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# Overview on Fermentation technique and its Application towards industry

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## Abstract
Fermentation is the process of chemical change caused by organism or their product, usually producing effervescent and heat. It is an ancient process dating back thousands of years. Fermentation derives from the Latin work fever meaning "to ferment." It was the means by which bread, wine, beer, and cheese were made. The Chinese used micro-organisms in the production of yogurt, cheese, wine, vinegar, and different types of sauces. It is a powerful economic incentive for semi-industrialized countries, in their willingness to produce bio-ethanol.
**Keywords:** Fermentation, Downstream processing, Industrial application

## Introduction
Fermentation is the process of chemical change caused by organism or their product, usually producing effervescent and heat. Microbiologist considers fermentation as, any process for production of product by means of culture of micro-organism. Biochemist consider fermentation as, an energy generating process in which organic compound act both electron donor and acceptor; hence fermentation is an anaerobic process where energy produced without the participation of oxygen or other inorganic acceptor. Fermentation is process by which the living cell is able to obtain energy through the breakdown of glucose and other simple sugar molecules.

Fermentation can be defined as the breakdown or catabolism of organic compounds by micro-organisms under both aerobic and anaerobic conditions. This breakdown yields end products. Fermentation can help generate ATP from glucose by substrate-level phosphorylation as long as there is a supply of NAD+ to accept electrons.

- If the NAD+ pool is exhausted, glycolysis shuts down.
- Under aerobic conditions, NADH transfers its electrons to the electron transfer chain, recycling NAD+.
- Under anaerobic conditions, various fermentation pathways generate ATP by glycolysis and recycle NAD+ by transferring electrons from NADH to pyruvate or derivatives of pyruvate.

**History:**
Fermentation is an ancient process dating back thousands of years .The term "fermentation" derives from the Latin work *fevere* meaning "to ferment." It was the means by which bread, wine, beer, and cheese were made. Egyptians found that uncooked dough left standing became lighter and softer. They and the Romans discovered that yeast produced lighter and leavened bread. Around 4000 BC wine was made from grape juice through a fermentation process. Beer making by the ancients came about by the soaking of barley in water, probably a serendipitous by-product of bread making. The Chinese used micro-organisms in the production of yogurt, cheese, wine, vinegar, and different types of sauces. Cheese was made by storing milk in animal skins or bladders made from animal stomachs. The bacteria and enzymes present in these containers would cause separation of casein (milk protein) to form curd. Fermented rice, vegetables, and fruits were extensively used by Ecuadorians. Not until the work of Louis Pasteur in the late 19 century was it understood how the process, which to that point was based on experience and tacit knowledge, actually worked. Antony van Leeuwenhoek, a Dutch biologist in 1680 was the first to see micro-organisms in samples of fermenting beer through a microscope. Pasteur, a French chemist, discovered that yeasts convert sugars to alcohol and carbon dioxide during fermentation. Fermentation is the process by which alcoholic

beverages or acidic dairy products (cheeses, yogurt) are manufactured. It is a way for a cell to obtain energy without using oxygen. During the process, complex organic substances are broken down into simpler ones. The cell (microbial or animal) obtains energy through glycolysis– the splitting of a sugar molecule to extract its electrons. The by-product of this process is excreted from the cell in the form of substances such as alcohol, lactic acid, and acetone. With advances in the science of microbiology and technologies like biotechnology, micro-organisms are exploited to produce a wide variety of products using fermentation. These include:

- Dairy products– Cheese, yogurt
- Beverages– Beer, wine
- Single Cell Proteins (SCP) – SCP are a cell monoculture of bacteria, fungi, and algae. Since the cells contain large amounts of protein, SCP is used as food or food supplement for humans and cattle. It is regarded as a cheap source of dietary protein and is produced from methanol and by-products of cheese production and paper making.
- Antibiotics– Antibiotics are one of the most important compounds produced by fermentation. Alexander Fleming in 1929 was the first to discover "penicillin", an antibiotic. Large numbers of antibiotics are being produced now by fermentation using various bacteria and fungi.

