## Microbiology culture techniques and their applications:

Microorganisms are found in abundance wherever there is food, moisture and temperature suitable for their growth and reproduction. Given that the encouraging conditions for the living and growth of many microorganisms are those under which humans live, it is certain that we live in the midst of a very large crowd of microbes, as they are present in the air that we blow up and are found on the surfaces of our bodies and in All our clothes, our mouths, our noses and any other bodily openings. Unfortunately, some microorganisms are harmful to us, but we have ways to resist the invasion of these very harmful organisms. However, many microorganisms have proven of great industrial, commercial and medical importance, whether they are bacteria, yeasts, molds or algae.

## Microorganisms are divided into:

١- أحياء حقيقية النواة Eukaryotic (الطحالب Algae، الفطريات Fungi "الاعفان والخمائر"، الابتدائيات Protozoa)

٢- أحياء بدائية النواة Prokaryotic (الاكتينومايسيتات Actinomycetes، البكتريا Bacteria)

٣- الفيروسات Viruses

### The role of microbial systems in biomanufacturing:

At the present time, many vital industries tend to use microorganisms, but this trend is not absolute and if it is possible to produce some animal cell products in microorganisms, where there are many obstacles that make each type of cell stand in its place. Among the specifications that drew attention to microbial systems and may have led to an increase in the use of microbial cells in biomanufacturing are the following: ومن المواصفات التي أدت إلى جذب الانتباه إلى الأنظمة الميكروبية وربما أدت إلى زيادة استعمال الخلايا الميكروبية في التصنيع الحيوي الآتي:

• Microorganisms are fast growing/ their growth rates are very high due to the high surface to volume ratio.الأحياء المجهرية **سريعة النمو**

• It is possible to grow large quantities of microbial cells from bacteria and fungi without the emergence of problems in dealing with this quantity of bacteria.

• Microorganisms use cheap and available materials for their growth, such as agricultural waste, while high-end cells have complex nutritional needs. الأحياء المجهرية **تستخدم لنموها موادا رخيصة الثمن**

• Microorganisms have many chemical reactions and synthesis pathways in contrast to animal and plant cells, which provides a fertile side for obtaining different products.

• The genetic systems in microorganisms are simpler than the genetic systems in higher cells, which leads to the ease of genetic manipulation.

- • Microorganisms do not obey the laws of aging, that is, they continue to grow and divide as long as there is a supply of nutrients. خلايا الأحياء المجهرية **لا تخضع لقوانين الشيخوخة** أي أنها تستمر بالنمو والانقسام طالما كان هناك تزويد بالمواد الغذائية.

## Fermentation Processes

The term fermentation is derived from the Latin name Ferever, which means "to **boil**", thus clarifying the appearance of the action of yeast on extracts of fruits or sprouted grains, and the appearance of boiling is due to **bubbles of carbon dioxide or carbon dioxide produced due to anaerobic catabolism of the sugars present in the extract.** However, the name of the fermentation process means different things to a person whether they are a biochemist or an industrial microbiologist.

The meaning of fermentation from a biochemical point of view is related to the generation of energy by the catabolism of organic compounds, but its meaning for industrial microbiology is much broader: it includes almost any process mediated or shared by microorganisms in which the product of economic value is accumulated. There is more than one concept of fermentation at the present time. It is basically defined as: **Biological activities that occur in the absence of oxygen, that is, the recipient of electrons is not external, but rather one of the substances resulting from reactions, but this concept has expanded at the present time to include almost all vital activities, whether they take place in the presence or absence of oxygen. It also includes transformations that take place by enzymes and not living cells. It is noted that the vessels in which these activities take place took the derivative name, which is fermenters, although it is better to be called bioreactors.المفاعلات الحيوية**

Accordingly, the fermentation processes adopted in biomanufacturing or biotechnology are nothing but the vital activities of living cells or their parts or enzymes extracted from them, so for the purpose of producing any substance it is necessary to understand:

• Physiology of the organism or its derivatives..فسلجة الكائن الحي أو مشتقاته

• Full knowledge of the control processes and the appropriate conditions for production.
الإلمام التام بعمليات السيطرة والظروف الملائمة للإنتاج.

Thus, the manufacturing process can be dealt with better.

