Industrial fermentation is the intentional use of fermentation by microorganisms such as bacteria and fungi to make products useful to humans. Fermented products have applications as food as well as in general industry. Some commodity chemicals, such as acetic acid, citric acid, and ethanol are made by fermentation. The rate of fermentation depends on the concentration of microorganisms, cells, cellular components, and enzymes as well as temperature, pH and for aerobic fermentation oxygen. Product recovery frequently involves the concentration of the dilute solution. Nearly all commercially produced enzymes, such as lipase, invertase and rennet, are made by fermentation with genetically modified microbes. In some cases, production of biomass itself is the objective, as in the case of baker's yeast and lactic acid bacteria starter cultures for cheese making. In general, fermentations can be divided into four types:

- Production of biomass (viable cellular material)
- Production of extracellular metabolites (chemical compounds)
- Production of intracellular components (enzymes and other proteins)
- Transformation of substrate (in which the transformed substrate is itself the product)

These types are not necessarily disjoint from each other, but provide a framework for understanding the differences in approach. The organisms used may be bacteria, yeasts, molds, algae, animal cells, or plant cells. Special considerations are required for the specific organisms used in the fermentation, such as the dissolved oxygen level, nutrient levels, and temperature.

General process overview.

In most industrial fermentations, the organisms are submerged in a liquid medium; in others, such as the fermentation of cocoa beans, coffee cherries, and miso, fermentation takes place on the moist surface of the medium. There are also industrial considerations related to the fermentation process. For instance, to avoid biological process contamination, the fermentation medium, air, and equipment are sterilized. Foam control can be achieved by either mechanical foam destruction or chemical anti-foaming agents. Several other factors must be measured and controlled such as pressure, temperature, agitator shaft power, and viscosity. An important element for industrial fermentations is scale up. This is the conversion of a laboratory procedure to an industrial process. It is well established in the field of industrial microbiology that what works well at the laboratory scale may work poorly or not at all when first attempted at large scale. It is generally not possible to take fermentation conditions that have worked in the laboratory and blindly apply them to industrial-scale equipment. Although many parameters have been tested for use as scale up criteria, there is no general formula because of the variation in fermentation processes. The most important methods are the maintenance of constant power consumption per unit of broth and the maintenance of constant volumetric transfer rate.

Phases of microbial growth

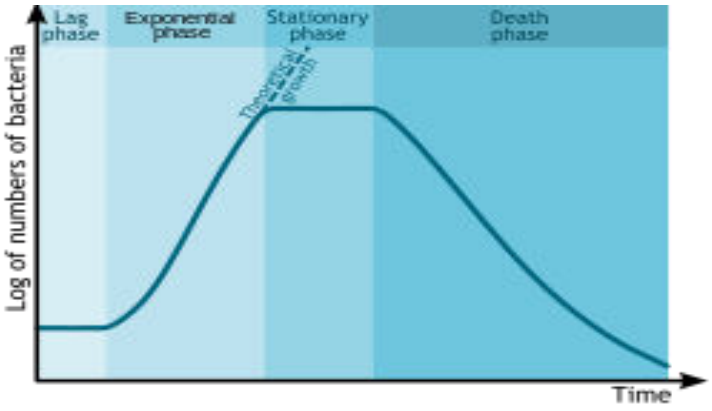

Fig: Bacterial growth curve\Kinetic Curve

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. Following the lag phase, the rate of growth of the organism steadily increases, for a certain period—this period is the log or exponential phase. After a certain time of exponential phase, the rate of growth slows down, due to the continuously falling concentrations of nutrients and/or a continuously increasing (accumulating) concentrations of toxic substances. This phase, where the increase of the rate of growth is checked, is the deceleration phase. After the deceleration phase, growth ceases and the culture enters a stationary phase or a steady state. The biomass remains constant, except when certain accumulated chemicals in the culture lyse the cells (chemolysis). Unless other micro-organisms contaminate the culture, the chemical constitution remains unchanged. If all of the nutrients in the medium are consumed, or if the concentration of toxins is too great, the cells may become scenescent and begin to die off. The total amount of biomass may not decrease, but the number of viable organisms will decrease.

