Fermentation by microorganisms

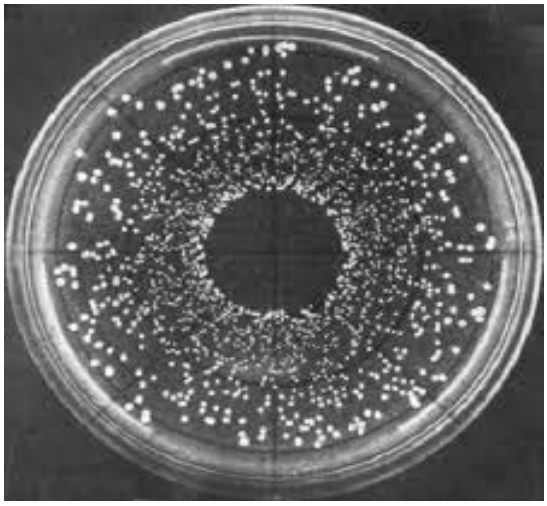
Bioprocess or fermentation technology is an important component in biotechnology process and involve complete living cells (microbe, mammalian or plant), organelles or enzymes as the biocatalyst and will aim to bring about specific chemical and/or physical changes in organic materials.

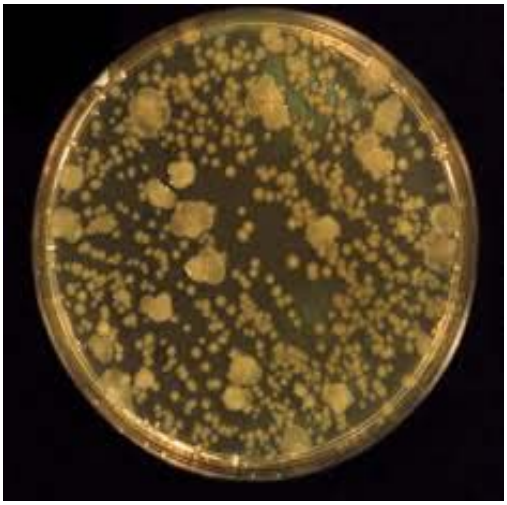
Stages of fermentation

1-Screening and isolation of microorganisms

Screening may be defined as the use of highly selective procedures to allow the detection and isolation of only those microorganisms of interest from among a large microbial population. The techniques that used for screening:

1-Crowded plate technique: The crowded plate technique is the simplest screening technique employed in detecting and isolating antibiotic producers.





2-Auxanography technique: This technique is largely employed for detecting microorganisms ability to produce growth factors (eg. Amino acid and Vitamins) extracellularly.

3-Enrichment culture technique: This technique used to isolate the desired microorganisms from a heterogeneous microbial population present in the sample. Either medium or incubation conditions are adjusted so as to favor the growth of the desired microorganism.

The wild strains isolated from the nature have low production efficiency, therefore; many ways were used for enhance the productivity such as:

1- Ecological ways:

Provision of the optimal growth conditions for microorganism such as temperature, pH, aeration, humidity, media.....etc.

2-Genetic ways:

Any alteration in the inherited nucleic acid sequence of the genotype of an organism by using:

1-Genetic mutation

The mutation is defined as a permanent change in the sequence of DNA that alter the sequence of amino acids in the protein. There are two types of mutations:

a- Spontaneous mutation

Spontaneous mutations occur without exposure to any obvious mutagenic agent. Sometimes DNA nucleotides shift without warning to a different chemical form. This leads to mistakes at the time of DNA replication. The frequency and productivity were low so that don't depend on it.

b-Induced mutation

Induced mutation occur with treatment the cells with mutagens such as physical mutagens which including ultraviolet and X-rays and chemical mutagens such as mitomycin C, nitrosoguanidine..etc. The cells that result from mutation called mutants which divided to:

1-Majer mutants: The mutant strains have appeared a big and clear change in biochemical characteristics. The mutation had easily lost. These mutants were important in genetic studies.

2-Minor mutants: The mutant strains have appeared a little change in some features and don't recognize in the external shape. This mutation is genetically constant and important in development the productivity of strains.

2-Transfer of genetic material(hybridization)

The process of transfusion the genetic material between genetically different two bacterial cells and produced hybrid cell. This process was done by:

a-Transformation:

Transformation is the genetic alteration of a cell resulting from the direct uptake, incorporation and expression of exogenous genetic material from its surroundings and taken up through the cell membrane(s). Transformation occurs naturally in some species of bacteria, but it can also be effected by artificial means in other cells.

b-Transduction

Transduction is the process by which DNA is transferred from one bacterium to another by a virus.When bacteriophages infect a bacterial cell, their normal mode of reproduction is to translation machinery of the host bacterial cell to make numerous virions, including the viral DNA or RNA and the protein coat.

c- conjugation

conjugation is transfer of genetic material between two bacterial cells in direct contact and formation of bridge between them, one of these cells is donating cell and the other is receiving cell.

