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. The reasons for using microorganisms in fermentation:

1-The ratio of surface area to volume is high, so that the nutrients in the medium consumed quickly forced the metabolic reactions.

2- Adaptation for different ecological conditions that facilitates its transfer from natural habitat to the lab and growth on cheap carbon and nitrogen sources then production compounds that higher economical value.

3-The ability to achieve huge chemical reactions.

4- By easydealing with microorganisms in field genetic mutation and genetic engineering easing fordesigning genetically modified organisms produced higher amounts of product in comparison with wild type.

Stages of fermentation

1-Screening and isolation of microorganisms

Microorganisms holds the key to the success or failure of a fermentation process. It is therefore important to select the most suitable microorganisms to carry out the desired industrial process.The most important factor for the success of any fermentation industry is a production strain. It is highly desirable to use a production strain possessing the following four characteristics:

1-It should be high-yielding strain.

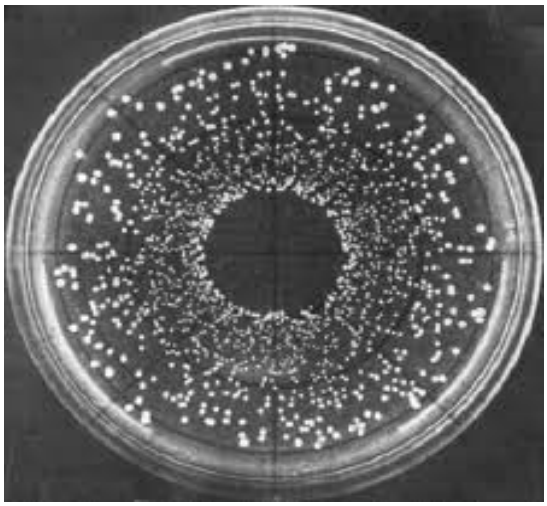
2-It should have stable biochemical/genetical characteristics.

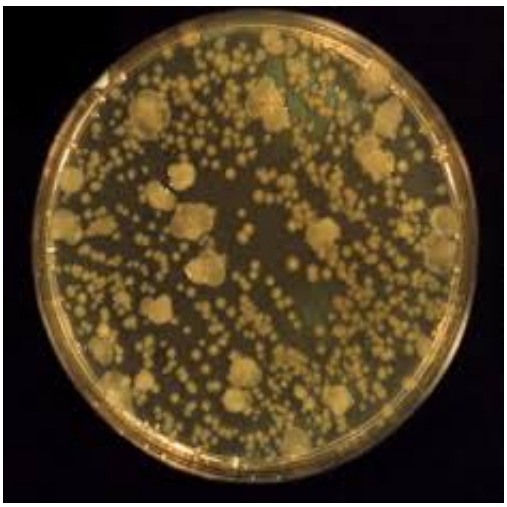
3-It should not produce undesirable substances.

4-It should be easily cultivated on large-scale.

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.It consists of preparing a series of dilution of the source material for the antibiotic producing microorganisms, followed by spreading the dilution on the agar plates.The agar plates having 300- 400 or more colonies per plate after incubation for 2-4 days are observed since they are helpful in locating the colonies producing antibiotic activity. Colonies showing antibiotic activity is indicated by the presence of a zone of inhibition surrounding the colony. Such a colony is sub- cultured to a similar medium and purified.





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

3-Enrichment culture technique: This technique was used to isolate the desired microorganisms form 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. On the other hand, unwanted microbes are eliminated or develop poorly since they do not find suitable growth conditions in the newly created environment.