**Types of Fermentation:**

1) Alcoholic fermentation
2) Lactic acid fermentation

**Alcoholic fermentation**

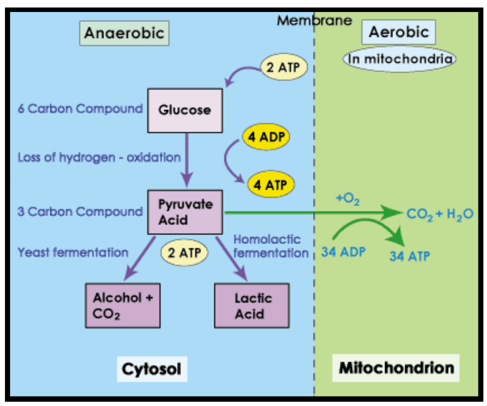


**Fig.1 Alcoholic Pathway**

Various bacteria and yeasts metabolize sugars into ethanol through different pathways using different enzyme systems. Alcohol fermentation is used for the industrial production of alcohols and alcoholic beverages. In the preparation of alcoholic beverages several factors have to be considered, such as flavor, taste, appearance, and safety. These require special procedures and standards. The commercial producers of alcoholic beverages each have their own protocols which give their product a distinct taste and flavor, and these are often kept confidential. The process is as follows:

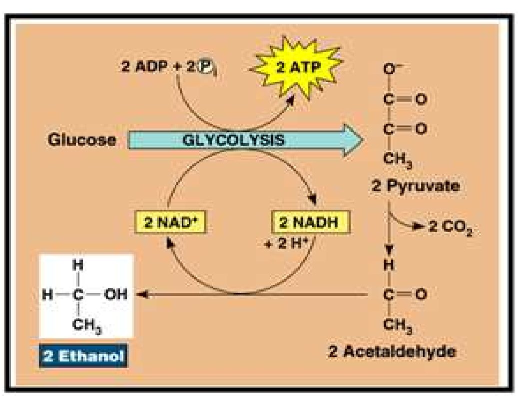


**Fig. 2 Alcohol Fermentation**

Sugar (Carbohydrate) =2Ethanol + 2Carbon Dioxide

There are four different phases in bacterial growth during fermentation. A good understanding of these phases is very important for effective management of the whole fermentation process.

**Lag Phase**
At the start of the process microorganisms are added to the nutrient medium and allowed to grow. The number of microorganisms will not increase because they try to adapt to the environment.

**Log Phase**
The microorganisms are adjusted to the new environment and they multiply at a very rapid pace thus increasing the cell number exponentially.

**Stationary Phase**
As the microorganisms grow they produce metabolites which are toxic to microbial growth. Also, the nutrient medium is used up, slowing down or stopping cell growth.

**Death Phase**
Microorganisms produce toxic metabolites to the extent that they cause the death of the microorganisms.

In alcohol fermentation, pyruvate is converted to ethanol in two steps.

1) Pyruvate is converted to a two-carbon compound, acetaldehyde by the removal of CO2.
2) Acetaldehyde is reduced by NADH to ethanol.Alcohol fermentation by yeast is used in brewing and winemaking.

**Lactic acid fermentation**
During lactic acid fermentation, pyruvate is reduced directly by NADH to form lactate (Ionized form of lactic acid).