Fermentation medium

The microbes used for fermentation grow in (or on) specially designed growth medium which supplies the nutrients required by the organisms. A variety of media exist, but invariably contain a carbon source, a nitrogen source, water, salts, and micronutrients. In the production of wine, the medium is grape must. In the production of bio-ethanol, the medium may consist mostly of whatever inexpensive carbon source is available.

Carbon sources are typically sugars or other carbohydrates, although in the case of substrate transformations (such as the production of vinegar) the carbon source may be an alcohol or something else altogether. For large scale fermentations, such as those used for the production of ethanol, inexpensive sources of carbohydrates, such as molasses, corn steep liquor, sugar cane juice, or sugar beet juice are used to minimize costs. More sensitive fermentations may instead use purified glucose, sucrose, glycerol or other sugars, which reduces variation and helps ensure the purity of the final product. Organisms meant to produce enzymes such as beta galactosidase, invertase or other amylases may be fed starch to select for organisms that express the enzymes in large quantity.

Fixed nitrogen sources are required for most organisms to synthesize proteins, nucleic acids and other cellular components. Depending on the enzyme capabilities of the organism, nitrogen may be provided as bulk protein, such as soy meal; as pre-digested polypeptides, such as peptone or tryptone; or as ammonia or nitrate salts. Cost is

also an important factor in the choice of a nitrogen source. Phosphorus is needed for production of phospholipids in cellular membranes and for the production of nucleic acids. The amount of phosphate which must be added depends upon the composition of the broth and the needs of the organism, as well as the objective of the fermentation. For instance, some cultures will not produce secondary metabolites in the presence of phosphate.

Growth factors and trace nutrients are included in the fermentation broth for organisms incapable of producing all of the vitamins they require. Yeast extract is a common source of micronutrients and vitamins for fermentation media. Inorganic nutrients, including trace elements such as iron, zinc, copper, manganese, molybdenum and cobalt are typically present in unrefined carbon and nitrogen sources, but may have to be added when purified carbon and nitrogen sources are used. Fermentations which produce large amounts of gas (or which require the addition of gas) will tend to form a layer of foam, since fermentation broth typically contains a variety of foam-reinforcing proteins, peptides or starches. To prevent this foam from occurring or accumulating, antifoaming agents may be added. Mineral buffering salts, such as carbonates and phosphates, may be used to stabilize pH near optimum. When metal ions are present in high concentrations, use of a chelating agent may be necessary.

BEER PRODUCTION (From Barley and Hops to Beer)

Making beer is a surprisingly complicated process. Beer production encompasses five main stages: malting, the germination of barley grains; mashing, a stepwise heating process to promote starch hydrolysis; wort boiling with hops; fermentation; and postfermentation treatments.

Malting of barley. The barley grains are malted, or soaked in water, to encourage germination. During germination, the endosperm (stored food) of the grain secretes gibberellin, a hormone that stimulates the growth of rootlets and the emerging stem. Gibberellin induces the aleurone (lining of the endosperm) to produce hydrolases, enzymes that break down starch to maltose and proteins to amino acids for use by the growing plant. The hydrolases actually become activated during the second stage, called mashing, when the grains are crushed and stirred in huge vats of water.

Mashing. While the grain is being mashed, the temperature is raised in steps, each of which optimizes the activity of a different hydrolase. At 52°C, the protein hydrolases are activated. Then at 68°C, the starch hydrolases convert long-chain sugars to the disaccharide maltose, which can support yeast fermentation. The final temperature (77°C) inactivates all enzymes; then the mash is cooled, pressed, and filtered. The liquid filtrate of the mash is called wort.

