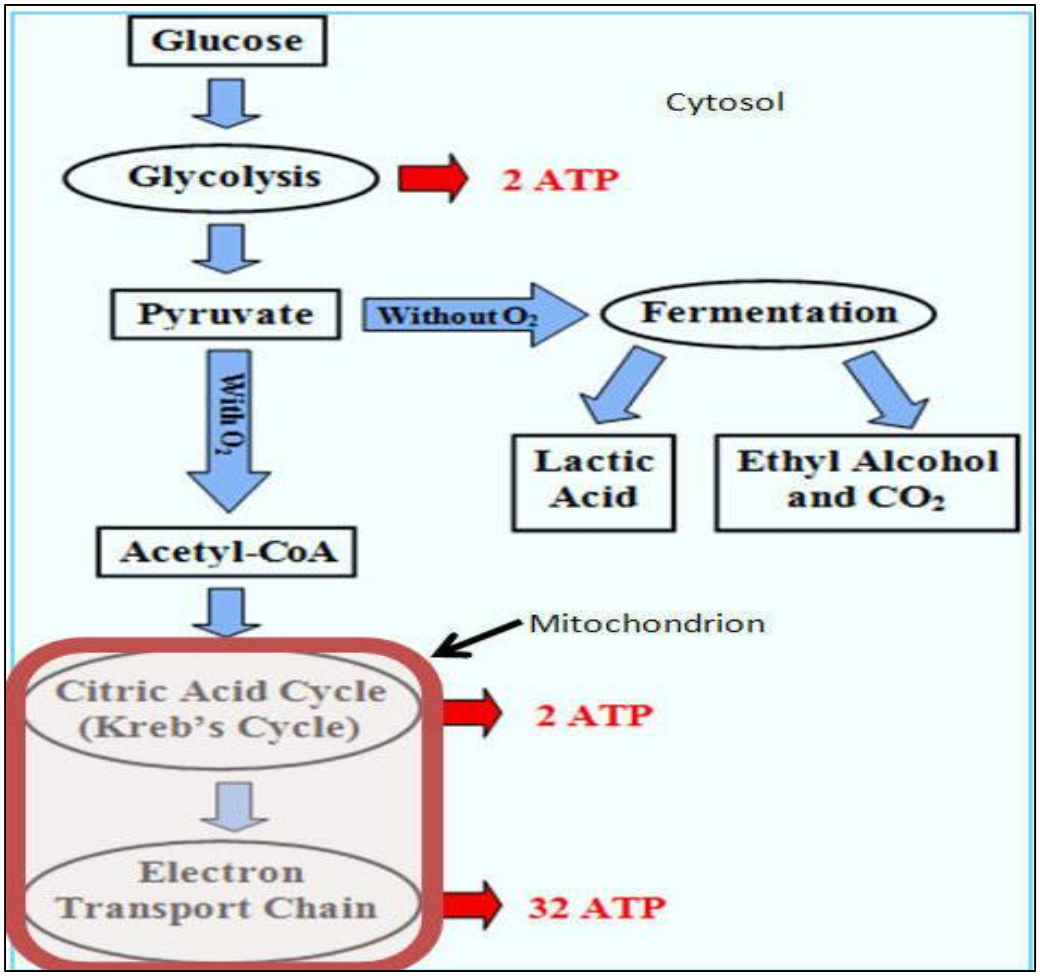
Fermentation:

It is a chemical act or process, caused by a fermenting agent, of converting a carbohydrate into alcohol, acids, gas and other compounds, as yeast converts the sugar in grape juice into alcohol, producing wine. The *fermentor* is a tightly sealed reaction chamber in which a controlled reaction can take place. It keeps any contaminants (biotic and abiotic alike) from disturbing or even spoiling the enclosed reaction mechanism. *A strict definition of fermentation states that it is an anaerobic catabolism especially by microorganisms such as bacteria and yeasts in which an organic compound serves as both an electron donor and an electron acceptor and in which ATP is produced.*



Aerobic and anaerobic breakdown of sugar

Aerobic process (Kreb's cycle, where pyruvic acid is converted into acetylCoA, and finally broken down completely into carbon dioxide; electron transport chain reactions produce water and energy): in this process glucose is broken down in the presence of oxygen into carbon dioxide water and energy in the mitochondria of the cell.

Design features and key aspects of a fermentation process:

Keeping out unwanted microorganisms that might interfere with the production – sterility is the key word in biotechnology.