## Fermentation products and their importance:

There are four main groups of commercially important ferments:

### 1) Microbial Biomass الكتلة الحيوية للأحياء المجهرية

Commercial production of biomass is divided into **two main processes**:

- Yeast production for the purpose of use in bread and pastries.
- Production of microorganism cells for the purpose of using them as food for humans and animal feed, which is known as Single Cell Protein-SCP.

### 2)Microbial Enzymes: انزيمات الاحياء المجهرية

Most commercial enzymes are produced from microbial sources and are used in the food industry and other uses. Microorganisms precisely control the enzyme production process, and their production can be improved by modifying control systems with genetic modification techniques and strain selection.

### 3) Microbial Bioconversion التحولات الحياتية بواسطة الأحياء المجهرية

Microorganisms cells are used for the purpose of transforming the compounds added to the culture medium into compounds close to them at the structural level, but more valuable at the commercial level. A familiar example of microbial conversion processes is the conversion of ethanol to acetic acid. These transformations include **hydrogenation** and **oxidation reactions**, **hydroxylation**, **dehydrogenation**, removal of the carboxylic group, addition and removal of an amine group, **dehydration** and others.

### 4) Microbial Metabolites المنتجات الايضية للأحياء المجهرية

Microorganisms during their growth on farms pass through phases known as growth phases, and two groups of products can be distinguished during these phases:

## First: Primary Metabolites

These activities mainly include growth processes, with the first two parts: building materials necessary for growth, most of which are of low molecular weight. The second prong is the demolition of nutrients to obtain energy and basic building units. In both cases, the resulting materials are called primary metabolites, and they are also called growth-related materials and are important from the factory's point of view as well. The primary metabolites are created by cells during the first growth phase, which is the acclimation phase and the logarithmic phase, which together are called the building or طور البناء أو التغذية Trophophase

(Note Figure -1-) It includes building materials such as **amino acids**, **vitamins, nucleotides** and others. These materials are usually produced in sufficient quantities for the cells' needs and used by cells in building. As **for primary metabolic products, which are produced during the feeding and building phase**, which result from demolition processes عمليات الهدمto obtain The energy that occurs most often under anaerobic conditions includes various groups of substances such as: organic acids (such as Citric acid and Acetic acid), some alcohols such as glycerol, butanol and other alcohols, in addition to other substances that remain inside the cells or are excreted into the cells. outside the cells.

**Ways to increase the production of primary metabolites:**

• Genetic manipulation for the purpose of obtaining strains with excessive production.

• Manipulating the composition of the nutrient medium so that it leads to changes in the maturity of the cells or any means that lead to an increase in production.

## Second: Secondary Metabolites

These are produced in phases in which the growth rate is reduced or stopped completely, so it mainly includes the **stationary phase** and this is **called the Idiophase** (note Figure 1). During this phase, secondary metabolites are produced, which are also called non-growth-related substances. It seems that secondary metabolites do not play an important role in the processes of cell growth or energy production in general, but are produced when the first (primary metabolism) is inhibited. **The secondary metabolites are mainly antibiotics, which are important from an industrial point of view, as well as Giberellic acid, Allkaliods, Antitumours, Insecticides, some dyes, and Toxins.**

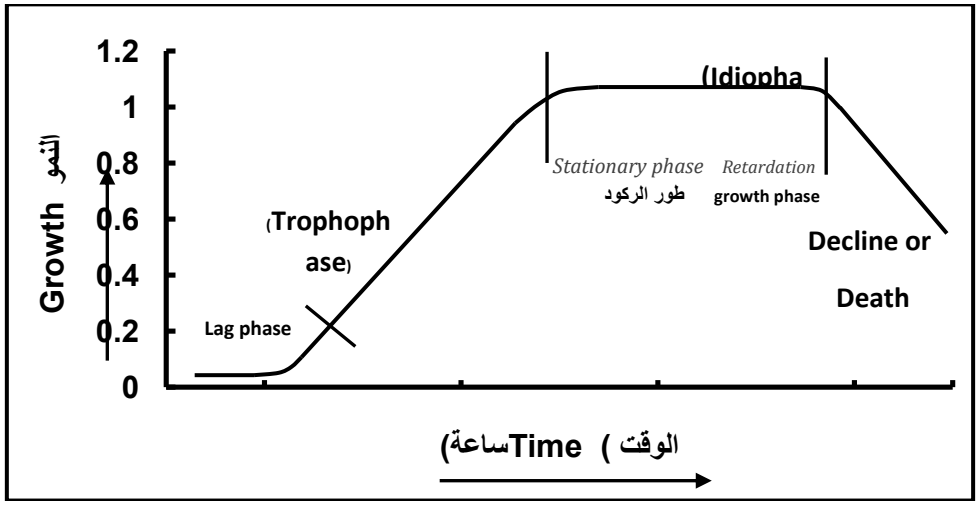



Figure -1- Shows the Growth Phases in Intermittent Cultures (Batch culture)