Wort boiling. The wort is supplemented with hops, a traditional herb used for centuries in Europe to contribute a distinctive flavor of beer. After boiling with hops, the wort is again filtered.

Fermentation. The wort is inoculated with a special strain of *Saccharomyces cerevisiae* known as brewer's yeast. The yeast conducts ethanolic fermentation on the maltose from hydrolyzed starch. At the same time, minor by-products, such as long-chain alcohols, impart good flavors. The time of fermentation is a key factor in the quality of the beer.

Postfermentation treatment. This step includes filtering of the wort to remove the bulk of the yeast. The product, now recognizably beer, still contains undesirable levels of acetaldehyde and diacetyl generated by partial oxidation. These oxidized by-products can be reduced by the few remaining yeast cells during a period of

secondary fermentation. During secondary fermentation, oxygen is completely excluded and the temperature is decreased to 15°C or lower. The best German beers are aged at 2°C for several months.

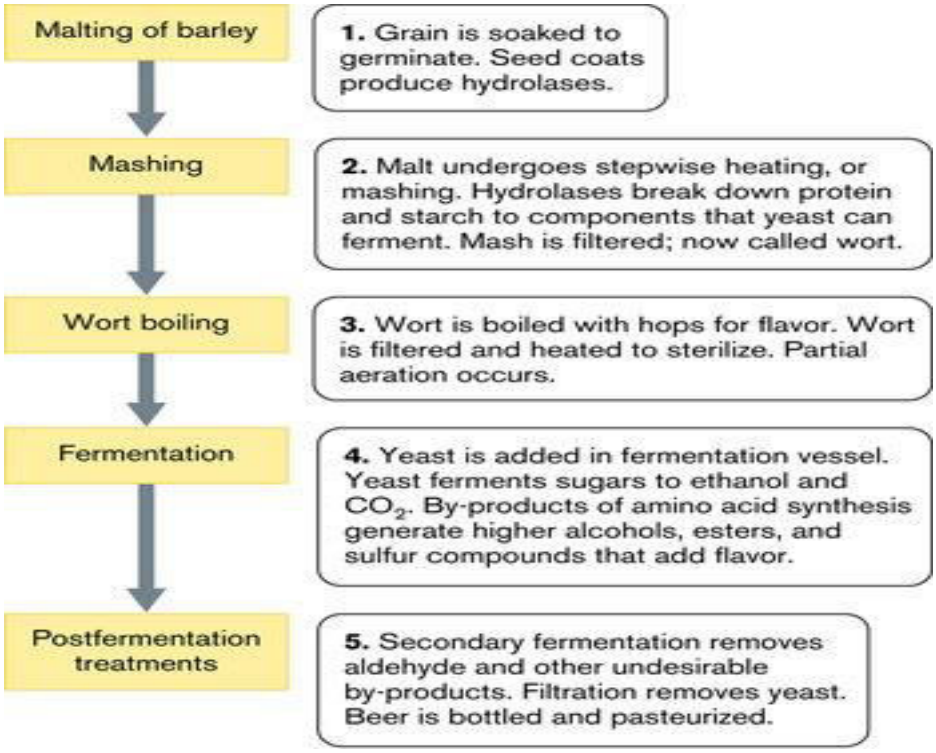

Figure: Process of beer production. Barley is malted, milled, and mashed to break down long-chain sugars and proteins into short chains and monomers that yeast can digest, forming wort. The wort is fermented by yeast to make beer.

