Chapter # 4 Insight into Fermentation

FERMENTATION

The use of microorganisms such as bacteria and fungi to make products useful to humans is called fermentation. The process can either be aerobic or anaerobic. Fermented products have applications as food as well as in general industry.

FERMENTERS

A fermentor is a device which provide controlled environment to the organisms for the production of desired product. Factors like temperature, pH and other media requirements are controlled in a fermentor. The fermentor is termed as bioreactor if plant, animal or bacterial cells are used to make the products.

FEATURES OF A FERMENTER

1. *Design should be aseptically designed in order to avoid undesirable microbes which otherwise would lead to high cost downstream processing. It should also consume less power.*
2. *Fermentor should have adequate aeration / agitation system for uniform mixing of media.*
3. *Facilitate the growth of a wide range of organisms.*
4. *There should be low evaporation from fermentor.*
5. *Operation of bioreactor must involve minimal labor in order to reduce chances of contamination and make it economical.*
6. *Construction should be cheap and from best material.*
7. *There should be temperature and pH control along with proper sterilization system.*
8. *Inlet and outlet should be properly monitored.*

DESIGN OF FERMENTOR

Construction

Depending on the construction type, the bioreactor vessel is made of steel, metal or their combination. The relation between the height H and diameter D of the bioreactor is within 1.5-2.5. The reactor filling is about 70%. There are high requirements for the reactor vessel materials to prevent the inhibition of the microorganism growth. The same applies also to any other part (sensors, pipes, etc.), which are installed inside the bioreactor vessel. The glass should be 100% borosilicate. All the metal parts should be made from stainless steel. The inner surface of the stainless steel bioreactor should be polished to about a mirror surface quality to facilitate the washing and sterilization process. Welding should be carried out in a fully inert gas medium. The inert gas should be argon, which fully replaces the air.

Temperature Control System

Most of the times temperature inside the bioreactor increases due to agitation and metabolic activity of microbes. High temperature can thus deactivate enzymes resulting in their loss of function. So it is important to maintain temperature inside the vessel. For this purpose water jackets are used which absorbs heat during an exothermic reaction.

Aeration/Agitation

Agitation is required for uniform mixing of the media. It is carried out with the help of impellers or propellers. Number of impellers depends upon the size of the vessel. The speed of impellers should be from 50-150 rpm. It depends upon the volume of the media. For Aeration two types of spargers are used. These are Porous sparger and nozzle sparger.

pH Control System

pH is controlled by the pH control system which consists of an optical pH sensor, a buffer, and the necessary hardware. The pH sensor measures the pH of the culture medium in the bioreactor where cells are grown. A buffer, consisting of a mixture of sodium and potassium hydroxide and bicarbonate, is added to the cellular culture medium to keep the pH within a known range.

Baffles

Baffles are flow-directing or obstructing vanes used in chemical reactors, and static mixers. Baffles are an integral part of the bioreactors design. A baffle is designed to direct the flow of fluids for maximum efficiency. They also help in preventing the formation of bubbles and foam in the media by using anti foaming agents like Pluronic 20 & Tween 80.

Computer

For good quality practices computer are attached to the fermentor if our desired product in the form of standard spectrum. It is useful in storing data in batch or continuous process. It also helps in detecting errors during the process.

Inlet & Outlet

These are used for the introduction and release of the media into the culture vessel.

Aseptic Conditions

Sterilization is done for maintaining aseptic conditions. It is normally done through steam with pressure. There should be piping system. The air that comes into the fermentor should be filtered using heat filters. Granular materials can also be used for sterilizing the bioreactor.

TYPES OF FERMENTORS

1. AIR LIFT FERMENTOR

It is a bioreactor in which the reaction medium is kept mixed and gassed by the introduction of air or another gas at the base of a column-like reactor. It uses a draft tube which may be an inner tube (this kind of air-lift bioreactor is called "air-lift bioreactor with an internal loop) or an external tube (this kind of air-lift bioreactor is called "air-lift bioreactor with an external loop) which improves circulation and oxygen transfer and equalizes shear forces in the reactor. But the problem with these bioreactors is that the draft tube decreases the capacity. It should be noted that the largest type of bioreactors are airlift bioreactors.

2. FLUIDIZED BED FERMENTOR

It was designed by Von Winkler for the first time in 1922. A fluidized bed is formed when a quantity of a solid particulate substance is placed under appropriate conditions to cause the solid/fluid mixture to behave as a fluid. It is the best choice for immobilizing cells or enzymes. No baffles or impellers are used in these bioreactors. Its advantages are that the product is free from cells and enzymes resulting in less cost price of downstream processing. But the repacking of the enzyme is very difficult as toxic substances may stick to it.

3. MEMBRANE BIOREACTOR

Membrane bioreactor (MBR) is the combination of a membrane process like microfiltration or ultrafiltration with a suspended growth bioreactor, and is now widely used for municipal and industrial wastewater treatment. The filtration element is installed in the main bioreactor vessel. The product passes from the membrane but the media remains in the vessel. But its disadvantage is that the membrane gets block after sometime.

4. PHOTOBIOREACTOR

A photobioreactor is a bioreactor that incorporates some type of light source to provide photonic energy input into the reactor. A closed photobioreactor can be described as an enclosed, illuminated culture vessel designed for controlled biomass production of phototrophic liquid cell suspension cultures. Despite its cost, it has several major advantages over open systems. They can prevent or minimize contamination, permitting cultivating of algal monocultures. It also offers better control over bio-cultural conditions (pH, light intensity, carbon dioxide, and temperature). Open Photobioreactor, on the other hand increases chances of contamination and evaporation.

5. STIRRER TANK BIOREACTOR

It is a common ideal reactor type in chemical engineering. It consists of a tank containing impellers which move constantly for continuous agitation. The size of this type of bioreactor depends upon media. Spargers are used for aeration while anti foaming agents are used to reduce foam in the vessel. The height and width should be in 1 ratio 2. They are always operated in continuous mode. But the impellers can damage the cells. It also covers large space due to giant sizes.

SCALE UP PROCESS

The commercial application of technology developed in the laboratory to industrial application requires the scaling up from the lab scale (0.1 to 1L) to the industrial scale (10,000 – 1,000,000+ L), representing an increase. This represents a scale-up process, equipment design, and technology risks. The scale-up process performed at three scales.

- ***LAB SCALE***

The process is first evaluated on lab scale. Conditions are optimized and analyzed during development. Lab scale uses different instruments.

1. Use of Erlenmeyer flask

An Erlenmeyer, also known as a conical flask, is a widely used type of laboratory flask which features a flat bottom, a conical body, and a cylindrical neck. It is named after the German chemist Emil Erlenmeyer, who created it in 1861.The Erlenmeyer is usually marked on the side (graduated) to indicate the approximate volume of contents, and has a spot of enamel where it can be labeled with a pencil. It differs from the beaker in its tapered body and narrow neck. It has a capacity of 250 ml-1 liter.