3-Gene amplification

Gene amplification, also known as gene duplication or chromosomal duplication, is a cellular process in which multiple copies of a gene are produced. Drug resistance in cancer cells is linked to amplification of the gene that prevents absorption of the chemotherapeutic agent by the cell, such an increase induced by a polymerase chain reaction.

4-Genetic recombination

Genetic Recombination is the process by which an organism's offspring's combination of genes becomes different than the organism's combination of genes. This process is a natural process or artificially by applying genetic engineering techniques.

5-Protoplast fusion

A protoplast is a plant, bacterial or fungal cell that had its cell wall completely or partially removed using either mechanical or enzymatic means.

Protoplasts can be made by degrading cell walls with a mixture of the appropriate polysaccharide-degrading enzymes:

Type of cell	**Enzyme**
Plant cells	Cellulase, pectinase, xylanase
Gram-positive bacteria	Lysozyme (+EDTA)
Fungal cells	Chitinase

Protoplast becomes very sensitive to osmotic stress, therefore; it treats with chemical stabilizers such as inorganic salts, sugars as sucrose and alcohols to give the plasma membrane osmotic helpful to prevent rupture of the plasma membrane.

However, isolated protoplasts will not aggregate and fuse easily in the absence of a fusogenic agents. The most successful and most widely used is polyethylene glycol (PEG). Also Ca^{+2} was necessary to obtain the fusion at high frequency.

The process was observed in *Bacillus and Streptomyces griseus*. And the first attempt in protoplast fusion was done for *Geotrichum candidum* then for *Cephalosporium* that produced Cephalosporin. Fusion was done between different genera such as *Sacccharomyces* and *Candida* or between yeast and fungi as *Trichoderma reesei* and *Sacccharomyces cerevisiae.*

2- <u>Fermentation medium (raw material)</u>

Most fermentations, except those involving solid substrates, require large quantities of water in which the medium is formulated. General media requirements include a carbon source provides both energy and carbon units for biosynthesis, and sources of nitrogen, phosphorus and sulphur. Other minor and trace elements must also be supplied, and some

microorganisms require added vitamins, such as biotin and riboflavin. Many considerations have made when choosing media for fermentation such as cheapness and availability of material.

3-Controlled favorable environment

Bioreactors are the containment vehicles of any biotechnology- based production process.To achieve optimization of the fermentation process the following must be adhered:

1-Biological environment: Excluding entrance of contaminating organisms and using the desired organisms.

2-Physical environment: supplement the optimal temperature for production and agitation for aerobic organisms.

3-Chemical environment: including pH, dissolved oxygen and excluding the inhibitors.

Fermentation products

1-Microbial biomass: The production of SCP that used as food for human and animals, also the yeast was used in the bread industry.

2-Microbial enzymes: Animal, plant and microorganisms produce different enzymes and huge amounts of enzymes produced by fermentation process.

3-Microbial metabolites:

a-Primary metabolites:

A primary metabolite is a kind of metabolite that is directly involved in normal growth, development, and reproduction. It produces during lag and log phases that together called trophophase and including proteins, lipids, carbohydrates, nucleic acids and amino acids.

b-Secondary metabolites:

The metabolites that don't appear to have an obvious role in the metabolism of the producing organism, but usually has an important ecological function.They produce during stationary phase and including antibiotics, toxins and hormones.

4- Bioconversion:

Also known as biotransformation refers to the use of live organisms to convert a substance found in the medium to a chemically modified form that has high commercial level. An example is bioconversion of progesterone to 11-alpha-Hydroxy progesterone by *Rhizopus nigricans.* Bioconversion differ from chemical conversion in highly specificity,

needing to low temperature and don't need to use the heavy metals.

Inoculum

The inoculum was very important to success the fermentation process.

Inoculum parameters

1- The cells should be active

2-The volume of inoculum proportional with the volume of culture medium

3-Approperate phenotype

4-Free from contamination

5-The cells should be having genotype that gives the desired product.

Factors affecting on the efficiency of the inoculum

1-Inoculum volume

The volume of bacterial inoculum was 1-3% dependent on the type of product. The fungi and actinomycetes were added to the medium at 5-10%, but the spore suspension was added at about 1-2 $x10^5$ spore/liter of the medium.

2-Age volume

In the case of primary metabolite production the cells in inoculum should be active and in logarithmic phase, while the production of secondary metabolites need cells in the case of non-division.

3-Nutrition medium

The medium in the last stage for inoculum production similar to production medium, but it poorer in all contents.

There are three reasons lead to failure the inoculation process:

1-Contamination: The contamination was recognized by microscopic examination and culturing in solid media.

2-Bacteriophage: The bacteriophage infection was recognized by decreasing or stopping the growth of cells.

3-Mutation: The mutation was recognized by decreasing the productivity, since the most of mutations lead to decrease of the productivity.