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 (know as an **isomer**) which in turn will form a different series of hydrogen bonds with its partner. This leads to mistakes at the time of DNA replication. The frequency and productivity were low so that don't dependent on it.

b-Induced mutation

Induced mutation occur by treatment the cells with mutagens such as physical mutagens which includingultraviolet andX-rays and chemical mutagens such as mitomycin C, nitrosoguanidine..etc. The cells that result from mutation called mutantswhich 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 (exogenous DNA) 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. For transformation to happen, bacteria must be in a state of competence, which might occur as a time-limited response to environmental conditions such as starvation and cell density.

b-Transduction

Transduction is the process by which DNA is transferred from one bacterium to another by a virus. It also refers to the process whereby foreign DNA is introduced into another cell via a viral vector. .When bacteriophages (viruses that infect bacteria) infect a bacterial cell, their normal mode of reproduction is to harness the replicational, transcriptional, and translation machinery of the host bacterial cell to make numerous virions, or complete viral particles, 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. The result is an elevation in the level of the RNA or protein encoded for by the gene and a corresponding amplification of the phenotype that the gene confers on the cell. 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, such as the crossing over between homologous chromosomes during meiosis. It can also be done artificially by applying genetic engineering techniques. Recombination may be caused by loci on different chromosomes that sort independently or by a physical crossing over between two loci on the same chromosome, with breakage and exchange.

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.

Cell walls are made of a variety of polysaccharides. 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

During and subsequent to digestion of the cell wall, the 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 an fusogenic agents. Several chemicals have been used successfully to induce fusion but the most successful and the one most widely used at the present time is polyethylene glycol (PEG). The effects of PEG are not specific and it will promote the aggregation and fusion of protoplasts from the same or different species also Ca^{+2} was necessary to obtain the fusion at

high frequency.

Fusion in bacteria:

The process was observed between two protoplast of *Bacillus* at high frequency(10^4-10^5)by using PEG and $CaCl_2$.It also used for production of indolyzomycin by *Streptomyces griseus* .

Fusion in fungi:

The first attempt in protoplast fusion was done for *Geotrichum candidum* then for *Cephalosporium* that produced sephalosporin. In the first the fusion was done among strains inside the same species for *Aspergillus* and *Penicillium*,then among species inside the same genus, then developed 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, which in virtually all industrial fermentations 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. Usually, media incorporate-buffers, or the pH is controlled by acid and alkali additions. Many considerations have made when choosing media for fermentation such as cheapness and availability of material. Besides material cost and product yield, it must be considered whether materials used are readily available in sufficient supply without high transportation costs.

3-<u>Controlled favorable environment</u>

Bioreactors are the containment vehicles of any biotechnology- based production process, be it for brewing, organic or amino acids, antibiotics, enzymes or vaccines .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 bread industry.

2-Microbial enzymes: Animal, plant and microorganisms produce different enzymes but the last produce huge amounts of enzymes 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 usually performs a physiological function in the organism (i.e. an intrinsic function). A primary metabolite is typically present in many organism or cell. It is also referred to as a central metabolite. 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 producer organism, but usually has an important ecological function (i.e. a relational function). A secondary metabolite is typically present in a taxonomically restricted set of organisms or cells (Plants, Fungi, Bacteria...).They produce during stationary phase and including antibiotics,toxins and hormones.

4- Bioconversion:

Bioconversion, also known as biotransformation refers to the use of live organisms often microorganisms to carry out a chemical reaction that is more costly. These organisms convert a substance found in the medium to a chemically modified form that has high commercial level. An example is the industrial production of cortisone. One step is the bioconversion of progesterone to 11-alpha-Hydroxyprogesterone by *Rhizopus nigricans.* Another example of this is the conversion of organic materials, such as plant or animal

waste, into usable products or energy sources. 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. The laboratory inoculum differs from an industrial inoculum in:

1- Industrial inoculum prepares in large amounts.

2-Industrial inoculum passes with different conditions in particular nutritional media and supplement with oxygen in preproductivity and productivity stages.

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 $1\text{-}2^{x}10^{5}$ spore/liter of the medium.

2-Age volume

In the case of primary metabolites 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 in order to increase the induction that lead to decrease or remove the lag phase.

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.