Kingdom of Saudi Arabia
Ministry of Education
Northern Border University
Faculty of Science and Arts
Department of Biology
(Microbiology)


Industrial Microbiology Course (3303-411)


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Contents

1. Introduction to Industrial Microbiology

❖ Glossary of terms

No.	Term	Meaning (Arabic)
1	Industrial Microbiology	الميكروبيولوجيا الصناعية
2	Applied Microbiology	الميكروبيولوجيا التطبيقية
3	Biotechnology	التكنولوجيا الحيوية
4	Fermentation	التخمير (التخمر)
5	Fermentor	المُخمر (جهاز التخمير)
6	Fermenter	المُخمر (الميكروب القائم بالتخمير)
7	Bioreactor	مُفاعل حيوي
8	Primary metabolites	نواتج الأيض الأولية
9	Secondary metabolites	نواتج الأيض الثانوية
10	Biofuel	الوقود الحيوي
11	Biogas	الغاز الحيوي
12	Antibiotics	المضادات الحيوية
13	Enzymes	الانزيمات
14	Pigments	أصباغ
15	Alcohols	الكحول
16	Beer	البيرة
17	Vinegar	الخل
18	Batch	الكمية المعدة (دُفعة)
19	biopolymers	البوليمرات الحيوية
20	Bioplastics	البلاستيك الحيوي

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❖ What is the Industrial Microbiology?

Industrial Microbiology: is a branch of applied microbiology in which microorganisms are used in industrial processes; for example, in the production of high-value products such as drugs, chemicals, fuels and electricity.

Or:

Industrial Microbiology: is a branch of biotechnology that applies microbial sciences to create industrial products in mass quantities.

The field of industrial microbiology has been undergoing rapid change in recent years because of:

First, powerful new tools and technologies especially genetic engineering, genomics, proteomics, bioinformatics and such like new areas promise exciting horizons for man's continued exploitation of microorganisms. Second, new approaches have become available for the utilization of some traditional microbial products such as immobilized enzymes and cells, site-directed mutation and metabolic engineering. Even the search for organisms producing new products has now been broadened to include unculturable organisms which are isolated mainly on genes isolated from the environment.

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There are multiple ways to manipulate a microorganism in order to increase maximum product yields. Introduction of mutations into an organism many be accomplished by introducing them to mutagens. Another way to increase production is by gene amplification, this is done by the use of plasmids, and vectors.

The manipulation of organisms in order to yield a specific product has many applications to the real world like the production of some antibiotics, vitamins, enzymes, amino acids, solvents, alcohol and daily products.

❖ History of Industrial Microbiology

Industrial microbiology came into existence, primarily, based on a naturally occurring microbiological process called fermentation. There are many evidences which clearly shows that ancient man knew fermentation process and practiced it more as an art rather than as a science.

Early fermentation process practiced by man included the leavening of bread, retting of flax, preparation of vinegar from wine, production of various alcoholic beverages like beer, wine, mead and the production of various fermented foods and milk.

The history of industrial microbiology can be divided into five phases, which are précised in the following table:

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Table 1: The phases in the history of industrial microbiology

Phase	Main products	Fermenters	Culture method	Strain selection
(I) Period before 1900	Alcohol	Wooden	Batch	Pure yeast culture
	Bakers yeast	Steel vessels	Batch and fed-batch systems	Pure cultures used
(II) Period between 1900-1940	Penicillin, streptomycin other antibiotics	Mechanical stirring used in small vessels	Batch and fed-batch	Mutant strain
(III) Period between 1940-1964	Gibberellins, amino acids, nucleotides, enzymes, transformations	Vessels operated aseptically, true fermentations	Continuous culture Introduced for brewing and some Primary metabolites	Mutations and Selection programme essential
(V) 1979-onward	Production of proteins by microbial cells Monoclonal antibodies produced by animal cells	Vessels operated Aseptically	Batch, fedbatch Or continuous Fermentation developed for animal cell processes	Introduction Of foreign genes into Microbial cells

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❖ Microorganisms Commonly Used In Industrial Microbiology

The microorganisms currently used in industrial microbiology and biotechnology are found mainly among the bacteria and eukarya; the Archae are not used.

Many organisms in Archae are able to grow under extreme conditions of temperature or salinity and these conditions may be exploited in industrial processes where such physiological properties may put a member of the Archae at an advantage over contaminants.

Plants and animals as well as their cell cultures are also used in the industrial biotechnology. Microorganisms have the following advantages over plants or animals as inputs in biotechnology:

(1) Microorganisms grow rapidly in comparison with plants and animals.

The generation time (the time for an organism to mature and reproduce) is about 12 years in man, about 24 months in cattle, 18 months in pigs, 6 months in chicken, but only 15 minutes in the bacterium, *E coli*.

(2) The space requirement for growth of microorganisms is small.

A 100,000 liter fermenter can be housed in about 100 square yards of space, whereas the plants or animals needed to generate the equivalent of products in the 100,000 fermenter would require many acres of land.

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(3) Microorganisms are not subject to the problems of the vicissitudes of weather which may affect agricultural production especially among plants.

(4) Microorganisms are not affected by diseases of plants and animals, although they do have their peculiar scourges in the form phages and contaminants, but there areprocedure to contain them.

❖ **Examples of Microorganisms Used**

(1) Bacteria

The bacterial phyla used in industrial microbiology and biotechnology are found in the Proteobacteria, the Firmicutes and the Actinobacteria.

a- The Acetic Acid Bacteria: *Acetobacter* and *Gluconobacter* which used for production of acetic acid, and are used in the manufacture of vinegar.

b- The Lactic Acid Bacteria: *Lactobacillus bulgaricus* *Lactococcus lactis* which produce lactic acid, ethanol, as well as CO_2.

c- The Actinobacteria: *Streptomyces*, from which many antibiotics as well as non-anti-microbial drugs have been obtained.

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(2) Fungi

a- *Rhizopus* and Mucor are used for producing various enzymes.

b- Yeasts are used for the production of ethanol and alcoholic beverages.

c- *Penicillium* is well-known for the antibiotic penicillin which it produces.

d- *Agaricus* produces the edible fruiting body or mushroom.

❖ Characteristics of Microbes Used In Industrial Microbiology

Microorganisms which are used for industrial production must meet certain requirements such as:

(1) *The organism must be able to grow in a simple medium and should preferably not require growth factors* (i.e. pre-formed vitamins, nucleotides, and acids) outside those which may be present in the industrial medium in which it is grown.

(2) *The organism should be able to grow vigorously and rapidly in the medium in use.* A slow growing organism no matter how efficient it is, in terms of the production of the target material, could be a liability. In the first place the slow rate of growth exposes it, in comparison to other equally effective producers which are faster growers, to a greater risk of contamination.

(3) *Not only should the organism grow rapidly, but it should also produce the desired materials rapidly,* whether they be cells or

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metabolic products, in as short a time as possible, for reasons given above.

(4) *Its end products should not include toxic and other undesirable materials,* especially if these end products are for internal consumption.

(5) *The organism should have a reasonable genetic, and hence physiological stability.* An organism which mutates easily is an expensive risk. It could produce undesired products if a mutation occurred unobserved. The result could be reduced yield of the expected material, production of an entirely different product or indeed a toxic material.

(6) *The organism should be reasonably resistant to predators such as bacteriophages.* It should therefore be part of the fundamental research of an industrial establishment using a phage-susceptible organism to attempt to produce phage-resistant but high yielding strains of the organism.

(7) *The organism should not be too highly demanding of oxygen as aeration* (through greater power demand for agitation of the fermentor impellers, forced air injection etc) contributes about 20% of the cost of the finished product.

(8) *The organisms used must have physiological requirements which protect them against competition from contaminants.* An

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organism with optimum productivity at high temperatures, low pH values or which is able to elaborate agents inhibitory to competitors has a decided advantage over others.

(9) Lastly, the organism should be fairly easily amenable to genetic manipulation to enable the establishment of strains with more acceptable properties.

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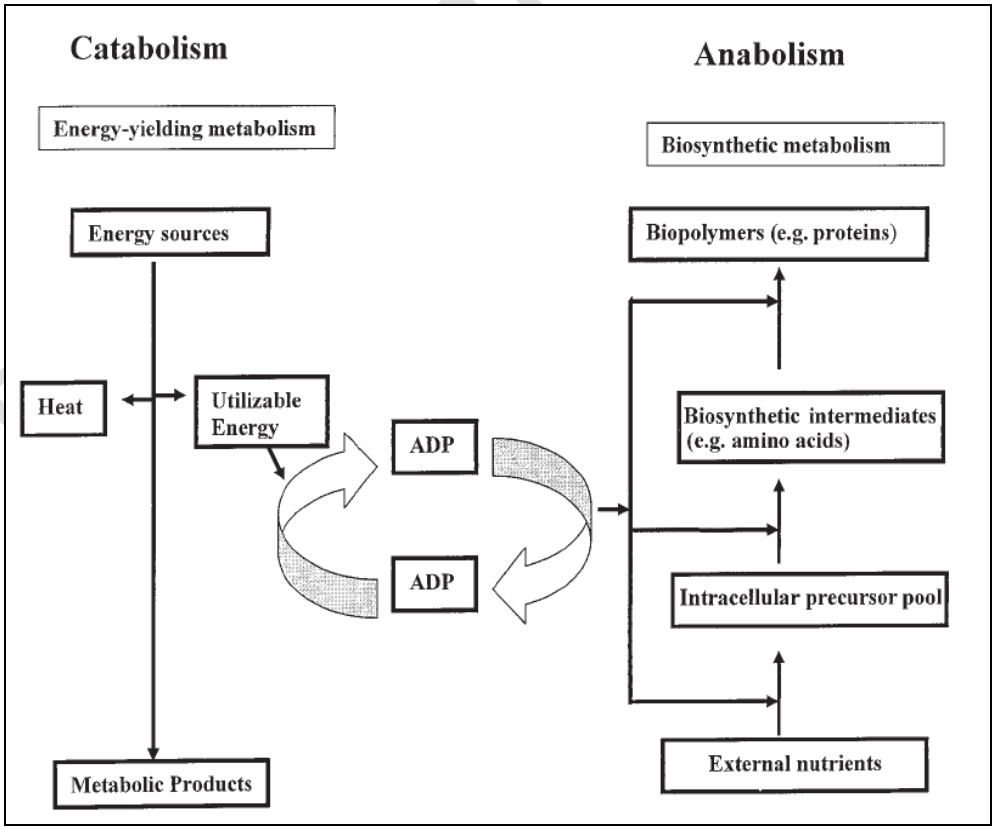
❖ Metabolic Pathways for the Biosynthesis of Industrial Microbiology Products

- **Metabolic Pathways is defined as:** *The series of chemical reactions involved in converting a chemical (or a metabolite) in the organism into a final product.*

  There are two mains metabolic pathways that are:

(1) **Anabolic pathways:** *in which the reactions lead to the formation of a more complex substance*.

(2) **Catabolic pathways:** *in which series of reactions lead to less complex compounds.*



The compounds involved in a metabolic pathway are called intermediates and the final product is known as the end-product.

❖ Industrial Microbiological Products As Primary And Secondary Metabolites

Products of industrial microorganisms are divided into two broad groups, primary metabolism and secondary metabolism.

(1) Primary Metabolism

Primary metabolism is the inter-related group of reactions within a microorganism which are associated with growth and the maintenance of life.

Primary metabolism is essentially the same in all living things and is concerned with the release of energy, and the synthesis of important macromolecules such as proteins, nucleic acids and other cell constituents. When primary metabolism is stopped the organism dies.

Products of primary metabolism are associated with growth and their maximum production occurs in the logarithmic phase. Examples of these metabolites are listed in Table below:

Table (2): Some industrial products of microbial secondary metabolism

Anabolic Products	*Catabolic Products*
1. Enzymes	1. Ethanol and ethanol-containing products, e.g. wines
2. Amino acids	2. Butanol
3. Vitamins	3. Acetone
4. Polysaccharides	4. Lactic acid
5. Yeast cells	5. Acetic acid (vinegar)
6. Single cell protein	
7. Nucleic acids	
8. Citric acid	

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(2) Secondary Metabolism

Secondary metabolism has no apparent function in the organism. Secondary metabolites are produced in response to a restriction in nutrients. They are therefore produced after the growth phase, at the end of the logarithmic phase of growth and in the stationary phase (in a batch culture). They can be more precisely controlled in a continuous culture.

Secondary metabolism appears to be restricted to some species of plants and microorganisms. Microbial secondary metabolites include antibiotics, pigments, toxins, enzyme inhibitors, pesticides, antitumor agents (table below).

Table (3): Some industrial products of microbial secondary metabolism

Product	*Organism*	*Use/Importance*
Antibiotics		
Penicillin	*Penicillium chrysogenum*	Clinical use
Streptomycin	*Streptomyces griseus*	Clinical use
Anti-tumor Agents		
Actinomyin	*Streptomyces antibioticus*	Clinical use
Bleomycin	*Streptomyces verticulus*	Clinical use
Toxins		
Aflatoxin	*Aspergiulus flavous*	Food toxin
Amanitine	*Amanita* sp	Food toxin
Alkaloids		
Ergot alkaloids	*Claviceps purpurea*	Pharmaceutical
Miscellaneous		
Gibberellic acid	*Gibberalla fujikuroi*	Plant growth hormone
Kojic acid	*Aspergillus flavus*	Food flavor
Muscarine	*Clitocybe rivalosa*	Pharmaceutical
Patulin	*Penicillium urticae*	Anti-microbial agent

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2. Regulation of Microbial Growth

One of the most essential aspects of living cells is growth. Fast growth represents an effective strategy for microbial organisms to survive in competitive environments. To accomplish this task, cells must adapt their metabolism to changing nutrient conditions in a way that maximizes their growth rate.

❖ Factors Regulating Growth:

Microbial growth is affected by two major factors:

(1) Physical (environmental): such as temperature, pH, radiation in addition to solutes and water activity.

(2) Chemical (nutritional): such as proper concentrations of carbon (C), nitrogen (N), sulfur (S), phosphors (P) and trace elements.

(1) Influence of physical (environmental) factors on the microbial growth

Microorganisms grow in a wide range of environments. Some like it hot while others like it cold. Some are acidic loving. Some require high moisture. Others do not. Some can tolerate high-salt (saline) environments. Many require the presence of oxygen, but some do not.