2. Optimization of Conditions

It mainly includes temperature, pH, media and gas requirements. Different conditions are used and the one giving the best results are selected on trial and error basis. E.g. Temperature ranging from 25C-45C is used in synthesis of lactic acid by lactobacillus Bulgaricus. The one with better result is selected for future use. Likewise pH and media are also optimized.

PILOT SCALE

A pilot scale is a small scale preliminary study conducted in order to evaluate feasibility, time, cost, adverse events, and effect size (statistical variability). They are designed to predict an appropriate sample size and improve upon the study design prior to performance of a full-scale research project. Pilot studies, therefore, may not be appropriate for case studies. They are frequently carried out before large-scale quantitative research, in order to avoid time and money being wasted on an inefficiently designed project. About 1-12 liters of media is used in pilot scale and all the conditions are calibrated as per laboratory scale. If the results are satisfactory the process is practiced on industrial scale, otherwise not.

INDUSTRIAL SCALE

The approved process is then practiced on industrial scale for production of our desired product in higher amount. All the conditions remain the same with exception of time which increases as per media. 100-10,000 liters of media is processed at a single time in industries.

UPSTREAM PROCESSING

The upstream processing refers to the first step in which biomolecules are grown, usually by bacterial or mammalian cell lines in bioreactors. When they reach the desired density (for batch and fed batch cultures) they are harvested and moved to the downstream section of the bioprocessing.

DOWNSTREAM PROCESSING

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. It also includes 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; natural fragrance and flavor compounds. Downstream processing is usually considered a specialized field in biochemical engineering. Downstream processing is accomplished in the following steps.

1. DISRUPTION OF THE CELL

If our desired product is intracellular then we first need to disrupt the cell. For this purpose three methods are used.

PHYSICAL METHOD

1. Mechanical Disruption

Mechanical methods rely on the use of rotating blades to grind and disperse large amounts of cellular media. The Waring blender and the Polytron are commonly used for this purpose. Waring blender is similar to a standard household blender. Polytron can be used for samples as small as 1 ml.

2. Mortar and Pestle

Manual grinding is the most common method used to disrupt plant cells. Tissue is frozen in liquid nitrogen and then crushed using a mortar and pestle. Because of the tensile strength of the cellulose and other polysaccharides comprising the cell wall, this method is the fastest and most efficient way to access plant proteins and DNA.

3. Sonication

Sonication is the third class of physical disruption commonly used to break open cells. The method uses pulsed, high frequency sound waves to agitate and lyse cells, bacteria, spores and finely diced tissue. The sound waves are delivered using an apparatus with a vibrating probe that is immersed in the liquid cell suspension. Mechanical energy from the probe initiates the formation of microscopic vapor bubbles that form momentarily and implode, causing shock waves to radiate through a sample. To prevent excessive heating, ultrasonic treatment is applied in multiple short bursts to a sample immersed in an ice bath. Sonication is best suited for volumes <100 ml.

CHEMICAL METHOD

Different chemicals can be used to interact with cell wall and cell membrane of cells resulting in their disruption. We use organic solvents like methanol, ethanol and acetone for this purpose. Surfactants like soap, detergents etc are also used.

BIOLOGICAL METHOD

The use of enzymatic methods to remove cell walls is well-established for disrupting cells for to get intracellular products. The enzymes are generally commercially available and, in most cases, are originally isolated from biological sources. The enzymes commonly used for gram negative bacterial lysis are lysozyme and cellulase. While for yeast/fungi we use glycoprotein, chitin, mannonase, gluconase etc.

2. REMOVAL OF INSOLUBLES

Cell debris is considered as insolubles in case we are interested in intracellular products. While in case of extracellular products whole cells are considered as insolubles. These insoluble are removed from the fermentation broth by the following methods.

FILTRATION

Filtration is commonly the mechanical or physical operation which is used for the separation of solids from fluids (liquids or gases) by interposing a medium through which only the fluid can pass.

1. Dead End Filtration

A type of membrane filtration where the water being filtered flows through the membrane, but there is no wastestream from the system. All solids accumulate on the membrane during filtration and are removed during backwash. It has high collection rate (almost 100%) with a low cost. But filters must be replaced after some usage and also it cannot be used if large amounts of insoluble materials are present.

2. Cross Flow Filtration

Cross flow filtration is a type of filtration. The principal advantage of this is that the filter cake (which can blind the filter) is substantially washed away during the filtration process due to cross flow of cellular media, increasing the length of time that a filter unit can be operational. It can be a continuous process, unlike batch-wise dead-end filtration. The filter maintenance frequency is low and can be used even if large amounts of insoluble materials are included. But its disadvantage is that the unit is large and complicated with relatively high cost. Industrial examples of this include the extraction of soluble antibiotics from fermentation liquors.

CENTRIFUGATION

Centrifugation is a process that involves the use of the centrifugal force for the sedimentation of mixtures with a centrifuge, used in industry and in laboratory settings. More-dense components of the mixture migrate away from the axis of the centrifuge, while less-dense components of the mixture migrate towards the axis. At the end, the dense components settle at the bottom and is called pellet while the lighter components float on the surface of pellet and is called supernatant. The supernatant liquid is then either quickly decanted from the tube without disturbing the precipitate, or withdrawn with a Pasteur pipette.

1. Decantation

Decantation is a process for the separation of mixtures. This is achieved by carefully pouring a solution from a container in order to leave the precipitate in the bottom of the container.

2. Pipetting

Pipetting is a process in which the supernatant part of the mixture is carefully removed with the help of a pipette.

FLOCCULATION

Flocculation is a process of contact and adhesion whereby the dispersed particles form larger-size clusters. It is a process wherein colloids come out of suspension in the form of flakes by the addition of a clarifying agent. The action differs from precipitation in that, prior to flocculation, colloids are merely suspended in a liquid and not actually dissolved in a solution. Those agents which are used for flocculation are called flocculating agents. The commonly used flocculating agents are Aluminium sulphate, calcium hydride, sodium aluminate and sodium silicate.

3. PRODUCT ISOLATION

Once the cells are disrupted and insolubles are removed, the product can be isolated by the following methods.

SOLVENT EXTRACTION

Solvent extraction is a method to separate compounds based on their relative solubilities in two different immiscible liquids, usually water and an organic solvent like n-hexane. It is an extraction of a substance from one liquid phase into another liquid phase. These liquids must be immiscible and must form a separation layer in between them. It is a basic technique using a separatory funnel. Solvent extraction is used in nuclear reprocessing, ore processing, the production of fine organic compounds, the processing of perfumes, the production of vegetable oils and biodiesel, and other industries.