· Double mechanical seals.

· Filters on exhaust pipes.

· Sampling outlets; safety precautions at safety outlets (outlets are considered the weakest point in the system - spills are very common).

· Aerosol prevention; aerosols are perfect carriers of contaminants from/to the environment especially if aerobic microorganisms are involved in the production process (O_2 encourages growth); aerosols can transport contaminants over large distances.

· Effluents: maintaining aseptic conditions at all stages (continuous sterilization) requires a multiple cleaning process; this is partly achieved with on-site installed killer tanks that contain toxic chemicals.

· Heat exchangers; the fermentation of microbial matter generates a substantial amount of heat; maintaining optimal temperature conditions is necessary to keep the system process going; if the cooling system is not properly set up, an excessive down-cooling mechanisms may cause the fermentor to burst (freezing).

· Centrifuges are notorious for leakage and aerosol generation; therefore, air-tight seals must be used.

· Vents (a small opening that allow air, smoke or gas to enter or leave a closed space) require microbiological filters in order to avoid any escape of GM-organisms into the environment.

· Pipes and tubing - connective equipment must be leak proof.

· Integrated sterilization system for the entire factory – usually a super-heated steam system is used to flush and heat-sterilize the pipes, tubes, and the fermentor; insertion of *in-situ* hot steam as some pockets are hard to reach. This also requires an aerodynamic design and polished surfaces of the tubes (in the experimental lab-phase, such expensive means are not required).

Running - Aseptic Operations:

Successful fermentation means zero contamination; for every item that is used, it must be sterile before inoculation. Practically this implies that:

· Fermentor, auxiliary equipment plus medium must be sterile;

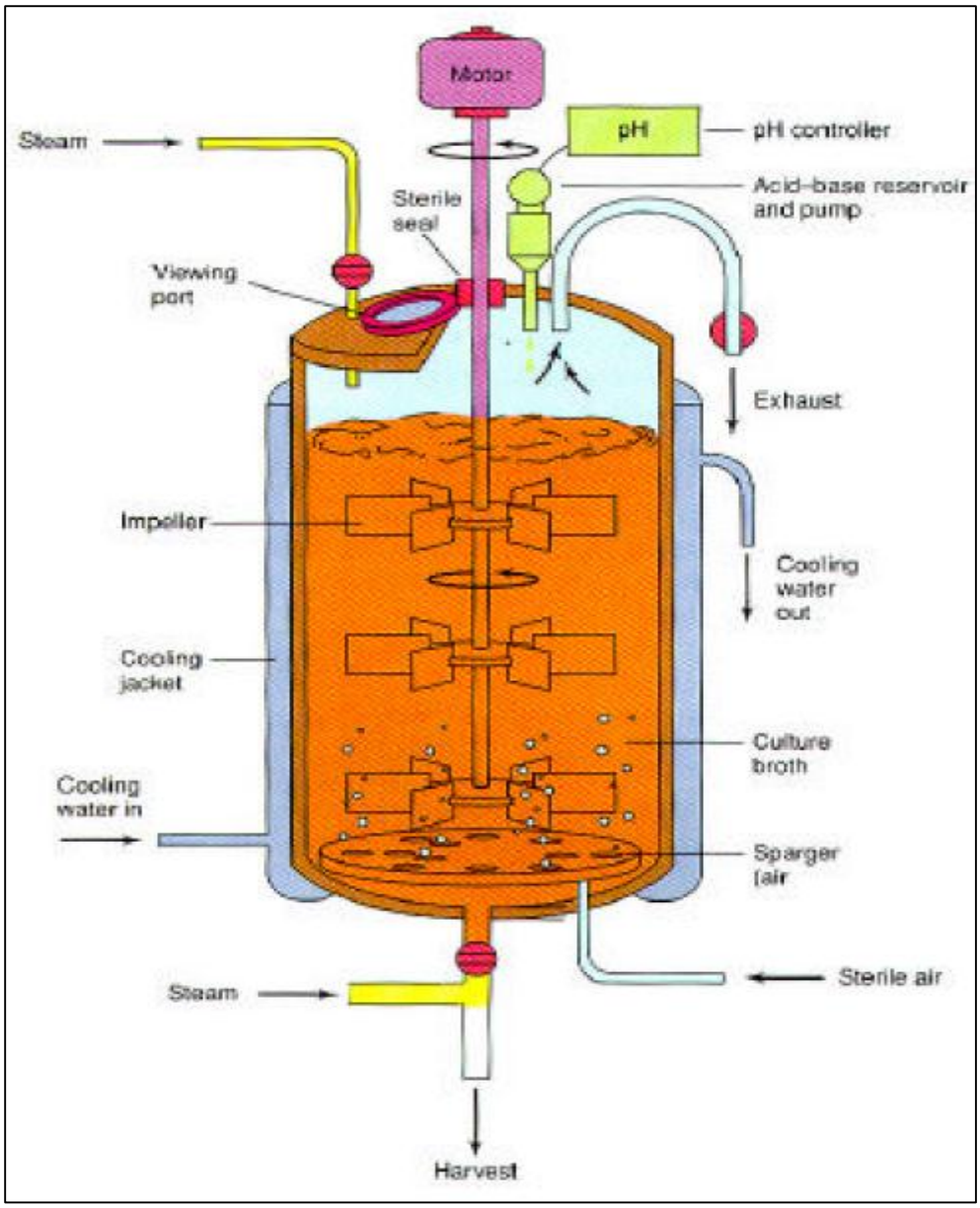
· Sterilization of air-supply (HEPA filters*); bacteriophags cannot pass such narrow filters (except nanobacteria); a common practice is to heat and subsequently cool down the air before it passes the filter; - nothing must come in or leave the plant via the venting system - gases must be monitored carefully.