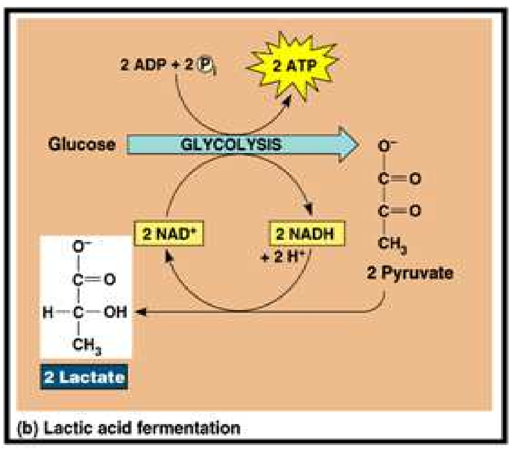


**Fig. 3 Lactic Acid Fermentation**

- Lactic acid fermentation by some fungi and bacteria is used to make cheese and yogurt.
- Muscle cells switch from aerobic respiration to lactic acid fermentation to generate ATP when O2 is scarce.
- The waste product, lactate, may cause muscle fatigue, but ultimately it is converted back to pyruvate in the liver.

**Industrial Fermentation:**
Growth media are required for industrial fermentation, since any microbe requires water, (oxygen), an energy source, a carbon source, a nitrogen source and micronutrients for growth.

Carbon & energy source + nitrogen source + $O_2$ + other requirements → Biomass + Product + byproducts + $CO_2$ + $H_2O$ + heat

| Nutrient | Raw material |
|---|---|
| **Carbon** | |
| Glucose | corn sugar, starch, cellulose |
| Sucrose | sugarcane, sugar beet molasses |
| Lactose | milk whey |
| fats | vegetable oils |
| Hydrocarbons | petroleum fractions |

| **Nitrogen** | |
|---|---|
| Protein | soybean meal, corn steep liquor, distillers' soluble |
| Ammonia | Pure ammonia or ammonium salts urea |
| Nitrate | nitrate salts |
| Phosphorus source | phosphate salts |

Trace elements: Fe, Zn, Cu, Mn, Mo, Co
Antifoaming agents : Esters, fatty acids, fats, silicones, sulphonates, polypropylene glycol
Buffers: Calcium carbonate, phosphates
Growth factors: Some microorganisms cannot synthesize the required cell components themselves and need to be supplemented, e.g. with thiamine, biotin, calcium pentothenate
Precursors: Directly incorporated into the desired product: Phenyl ethylamine into Benzyl penicillin, Phenyl acetic acid into Penicillin G
Inhibitors: To get the specific products: e.g. sodium barbital for rifamycin
Inducers: The majority of the enzymes used in industrial fermentation are inducible and are synthesized in response of inducers: e.g. starch for amylases, maltose for pollulanase, pectin for pectinase,olive oil and twin are also used at times.
Chelators: Chelators are the chemicals used to avoid the precipitation of metal ions. Chelators like EDTA, citric acid, polyphosphates are used in low concentrations.

**Micro- organism:**
Several species belonging to the following categories of micro-organism are used in fermentation process.

| | |
|---|---|
| 1) Prokaryotic | Unicellular: bacteria, cyanobacteria<br>Multicellular: cyanobactria |
| 2) Eukaryotic | Unicellular: yeast, algae<br>Multicellular: fungi, algae |

**Microbial metabolite**
**1. Primary metabolite**
During the log or exponential phase organism produce variety of substance that are essential for their growth, such as nucleotide, amino acid protein, carbohydrate, lipids, etc or by products of energy yielding metabolism such as ethanol, acetone, butanol etc. This phase described as troposphere and the products are usually called primary metabolite.

**Examples of commercially produced primary metabolite**

| Primary metabolite | Organism | Significance |
|---|---|---|
| Ethanol | Saccharomyces cerevisiae<br>Kluyveromyces fragilis | Alcoholic beverage |
| Citric acid | Aspergillus niger | Food industry |
| Acetone and butanol | Clostridium acetobutyricum | Solvent |
| Lysine | Corynebacterium | Nutritional additive |
| Glutamic acid | Glutamacium | Flavour enhancer |