ULTRAFILTRATION

Ultrafiltration is a membrane filtration in which hydrostatic pressure forces a liquid against a semi permeable membrane. Pore size used is 0.1-0.001 um. Suspended solids and solutes of high molecular weight are retained and is called retentate, while water and low molecular weight solutes pass through the membrane and is called permeate. Both dead-end and cross flow methods can be used for ultrafiltration.

PRECIPITATION

Precipitation is the formation of a solid in a solution or inside another solid during a chemical reaction or by diffusion in a solid. When the reaction occurs in a liquid, the solid formed is called the precipitate, or when compacted by a centrifuge, a pellet. The liquid remaining above the solid is in either case called the supernatant. Precipitation can also be obtained by making a super saturated solution through heating. The heat is then removed and the excess amount of solutes gets settle in the bottom resulting in precipitation.

4. PRODUCT PURIFICATION

It is the most expensive step of downstream processing and it involves sophisticated techniques. Mostly chromatography is practiced for product purification. These may be:

AFFINITY CHROMATOGRAPHY

Affinity chromatography is a method of separating biochemical mixtures and is based on a highly specific interaction such as that between antigen and antibody, enzyme and substrate, or receptor and ligand. The stationary phase is typically a gel matrix, often of agarose. The molecule of interest will have a well known and defined property which can be exploited during the affinity purification process. The process itself can be thought of as an entrapment, with the target molecule becoming trapped on a solid or stationary phase. The other molecules in solution will not become trapped as they do not possess this property. The solid medium can then be removed from the mixture, washed and the target molecule released from the entrapment in a process known as elution. Possibly the most common use of affinity chromatography is for the purification of recombinant proteins.

SIZE EXCLUSION CHROMATOGRAPHY

Size-exclusion chromatography is a chromatographic method in which molecules in solution are separated by their size, and in some cases molecular weight. It is usually applied to large molecules or macromolecular complexes such as proteins and industrial polymers. Typically, when an aqueous solution is used to transport the sample through the column, the technique is known as gel-filtration chromatography, versus the name Gel permeation chromatography, which is used when an organic solvent is used as a mobile phase. Size Exclusion Chromatography works on the principle that larger molecules will come first leaving smaller molecules behind. The time taken by the sample component to its end is called retention time. We can detect different components of sample from its retention time. The spectrum obtained is compared with the standard spectrum and desired product is determined.

REVERSE PHASE CHROMATOGRAPHY

Reversed-phase chromatography includes any chromatographic method that uses a non-polar stationary phase. The name "reversed phase" has a historical background. In the 1970s most liquid chromatography was done on non-modified silica or alumina with a hydrophilic surface chemistry and a stronger affinity for polar compounds - hence it was considered "normal". Now in Reverse Phase Chromatography, polar compounds are eluted first while non-polar compounds are retained, hence called "reversed phase". All of the mathematical and experimental considerations used in other chromatographic methods apply. Today, reversed-phase column chromatography accounts for the vast majority of analysis performed in liquid chromatography. Reverse phase chromatography can also be called hydrophobic chromatography. It is similar to ion exchange chromatography. Lipophilic groups are attached to the stationary phase of the column. When a solution of proteins or molecules is passed through the column, hydrophilic proteins will flow through the column, while lipophilic proteins will remain in the column.

5. PRODUCT POLISHING

In this step the product is brought to a commercial form for consumer usage.

CRYSTALLIZATION

Crystallization is a technique which is used to purify solid compounds. It is one of the fundamental procedures based on the principles of solubility: compounds (solutes) tend to be more soluble in hot liquids (solvents) than they are in cold liquids. If a saturated hot solution is allowed to cool, the solute is no longer soluble in the solvent and forms crystals of pure compound. Impurities are excluded from the growing crystals and the pure solid crystals can be separated from the dissolved impurities by filtration.

USE OF ANTI-SOLVENT

The use of anti-solvent achieves supersaturation and solidification by exposing a solvent of the product to another solvent in which the product is not soluble. This will result in solvent-solvent interaction and the solutes will settle down at the bottom. The process can be semi-batch or continuous.

DESICCATION

Desiccation is the state of extreme dryness, or the process of extreme drying. A desiccant is a hygroscopic substance that holds and attracts water from its surroundings. A desiccator is a heavy glass or plastic container used for making or keeping small amounts of material very dry. The material is placed on a shelf, and a drying agent or desiccant, such as dry silica gel or anhydrous sodium hydroxide, is placed below the shelf. The desiccator absorbs water content leaving pure product. This method increases the shelf life of product since there in no water present in it.

6. PACKING OF THE PRODUCT

The purified products are then packed and labeled for market use. Following precaution should be practiced while packing a product.

- *The objects enclosed in the package may require protection from mechanical shock, vibration, electrostatic discharge, compression, temperature, etc.*
- *A barrier from oxygen, water vapor, dust, etc. is often required. Permeation is a critical factor in design. Some packages should contain desiccants or Oxygen absorbers to help extend shelf life.*
- *Small objects are typically grouped together in one package for reasons of efficiency. Liquids, powders, and granular materials need containment.*
- *Packages and labels should depict how to use, transport, recycle, or dispose of the package or product. For pharmaceuticals, food, medical, and chemical products, different types of information are required by governments.*
- *The packaging and labels could be used by marketers to encourage potential buyers to purchase the product. Package graphic design and physical design must be attractive for consumer concentration.*

7. ADVERTISEMENT

Advertising is a form of communication used to persuade consumers to take some action with respect to your products. Most commonly, the desired result is to make consumer buy your product. The product can be advertised by sponsors and viewed via various traditional media; including mass media such as newspaper, magazines, television commercial, radio advertisement, outdoor advertising or direct mail; or new media such as websites and text messages.

Chapter # 5 **Produce some vital Organic Compounds**

ETHANOL PRODUCTION

Ethanol (ethyl alcohol) is a clear, colorless liquid with a characteristic, agreeable odor. In dilute aqueous solution, it has a somewhat sweet flavor, but in more concentrated solutions it has a burning taste. Ethanol, CH_3CH_2OH, is an alcohol, a group of chemical compounds whose molecules contain a hydroxyl group, –OH, bonded to a carbon atom. The word alcohol derives from Arabic "al-kuhul" which means fine powder Ethanol has been made since ancient times by the fermentation of sugars. All beverage ethanol and more than half of industrial ethanol is still made by this process. Simple sugars are the raw material. Zymase, an enzyme from yeast, changes the simple sugars into ethanol and carbon dioxide.

SUBSTRATES:

1. ***STARCHY CROPS:***
   Corn, barley, sorghum etc.

2. ***SWEET CROPS:***
   Sugar cane, Sugar beet.

3. ***WOOD PULP:***
   Paper is prepared from wood pulp and it is used for production of ethanol. All of these 3 are cellulosic materials.