Other aspect that has to be taken in consideration – the plant should not be located at a site where:

i) Persistent winds redirect exhaust gases towards densely populated areas (smells cause problems).

ii) Farming communities should not be located nearby as contaminated aerosols may disrupt the production facilities (i.e. *E.coli* grows and reproduces perfectly in soils).

· Regular checks for mechanical micro-fractures;



Simple fermentor

***High-Efficiency Particulate Air** is types of air filter have many applications, including use in medical facilities, automobiles and aircraft.*

Microbial growth

A. Requirements for artificial culture

The growth of organisms involves complex energy based processes. The rate of growth of micro-organisms is dependent upon several **culture conditions,** which should provide for the energy required for various chemical reactions. The production of a specific compound requires very precise cultural conditions at a particular growth rate. Many systems now operate under computer control.

The rate of growth of micro-organisms and hence the synthesis of various chemical compounds under artificial culture, requires organism specific chemical compounds as the **growth (nutrient) medium**. The kinds and relative concentrations of the ingredients of the medium, the pH, temperature, purity of the cultured organism, etc., influence microbial growth and hence the production of **biomass** (the total mass of cells or the organism being cultured), and the synthesis of various compounds. The nutrient sources for industrial fermentation are given in table below.

Nutrient	Raw materials
Carbon source	
Glucose	Corn sugar, Starch and Cellulose
Sucrose	Sugarcane, sugar beet molasses
Lactose	Milk whey
Fat	Vegetable oil
Hydrocarbon	Petroleum fractions
Nitrogen source	
Protein	Soybean meal, Corn steep liquor, Distillers soluble
Ammonia	Pure ammonia or ammonium salts
Nitrate	Nitrate salts
Nitrogen	Air
Phosphorus sources	Phosphate salts

B. Phases of microbial growth

When a particular organism is introduced into a selected growth medium, the medium is **inoculated** with the particular organism. Growth of the inoculum does not occur immediately, but takes a little while. This is the period of adaptation, called the **lag phase** (the first major phase).

The second major phase of microbial growth is the **log** or **exponential phase**. Cells have adjusted to their new environment and dividing at a constant rate resulting in an exponential increase in the number of cells present. This is known as the specific growth rate and is represented mathematically by first order kinetics as the following:

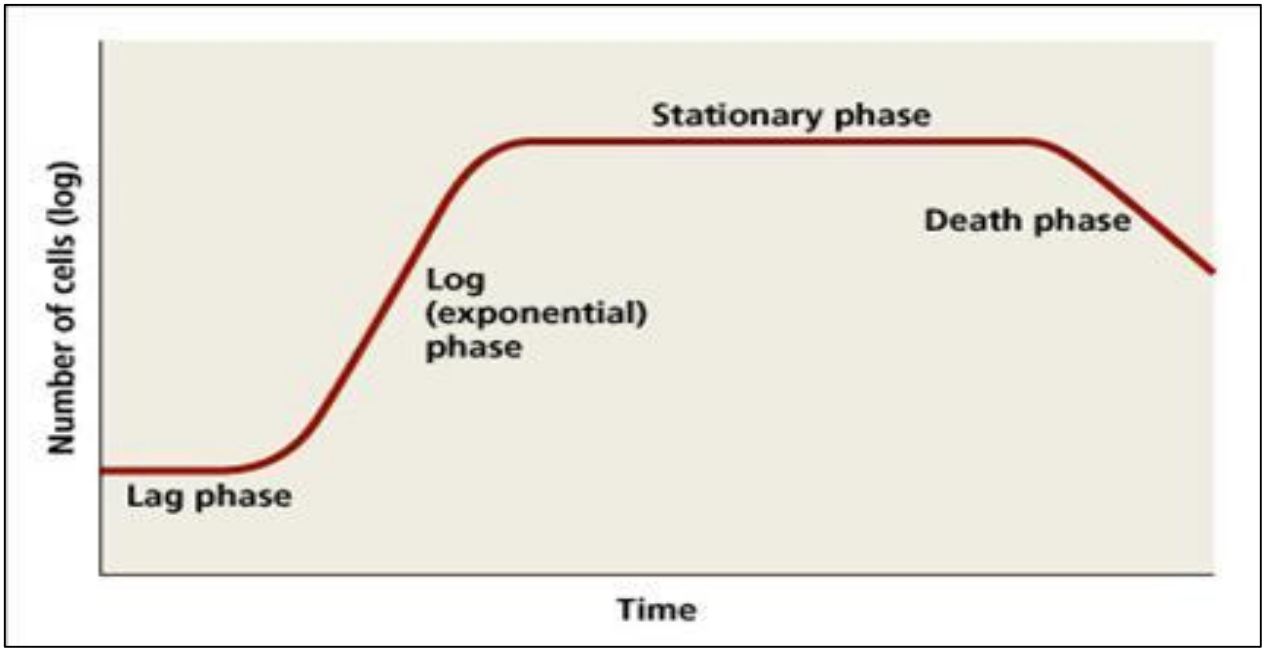
$dx/dt=(\mu-k_d)x$

Where x is the cell concentration, μ is the cell growth rate, and is k_d the cell death rate. The term μ-k_d can be referred to as μ_{net}. The cell death rate is sometimes neglected if it is considerably smaller than the cell growth rate.

Stationary phase is the third major phase of microbial growth .Occurs when the number of cells dividing and dying is in equilibrium and can be the result of the following: depletion of one or more essential growth nutrients, accumulation of toxic growth associated by products and stress associated with the induction of a recombinant gene.

Finally growth enters to fourth phase which called **decline of death phase**. The rate of cells dying is greater than the rate of cells dividing. Similar to exponential phase, it is represented mathematically by first order kinetics as the following:

$dx/dt=-k_dx$



Microbial growth curve

Fermentors and Bioreactors

A fermenter is the set up to carry out the process of fermentation. The fermentors vary from laboratory experimental models of one or two liters capacity, to industrial models of several hundred liters capacity, which refers to the volume of the main fermenting vessel.
A **bioreactor** differs from a fermentor in that the former is used for the mass culture of plant or animal cells, instead of micro-organisms. The chemical compounds synthesized by these cultured cells, such as therapeutic agents, can be extracted easily from the cell biomass. The design engineering and operational parameters of both fermentors and bioreactors are identical.

The fermentation process requires the following:

a) a pure **culture** of the chosen organism, in sufficient quantity and in the correct physiological state; b**) sterilized**, carefully composed **medium for growth** of the organism; c) a **seed fermenter**, a mini-model of production fermenter to develop an inoculum to initiate the process in the **main fermenter**; d) a **production fermenter**, the functional large model; and e) equipments for i) drawing the culture medium in steady state, ii) cell separation, iii) collection of cell free supernatant, iv) product purification, and v) effluent treatment.

Applications of Fermentation

1) Production of cells (biomass) such as yeasts.
2) Extraction of metabolic products such amino acids, proteins (including enzymes), vitamins, alcohol, etc., for human and/or animal consumption or industrial use such as fertilizer production.
3) Production of organic solvents e.g. acetone, butanol, ethanol etc.
4) Production of acids such as citric and lactic acids.
5) In pharmaceutical industries for producing compound such as antibiotics and vaccines.
6) Production of dairy products such as yoghurt, butter milk, sour cream.
7) In production of beverages and related products.
8) Production of recombinant products.

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Bioprocess Engineering Basic, (2002), Shuler, M. L. and F. Kargi.
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