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**Fermentation by microorganisms**

**Bioprocess or fermentation technology is a process that 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 easy dealing with microorganisms in field genetic mutation and genetic engineering easing for designing genetically modified organisms produced higher amounts of product in comparison with wild type.**

**Stages of fermentation**

**1-Screening and isolation of microorganisms**

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.

**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.

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.

**b-Induced mutation**

Induced mutation occur by treatment the cells with mutagens such as physical mutagens which including [ultraviolet](http://en.wikipedia.org/wiki/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.

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| **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](http://en.wikipedia.org/wiki/Introduction_to_genetics) alteration of a [cell](http://en.wikipedia.org/wiki/Cell_(biology)) resulting from the direct uptake, incorporation and [expression](http://en.wikipedia.org/wiki/Gene_expression) of exogenous [genetic](http://en.wikipedia.org/wiki/Gene) material ([exogenous DNA](http://en.wikipedia.org/wiki/Exogenous_DNA)) from its surroundings and taken up through the cell membranes  **b-Transduction**  Transduction is the process by which [DNA](http://en.wikipedia.org/wiki/DNA) is transferred from one [bacterium](http://en.wikipedia.org/wiki/Bacterium) to another by a [virus](http://en.wikipedia.org/wiki/Virus).  **c-** [**conjugation**](http://en.wikipedia.org/wiki/Bacterial_conjugation)  [conjugation](http://en.wikipedia.org/wiki/Bacterial_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](http://www.wisegeek.com/what-is-gene-duplication.htm) or chromosomal duplication, is a cellular process in which multiple copies of a gene are produced.  **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](http://www.biology-online.org/dictionary/Crossing_over) between [homologous chromosomes](http://www.biology-online.org/dictionary/Homologous_chromosomes) during [meiosis](http://www.biology-online.org/dictionary/Meiosis). It can also be done artificially by applying [genetic engineering](http://www.biology-online.org/dictionary/Genetic_engineering) techniques.  **5-Protoplast fusion**  A protoplast is a [plant](http://en.wikipedia.org/wiki/Plant), [bacterial](http://en.wikipedia.org/wiki/Bacterium) or [fungal](http://en.wikipedia.org/wiki/Fungus) cell that had its [cell wall](http://en.wikipedia.org/wiki/Cell_wall) completely or partially removed using either mechanical or enzymatic means such as:   |  |  | | --- | --- | | **Type of cell** | **Enzyme** | | [Plant cells](http://en.wikipedia.org/wiki/Plant_cell) | [Cellulase](http://en.wikipedia.org/wiki/Cellulase), [pectinase](http://en.wikipedia.org/wiki/Pectinase), [xylanase](http://en.wikipedia.org/wiki/Xylanase) | | [Gram-positive](http://en.wikipedia.org/wiki/Gram-positive) bacteria | [Lysozyme](http://en.wikipedia.org/wiki/Lysozyme) (+[EDTA](http://en.wikipedia.org/wiki/EDTA)) | | [Fungal](http://en.wikipedia.org/wiki/Fungus) cells | [Chitinase](http://en.wikipedia.org/wiki/Chitinase) |   During and subsequent to digestion of the cell wall, the protoplast becomes very sensitive to [osmotic](http://en.wikipedia.org/wiki/Osmosis) stress, therefore; it treats with chemical stabilizers such as inorganic salts, sugars as sucrose and alcohols to give the [plasma membrane](http://en.wikipedia.org/wiki/Plasma_membrane) osmotic helpful to prevent rupture of the [plasma membrane](http://en.wikipedia.org/wiki/Plasma_membrane).  The isolated protoplasts will not aggregate and fuse easily in the absence of an fusogenic agents thus polyethylene glycol (PEG) was used successfully to induce the fusion 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  **Fusion in fungi:**  The first attempt in protoplast fusion was done for *Geotrichum candidum* then for *Cephalosporium* that produced sephalosporin.  **2- Fermentation medium(raw material)**  General media requirements include a carbon source which 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, availability of material and the yield of product.  **3-Controlled favorable environment**  To achieve optimization of the fermentation process the following must be controlled:  **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](http://en.wikipedia.org/wiki/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 producer organism, but usually has an important [ecological](http://en.wikipedia.org/wiki/Ecology) function. They produce during stationary phase and including antibiotics, toxins and hormones.  **4- Bioconversion:**  Bioconversion 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 such as the bioconversion of [progesterone](http://en.wikipedia.org/wiki/Progesterone) to 11-alpha-Hydroxyprogesterone by [*Rhizopus nigricans*](http://en.wikipedia.org/wiki/Rhizopus_nigricans)or the [conversion](http://en.wikipedia.org/wiki/Conversion_(chemistry)) of plant or animal wastes into useful products or [energy sources](http://en.wikipedia.org/wiki/Energy_source). 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 give 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 1-2ᵡ105 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 stages 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. |

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