The major physical (environmental) factors which affect microbial growth are:

(a) Solutes and water activity (b) Temperature (c) pH

(d) Oxygen concentration (e) Pressure (f) Radiation

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(A)Solutes and Water activity

Water is one of the most essential requirements for life. Thus, its availability becomes most important factor for the growth of microorganisms. Water is required to dissolve most cell substances that microorganisms use: minerals, ions, gases and numerous organic compounds. Some bacteria can survive under extremely dry conditions by forming spores.

Water activity (a_w) is the amount of water available to microorganisms and this can be reduced by interaction with solute molecules (osmotic effect).

Changes in osmotic concentration of the surroundings can affect microbial growth as a selectively permeable plasma membrane separates the microorganisms from their surroundings.

Dehydration is a method to decrease water content. Formula used to determine amount of free water:

-a_w = water activity = vapor pressure of food vapor pressure of water.

-a_w below 0.70 greatly reduces microbial growth, Most bacteria grow at a_w 0.85-1.0. Many molds are osmotically tolerant and can grow at a_w value of 0.6.

Microorganisms like *Staphylococcus aureus* can survive over a wide range of water activity and are called as osmotolerant (as water activity is inversely related to osmotic pressure).

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(B) Temperature

All forms of life are greatly influenced by temperature. In fact, the microorganisms are very sensitive to the temperature where temperature influences the rate of chemical reactions and protein structure integrity thus affecting rates of enzymatic activity. At low temperature enzymes are not denatured, therefore, every 10˚C rise in temperature results in rise of metabolic activity and growth of microorganisms.

Temperature profoundly affects and damages the microorganisms by:

1. Inhibition of enzyme-catalyzed reactions.
2. Slowing the growth.
3. Denaturation of enzymes.
4. Denaturation of transport carriers and other proteins.
5. The plasma membrane also is disrupted.

Microorganisms are classified into three major categories (psychrophiles, mesophiles and thermophiles) and five subcategories based on their temperature ranges for growth as follows:

(1) Psychrophiles:

Microorganisms grow well at 0˚C and the optimum growth temperature of 15˚C or lower and maximum at around 20˚C. Examples of psychrophilic microorganisms:

- Bacteria: *Pseudomonas, Vibrio, Alcaligenes and Bacillus.*

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- Fungi: *Aureobasidium pullulans and Aspergillus niger.*
- Algae: *Chlamydomonas nivalis.*

(2) Mesophiles:

Growth optimum around 20˚C to 40˚C, minimum at 15˚C to 20˚C and maximum at 45˚C or lower.

Most of the organisms fall under or within this category including human pathogens as might be expected because the human body is a fairly constant 37˚C. Example of mesophilic microorganisms:

- Bacteria: *Escherichia, Staphylococcus and Salmonella.*
- Fungi: *Candid albicans and Humicola stellate.*
- Algae: *Spirogyra.*

(3) Thermophiles

The microorganisms in this group can grow at temperature of 55˚C or higher, minimum is usually around 45˚C and growth optima at around 55˚C to 65˚C.

Mostly prokaryotes and a few algae and fungi belong to this group. The habitats in which they grow include, composts, self-heating haystacks, hot water lines and hot springs. Example of thermophilic microorganisms:

- Bacteria: *Bacillus stearothermophilus and Pyrococcus furiosus.*
- Fungi: *Mucor pusillus.*

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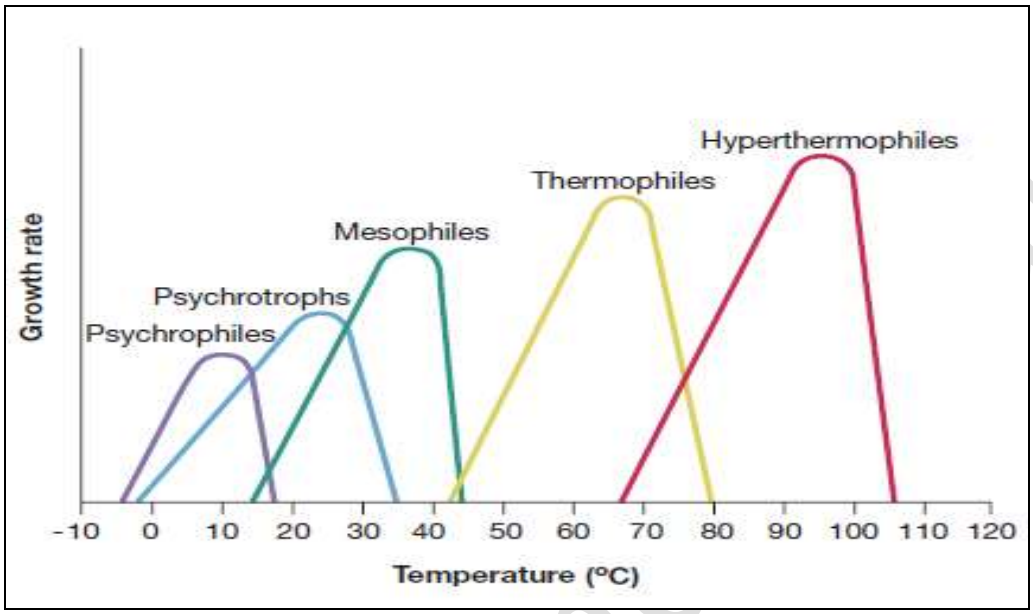

Fig. (1): Temperature ranges for microbial growth

(C) pH

pH: It refers to the acidity or alkalinity of a solution. It is a measure of the hydrogen ion activity of a solution.

pH is defined as the negative logarithm of the hydrogen ion concentration. $pH = -\log [H^+] = \log (1/H^+)$

The pH can affect the growth of microorganisms and each species has a definite pH growth range and pH growth optimum.

Depending on the optimum pH for growth, microorganisms can be classified into:

(1) Acidophiles: their optimum growth between pH 0 and 5.5

(1) Neutrophiles: between 5.5 and 8.0

(2) Alkalophiles: prefer pH range of 8.5 to 11.5.

Most bacteria and protozoans are neutrophiles, fungi prefer acid surroundings about pH 4 to 6; algae also seem to favour slight acidity. *Cyanidium caldarium* (algae) and archaeon *Sulfolobus acidocaldarium* are inhabitants of acidic hot springs; both grow well around pH 1 to 3 and at high temperature.

Drastic changes/variations in cytoplasmic pH that harm microorganisms (Effect of pH on the growth of the microorganism can be explained) through:

(1) Disrupting the plasma membrane

(2) Inhibiting the activity of the enzymes

(3) Inactivation of plasma membrane transport proteins

(D)Oxygen concentration

The importance of oxygen to the growth of an organism correlates with its metabolism in particular, with the processes it uses to conserve the energy supplied by its energy source.

Depending on the requirement of Oxygen/Air concentration, microorganisms can be classified into:

(1) Aerobic (Aerobes): Microorganisms are able to grow in the presence of atmospheric O_2. Examples: *Bacillus, Micrococcus.*

(2) Obligate aerobic: Microorganisms those are completely dependent on atmospheric oxygen for growth. Oxygen serves as the terminal electron acceptor for the the metabolic process. Example: *Mycobacterium tuberculosis and Nocardia.*

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(3) Microaerophiles: Microorganisms that require a few levels of oxygen (2-10%). Example: *Campylobacter* are damaged by the normal atmospheric level of O_2 (20%) and require O_2 levels in the range of 2 to 10% for growth.

(4) Aerotolerants: Microorganisms ignore O_2 and can grow in its presence or absence. Examples: *Enterococcus faecalis.*

(5) Facultative anaerobes: Microorganisms that do not require oxygen for growth but grow well in its presence. Examples: *Salmonella, Vibrio, Listeria.*

(6) Anaerobic (Anaerobes): which can grow only in the absence of free oxygen. They are killed by oxygen. Example: *Actinomyces.*

(7) Obligate anaerobes: Microorganisms do not tolerate the presence of oxygen at all and ultimately die. Example: *Clostridium and Furobacterium.*

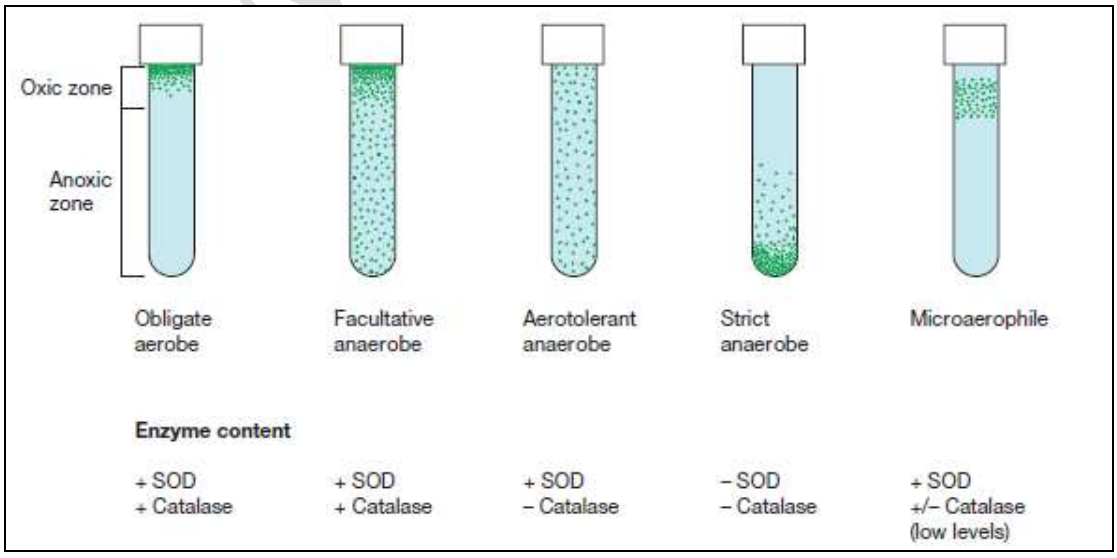

Fig. (2): Oxygen and bacterial growth

- Culturing of aerobic microorganisms:

Aerobic microorganisms are cultured, either the culture is shaken to aerate the medium or sterile air is pumped.

- Culturing of anaerobic microorganisms:

Anaerobic microorganisms require special technique for their culturing as follows:

(1) Using anaerobic media containing reducing agents such as thioglycollate or cysteine.

(2) Removing air with a vacuum pump and flushing out residual oxygen with nitrogen gas.

(3) Using anaerobia sealed jar: CO_2 and nitrogen is added to the chamber since many anaerobes require a small amount of CO_2 for best growth.

Effect of O_2 on growth of the microorganism can be summarized in:

(1) Inactivation of proteins.

(2) Inactivation of enzymes when sensitive groups like sulfydryls are oxidised.

(3) Accumulation of toxic O_2 derivatives (H_2O_2) that rapidly destroy cellular constituents of the microbial cell.

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(2) Influence of chemical (nutritional) factors on the microbial growth

The chemicals and elements of this environment that are utilized for microbial growth are referred to as nutrients. The major chemical (nutritional) factors which affect microbial growth are divided into two major types:

(A) Macronutrients or macro elements:

These are required by microorganisms in relatively large amounts e.g: Carbon, oxygen, hydrogen nitrogen, sulfurs and phosphorous which are components of carbohydrates, lipids, proteins and nucleic acids. The remaining four macro elements (K, Ca, Mg and Fe) exist in the cell as cations.

(B) Micronutrients or Trace elements:

These are required by microorganisms in relatively samll (trace) amounts e.g: manganese, zinc, cobalt, molybdenum, nickel and copper. These are normally part of enzymes and cofactors, and they aid in the catalysis of reactions and maintenance of protein structure.

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Table (3): Major elements, their sources and functions in microbial cells.

Element	% of dry weight	Source	Function
Carbon (C)	50	Organic compounds or CO_2	Main constituent of cellular material
Oxygen (O_2)	20	H_2O, organic compounds, CO_2, and O_2	Constituent of cell material and cell water
Nitrogen (N_2)	14	NH_3, NO_3, organic compounds, N_2	Constituent of amino acids, nucleic acids and coenzymes
Hydrogen (H_2)	8	H_2O, organic compounds, H_2	Main constituent of organic compounds and cell water
Phosphorus (P)	3	Inorganic phosphates (PO_4)	Constituent of nucleic acids, nucleotides, phospholipids, LPS, teichoic acids
Sulfur (S)	1	SO_4, H_2S, So, organic sulfur compounds	Constituent of cysteine, methionine, glutathione, several coenzymes
Potassium (K^+)	1	Potassium salts	Main cellular inorganic cation and cofactor for certain enzymes
Magnesium (Mg^{2+})	0.5	Magnesium salts	Inorganic cellular cation, cofactor for certain enzymatic reactions
Calcium (Ca^{2+})	0.5	Calcium salts	Inorganic cellular cation, cofactor for certain enzymes and a component of endospores

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3. Screening of Industrially Important Microbes

❖ Screening:

Screening can be defined as: the use of highly selective procedures to allow the detection & isolation of only those microorganisms which are of interest from among a large microbial population.

- Screening allows the discarding of many valueless microorganisms, at the same time it allows the easy detection of the useful microorganisms that are present in the population in very less number.

❖ Types of screening techniques:

There are two main types of the screening techniques that are primary and secondary screening.

(1) **Primary screening (PS)**:

Primary screening allows the detection & isolation of microorganisms that possess potentially interesting industrial application.