**The biological fermentation that takes place by living cells can be carried out using:**

**First: Liquid Substrate**, and the fermentation method in this case is divided into:

- **Batch Fermentations**
- **Continuous Fermentations**

**Second: Solid Substrate Fermentation**

First: fermentation of liquid substances:

**a) Batch Fermentations المزارع المتقطعة**

One-meal farms are considered **closed systems**, except for nutrition, heat removal and CO2, and they contain a specific amount of the nutrient medium, where vaccines are added to it from the start. Therefore, the concentration of nutrients is in constant change, as well as other factors, which leads to making the neighborhoods under unstable and unstable conditions. controlled by it. The growth of the microorganism during the intermittent fermentation or the fermentation of a single meal matches the characteristics of the previous growth curve as it performs the lag phase Or the period of imprinting on the environment to the logarithmic phase in which the organism divides exponentially, and this in turn ends with an increasing decrease in growth rates as a result of a deficit in one or more basic nutrients until reaching the stable growth phase, when the amount of protoplasm in the farm remains constant. It is clear that the time spent in the stagnant phase and the stationary phase is lost in the production of microbial cells and for this reason the commercial yeast industry and the beer industry have gradually shifted to modern and advanced methods of continuous fermentation.

**Advantages of Batch cultures:**

- Low possibility of contamination.
- Conditions can be controlled to a certain extent for the purpose of producing materials, whether they are related to primary or secondary metabolism.
- In such farms there is no problem of strain-degeneration, because the cells do not grow constantly, and this leads to the stability of the genetic characteristics of the strains used.
- The used containers can be used for more than one purpose as well as for the production of different types of materials.

**Disadvantages of Batch cultures:**

- The conditions on the farm are constantly changing for the reasons mentioned above.

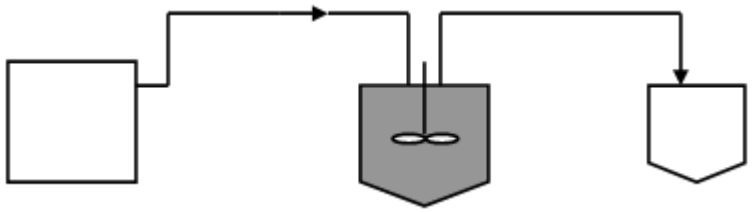
• Significant differences between the different manufacturing processes.

• There is a long time in which no production takes place, and it is the stage of adaptation, which in turn affects productivity.

**B.Continuous Fermentations**

In order to keep the cells always in a balanced state of growth, i.e. in a logarithmic phase, continuous farms can be used, which are always provided with new nutrients and removing equivalent quantities from the fermentation medium at the same rate. Therefore, the balanced growth can continue in these **open systems.**

This type of farm is not suitable for the production of many of the required materials, which limited their use in most cases in the production of biomass and the production of other **primary metabolites** related to growth processes and the inability to use them to produce secondary metabolites that are of great industrial importance. ان هذا النوع من المزارع لا تكون ملائمة لإنتاج العديد من المواد المطلوبة مما **حدد استعمالها** في اغلب الاحيان بإنتاج الكتلة الحيوية وإنتاج مواد الايض الاولي الاخرى ذات العلاقة بعمليات النمو **وعدم امكانية استعمالها لإنتاج نواتج الايض الثانوي** التي تكون ذات اهمية صناعية كبيرة.