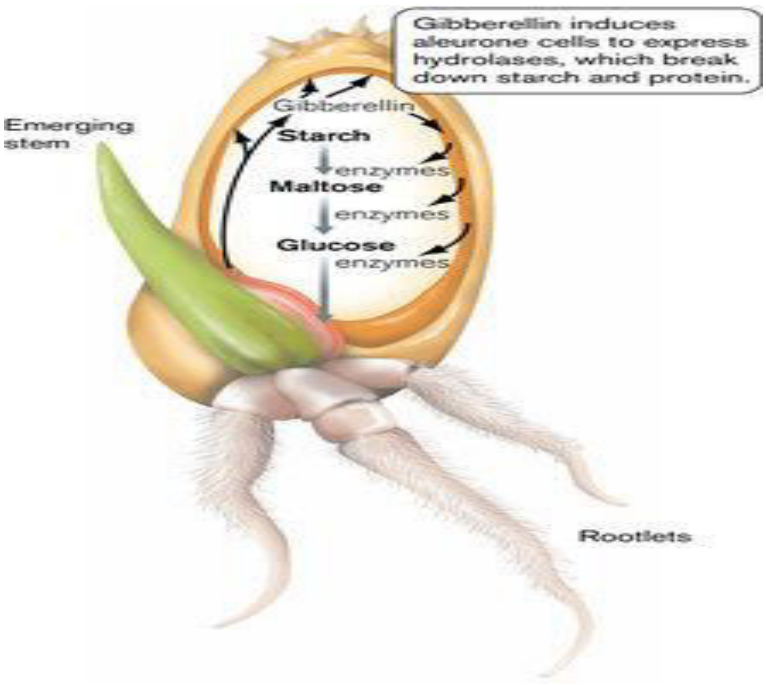

Figure: Malting the grain. Malting involves germination of the grain to induce expression of hydrolase enzymes.

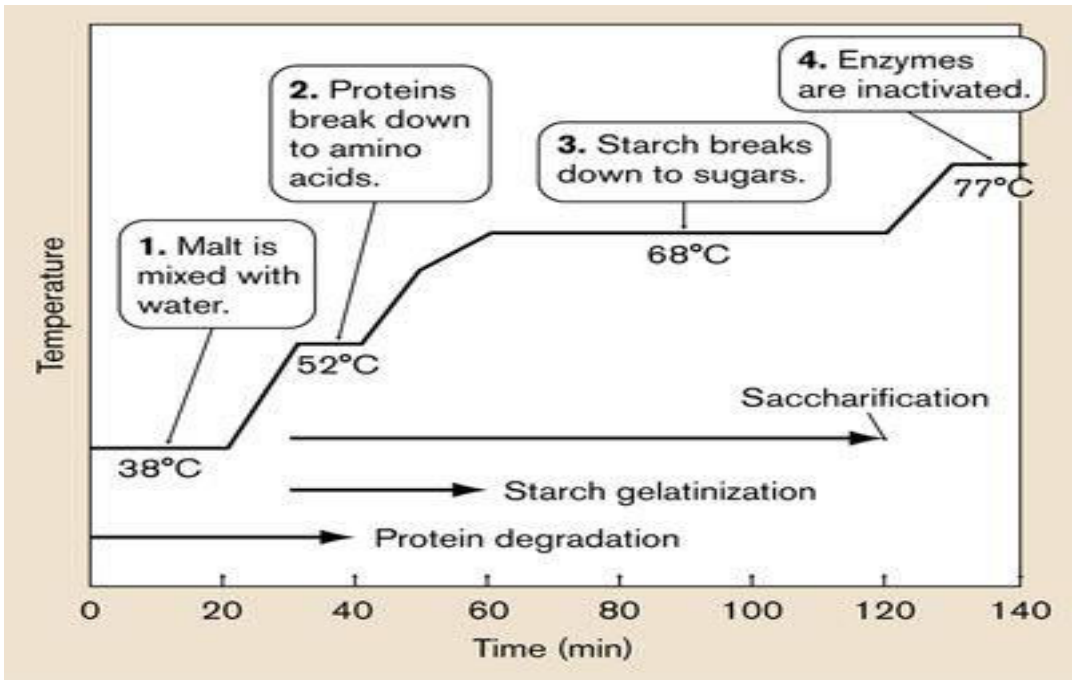

Figure: Mashing the wort. During mashing, stepwise heating activates the hydrolases to break down carbohydrates and proteins.