**2. Secondary metabolite**
Organism produces a number of products, other than the primary metabolite. Examples of commercially produced secondary metabolite

| Metabolite | Species | Significance |
|---|---|---|
| Penicillin | Penicillium chrysogenum | Antibiotic |
| Erythromycin | Streptomyces erythreus | Antibiotic |
| Streptomycin | Streptomyces griseus | Antibiotic |

**Production of enzyme**

Enzymes are used in clinical or industrial analysis and now they are even added to washing powder. Enzymes may be produced by microbial, plant or animal culture. Even plant and animal enzyme can be produced by microbial fermentation.
Examples of commercially produced enzymes:

| **Organism** | **Enzymes** |
|---|---|
| Bacillus species | Protease |
| Aspergillus oryzae | Amylases |
| Aspergillus niger | Glucamylase |
| Trichoderma reesii | Cellulose |

**Downstream Process[3]:**
Downstream processing refers to the recovery and purification of biosynthetic products, particularly pharmaceuticals, from natural sources such as animal or plant tissue or fermentation broth, including the recycling of salvageable components and the proper treatment and disposal of waste. It is an essential step in the manufacture of pharmaceuticals such as antibiotics, hormones (e.g. insulin and human growth hormone), antibodies and vaccines; antibodies and enzymes used in diagnostics; industrial enzymes; and natural fragrance and flavor compounds. Downstream processing is usually considered a specialized field in biochemical engineering, itself a specialization within chemical engineering, though many of the key technologies were developed by chemists and biologists for laboratory-scale separation of biological products.
The various stages of processing that occur after the completion of the fermentation or bioconversion stage, including separation, purification, and packaging of the product

**Stages in Downstream Processing:**

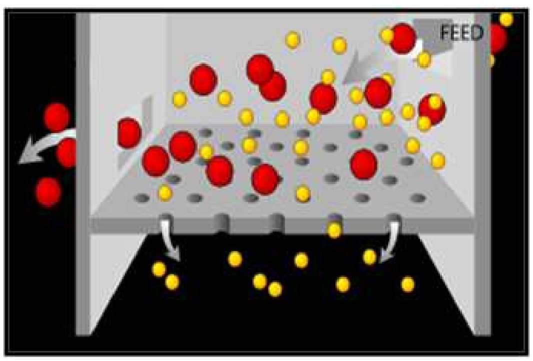


**Fig. 4 Stages in Downstream Process**

Removal of insoluble's
- Product Isolation
- Product Purification
- Product Polishing

**Removal of insoluble's**
- Capture of the product as a solute in a particulate-free liquid
- Separation of cells, cell debris or other particulate matter from fermentation broth containing an antibiotic.
- Typical operations
- Filtration
- A mechanical operation used for the separation of solids from fluids (liquids or gases) by interposing a medium to fluid flow through which the fluid can pass, but the solids in the fluid are retained

**Filter media**
Two main types of filter media are solid sieve which traps the solid particles bed of granular materials retains the solid particles
Points to be considered while selecting the filter media:
- Ability to build the solid.
- Minimum resistance to flow the filtrate.
- Resistance to chemical attack.
- Minimum cost.
- Long life

**Centrifugation**

- Use of the centrifugal force for the separation of mixtures
- More-dense components migrate away from the axis of the centrifuge
- Less-dense components migrate towards the axis.

**Flocculation**

- Process where a solute comes out of solution in the form of flocs or flakes.
- Particles finer than 0.1 µm in water remain continuously in motion due to electrostatic charge which causes them to repel each other
- Once their electrostatic charge is neutralized (use of coagulant) the finer particles start to collide and combine together.
- These larger and heavier particles are called flocs.