➢ In the PS you must know:

- Primary screening separate out only a few microorganisms having real commercial value.
- Primary screening determines which microorganisms are able to produce a compound without providing much idea of the production or yield potential of the organisms.
- Example of the primary screening techniques is:

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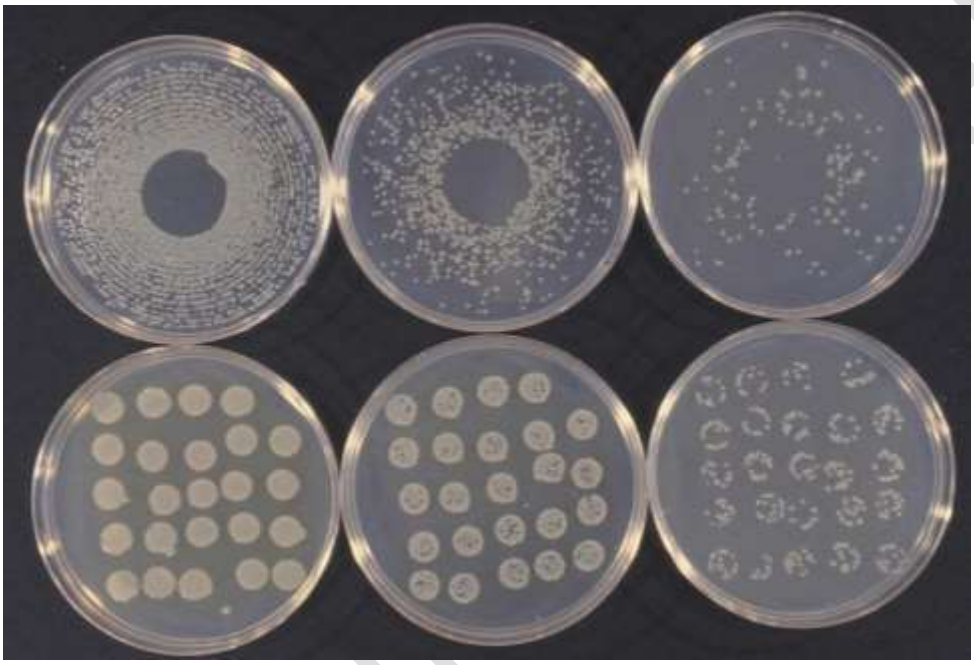
◆ Primary screening of antibiotic producing microorganisms

- The simplest screening technique for antibiotic producers is: Crowded Plate technique (Figures below).



- The technique is used to find out the microorganisms that produce an antibiotic without giving much information of sensitivity towards other microorganisms.

- Procedure include dilution and spreading or pouring of soil samples that give 300 or 400 or more colonies per plate
- Colonies producing antibiotic activity are indicated by an area of agar around the colony
- Such a colony is sub-cultured to a similar medium and purified by streaking, before making stock cultures. The purified culture is then tested to find what types of microorganisms are sensitive in the presence of these the antibiotics i.e. "Microbial Inhibition Spectrum" (MIS).
- The crowded plate procedure also does not necessarily select an antibiotic producing microorganism, because the inhibition area around the colony sometimes can be due to other reason like:
    (1) Marked change in the pH of the medium resulted due the metabolism of the colony.
    (2) Rapid utilization of critical nutrients in the vicinity of the colony etc.
- Thus further testing is required to confirm the inhibitory activity associated with a microorganisms is whether attributed to the presence of an antibiotic or not.
- Screening of antibiotic producing microorganisms can be improved by using a "test organism" and Wilkins method.

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(2) Secondary screening (SS):

Secondary screening allows further sorting out of microorganisms obtained from PS having real value for industrial processes and discarding of those lacking this potential.

- **In the SS you must know:**
  - SS is conducted on agar plates, in flasks or small fermenter containing liquid media.
  - SS can be qualitative or quantitative in its approach.
  - SS should give information about the evaluation of the true potential of the microorganisms for industrial usage.
  - SS should determine whether microorganisms are actually producing new chemical compounds not previously described.
  - SS should reveal whether there is pH, aeration or other critical requirements associated with particular microorganisms, both for the growth of the organism and for the formation of chemical products.
  - SS should also detect gross genetic instability in microbial cultures.
  - SS should show whether certain medium constituents are missing or possibly, are toxic to the growth of the organisms or its ability to accumulate fermentation products.

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❖ Strategies for isolation of industrially important microbes

- The diversity of microorganisms may be exploited still by searching for strains from the neutral environment able to produce products of commercial value.
- The first stage in the screening of microorganisms of potential industrial is their "isolation".
- Isolation involves obtaining either pure or mixed cultures followed by their assessment to determine which carry out the desired reaction or produce the desired product.
- In some cases it is possible to design the isolation procedure in such a way that the growth of producers is encouraged or that they may be recognized at the isolation stage, whereas in other cases organisms must be isolated and producers recognized at a subsequent stage.
- It should be remembered that the isolate must carry out the process economically and therefore the selection of the culture to be used is a compromise between the productivity of the organism and the economic constraints of the process.

❖ Criteria used for choice of organisms

(1) The organism should be capable to utilize the ingredients present in the medium to produce interested product.

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(2) The organism must having an optimistic temperature above 40°C considerably reduces the cooling costs of a large-scale fermentation, and therefore, the use of such a temperature in the isolation procedure may be beneficial.

(3) The stability of the organism and its amenability to genetic manipulation.

(4) The productivity of the organism, measured in its ability to convert substrate into product and to give a high yield of product per unit time.

(5) The easy product recovery from the cultures.

(6) It should be a high yielding strain.

(7) It should have stable biochemical characteristics.

(8) It should not produce undesirable substances.

(9) It should be easily cultivated on a large scale.

(10) The reaction of the organism with the equipment to be employed.

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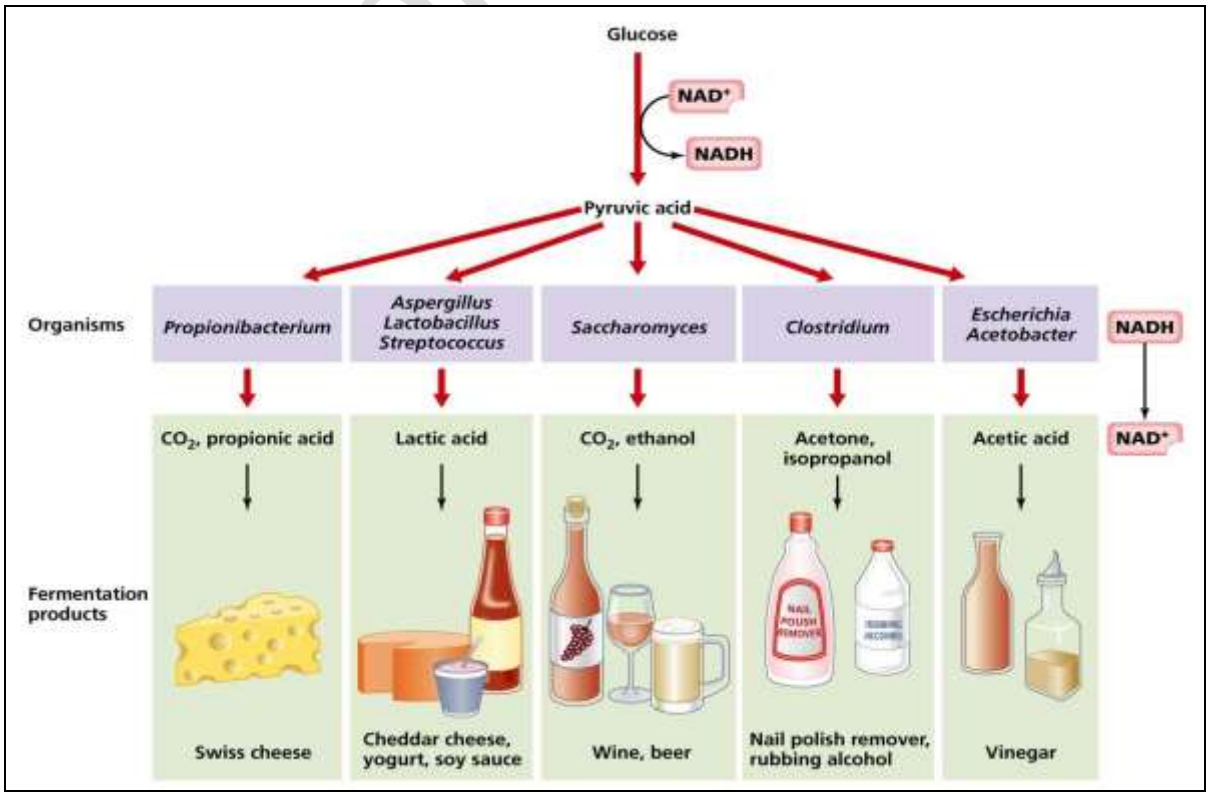
4. Fermentation

Fermentation: is a metabolic process by which the living cell is able to obtain energy through the breakdown of glucose and other simple sugar molecules in absence of oxygen.

- **Fermentation** is the term used by microbiologists to describe any process for the production of a product by means of the microorganism.

Or:

- **Fermentation** is the intentional use of microorganisms such as bacteria, yeast, and fungi to make products useful to humans (biomass, acids, alcohol antibiotics, enzymes, etc.) on an industrial scale (Figure below).



Products of Microbial Fermentation

❖ **Fermentation products include:**

(1) Food products: from milk (yogurt, kefir, fresh and ripened cheeses), fruits (wine, vinegar), vegetables (pickles, sauerkraut, soy sauce), meat (fermented sausages, salami).

(2) Industrial chemicals: (solvents: acetone, butanol, ethanol, enzymes, amino acids).

(3) Specialty chemicals: (vitamins, pharmaceuticals).

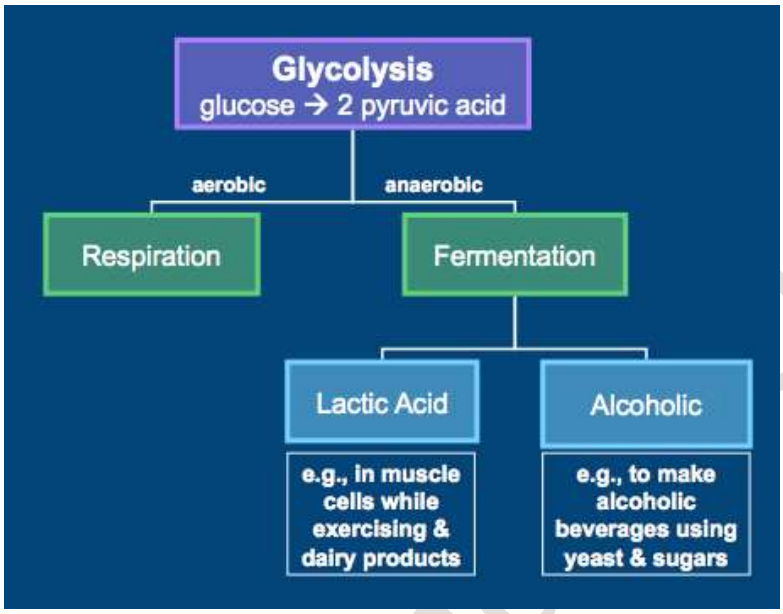
❖ **History of Fermentation:**

Louis Pasteur in the 19th century used the term fermentation in to describe the changes brought about by yeasts and other microorganisms growing in the absence of air (anaerobically).

He also recognized that ethyl alcohol and carbon dioxide are not the only products of fermentation.

In microorganisms, fermentation is the primary means of producing ATP by the degradation of organic nutrients anaerobically.

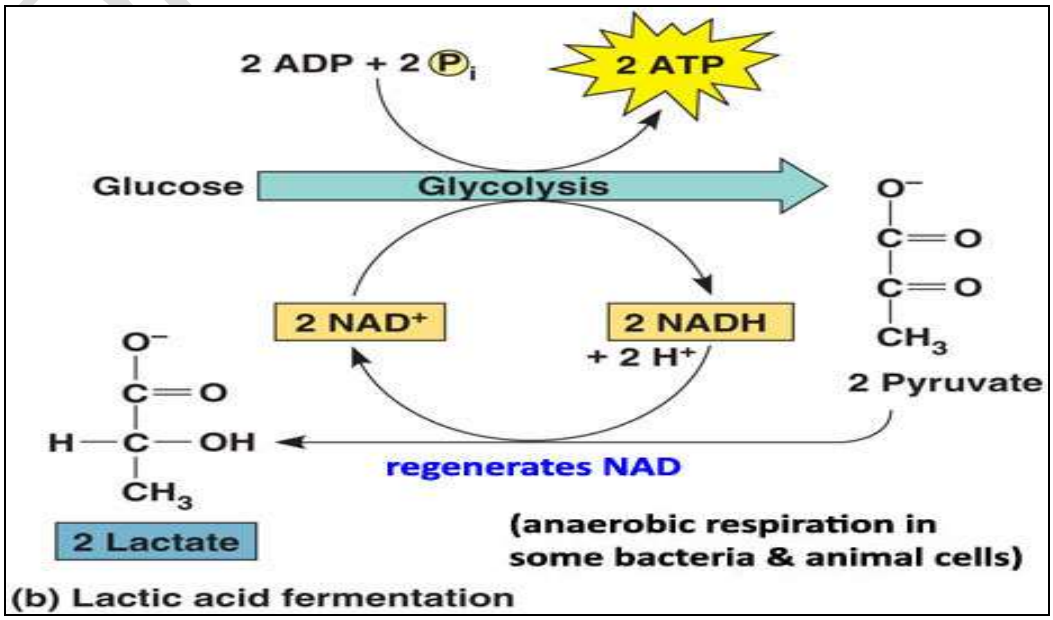
❖ **Types of Fermentation:** there are two types of fermentation:



Types of Fermentation

(A) Lactic Acid Fermentation

- Pyruvate is broken into lactic acid.
- Occurs in animal muscles when the tissue requires energy at a faster rate than oxygen can be supplied.
- Occurs in some bacteria and fungi to convert lactose into lactic acid in yogurt and cheese production.