In Starchy crops: Ethanol production involves the following steps:

***Step 0I: SACCHARIFICATION**: Production of simple sugars from starch polymer.*

***Step II: FERMENTATION**: Ethanol is produced when microorganisms use simple sugars.*

COMMERCIAL PRODUCTION

At Industrial level production involve the following steps:

(I) ***Milling:*** *Small pieces of substances are formed by the process of milling.*

(II) ***Mash Formation**: Small pieces which are obtained by milling are used and water is added into them. Saccharification is also carried out by means of 2 methods.*

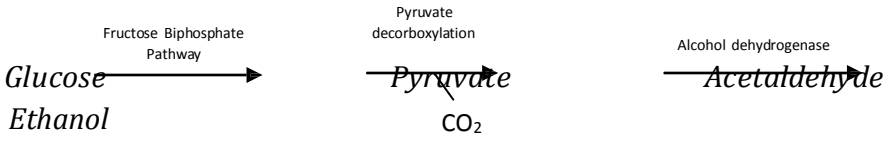
- *Biological Method*
- *Chemical Method*

- *Biological Method:*
  If we take the example of starch, enzyme called α-amylase act upon it and oligosaccharides are formed. Microbes are not capable to use these oligosaccharides. So another enzyme glucoamylase convert it is used by microbes.
- *Chemical Method:*
  Acid convert polymers into small units which can be used for production process. High temperatures can also be used for obtaining small units of polymers.

(III) Fermentation:

2 types of microorganisms can be used: Yeast, Bacteria

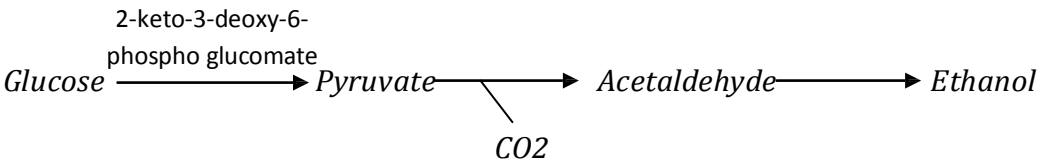
Yeast:



2 molecules of ethanol are obtained and 2 molecules of CO2 are released during this process.

Yeast eg, Saccharomyces Cereviase, Saccharomyces ovarum, can be sued industrially.

BACTERIA:



6 carbon glucose → 2 ethanol (4C)

→ $2CO_2$ (2C)

Zymomonas mobilis is mostly used at industrial level. Yeast uses 1,2,5,6 (carbon mo.) for the production of ethanol and bacteria uses carbon no 2,3,5,6 for ethanol production.

(IV) Liquid Solid Separation:

It is carried out after ethanol production. Best procedure for it is filtrations where both these are separated.

(V) Distillation:

By 2 methods:

1→ Water is chief impurity so it is removed from ethanol. At 78 °C, both these are separated. Boiling point of ethanol is 78 °C. Providing this temperature these two.

2→ Zeolite is composed of silicon, Al and oxygen. And it is used as absorbent. It is passed through the solution and it will absorb all the water so ethanol is left behind.

By Products formed during process:

- ➔ *CO2 is also formed as an impurity and it is used for carbonated beverages.*
- ➔ *Distiller's grain: Rich source of proteins, lipids and nutrients p It is formed in liquid-solid separation step – used for cattle food.*

USES OF ETHANOL

- ❖ *Ethanol is used extensively as a solvent in the manufacture of varnishes and perfumes.*
- ❖ *It is used as a preservative for biological specimens.*
- ❖ *It is also used in the preparation of essences and flavorings.*
- ❖ *It is used in many medicines and drugs; as a disinfectant and in tinctures.*
- ❖ *The most popular use of ethanol alone is in the automotive fuel industry where it is used as a fuel and additive.*

LACTIC ACID PRODUCTION

Lactic acid, also known as milk acid, is a chemical compound that plays a role in various biochemical processes and was first isolated in 1780 by the Swedish chemist Carl Wilhelm Scheele. Lactic acid is a carboxylic acid with the chemical formula $C_3H_6O_3$. Lactic acid is miscible with water or ethanol, and is hygroscopic. In industry, lactic acid fermentation is performed by lactic acid bacteria. These bacteria can also grow in the mouth; the acid they produce is responsible for the tooth decay known as caries.

COMMERCIAL PRODUCTION

Commercial production of this acid started in 1881 by M/S Clinton processing company, Clinton, Iowa (USA) by the process of fermentation.

Lactic Acid has 2 isomers:

- ➔ *L-lactic acid*
- ➔ *D-lactic acid*

Humans can use L isomer but cannot use D isomer.

Fermentation of Lactic Acid:

Fermentation of lactic acid can be done by processes such as:

1. ***Homofermentative process:***
   Product formed is more than 90% lactic acid and very less amounts of by products are formed. This process is carried out by bacteria which are Lactobacillus blgaricus, Lactobacillus pentosus and Streptococcus lactis.

2. ***Heterofermentative Process:***
   By-products are formed along with lactic acid. Products formed are, lactic acid, ethanol, water, CO2 and acetic acid. Microorganisms which can carry out this process are leuconostoc mesenteroides, lactobacillus fermentum. Homofermentative process is mostly carried out in industries because more lactic acid is produced and less by products is formed so it is an advantageous process.

Media Composition Carbon Source:

It can be sucrose lactose, molasses, sugarcane, whey. Whey is mostly preferred. After the production of yogurt, whey is discarded in the environment. If discarded in water reservoirs, lower down the oxygen level and disturb the whole ecosystem.

So, it is used as C-source. Whey is having 3-4 % sucrose.

Calcium Carbonate:

When lactic acid reaches a particular level, due to feed back inhibition its production is stopped.

So, in order to prevent feedback inhibition, calcium carbonate is added in the media.

$$LA + CaCO_3 \longrightarrow Ca\text{-}lactate$$

AMMONIUM HYDROGEN PHOSPHATE:

PH of media is maintained at 5.5 to 6.5 while temperature is kept at 45 to 55 °C.

Fermentor should not be of stainless steel because the acid is corrosive. So, woody fermentors are used. Process of fermentation is done for 5 to 10 days. Inoculums are 5%. It means for 95 ml media, 5ml broth containing (bacteria, microorganisms is added. Lactic acid is produced by upstream process.

After that, downstream processes are carried out.

Isolation of product and removing by products:

1st Method: ***For extraction of lactic acid:***

During downstream process, quantity of CaCO3 is increased. As a result, all the lactic acid is converted into Ca-lactate. To separate Ca from lactate, Sulphuric acid is used.

$$Ca\text{-}lactate + H_2SO_4 \longrightarrow Ca\,SO_4 + Lactic\ acid$$

2nd Method: ***Solvent-Solvent Extraction****:*

Solvent used is Isopropyl ether. After fermentation, this isopropyl ether is introduced in the media and all the lactic acid attaches with isopropyl ether and leaves the broth.