**Product Isolation**

Reducing the volume of material to be handled and concentrating the product.
the unit operations involved
- Solvent extraction
- Ultra filtration
- Precipitation

**Precipitation**

Formation of a solid in a solution during a chemical reaction.
Solid formed is called the precipitate and the liquid remaining above the solid is called the supernatant**.**

**Product Purification**

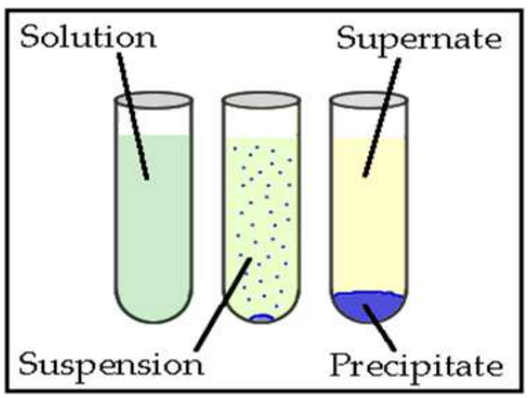


**Fig.5 Product Purification**

- To separate contaminants that resembles the product very closely in physical and chemical properties.
- Expensive and require sensitive and sophisticated equipment.
- Crystallization
- Process of formation of solid crystals precipitating from a solution, melt or more rarely deposited directly from a gas.
- Chemical solid-liquid separation technique, in which mass transfer of a solute from the liquid solution to a pure solid crystalline phase occurs.

**Product Polishing**

- Final processing steps which end with packaging of the product in a form that is stable, easily transportable and convenient.
- Crystallization, desiccation, lyophilization and spray drying are typical unit operations
- Lyophilization
- freezing the material
- reducing the surrounding pressure and adding enough heat to allow the frozen water in the material to sublime directly from the solid phase to gas

**Fermentation Equipments[2, 3]:**

**Stirred Tank Fermenters[8]**

These are the most commonly used fermenters. They are cylindrical vessels with a motor driven agitator to stir the contents in the tank. The Top-entry stirrer (agitator) model is most commonly used because it has many advantages like ease of operation, reliability, and robustness. The Bottom-entry stirrer (agitator) model is rarely used.

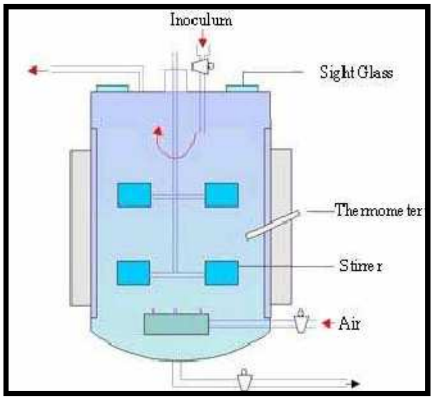


**Fig. 6:-Stirred tank fermenter**

Laboratory scale stirred tank fermenters are made of borosilicate glass with a stainless steel lid and top-entry stirrer. Typical volume of these fermenters is 1 to 100 liters. Stirrers consist of a motor attached to the shaft. The shaft contains impellers. Stainless steel fermenters are also used in laboratories and have special requirements. They should be made of high grade stainless steel, have an internal surface that should be polished to reduce adhesion of contents to the walls of the fermenter, and have joints that should be smooth and free from pin-holes.

**1) Agitator**

This consists of shaft, impellers with 4 to 6 blades and motor to drive. Shafts should have double seals to prevent leakage of the contents. The main function of the agitator is mixing of the contents, aeration, and removal of carbon dioxide produced during fermentation process by mixing action.

Different types of impellers are:

1. Ruston blade or disc turbine: This is the most commonly used impeller because of its simple design, robustness and ease of operation. It has 4 to 6 blades.
2. Open turbine impellers
3. Marine impellers

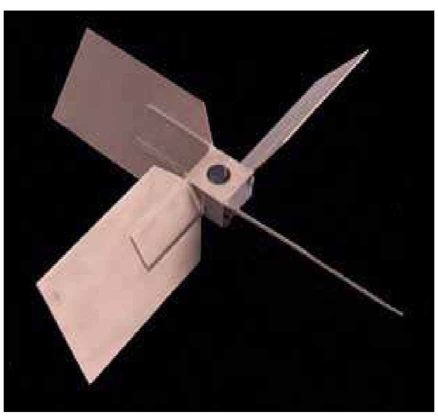

**Fig No. 7:-Ruston blade/disc turbine**

**Fig No. 8:-Marine impeller**

1) **Baffles:** Four baffles are fixed on the walls of the fermenter which are used to prevent formation of a vortex. Impellers and baffles produce axial or radial flow patterns of the contents in the fermenter.
2) **Autoclave**