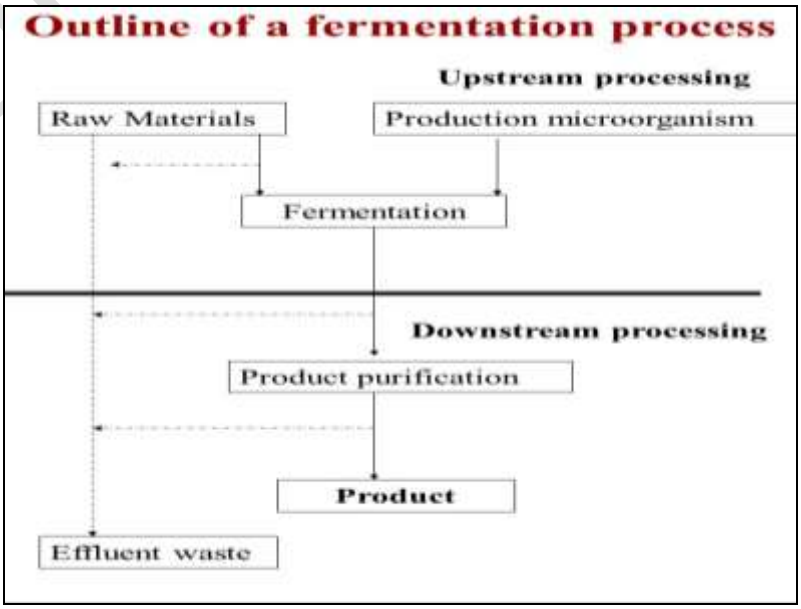


(B) Alcoholic Fermentation

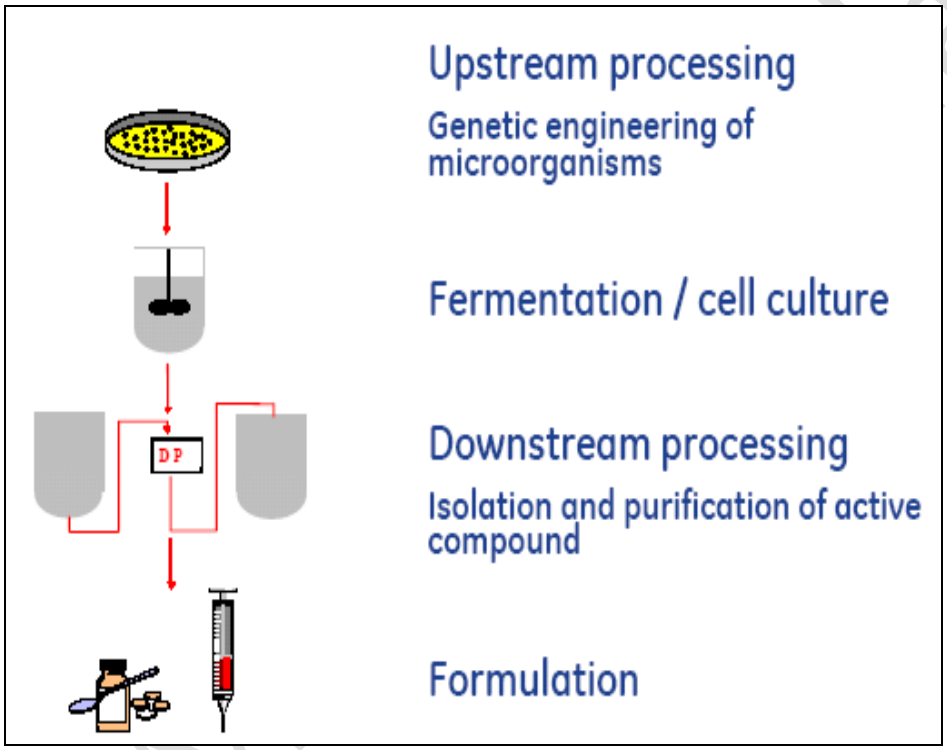
- Pyruvate is broken into alcohol and carbon dioxide.
- Occurs in yeasts and certain bacteria.
- Used in the production of beer, wine and bread (Figure below).

❖ Basis of Fermentation Process:

A typical operation in the fermentation process involves both upstream processing (USP) and downstream processing (DSP) stages (Figure below).



The fermentation process requires major contributions from a wide range of other disciplines, particularly biochemistry, genetics and molecular biology, chemistry, chemical and process engineering, and mathematics and computer technology.



Production cycle

❖ **Fermentation Medium**

Optimum balance of the media is mandatory for cells propagation and for the maximum production of target metabolite (end-product).

Media compositions:

- Carbon source.
- Nitrogen source.
- Minerals.

- Growth factors.
- Precursors (mutants).

The selection of suitable cost effective carbon and energy sources, and other essential nutrients, along with overall media optimization are vital aspects of process development to ensure maximization of yield and profit. In many instances, the basis of industrial media are waste products from other industrial processes, notably sugar processing wastes, lignocellulosic wastes, cheese whey and corn steep liquor.

❖ **The Component Parts of A Fermentation Process**

(1) The formulation of media to be used in culturing and in the production fermenter.

(2) The sterilization of the medium, fermentors and ancillary equipment.

(3) The production of an active, pure culture in sufficient quantity to inoculate the production vessel.

(4) The growth of the organism in the production fermentor under optimum conditions for product formation.

(5) The extraction of the product and its purification.

(6) The disposal of effluents produced by the process.

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❖ Fermentor (Bioreactor): History, Design and Its Construction

Meaning of Fermentor:

A fermentor (bioreactor) is a vessel for the growth of microorganisms which, while not permitting contamination, enables the provision of conditions necessary for the maximal production of the desired products.

A fermentor (bioreactor) is a closed vessel with adequate arrangement for aeration, agitation, temperature and pH control, and drain or overflow vent to remove the waste biomass of cultured microorganisms along-with their products.

A fermentor is used for commercial production in fermentation industries and is a device in which a substrate of low value is utilized by living cells or enzymes to generate a product of higher value. Fermentors are extensively used for food processing, fermentation, waste treatment, etc.

History of Fermentors:

De Beeze and Liebmann (1944) used the first large scale (above 20 litre capacity) fermentor for the production of yeast. But it was during the first world war, a British scientist named Chain Weizmann (1914-1918) developed a fermentor for the production of acetone. For the first time, large scale aerobic fermentors were used in central Europe in the year 1930's for the production of

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compressed yeast. The fermentor consisted of a large cylindrical tank with air introduced at the base via network of perforated pipes.

- Design of Fermentors:

All bioreactors deal with heterogeneous systems dealing with two or more phases, e.g., liquid, gas, solid. Therefore, optimal conditions for fermentation necessitate efficient transfer of mass, heat and momentum from one phase to the other. Chemical engineering principles are employed for design and operation of bioreactors.

- A bioreactor should provide for the following:

(1) Agitation (for mixing of cells and medium)

(2) Aeration (aerobic fermentors); for O_2 supply

(3) Regulation of factors like temperature, pH, pressure, aeration, nutrient feeding, liquid level etc.,

(4) Sterilization and maintenance of sterility

(5) Withdrawal of cells/medium (for continuous fermentors).

- Size of Fermentors:

The size of fermentors ranges from 1-2 litre laboratory fementors to 5,00,000 litre or, occasionally, even more, fermentors of upto 1.2 million litres have been used. The size of the fermentor

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used depends on the process and how it is operated. A summary of fermentor or size of fermentor (litres) Industrial product sizes for some common microbial fermentation is given in Table below.

Table: Fermentor sizes for various microbial fermentations

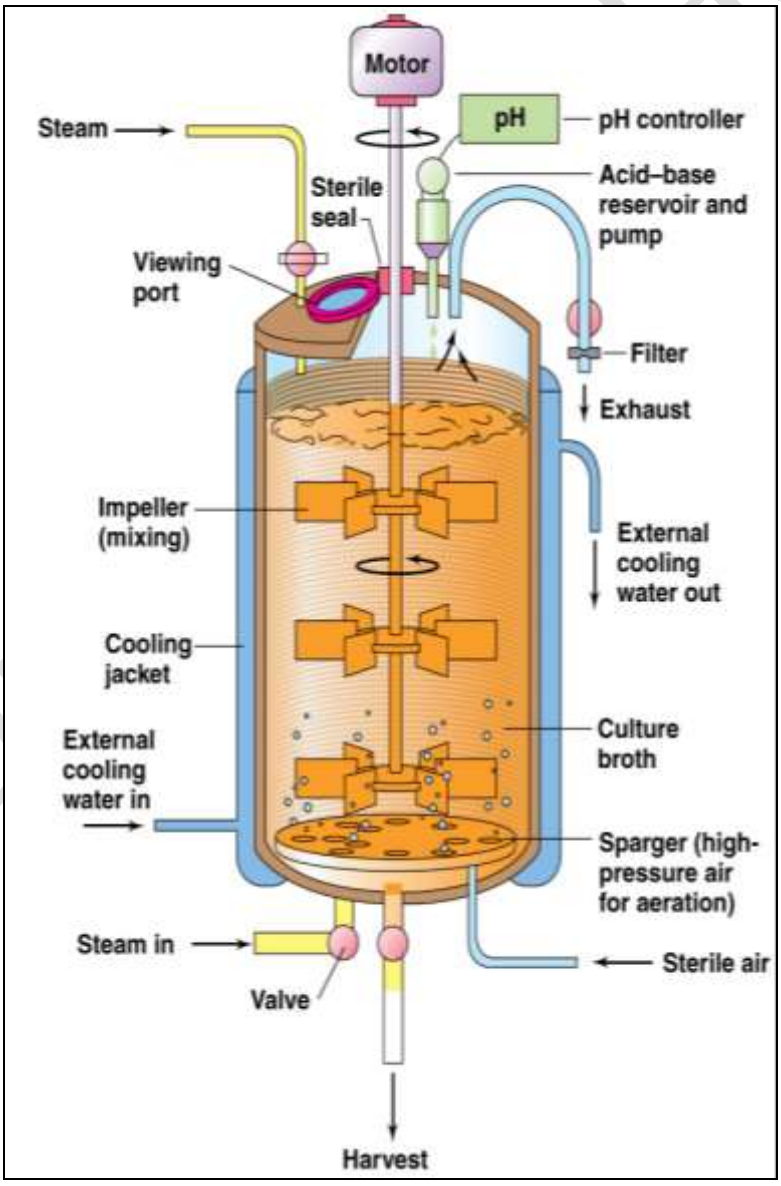
Size of fermentor (litres)	Industrial product
1-20,000	Diagnostic enzymes, substances for molecular biology.
40-80,000	Some enzymes, antibiotics.
100-1,50,000	Penicillium, aminolycoside, antibiotics, amyloses, proteases, amino acids, steriod transformations, wine, beer.
2,00,000-5,00,000	Amino acids(glutamate), wine, beer.

Construction of Fermentors:

Industrial fermentors can be divided into two major classes, anaerobic and aerobic. Anaerobic fermentors require little special equipment except for removal of heat generated during the fermentation process, whereas aerobic fermentors require much

more elaborate equipment to ensure that mixing and adequate aeration are achieved.

Since most industrial fermentation processes are aerobic, the construction of a typical aerobic fermentor (Figure below) is the following:



An industrial Aerobic Fermentor

❖ Types of Fermentation

There are two types of fermentation process: Solid State fermentation (SSF) and Liquid State fermentation.

(1) Solid State fermentation (SSF):

- SSF process can be defined as microbial growth on particles without presence of free water.

Solid State fermentation (SSF)

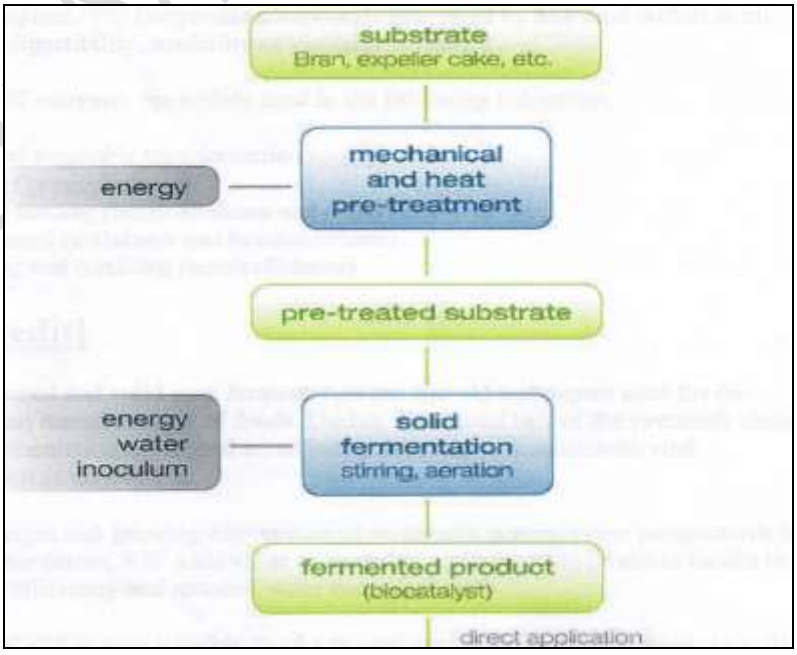
- Particles are a solid culture substrate such as rice or wheat bran saturated with water and inoculated with (mold, yeast, bacteria) in controlled room temperature.
- It is ideal for growing filamentous fungi.
- It has been used in Asia and developing nations.
- It is more cost effective (smaller vessels lower water consumption, reduced waste water treatment costs, lower energy consumption, and less contamination problems).

◆ **SSF process and applications:**

(1) Potentially many high value products such as extra-cellular enzymes, primary metabolites, and antibiotics could be produced in SSF.

(2) It is estimated that nearly a third of industrial enzyme produced in Japan is made by SSF process.

(3) Production of organic and ethanol from starchy substrates.



Flow-chart of SS fermentation

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(2) Liquid State fermentation (LSF):

- LSF is performed as Surface culture & submerged culture.
- Submerged culture is performed in tanks which can reach in size for over 100,000 gallons.
- It is ideal for the growing unicellular organisms such as bacteria and yeast.

Liquid State fermentation (LSF)

- **LSF have 4 methods:** Batch fermentation, Fed-batch fermentation, Continuous fermentation & Semi-continuous fermentation.

(A) Batch Fermentation

- Considered to be a closed system.
- The sterilized media in the fermenter is inoculated with the microorganism.
- Incubation is allowed under the optimum conditions (aeration, agitation, temperature).
- During entire fermentation nothing is added except air, antifoam and acid/base.

(B) Fed-Batch Fermentation

- It is enhancement of batch fermentation.
- Continue adding the nutrients (feeding) in a small doses during the fermentation.
- The method in controlling nutrients feeding process is by measuring methods.
- The main advantage of fed-batch fermentation is the elimination of catabolite repression (feed-back inhibition).

(C) Continuous Fermentation

- It is an open system.
- Continuously sterile nutrient is added and the converted nutrient is taken out from the fermentor.