H2O is denser so it comes down and then it is removed. To separate LA from isopropyl ether, distillation, evaporation can be carried out.

USES OF LACTIC ACID

- *Lactic acid is used as a humectant, or moisturizer, in some cosmetics and as a mordant.*
- *It is a chemical that helps fabrics accept dyes, in textiles.*
- *It is also used in making pickles and sauerkraut, foods for which a sour taste is desired.*
- *Lactic acid is used in the dairy industry not only in making yogurt but in making cheese as well.*
- *It is also used in tanning leather.*
- *Lactic acid is important in the pharmaceutical industry as a starting material for other substances and is involved in the manufacturing of lacquers and inks.*
- *A related compound that is made from lactic acid is calcium stearoyl-2-lactylate, which is used as a food preservative.*
- *Polylactic acid, a polymer of lactic acid is used in making biodegradable plastic.*

CITRIC ACID PRODUCTION

Citric acid is a weak organic acid. It chemical formula is $C_6H_8O_7$. It is a natural preservative and is also used to add an acidic or sour taste to foods and soft drinks. The conjugate base of citric acid, citrate, is important as an intermediate in the citric acid cycle, and therefore occurs in the metabolism of all living things. Citric acid is a commodity chemical, and more than a million tones are produced every year by fermentation. It is used mainly as an acidifier, as a flavoring, and as a chelating agent.

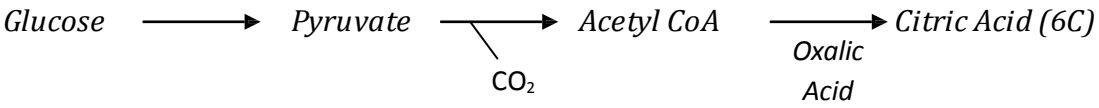
COMMERCIAL PRODUCTION

3 processes are there for commercial production of Citric acid.

1. ***Koji Process**: Also called as solid substrate method. Substrate used are sweet potatoes are used to grow aspergillus niger, reslting thing is called koji. This koji is used as inoculum for the production of citric acid.*

Steps:

We add 30 to 60 % water. Substrates used are starchy cros or any other source of sucrose so at the end we can get glucose. Then media and Koji are conbined in fermentor. Aseprgillus niger convert sugar soruce into citric acid.



Up to this, glycolysis steps are involved. Fermentor used is stainless steel or aluminum.

2. Liquid Surface culture process:

Fermentor used in this case is made up of aluminum. Acid can corrode othr materials. We prepare media using any of sugar source, then spores of Aspergillus niger are introduced on meida which then prow at the end. Citric acid is produced. It is called liquid process. Because Aspergillus niger grow on surface of liquid media.

3. Submerged Fermentation:

We grow specie of aspergillus niger called aspergillus japonicas. Remaining process is same except that specie goes sown in media and grows.

DOWNSTREAM PROCESSING:

We introduce Ca $(OH)_2$ as a result ca-citrate will from. Then fungus is removed. Now, we add H2SO4 in ca-citrate, citric acid crystals will form and CO2 react with H2SO4.

SOLVENT EXTRACTION:

Tris-n butyl phosphate is used as solvent to remove citric acid from media.

PROCEDURE

First boil the substrate – Polysaccharides convert into oligosaccharides when it cools down we introduce koji into it. Production is less there in LSCP because the pores rest on the top layer so products are formed on top only. Production at citric acid in Kreb's cycle, we add inhibitors

in it. These are Cu and H_2O_2 and it stops at citrate which is then converted into citric acid and for oxalic acid we do not add inhibitors to it the cycle go till oxaloacetate cycle.

PRODUCTION:

Carboxylic acid is the functional group of citric acid. In 1789, Schele isolated 4 ingredients from lemon and one of it was citric acid. And its production was first time done by a company of UK named; John and Edmund strusage company from the culture of Aspergillus niger.

Bacteria used For Isolation of Citric Acid:

1. *Aspergillus Niger*
2. *Aspergillus Wentii*
3. *Yeast*
4. *Penicillium Citrinum*

SUBSTRATES

1. *Sugar cane*
2. *Molasses*
3. *Sugar beat*
4. *Starchyl materials.*

MEDIA

1. *Ammonium salts*
2. *PH → 3-5*
3. *Temperature → 25 – 35*

DOWNSTREAM PROCESSING:

1. *Filtration:*

   Filters out the spores of aspergillus niger etc. from media and can be reused.
2. *Addition of Ca CO3:*

   It precipitates citric acid to make calcium citrate.

USES OF CITRIC ACID

- In the food industry, citric acid is used for flavoring and even for tenderizing meat.
- In sweet foods, citric acid is used to control the acidity of the other ingredients in the food.
- In addition to being used as a vitamin supplement, citric acid has various uses in the health-care industry.
- Citric acid, in the form of citrated calcium carbamide, is used to treat alcoholics.
- As the compound sodium citrate, citrus acid works to prevent blood from clotting.
- In photography, the sodium citrate is used to transform light-responsive paper into photographs.

PRODUCTION OF ACETIC ACID

Acetic acid (Ethanoic acid) is an organic compound with the chemical formula CH_3COOH. It is a colorless liquid that when undiluted is also called glacial acetic acid. Acetic acid is the main component of vinegar (apart from water), and has a distinctive sour taste and pungent smell. It is mainly produced as a precursor to polyvinyl acetate and cellulose acetate. Although it is classified as a weak acid, concentrated acetic acid is corrosive, and attacks the skin. The global demand of acetic acid is around 6.5 million tonnes per year (Mt/a), of which approximately 1.5 Mt/a is met by recycling; the remainder is manufactured from petrochemical feedstock. As a chemical reagent, biological sources of acetic acid are of interest but generally uncompetitive. Vinegar can be dilute acetic acid produced by fermentation.

COMMERCIAL PRODUCTION

Acetic acid has functional group carboxylic acid (CH_3COOH). Acetic acid is the main ingredient of vinegar. Vinegar is derived from French word "viangire" which means sour wine. Acetic acid production occurs in 2 steps.

Step I:

$$\text{Glucose} \longrightarrow \text{Ethanol}$$

Step II:

$$\text{Ethanol} \longrightarrow \text{Acetic Acid}$$

Glucose is converted to ethanol by yeast by anaerobic process. Acetobacter / Gluconobacter aerobically convert ethanol to acetic acid.

SUBSTRATES

i. *Fruit Juices*
ii. *Starchy Vegetables: In the case of starchy vegetables saccharification is performed.*
iii. *Malted (cereal): Those cereals whose grains whne place in water swell up but before germination, water is removed. Its advantage is that saccharification of starch become easier.*
iv. *Molasses*
v. *Honey:*

Based on the substrate used, vinegar can be classified.

a. ***Cider Vinegar:*** *when fruit juices are used as substrates, vinegar obtained is called cider vinegar.*
b. ***Malted Grains:*** *Give malted vinegar.*

Method for acetic acid production: Different methods are used.

