provide long-term cell growth and product development. In a continuous culture, the volume of media, cells, and product displaced or withdrawn is equal to the rate at which nutritional medium, including raw material, is delivered. The volume that is subtracted and added is equal. In reality, neither the culture's chemical environment nor its net volume has changed. The growing chamber is linked to a reservoir of sterile liquid in a chemo-stat.

Synchronous cultures

Synchronous cultures have cells at the same life stage. All culture cells will divide synchronously, grow for a generation, then divide again. As a result, there is no variation in the population's rate of growth or division. A single bacterial cell cannot be examined in order to learn about its organization, differentiation, or macromolecular production. The complete cell crop is produced in synchronized culture at the same stage of development. Such cultures may be measured in the same way that individual cells can be measured [10].

Conclusion

Microbial cultures have a huge potential for producing substances that are very beneficial. Once the microbial culture is formed, it may be utilized to produce a wide range of chemicals based on its metabolic activity. In general, there are six main methods that microbial cultures

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may be used to produce metabolites. Here is a list of them: synthesis of whole microbial cells (for food, vaccines). Synthesis of primary metabolites (acids, alcohol). making secondary metabolites (antibiotics). Reactions of biotransformation (enzymatic, steroid. taking advantage of metabolism (microbial leaching, biodegradable waste treatment). Recombinant protein synthesis (therapeutic proteins).

The most traditional use of microbial cultures is the creation of fermented foods like curd and cheese, where starter cultures are created using entire bacteria. Additionally, entire microbes are used in the creation of bacterial vaccines, such as those used to prevent typhoid and TB. Another instance of using entire microbes as a source of protein is single cell protein (SCP). Alcohol and acid production are examples of basic metabolic products, but the secondary metabolites created by many microbes include antibiotics. The microbial synthesis of vitamins has also made use of microbial metabolism. Microbial metabolism is also employed to transform inappropriate substrates into usable products in the extraction of metals from ores and the treatment of liquid waste, as examples.

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