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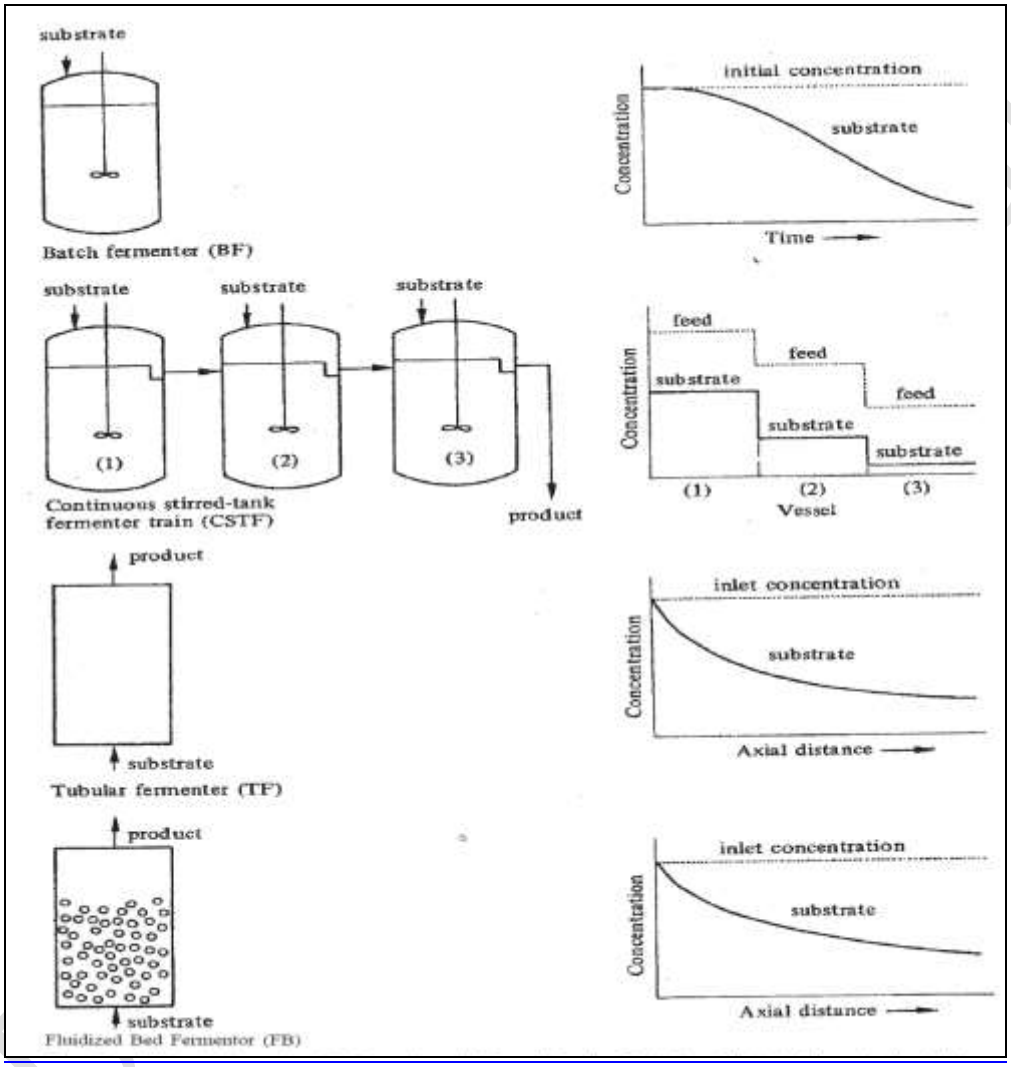
- In continuous process cell loss as a result of outflow must be balanced by growth of the microorganism.

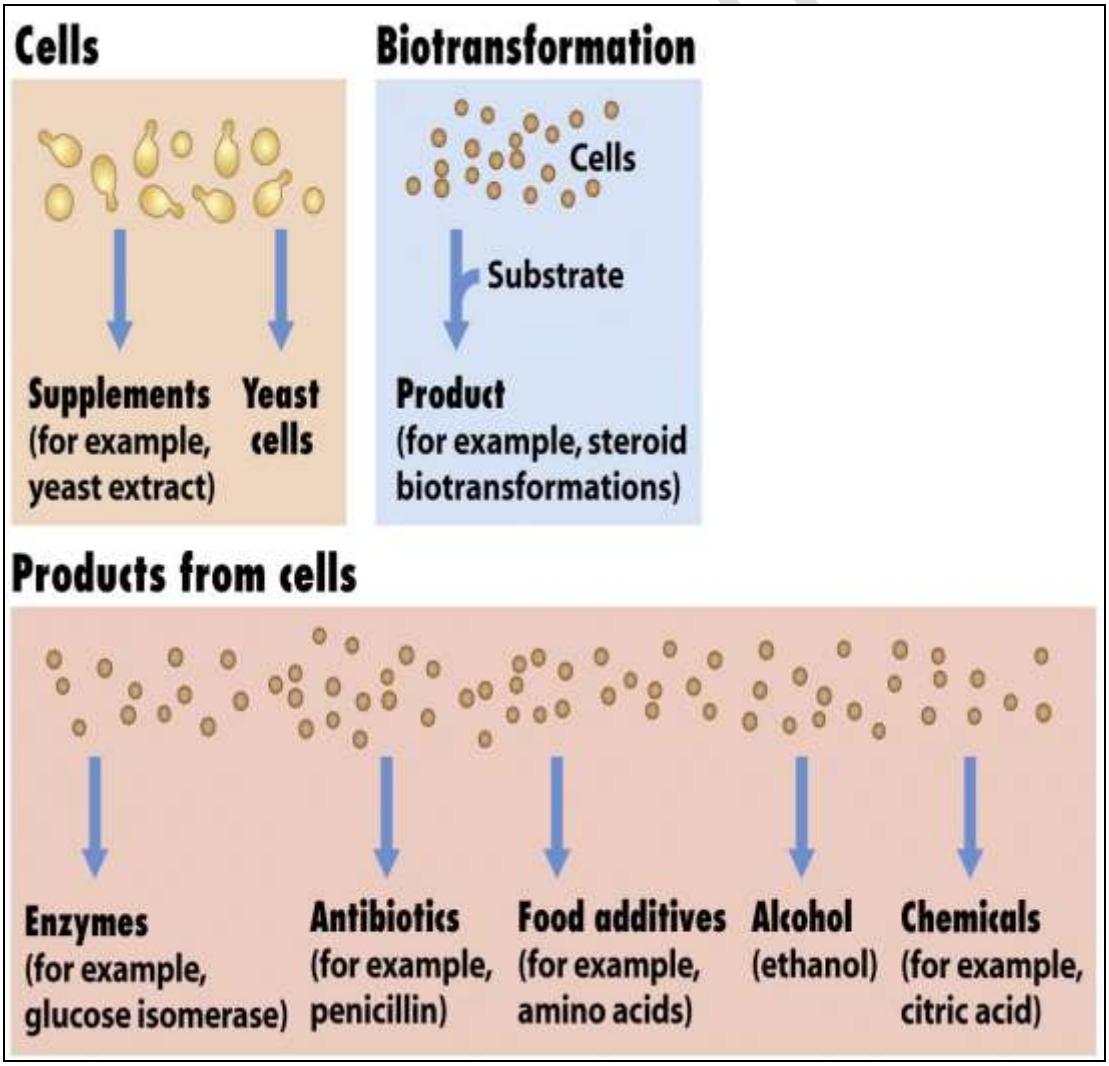


Different Fermentor Configuration

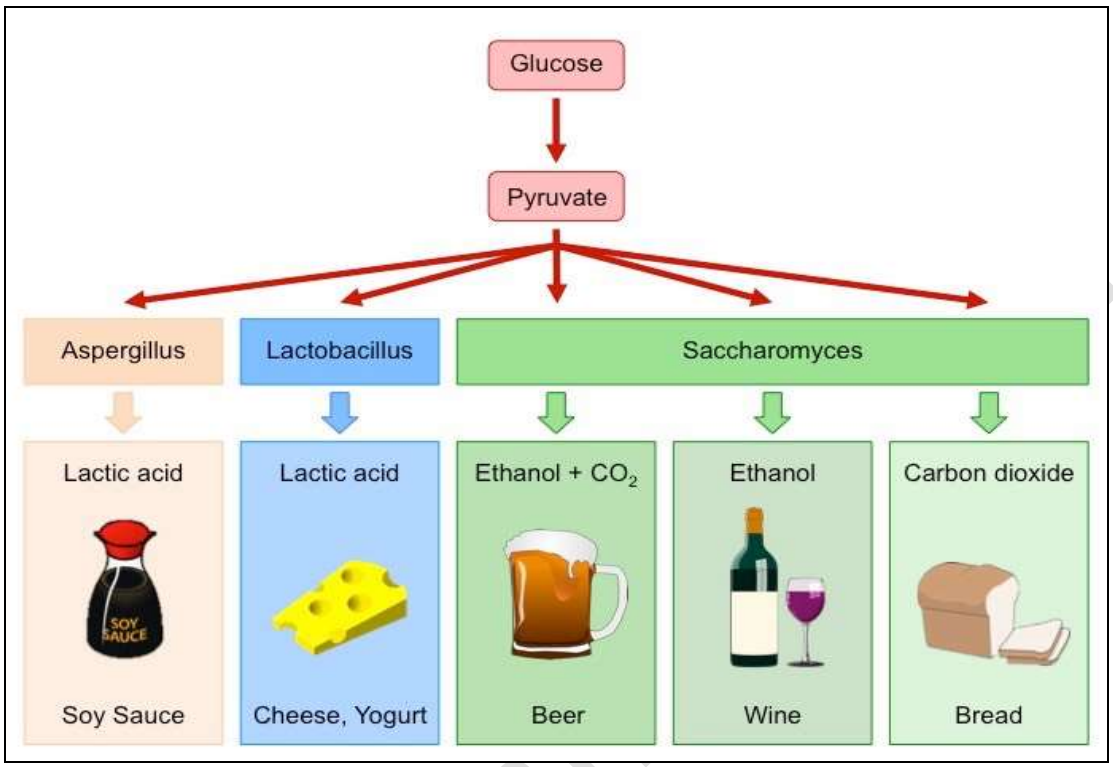
5. Microbial Bio-products

The product can either be:

- The cell itself: referred to biomass production.
- A microorganisms own metabolite: referred to as a product from a natural strain.
- A microorganisms foreign product: referred to as a product from recombinant DNA technology or genetically engineered strain, i.e. recombinant strain.



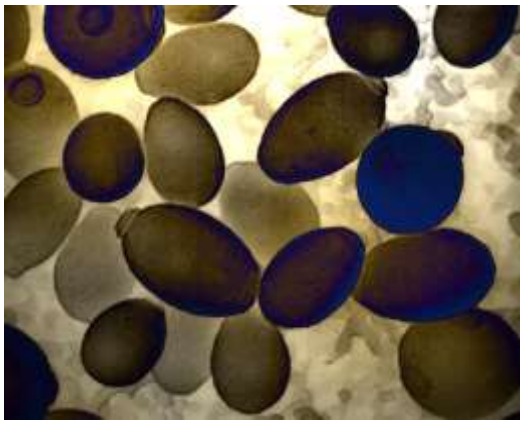
Types of Microbial Bio-products



Types of industrial microbial products and their producers

(1) Baker's Yeast Production

Yeasts are single-celled fungi. As fungi, they are related to the other fungi that people are more familiar with. These include common baker's yeast used to leaven bread, edible mushrooms available at the supermarket.



Yeast Cells

❖ History of Yeasts

Yeasts can be considered man's oldest industrial microorganism. It's likely that man used yeast before the development of a written language. Hieroglyphics suggest that that ancient Egyptians were using yeast and the process of fermentation to produce alcoholic beverages and to leaven bread over 5,000 years ago. The biochemical process of fermentation that is responsible for these actions was not understood and undoubtedly looked upon by early man.

- It was not until the invention of the microscope followed by the pioneering scientific work of Louis Pasteur in the late 1860's that yeast was identified as a living organism and the agent responsible for alcoholic fermentation and dough leavening. Shortly following these discoveries, it became possible to isolate yeast in pure culture form.
- Commercial production of baker's yeast began around the turn of the 20th century. Since that time, bakers, scientists and yeast manufacturers have been working to find and produce pure strains of yeast that meet the exacting and specialized needs of the baking industry.

❖ Production of Baker's Yeast:


Baker's yeast is used to leaven bread throughout the world and it is the type of yeast that people are most familiar with. Baker's yeast is produced from the genus and species of yeast called *Saccharomyces cerevisiae*.

The scientific name of the genus of baker's yeast, *Saccharomyces*, refers to "saccharo" meaning sugar and "myces" meaning fungus. The species name, *cerevisiae*, is derived from the name Ceres, the Roman goddess of agriculture. Baker's yeast products are made from strains of this yeast selected for their special qualities relating to the needs of the baking industry.

The typical yeast cell is approximately equal in size to a human red blood cell and is spherical to ellipsoidal in shape. Because of its small size, it takes about 30 billion yeast cells to make up to one gram of compressed baker's yeast.

Yeast reproduce vegetatively by budding, a process during which a new bud grows from the side of the existing cell wall. This bud eventually breaks away from the mother cell to form a separate

daughter cell. Each yeast cell, on average, undergoes this budding process 12 to 15 times before it is no longer capable of reproducing.

During commercial production, yeast is grown under carefully controlled conditions on a sugar containing media typically composed of beet and cane molasses. Under ideal growth conditions a yeast cell reproduces every two to three hours.

Yeasts can grow in the presence or absence of air. Anaerobic growth, is quite slow and inefficient. For instance, in bread dough, yeast grow very little. Instead, the sugar that can sustain either fermentation or growth is used mainly to produce alcohol and carbon dioxide. Only a small portion of the sugar is used for cell maintenance and growth.

In contrast, under aerobic conditions, in the presence of a sufficient quantity of dissolved oxygen, yeast grow by using most of the available sugar for growth and producing only negligible quantities of alcohol.

This means that the baker who is interested in the leavening action of carbon dioxide works under conditions that minimize the presence of dissolved oxygen. On the other hand, a yeast manufacturer that wants to produce more yeast cell mass, works under aerobic conditions by bubbling air through the solution in which the yeast is grown.

The problem posed to the yeast manufacturer, however, is not as simple as just adding air during the fermentation process. If the concentration of sugar in the fermentation growth media is greater than a very small amount, the yeast will produce some alcohol even if the supply of oxygen is adequate or even in abundance. This problem can be solved by adding the sugar solution slowly to the yeast throughout the fermentation process.

The rate of addition of the sugar solution must be such that the yeast uses the sugar fast enough so that the sugar concentration at any one time is practically zero. This type of fermentation is referred to as a fed-batch fermentation.