1. Slow process or Let Home Process:

Glucose $\xrightarrow{\text{Yeast}}$ *Ethanol*

A barrel is filled ¼ with ethanol. Acetobacter is introduced as inoculums in ethanol.

Acetobacter is called "mother of vinegar". From the previous batch of acetic acid, small amount of acetic acid is taken having mother of vinegar and added to barrel which converts ethanol to acetic acid.

This process is carried out in batch fermentation. Time duration is 7 days to 1month.

2. Quick Process:

Also known as french or leans process. A barrel is almost ¼ filled with vinegar having acetobacter which are acetic acid producer due to which other microbes i.e. competing microbes are killed and hence only acetobacter will remain.

Then ethanol is introduced which is converted into acetic acid by acetobacter. This process occurs at 21 to 29 °C and proceeds from weeks to months. It is carried out in the continuous fermentation. E.g. It 100 lit acetic acid is remove then 100 liters ethanol is introduced in barrel and this process continues.

3. Generator Process:

The acetobacter couture is introduced on wood shavings. Feed line is for ethanol. Sparger showers the ethanol on wood shavings having acetobacter due which ethanol is converted to acetic acid. Acetic acid is collected in collection chamber. Hea procutio occur which increase the temperature. Heat production occur which increase the temperature. This temperature is controlled by circulating water in cooling coils.

USES OF ACETIC ACID

- *Vinegar is typically 4-18% acetic acid by mass. Vinegar is used directly as a condiment, and in the pickling of vegetables and other foods.*
- *Glacial acetic acid is an excellent polar protic solvent, as noted above. It is frequently used as a solvent for re-crystallization to purify organic compounds.*
- *The major esters of acetic acid are commonly used solvents for inks, paints and coatings.*
- *Dilute solutions of acetic acids are also used as a stop bath during the development of photographicfilms.*
- *Acetic acid is a chemical reagent for the production of chemical compounds.*

OXALIC ACID PRODUCTION

Oxalic acid is an organic compound with the formula $H_2C_2O_4$. This colorless solid is a dicarboxylic acid. In terms of acid strength, it is about 3,000 times stronger than acetic acid. Oxalic acid is a reducing agent and its conjugate base is known as oxalate. Wohler prepared oxalic acid by hydrolysis of cyanogens in 1824. This experiment may represent the first synthesis of a natural product.

COMMERCIAL PRODUCTION

At industrial level Oxalic acid is mainly manufactured by the oxidation of carbohydrates or glucose using nitric acid or air in the presence of vanadium pentoxide by the fungus Aspergillus Niger. Pyruvate is formed in the first step which if provided with a pH of 2 can be converted into oxalic acid. In case of pH 3-5, pyruvate will yield citric acid.

USES OF OXALIC ACID

Oxalic acid has many uses in various industries, such as construction, joinery, furniture restoration, beekeeping and boating. Crystals of oxalic acid rubbed onto wooden beams and floors removes unsightly stains caused by age, exterior exposure or rust easily. It can also be used to treat, polish and repair other materials such as stone, marble and stainless steel.

Chapter #6 **Into the world of Antibiotics**

ANTIBIOTICS

An Antibiotic is a drug used to treat infections caused by bacteria and other microorganisms. Originally, an antibiotic was a substance produced by one microorganism that selectively inhibits the growth of another. These are actually secondary metabolites which are not directly involved in normal growth, development and reproduction, but usually have an important ecological function.

In 1926, Alexander Fleming discovered penicillin, a substance produced by fungi that appeared able to inhibit bacterial growth. In 1939, Edward Chain and Howard Florey further studied penicillin and later carried out trials of penicillin on humans. The term antibiotic was coined by Selman Waksman in 1942 to describe any substance produced by a microorganism that is antagonistic to the growth of other microorganisms in high dilution. Many antibiotic compounds are relatively small molecules with a molecular weight of less than 2000 atomic mass units. Accordingly, many antibacterial compounds are classified on the basis of chemical/biosynthetic origin into natural, semi-synthetic, and synthetic. Another classification system is based on biological activity; in this classification, anti-bacterials are divided into two broad groups according to their biological effect on microorganisms: bactericidal agents kill bacteria, and bacteriostatic agents slow down or stall bacterial growth.

COMMERCIAL PRODUCTION

The production of antibiotics has been widespread since the pioneering efforts of Florey and Chain in 1938. The importance of antibiotics to medicine has led to much research into their discovery and production.

Despite the wide variety of known antibiotics, less than 1% of antimicrobial agents have medical or commercial value. For example, whereas penicillin has a high therapeutic index as it does not generally affect human cells, this is not so for many antibiotics. Other antibiotics simply lack advantage over those already in uses, or have no other practical applications.

Useful antibiotics are often discovered using a screening process. To conduct such a screen, isolates of many different microorganisms are cultured and then tested for production of diffusible products that inhibit the growth of test organisms. Most antibiotics identified in such a screen are already known and must therefore be disregarded. The remainder must be tested for their selective toxicities and therapeutic activities, and the best candidates can be examined and possibly. penicillin. A more modern version of this approach is a rational design program. This involves screening directed towards finding new natural products that inhibit a specific target, such as an enzyme only found in the target pathogen, rather than tests to show general inhibition of a culture.

TECHNIQUES

Antibiotics are produced industrially by a process of fermentation, where the source microorganism is grown in large containers (100,000–150,000 liters or more) containing a liquid growth medium. Oxygen concentration, temperature, pH and nutrient levels must be optimal, and are closely monitored and adjusted if necessary. As antibiotics are secondary metabolites, the population size must be controlled very carefully to ensure that maximum yield is obtained before the cells die. Once the process is complete, the antibiotic must be extracted and purified to a crystalline product. This is simpler to achieve if the antibiotic is soluble in organic solvent. Otherwise it must first be removed by ion exchange, adsorption or chemical precipitation.

STRAINS

Microorganisms used in fermentation are rarely identical to the wild type. This is because species are often genetically modified to yield the maximum amounts of antibiotics. Mutation is often used, and is encouraged by introducing mutagens such as ultraviolet radiation, x-rays or certain chemicals. Selection and further reproduction of the higher yielding strains over many generations can raise yields by 20-fold or more. Another technique used to increase yields is gene amplification, where copies of genes coding for enzymes involved in the antibiotic production can be inserted back into a cell, via vectors such as plasmids. This process must be closely linked with retesting of antibiotic production and effectiveness. The effectiveness can be found out by two methods. These are:

1. SPOT PLATE METHOD

In spot plate method media is prepared and E-coli is introduced in this media plate. In other plate a Penicillium notatum is grown for 7 days. Small amount of it is taken and is grown in the former plate. After 24 hours of incubation zone of inhibition is measured. If the zone is observed, it means that the antibiotic is effective.