**وعاء الناتج وعاء المزرعة (المخمر) وعاء المواد المغذية (المستودع)**

**(Fermenter)**

**(شكل يوضح مزرعة مستمر)**

In continuous fermentation, the microorganism is grown in the farm container under the desired environmental conditions and it is constantly and at a constant rate supplied with the new food environment. This allows a constant level of fermentation fluid flow to flow into the product vessel at a rate equal to the rate at which the environment enters the fermentation vessel. The most famous types of continuous fermentation are the fermentation of beer production, the production of fodder yeasts (from the residual sulfur solution), vinegar and yeast bread (molasses), the production of some antibiotics such as penicillin, chlorophenicol, streptomycin, wine industry, the production of vitamin B12 and the continuous production of algae protein. residues. Here is a diagram showing a **continuous farm fermentation of Baker's Yeast:**

## Second: Solid Substrate Fermentation

This type of fermentation takes place in the absence of free water, provided that the humidity does not drop below 12%, which is considered very critical. The main difference when living organisms grow on solids or liquids is that in the second case the nutrients are available to the cells, while in the first case, the nutrients are not available to the cells at the beginning of fermentation, so some preliminary treatments must be done on the materials. As for the other difference, the nutrients in liquid media are always in a state of decreasing, but in the case of solid substances, they sometimes decrease, but mostly they are at the same level or increase as a result of disintegration processes. ان هذا النوع من التخمرات يتم بغياب الماء الحر free water على شرط عـدم انخفاض الرطوبة عن ١٢% التي تعتبر جدا حرجة. ان **الفرق الرئيسي** عند نمو الأحياء على المواد الصلبة أو السائلة هو انه في الحالة الثانية تكون المواد الغذائية متوفرة للخلايا، أما في الحالة الأولي فان **المواد الغذائية لا تكون متوفرة للخلايا في بداية التخمر**، لذا وجب إجراء بعض المعاملات الأولية على المواد. أما **الفرق الآخر** فان المواد الغذائية في الأوساط السائلة تكون دائما في حالة تناقص أما في حالة المواد الصلبة فإنها في **أحيان قليلة تتناقص** أما في الأغلب في تكون بنفس المستوى أو تزداد نتيجة لعمليات التفكك.

**Materials used in fermentation of solids and influencing enzymes:**

Most of the materials used in the fermentation processes of solid materials are of a polymeric nature (polymeric substances), including:

1. Polysaccharides: They are the most common materials, including cellulose, starch, semi-cellulose, and lignin. As for chitin, it is also one of the polymers used in solid fermentation and can be analyzed by the enzyme Chitinase.
2. Proteins and their derivatives: such as collagen and keratin, which are fibrous proteins or solid structures found in wool.

Most of the mentioned materials are found in nature as raw materials such as grains, including wheat bran or other grains, legume seeds, wood materials that represent agricultural wastes such as straw or forest waste in addition to hydrocarbons. ان اغلب المواد المذكورة Grains ومنها نخالة الحنطة Wheat bran، او غيرها من الحبوب **توجد في الطبيعة كمواد اولية** مثل الحبوب او فضلات الغابات Straw ، المواد الخشبية والتي تمثل فضلات الزراعة مثل التبن Legume seedsبذور البقوليات بالإضافة الى الهيدروكاربونات

The used organisms possess the necessary **enzymes to break down the polymers**, and most of them are induced enzymes, that is, they are created after exposing the organisms to food. The necessary enzymes, whether they **are extracellular**, present on the surfaces of the outer cells, or released when the cells die, must be close and in contact with the substances to be broken down. Fungi, yeasts and gram-positive bacteria are preferred in this case as they secrete a quantity of their enzymes to the outside, while gram-negative bacterial cells tend to

retain their **enzymes inside the intracellular cells or near the cell wall** from the inside and this is due to the difference in the nature of the composition of the different types of cells,

Whereas, gram-negative cells live in very dilute environments such as rivers and seas, while the other cells live in non-diluted environments. Solid materials fermentations are economical as the base materials are very concentrated and available in nature and cheap, as well as the extraction and purification processes are economical due to the small production process, in addition to that these fermentations do not need sterilization and energy expenditure because the materials used are low in moisture and do not encourage the growth of most organisms .

## Practical applications of solids fermentation:

1. Production of some organic acids such as citric acid.
2. Ethyl alcohol production.
3. Biomass production.
4. Commercial enzyme production.
5. Purification and extraction of metals.
6. Mushroom production (Agaricus bisporus) and truffles.
7. Biogas production such as methane.
8. Production of many oriental foods, such as: Soy sauce, tempeh, tamari, and miso.
9. Animal fodder production (silage) and fertilizer production .

The most important of these ferments are the **fermentations of cellulosic materials**, which depend mainly on the production of the cellulase enzyme, which is a system of three enzymes. The fermentation of cellulosic materials is one of the largest fields of the future due to the availability of the raw material in large and cheap quantities