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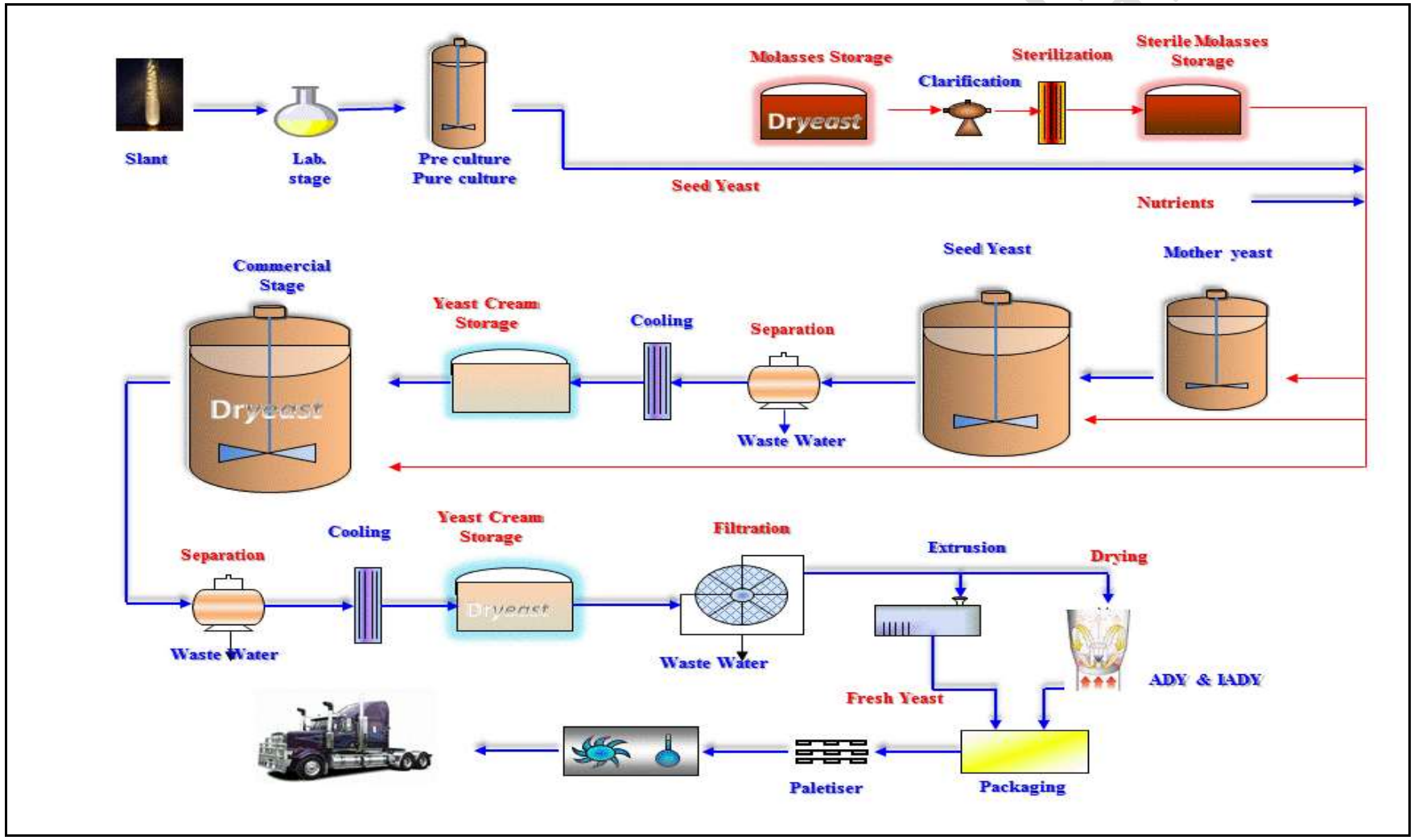

Flow-chart of the baker's yeast production process

❖ Functions of yaests in Backing


In the production of baked yeast have three primary functions:

(1) Production of carbon dioxide:

Carbon dioxide is generated by the yeast as a result of the breakdown of fermentable sugars in the dough. The evolution of carbon dioxide causes expansion of the dough as it is trapped within the protein matrix of the dough.

(2) Causes dough maturation:

This is accomplished by the chemical reaction of yeast produced alcohols and acids on protein of the flour and by the physical stretching of the protein by carbon dioxide gas.

(3) Development of fermentation flavor:

Yeast imparts the characteristic flavor of bread and other yeast leavened products. During dough fermentation, yeast produce many secondary metabolites such as ketones, higher alcohols, organic acids, aldehydes and esters. Some of these, alcohols escape during baking. Others react with each other and with other compounds found in the dough to form new and more complex flavor compounds.

(2) Alcohols Production

Alcoholic beverages have been produced throughout recorded human history.

They are manufactured worldwide from locally available fermentable materials, which are sugars derived either from fruit juices, plant sap and honey, or from hydrolyzed grain and root starch.

Although bacteria such as *Zymomonas* species may be involved in the production of certain products, yeasts are primarily used, either in single or mixed cultures.

Their fermentation products are ethanol, a range of desirable organoleptic (flavour and aroma) compounds and CO_2 (provides carbonation for some products).

The yeasts involved in these alcoholic fermentations are mostly strains of *Saccharomyces cerevisiae*, which cannot directly ferment starch. They require prior hydrolysis of the polysaccharide to simple sugars and small dextrins (not greater than three glucose units).

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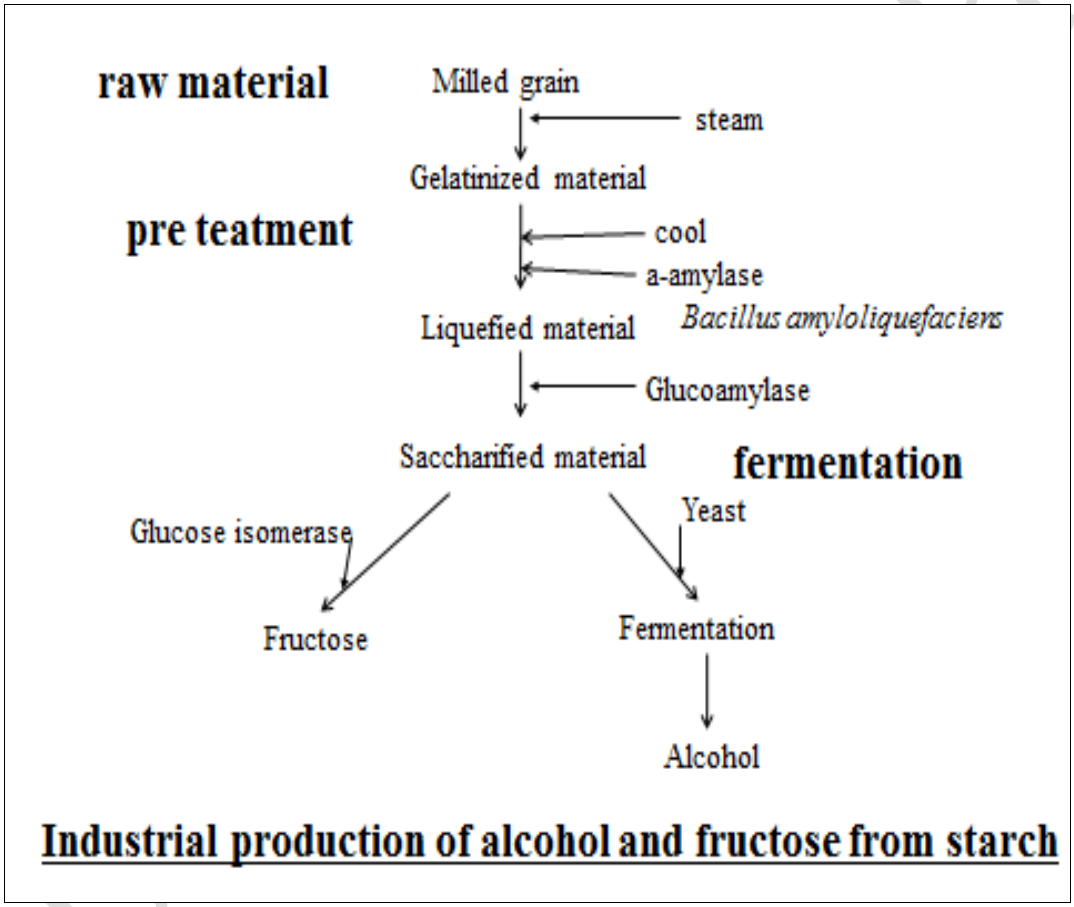
Traditionally, this is achieved by using fungal or plant amylases. These enzymes may be inherent elements of the carbohydrate source or added during processing.

Yeast + Glucose ➔ Alcohol (Ethanol) + CO_2



Industrial production of alcohol and fructose from starch

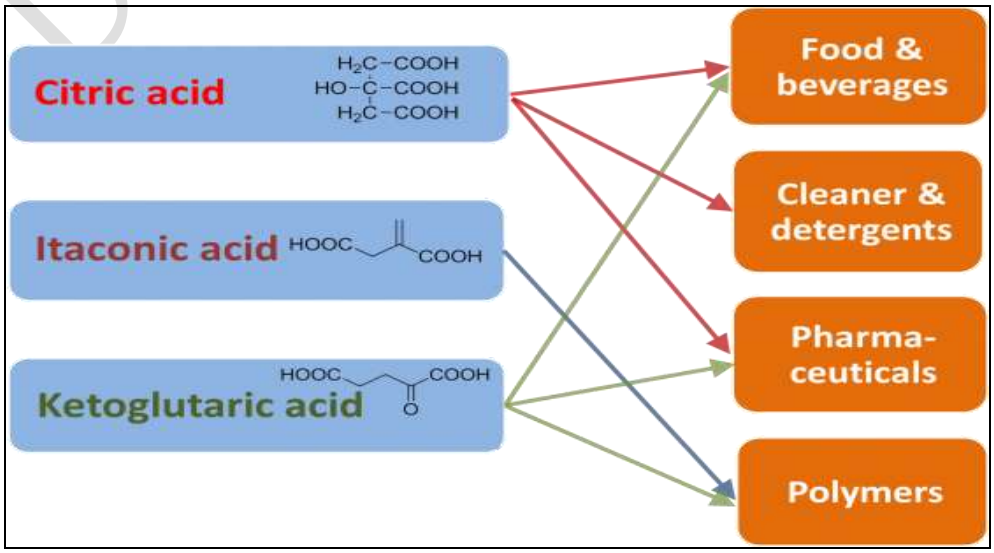
(3) Production of organic acids

The most common organic acids are the carboxylic acids whose acidity is associated with their carboxyl group -COOH. Organic acids have been used as food additives and preservatives.

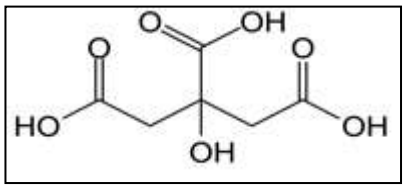
The organic acids produced by various microbes via fermentation. All acids of the tricarboxylic acid (TCA) cycle can be produced microbial in high yields (citric acid), other acids can be derived indirectly from the Krebs cycle such as itaconic acid, or can be derived directly from glucose (gluconic acid).

Some acids are formed as the end products from pyruvate or ethanol (lactic and acetic acid).

Large-scale commercial production of a number of organic acids are citric- gluconic- and itaconic acid. Other organic acids produced in lower scale are lactic acid, malic acid, gibberellic acid, and kojic acid.



❖ Citric Acid



Citric acid (2-hydroxypropane-1,2,3-tricarboxylic acid) is one of the world's major fermentation products with the annual production of over 550,000 tonnes, and its demand is increasing at the rate of 2-3% every year.

It was first isolated in 1784 from lemon juice and crystallized by Scheele. Until the 1920s, citric acid was extracted from the lemon juice and referred as 'natural citric acid'.

Whehmer first time in 1923 described that citric acid is a metabolic product of *Penicillium* and *Mucor*.

In 1923, Pfizer became the first industry to produce citric acid through fermentation based process in USA, by culturing *Aspergillus niger* in surface culture in a medium containing sucrose and mineral salts.

➢ Producing organisms:

Produced by many microorganisms including filamentus fungi, yeasts and bacteria. All of them could be used to produce citric acid, however, the mutants of *A. niger* are generally used for commercial use.

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Bacteria such as *Bacillus licheniformis, B. subtilis, Corynebacterium spp.*

Fungi *such as A. niger, A. awamori, A. foetidus, Penicillium restrictum*

Yeast such as *Candida lipolytica, C. intermedia* and *Saccharomyces cerevisiae*

However, ***A. niger*** a filamentous fungus remained the organism of choice for citric acid production due to ease of handling, its ability to ferment a variety of cheap raw materials, and high yields of citric acid.

- **Raw materials:**

Carbohydrate sources such as beet molasses, sucrose, commercial glucose, starch hydrolysis etc. The raw material is diluted to 20-25 per cent sugar concentration and mixed with nitrogen source and other salts.

- **Production conditions:**

The pH of the medium is maintained around 5.0, when molasses are used and pH is adjusted at 3.0 when sucrose is used. The fermentation is carried out either under the surface, submerged or under solid state conditions.

Citric acid fermentation by *Aspergillus niger* is greatly enhanced in the presence of trace metals such as iron, zinc, copper, manganese etc. in the medium.

The first stages of citric acid formation involve the breakdown of hexoses to pyruvate in glycolysis, followed by its decarboxylation to produce acetyl CoA

CO_2 released during this reaction is not lost, but is recycled by pyruvate carboxylase which is produced constitutively in *Aspergillus*.

Normally, oxaloacetate would largely be supplied through the completion of the TCA cycle, allowing recommencement of the cycle by condensing with acetyl CoA to form citrate, catalysed by citrate synthase. However, in order to accumulate citrate, continuation of the cycle must be blocked. This is achieved by inhibiting aconitase, the enzyme catalysing the next step in the TCA cycle. Inhibition is accomplished by removal of iron, an activator of aconitase.

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(4) Production of Antibiotics

Definition of Antibiotics

Antibiotics are secondary metabolites produced by many microorganisms (Actinomycetes, Bacteria, Fungi, & Algae) that at low concentration can kill or inhibit the growth of other microorganisms.

Penicillin was the first antibiotic discovered in September 1928 by an English Bacteriologist, late Sir Alexander Fleming who accidentally obtained the antibiotic from a soil inhabiting fungus *Penicillium notatum* but its discovery was first reported in 1929, and clinical trials first conducted on humans in 1940.