2. AGAR WELL DIFFUSION METHOD

In this method a loop full of bacterial strain is inoculated in 30 ml of Nutrient broth in a conical flask and incubated for 72 hrs to get active strain. Muller Hinton Agar is poured into Petri dishes. After solidification 0.25 ml of test strains are inoculated in the media separately. Care is taken to ensure proper homogenization. The experiment is performed under strict aseptic conditions. After the medium solidifies, a well is made in the plates with sterile borer (5mm). The extract compound (50 µl) is introduced into the well and plates are incubated at 37°C for 72 hrs. All samples are tested in triplicates. Microbial growth is determined by measuring the diameter of zone of inhibition. A control with standard antibiotic is also kept for all test strains and the control activity is deducted from the test and results are recorded.

CLASSIFICATION OF ANTIBIOTICS

Antibiotics can be classified on different basis.

ON THE BASIS OF EFFECT

1. Bactericidal Antibiotics

A bactericidal antibiotic, is a substance that kills bacteria and, ideally, nothing else. Common bactericidal antibiotics are daptomycin, fluoroquinolones, metronidazole, nitrofurantoin, co-trimoxazole, telithromycin.

2. Bacteriostatic Antibiotics

A bacteriostatic antibiotic is a biological or chemical agent that stops bacteria from reproducing, while not necessarily harming them otherwise. Bacteriostatic antibiotics limit the growth of bacteria by interfering with bacterial protein production, DNA replication, or other aspects of bacterial cellular metabolism Upon removal of the bacteriostat, the bacteria usually start to grow again. They include Tetracyclines, sulfonamides, Spectinomycin, Trimethoprim, Chloramphenicol, Macrolides and Lincosamides.

ON THE BASIS OF COVERAGE

1. Narrow Spectrum Antibiotics

Those antibiotics which are effective against particular families of micro organisms are called narrow spectrum antibiotics. The narrow-spectrum antibiotic will not kill as many of the normal microorganisms in the body as the broad spectrum antibiotics. So, It has less ability to cause super infection. They will cause less resistance of the bacteria as it will deal with only specific bacteria. But they can be used only if the causative organism is identified. These are Azithromycin, Clarithromycin, Clindamycin, Erythromycin and Vancomycin.

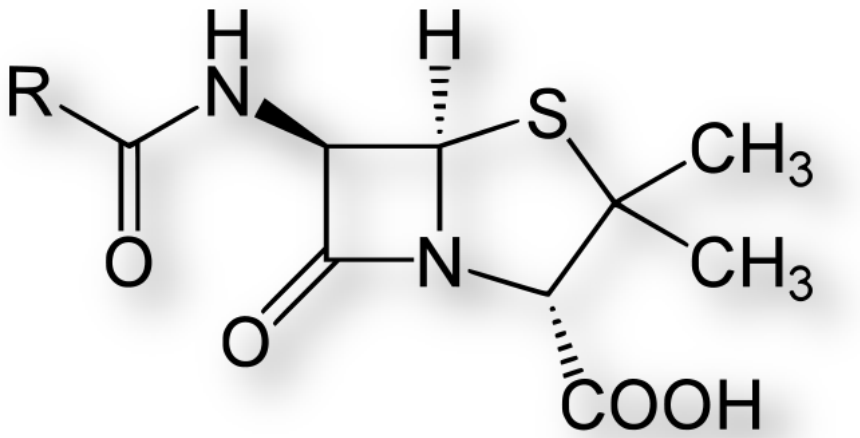
2. Broad Spectrum Antibiotics

The broad spectrum antibiotic is an antibiotic that acts against a wide range of disease-causing bacteria. A broad-spectrum antibiotic acts against both Gram-positive and Gram-negative bacteria. An example of a commonly used broad-spectrum antibiotic is ampicillin. Other examples include amoxicillin, levofloxacin, streptomycin, tetracycline, chloramphenicol.

PENICILLIN

Penicillin is a group of antibiotics derived from Penicillium fungi. They include penicillin G, procaine penicillin, benzathine penicillin, and penicillin V. Penicillin antibiotics are historically significant because they are the first drugs that were effective against many previously serious diseases such as syphilis and infections caused by staphylococci and streptococci. Penicillins are still widely used today, though many types of bacteria are now resistant. All penicillins are β-lactam antibiotics and are used in the treatment of bacterial infections caused by susceptible, usually Gram-positive, organisms.

STRUCTURE OF PENICILLIN



COMMERCIAL PRODUCTION

Penicillin is a secondary metabolite of fungus Penicillium that is produced when growth of the fungus is inhibited by stress. It is not produced during active growth. Production is also limited by feedback in the synthesis pathway of penicillin. The reaction can be written as:

α-ketoglutarate + AcCoA → homocitrate → L-α-aminoadipic acid → L-Lysine + β-lactam

MICROORGANISMS USED

1. *Penicillium Chrysogenum* 2. *Penicillium Notatum*

MEDIA

Media contains Ammonium Sulphate, Calsium carbonate, cornsteep liquor,

Calcium Hydroxide, Glucose and Sodium hydrogen phosphate.

PHYSICAL REQUIREMENTS

2. *pH (5.2)* *2. Temperature (23-25^{0}C)* *3. Aeration/ Agitation*

DEVELOPMENT OF INOCULUM

5 gm Barley seeds are added in a flask along with the spores of penicillium chrysogenum. They are left for 7 days with incubation at 25^0C. Mycelia of penicillium are formed which is used as inoculum in fermentors. Penicillium is produced in 3 phases after addition of inoculum.

1st Phase

- ➔ *Mycelial Growth*
- ➔ *Lactic acid is used as carbon source*
- ➔ *Antibiotic production is minimum.*

2nd Phase

- ➔ *Maximum antibiotic production*

3rd Phase

- ➔ *Autolysis occurs due to the deficiency of nutrients.*

DOWNSTREAM PROCESSING

It is carried out in the following steps.