Autoclaves are used to sterilize equipment, media, and other components of fermentation. They are similar to pressure cookers. Different sizes of autoclaves are available on the market.

3) ***Ovens***

Hot air ovens are used to sterilize or dry the equipment used in the fermentation process. The equipment to be dried or sterilized should withstand high temperatures, borosilicate or Pyrex glass equipment being good examples. The inner chamber is made of heat resistant stainless steel and has a fan to circulate the hot air evenly throughout the chamber to ensure proper heat transfer. Microwave ovens are used to perform drying and melting agar. The main advantage of micro wave ovens is they take a very short time to do the job.

**Fig9:-Microwave oven**

**Air-lift Fermenters**

These fermenters do not have mechanical agitation systems (motor, shaft, impeller blades) but contents are agitated by injecting air from the bottom. Sterile atmospheric air is used if microorganisms are aerobic and “inert gas” is used if microorganisms are anaerobic. This is a gentle method of mixing the contents and is most suitable for fermentation of animal and plant cell cultures since the mechanical agitation produces high shearing stress that may damage the cells. Air-lift fermenters are most widely used for large-scale production of monoclonal antibodies.

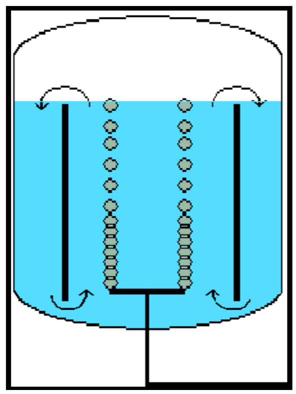

**Fig 10:-Air-Lift Fermenter**

**Fermentation Application[3-5]:**

In World War I, Germany needed to synthesise glycerol for manufacturing explosives. It was found that glycerol was generated in alcoholic fermentation. Nearberg discovered that the addition of sodium bisulphite to the fermentation broth was favoured and enhanced the production of glycerol at the expense of ethanol. German scientists quickly developed industrial-scale fermentation with a yield capacity of 1000 tons of glycerol per month.1,2 Ethanol is an essential chemical which is used as a raw material for a vast range of applications including chemicals, fuel (bioethanol), beverages, pharmaceuticals and cosmetics. The feedstock for ethanol generally comes from renewable sources such as starch (from wheat, barley, maize, potato, cassava, sweet potato, etc.) and molasses or syrups originating from sugar beet or sugar cane, etc. Bioethanol is produced by biological fermentation technology. The fermentation product stream is processed with subsequent enrichment by distillation/rectification and dehydration. Bioethanol is becoming a viable solution as a source of renewable energy, as it is a non-fossil fuel. It may originate from renewable agricultural sources, resulting in cleaner combustion without any emissions to the air.

**Production of Ethanol by Fermentation**

Carbohydrates obtained from grains, potatoes or molasses are fermented by yeasts to produce ethanol in the production of beer, alcohols and distilled spirits. Fermentation of sugar using *Saccharomyces cerevisiae* produces ethanol under anaerobic conditions. The batch fermentation system is affected by high substrate and product inhibition. Glucose concentration plays the major role in increasing the concentration of ethanol and cell growth rate in the fermentation broth. Cell density, ethanol concentration and glucose concentration are measured. Industrial grade sugars and molasses are used for the production of bioethanol. Today, alcohol technologies are well developed. Many extraction and purification techniques have enhanced ethanol production and the process is considered economically feasible.