Actinobacteria produce the majority of the naturally occurring antibiotics. The first antibiotics discovered in *Actinobacteria* were actinomycin from a culture of *Streptomyces antibioticus* in 1940, streptothricin from *Streptomyces lavendulae* in 1942, and streptomycin from *Streptomyces griseus* in 1944, all of which were discovered by Waksman and colleagues. Streptomycetes have been the major source of clinical antibiotics and are responsible for over 80% of all antibiotics of actinobacterial origin. That actinomycin, streptomycin, and streptothricin were the first to be found is not surprising, as these molecules occur at much higher frequencies than many other antibiotics.

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❖ Classification of antibiotics:

There are several ways of classifying antibiotics. The most common classification schemes are based on: their molecular structures, mode of action, spectrum of activity and type of activity.

Mode of Action of Antibiotics

The antimicrobial potency of most classes of antibiotic are directed at some unique feature of the bacterial structure or their metabolic processes. The most common targets of antibiotics are illustrated in Figure 22.

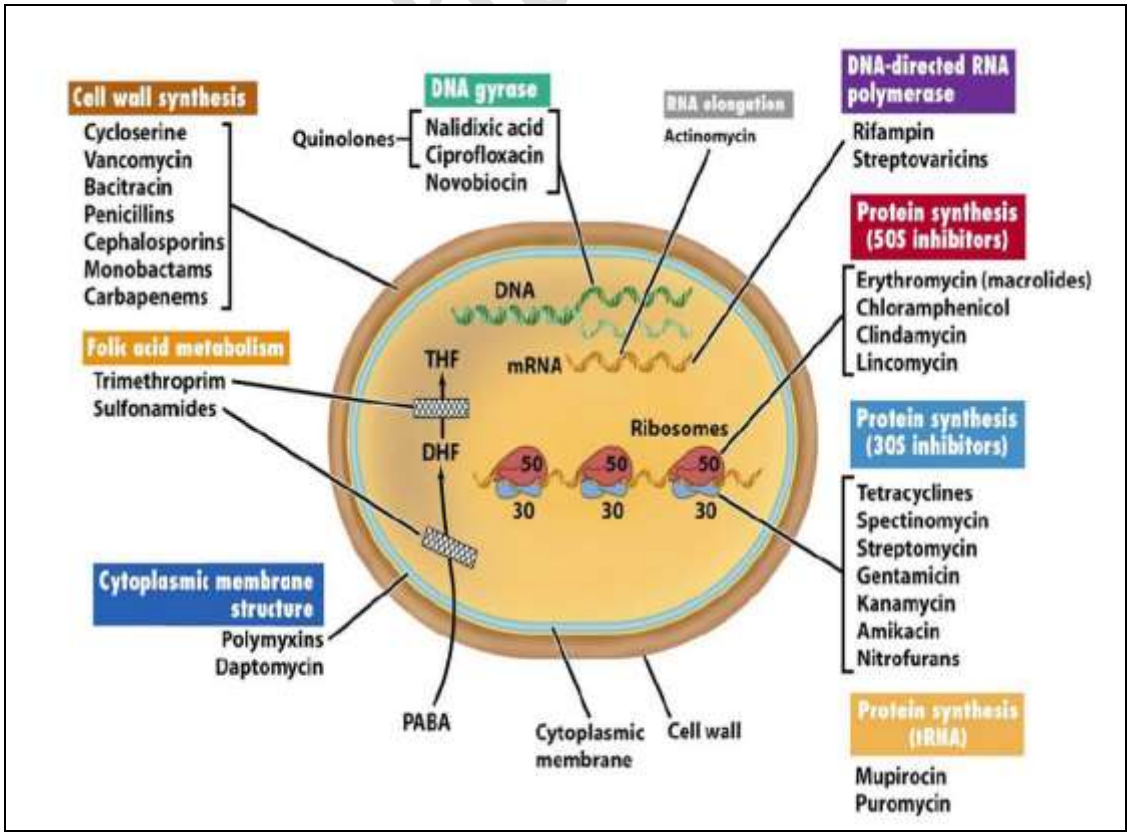


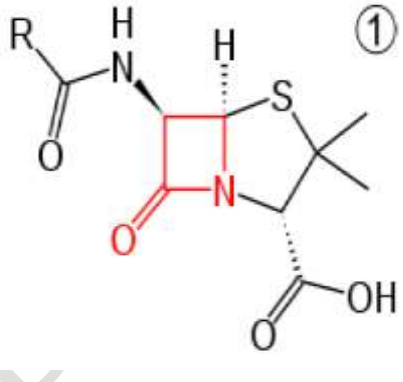
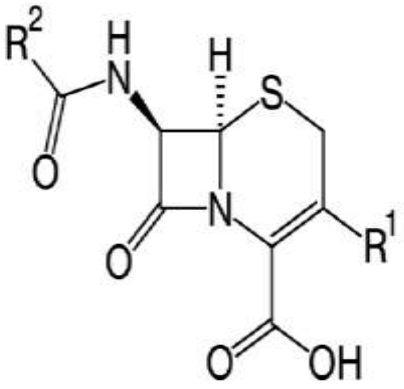
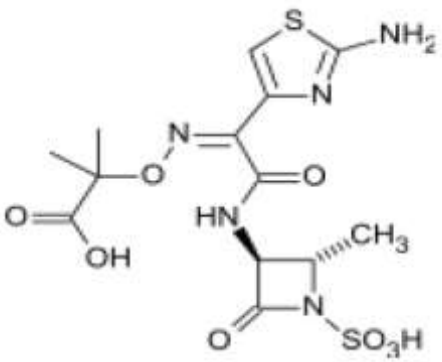
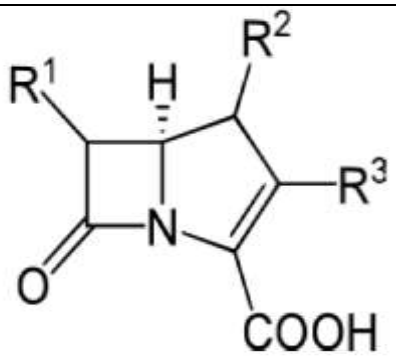
Figure: Antibiotic Target Sites (Mode of Action)

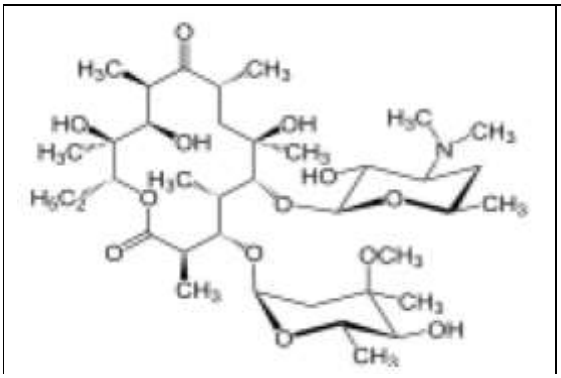
The mechanism of antibiotic actions are as follows:

- Inhibition of cell wall synthesis
- Breakdown of cell membrane structure or function
- Inhibition of the structure and function of nucleic acids
- Inhibition of protein synthesis
- Blockage of key metabolic pathways

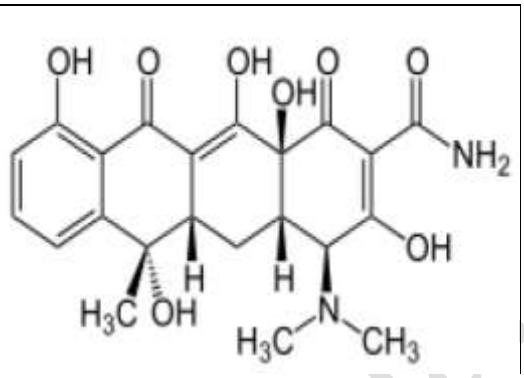
(1) Molecular structures of Antibiotics

The chemical or molecular structures (Figure 11) include Beta-lactams, Macrolides, Tetracyclines, Quinolones, Aminoglycosides, Sulphonamides, Glycopeptides and Oxazolidinones.

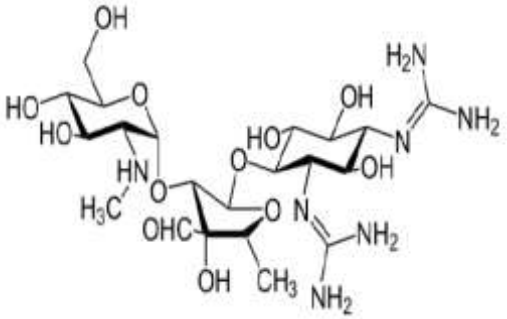
	
Beta-lactam	Cephalosporins
	
Monobactam	Carbapenem



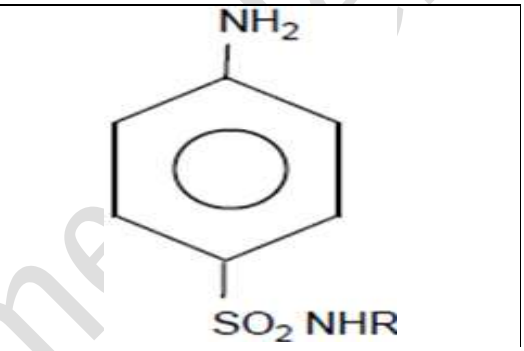
Macrolide



Tetracycline



Aminoglycoside



Sulphano mides

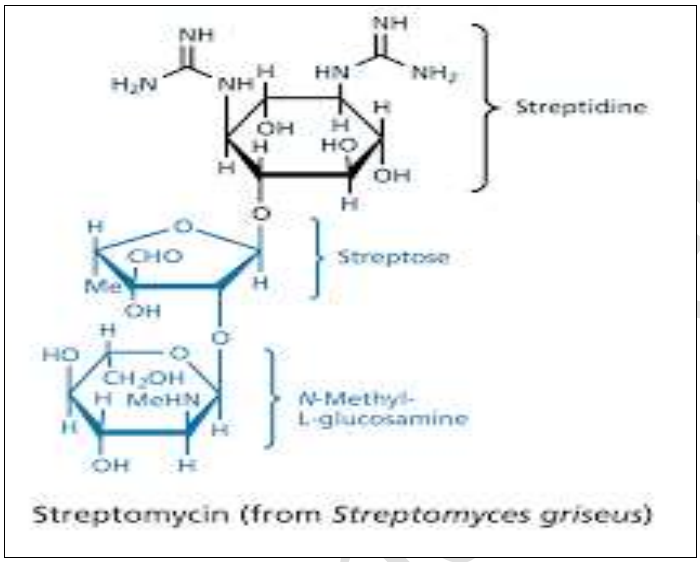
Molecular Structure of the Major classes of clinical antibiotics produced by actinomycetes.

(1) Aminoglycoside Antibiotics

Aminoglycoside is a medicinal and bacteriologic category of traditional Gram-negative antibacterial therapeutic agents that inhibit protein synthesis and contain as a portion of the molecule an amino-modified glycoside (sugar); the term can also refer more generally to any organic molecule that contains aminosugar substructures.

Streptomycin is the first-in-class aminoglycoside antibiotic. It is derived from *Streptomyces griseus* and is the earliest modern agent used against tuberculosis. Other examples of

aminoglycosides include antibiotics; kanamycin, tobramycin, gentamicin, and neomycin.



Streptomycin (from *Streptomyces griseus*)

Production of Streptomycin

The industrial production of streptomycin is carried out using submerged fermentation processes.

As the *Streptomyces* mutants are genetically unstable, the spores are maintained as soil stocks or lyophilized and are used for inoculating sporulation medium, which is then transferred to germinator where biomass is increased for inoculating fermenters.

The fermentation media consists of glucose, starch, dextrin, soy meal, corn steep liquor, sodium sulphate. The streptomycin fermentation requires high aeration and agitation.

The fermentation is carried out at 28-30ºC with pH maintained at 7.6-8 for good productivity. The fermentation lasts for 5-7 days with of yield of 1-3 g/L of the fermentation broth.

The streptomycin fermentation proceeds through three phases:

(1) In first phase: the organism produces proteases which digest the soybean meal and release ammonia and carbohydrates. These are utilized for increasing the biomass. Glucose is slowly utilized and net production of streptomycin is low during this phase. The pH of the medium increases from 6.7 or 6.8 to 7.5 or higher. This phase lasts for 24h.

(2) The next phase (the idiophase or the stationary phase): during which maximum streptomycin (secondary metabolite) is produced. It ranges from 24h to 6-7 days. Rapid utilization of ammonia and glucose occurs with no mycelial growth and pH during this phase remains fairly constant at 7.6 to 9.0.

(3) In the last phase (death phase): during which the sugars have been completely depleted in the medium and streptomycin production ceases completely. The ammonia released due to the cell lysis raises medium pH. Fermentation broth is generally harvested before the last phase begins.

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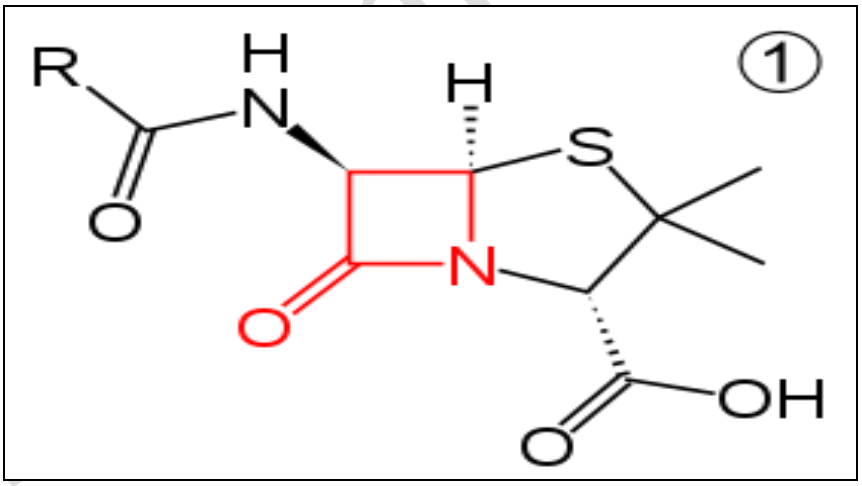
(2) β-lactam Antibiotics

β-lactam antibiotics are a class of broad-spectrum antibiotics, consisting of a beta-lactam ring in their molecular structures. This includes penicillin derivatives,(penams), cephalosporins (cephems), monobactams, and carbapenems.

Most β-lactam antibiotics work by inhibiting cell wall biosynthesis in the bacterial organism and are the most widely used group of antibiotics. Until 2003, when measured by sales, more than half of all commercially available antibiotics in use were β-lactam compounds.



Core structure of penicillins (β-lactam ring in red)

Mechanism of action

β-lactam antibiotics are bacteriocidal, and act by inhibiting the synthesis of the peptidoglycan layer of bacterial cell walls.

Production of Penicillin

The producing microorganism is *Penicillium.*

In general, commercial penicillin is produced by fed-batch fermentation process using bioreactors of 30K-250K capacity.

The fermentation medium is composed of a carbon source like lactose (from whey) and a nitrogen source like corn steep liquor (is an important ingredient in the broth; it supplies the fungus with nitrogen and growth factors).

The media used for the production of penicillin contains corn steep liquor solids 3.5%, lactose 3.5%, glucose 1%, calcium carbonate 1%, potassium di-hydrogen phosphate 0.4%, edible oil 0.25% and penicillin precursors. Additional supplements like yeast extract, soy meal, ammonium salts are also added. In addition, side chain precursors like phenyl acetic acid or phenoxyacetic acid are also added to the medium.

The media is placed at 25-27ºC with pH maintained at around 5.5-6.0 with a constant supply of oxygen.

Lyophilized spores of *Penicillium* are used as inoculum. For better production of penicillin, development, of loose mycelial pellets is however preferred.

The penicillin fermentation process can be divided into two phases: a vegetative growth phase (around 40 h) followed by penicillin production phase (150-180 h).

(1) Growth phase 1: The growth phase lasts for about 40 h during which the cell mass increases very rapidly and oxygen demand is very high.

(2) Penicillin production phase. The biomass production is greatly reduced and rate of penicillin production increases. As the lactose approaches limiting levels and cell densities in the fermenter become very high, addition of low levels of glucose maximize penicillin yield. Various other media components are fed during this phase to extend the penicillin production for 120-180h.

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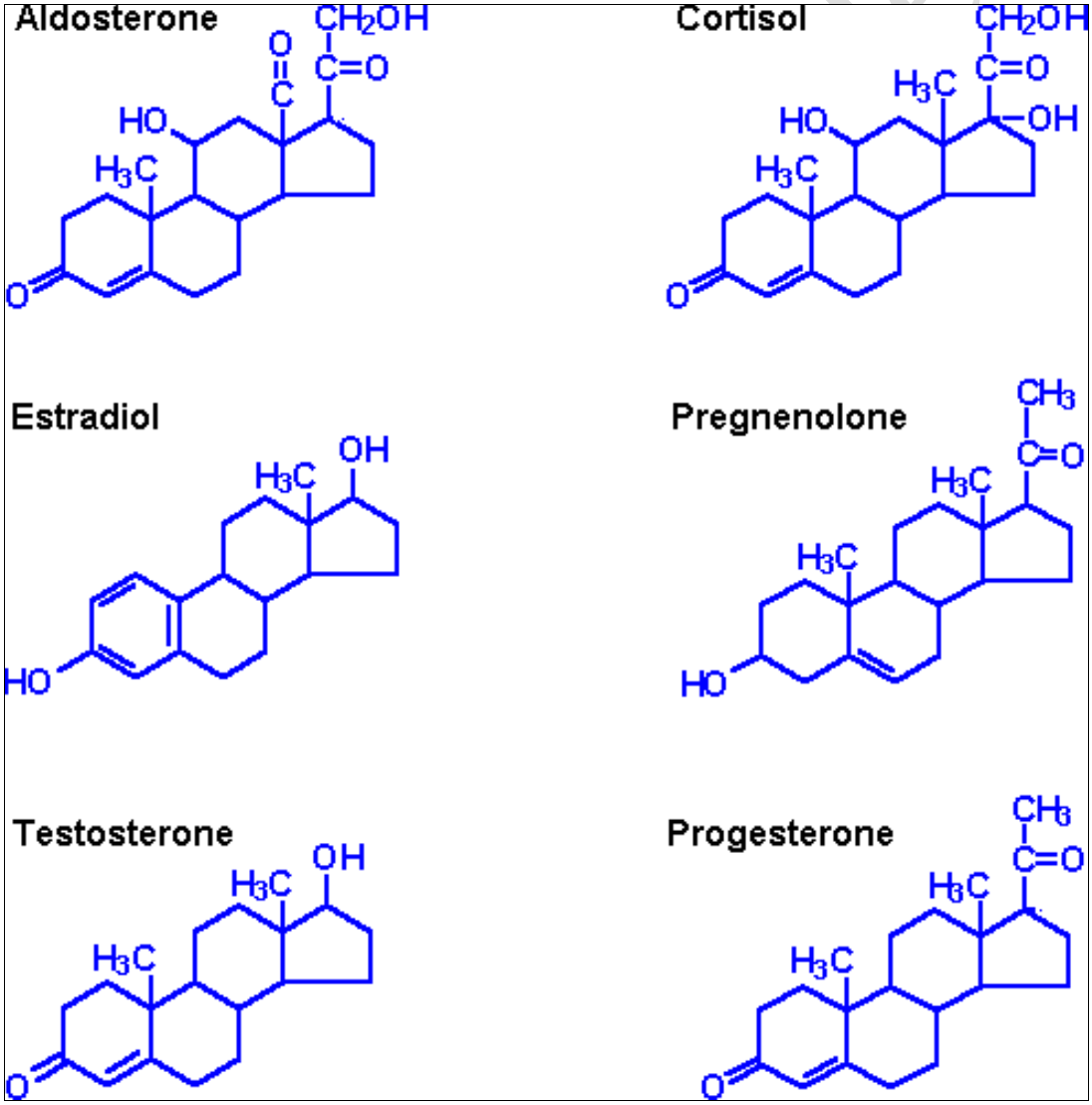
(5) Steroids biotransformation

Steroids

Are a large group of organic compounds with the perhydro-1,2 cyclopentano- phenanthrene nucleus, which consists of four fused rings (Figure below).



Structure of steroids

Steroids constitute a natural product class of compounds that is widely distributed throughout nature present in bile salts,

adrenal-cortical and sex hormones, insect molting hormones, sapogenins, alkaloids and some antibiotics.

- **Uses of Steroids:**

(1) Sex Hormones

The largest economic impact of synthetic estrogen and progestin production has been for use as contraceptive agents and for treatment and prevention of osteoporosis such as (Estrogens and Testosterone).

(2) Corticosteroids:

The greatest portion of steroid drug production is aimed at the synthesis of glucocorticoids which are highly effective agents for the treatment of chronic inflammation.

(3) Saponins:

These are used for their hypocholesterolemic (cholesterol lowering) activity.

- **Biotransformation**

Is a process by which organic compounds are transformed from one form to another to reduce the persistence and toxicity of the chemical compounds. This process is aided by major range of microorganisms and their products such as bacteria, fungi and enzymes.

Two of the earliest such microbial transformations were the conversion of progesterone to 11– a hydroxy progesterone by the

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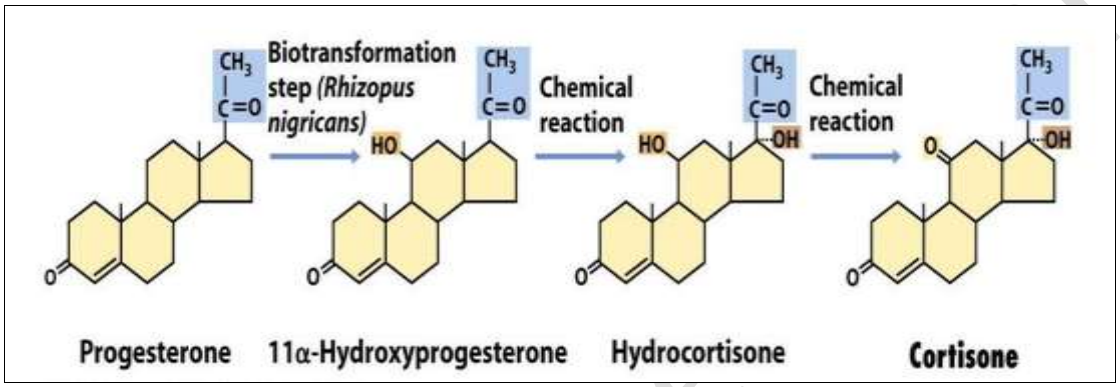
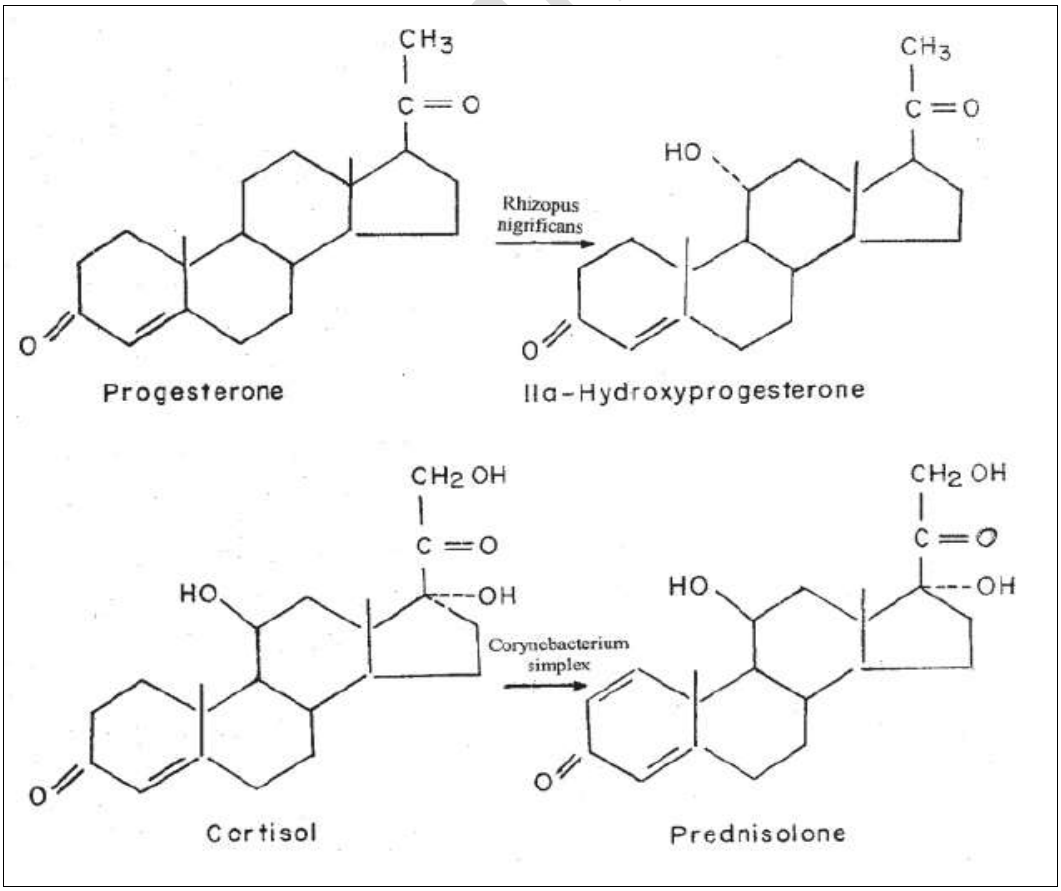
introduction of– OH at the position 11 using *Rhizopus nigricans* and the conversion of cortisol to prednisolone by *Corynebacterium simplex.*





Microbial Transformations of Some Steroids

- **Types of Microbial Transformations in Steroids:**

Transformations by microorganisms affecting various positions in a wide range of steroids and have been carried out. Although steroid hormones have been most widely studied, the transformation of bile acids, plant and animal steroid alkaloids have also occurred.

The transformation reaction include: hydroxylation, dehydrogenation, reduction, side chain degradation, lactone formation, aromatization, isomerization, epoxidation, hydrolysis esterification, halogenation, and cleavage of the steroid skeleton.

(1) **Hydroxylation**: Microbial hydroxylation is the involvement of direct replacement of the hydrogen atom on a given carbon. The oxygen atom in the hydroxyl group is derived from molecular oxygen (gaseous), not from water. The hydroxyl group thus formed always retains the stereo chemical configuration of the hydrogen atom that has been replaced.

(2) Dehydrogenation: Bacteria and fungi capable of dehydrogenizing the secondary alcohol group of steroids. They produce corresponding carbon derivatives.

(3) Hydrogenation: Ring hydrogenation involves the conversion of prednisone to cortisone, and prednisone to cortisol with *Bacillus megaterium*.

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❖ Conditions Used in Steroid Transformation:

- The media used are highly variable, but not very complex. They are basically mineral salts media containing some carbon source such as glucose, dextrin or glycerol. Nitrogen sources may be ammonium salts, corn steep liquor, soybean, or a protein digest. In some cases yeast extract is added.

- Steroid are lipids; they are not water soluble and therefore must be dissolved in a water-miscible lipid-solvent such as acetone, ethanol, propylene glycol, and methanol are suitable because they dissolve a reasonable amount of the steroid while being relatively non-inhibitory to the enzymes; dimethyl form amide dissolves a reasonable amount of the steroids but has only a minimum of toxicity.

- The level of steroid added is variable and depends both on the transforming ability of the organisms as well as its susceptibility to the toxic effects of the steroid. Normally 200-800 mg/litre are added. But much higher amounts are sometimes used. To solve the problem of the insolubility of steroids in water, non-ionic surface-acting agents which reduce surface tension e.g. Tween 80 are often added to the medium. Some poly-saccharides in the medium e.g. yeast cell wall mannan, bind to the steroids and cause them to be more available to the organism.

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- A wide range of microorganisms, mainly fungi and bacteria, are used in the transformation of steroids. Some of these include the fungi; *Rhizopus nigricans*, *Curvularia lunata*, *Fusarium spp. Cylindrocarpon radicicola* as well as the bacteria; *Mycobacterium* spp., *Corynedbacterium simplex*, and *Streptomyces* spp.

- Steroid transformations require vigorous aeration and a temperature of about 28˚C is usually employed. The fermentation is usually complete in four to five days.

- The transformation may occur at different stages of the growth and the steroid may be added to the growing cultures either simultaneously with the inoculation of the culture or the resting or stationary stage of the organism. Fungal spores may sometimes be inoculated as the steroid is introduced into the medium. In recent times immobilized cells have been employed in the transformations of steroids.

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6. References

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