**Optical Cell Density**

Cell growth is defined with cell density. Cell concentration is an indication of viability of Microorganism. Measure the optical cell density of *Saccharomyces cerevisiae* at a wavelength of 580 nm. Other wavelengths such as 600 nm or less may also be used, but one must be consistent. Draw a growth curve based on incubation time and cell dry weight. A standard calibration curve is needed before any actual experiment. Generate a calibration curve to relate the absorbance with cell dry weight. The usual rules of operating a spectrophotometer apply here, as well. For example, the accuracy of the method is greatest when the absorbance readings are in the range 0.1–1. For a given culture sample, a good spectrophotometer should yield a linear relation between the number of cells and the absorbance. However, optical density is also a function of cell morphology such as size and shape, because the amount of transmitted or scattered light depends strongly on these factors. Consequently, an independent calibration curve is required for each condition in accurate research work, as the cell size and shape depend on the specific growth rate and the nutrient composition. As a rule of thumb, an optical density of one unit corresponds to approximately 1 g of dry cell. This is also commonly referred to as the turbidity measurement.

**Industrial Application[6-7]:**

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.

**Food fermentation**

Ancient fermented food processes, such as making bread, wine, cheese, curds, idli, dosa, etc., can be dated to more than 6,000 years ago. They were developed long before man had any knowledge of the existence of the microorganisms involved. Fermentation is also a powerful economic incentive for semi-industrialized countries, in their willingness to produce bio-ethanol.

**Pharmaceuticals and the biotechnology industry**

There are 5 major groups of commercially important fermentation:

- Microbial cells or biomass as the product, e.g. single cell protein, bakers yeast, lactobacillus, E. coli, etc.
- Microbial enzymes: catalase, amylase, protease, pectinase, glucose isomerase, cellulase, hemicellulase, lipase, lactase, streptokinase, etc.
- Microbial metabolites :
- Primary metabolites – ethanol, citric acid, glutamic acid, lysine, vitamins, polysaccharides etc.
- Secondary metabolites: all antibiotic fermentation
- Recombinant products: insulin, HBV, interferon, GCSF, streptokinase
- Biotransformations: phenyl acetyl carbinol, steroid biotransformation, etc.

**Nutrient sources for industrial fermentation:**

- Growth media are required for industrial fermentation, since any microbe requires water, (oxygen), an energy source, a carbon source, a nitrogen source and micronutrients for growth.
- Carbon & energy source + nitrogen source + $O_2$ + other requirements → Biomass + Product + byproducts + $CO_2$ + $H_2O$ + heat
- Trace elements: Fe, Zn, Cu, Mn, Mo, Co
- Antifoaming agents: Esters, fatty acids, fats, silicones, sulphonates, polypropylene glycol
- Buffers: Calcium carbonate, phosphates
- Growth factors: Some microorganisms cannot synthesize the required cell components themselves and need to be supplemented, e.g. with thiamine, biotin, calcium pentothenate
- Precursors: Directly incorporated into the desired product: Phenyl ethylamine into Benzyl penicillin, Phenyl acetic acid into Penicillin G
- Inhibitors: To get the specific products: e.g. sodium barbital for rifamycin
- Inducers: The majority of the enzymes used in industrial fermentation are inducible and are synthesized in response of inducers: e.g. starch for amylases, maltose for pollulanase, pectin for pectinase,olive oil and tween are also used at times.
- Chelators: Chelators are the chemicals used to avoid the precipitation of metal ions. Chelators like EDTA, citric acid, polyphosphates are used in low concentrations.

## Conclusion

Fermentation process carried out by producing effervescent and heat. Different micro-organisms like bacteria, actinomycets, viruses are used as biocatalyst and different fermentation media like carbon sources, nitrogen sources,, buffers, antifoaming agent, minerals are used for fermentation of the product. Different pharmaceuticals such as antibiotics, enzymes, amino acids, insulin, vitamins etc.

## Acknowledgement

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