1. ***Filtration**: In filtration all the mycelia is removed from the fermentation broth.*
2. ***Absorption**: The penicillin is absorbed from the media.*
3. ***Precipitation**: Precipitation is carried out to settle down the product.*
4. ***Crystallization:** Penicillin is purified by crystallization.*

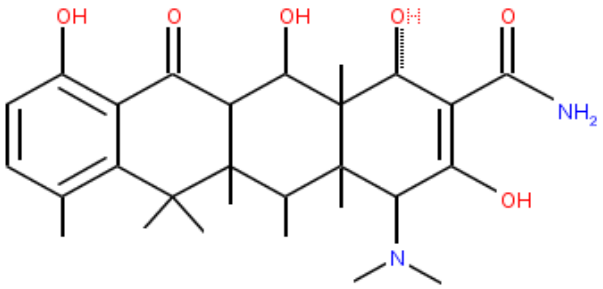
MECHANISM OF ACTION

Bacteria constantly remodel their peptidoglycan cell walls, simultaneously building and breaking down portions of the cell wall as they grow and divide. β-Lactam antibiotics inhibit the formation of peptidoglycan cross-links in the bacterial cell wall, but have no direct effect on cell wall degradation. The β-lactam moiety (functional group) of penicillin binds to the enzyme (DD-transpeptidase) that links the peptidoglycan molecules in bacteria. The enzymes that hydrolyze the peptidoglycan cross-links continue to function, which weakens the cell wall of the bacterium (in other words, the antibiotic causes cytolysis or death due to osmotic pressure). In addition, the build-up of peptidoglycan precursors triggers the activation of bacterial cell wall hydrolases and autolysins, which further digest the bacteria's existing peptidoglycan. This imbalance between cell wall production and degradation is responsible for the rapid cell-killing action of this class of drugs, even in the absence of cell division. In addition, the relatively small size of the penicillin molecule allows it to penetrate deeply into the cell wall, affecting its entire depth. This is in contrast to the other major class of cell wall synthesis inhibiting antibiotics, the glycopeptide antibiotics. Gram-positive bacteria are called protoplasts when they lose their cell wall. Gram-negative bacteria do not lose their cell wall completely and are called spheroplasts after treatment with penicillin.

TETRACYCLINE

Tetracyclines are a group of broad-spectrum antibiotics whose general usefulness has been reduced with the onset of bacterial resistance. Despite this, they remain the treatment of choice for some specific indications. They contains four rings that why they are called as tetracycline.

STRUCTURE OF TETRACYCLINE



MICROORGANISMS USED

1. *Streptomyces Aurofaciens*
2. *Streptomyces Ramosus*

MEDIA

Sugar (3%), Cornsteep Liquor (2%), CaCo3 (1%), NH4Cl (0.1%)

PHYSICAL REQUIREMENTS

1. *pH (6-7)*
2. *Temperature (23-25^{0}C)*
3. *Aeration/ Agitation*

PRODUCTION

It involves the same three phases as mentioned for penicillin.

DOWNSTREAM PROCESSING

a. ***Filtration**: In filtration all the mycelia is removed from the fermentation broth.*
b. ***Absorption**: The penicillin is absorbed from the media.*
c. ***Precipitation**: Precipitation is carried out to settle down the product.*
d. ***Crystallization:** Penicillin is purified by crystallization.*

MECHANISM OF ACTION

Tetracycline antibiotics are protein synthesis inhibitors, inhibiting the binding of aminoacyl-tRNA to the mRNA-ribosome complex. They do so mainly by binding to the 30S ribosomal subunit in the mRNA translation complex. Tetracyclines also have been found to inhibit matrix metalloproteinases. This mechanism does not add to their antibiotic effects, but has led to extensive research on chemically modified tetracyclines or CMTs (like incyclinide) for the treatmet of rosacea, acne, diabetes and various types of neoplasms. Since incyclinide was announced to be ineffective for rosacea in September 2007, no drugs of this group will be marketed in the near-future. Several trials have examined modified and unmodified tetracyclines for the treatment of human cancers, of those very promising results were achieved with CMT-3 for patients with Kaposi Sarcoma.

Chapter #7 **Have you heard of Amino acids?**

AMINO ACIDS

Amino acids are molecules containing an amine group, a carboxylic acid group and a side-chain that varies between different amino acids. The key elements of an amino acid are carbon, hydrogen, oxygen, and nitrogen. They are particularly important in biochemistry, where the term usually refers to alpha-amino acids. An alpha-amino acid has the generic formula $H_2NCHRCOOH$, where R is an organic substituent; the amino group is attached to the carbon atom immediately adjacent to the carboxylate group (the α–carbon). Other types of amino acid exist when the amino group is attached to a different carbon atom; for example, in gamma-amino acids (such as gamma-amino-butyric acid) the carbon atom to which the amino group attaches is separated from the carboxylate group by two other carbon atoms. The various alpha-amino acids differ in which side-chain (R-group) is attached to their alpha carbon, and can vary in size from just one hydrogen atom in glycine to a large heterocyclic group in tryptophan.

CLASSIFICATION OF AMINO ACIDS

Amino acids can be classified as under:

ESSENTIAL AMINO ACIDS

An essential amino acid or indispensable amino acid is an amino acid that cannot be synthesized de novo by the organism (usually referring to humans), and therefore must be supplied in the diet. The amino acids regarded as essential are phenylalanine, valine, threonine, tryptophan, isoleucine, methionine, leucine, lysine and histidine. Additionally, cysteine (or sulphur-containing amino acids), tyrosine (or aromatic amino acids), and arginine are required by infants and growing children.

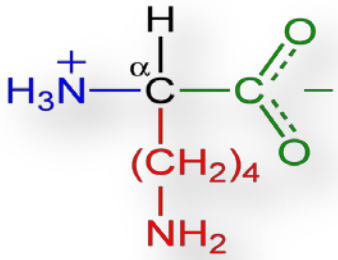
NON-ESSENTIAL AMINO ACIDS

Non essential amino acids are amino acids that can be produced in our body. Their uses and functions in our body are equally as important as the limiting amino acids. They include Alanine, arginine, asparagine, aspartic acid, cysteine, glutamic acid, glutamine, glycine, proline, serine, tyrosine.

LYSINE

Lysine is an α-amino acid with the chemical formula $HO_2CCH(NH_2)(CH_2)_4NH_2$. It is an essential amino acid, which means that the human body cannot synthesize it. Its codons are AAA and AAG. Lysine is a base, as are arginine and histidine. The ε-amino group often participates in hydrogen bonding and as a general base in catalysis. (The ε-amino group, which is attached to the NH3+ group, is the fifth carbon down from the α-carbon, which is attached to the carboxyl (C=OOH) group.

STRUCTURE OF LYSINE



BIOSYNTHESIS

- *As an essential amino acid, lysine is not synthesized in animals, hence it must be ingested as lysine or lysine-containing proteins. In plants and bacteria, it is synthesized from aspartic acid (aspartate):*
- *L-aspartate is first converted to L-aspartyl-4-phosphate by aspartokinase (or Aspartate kinase). ATP is needed as an energy source for this step.*
- *β-Aspartate semialdehyde dehydrogenase converts this into β-aspartyl-4-semialdehyde (or β-aspartate-4-semialdehyde). Energy from NADPH is used in this conversion.*
- *Dihydrodipicolinate synthase adds a pyruvate group to the β-aspartyl-4-semialdehyde, and two water molecules are removed forming Homoserine after a series of reaction.*
- *Homoserine give rise to Threonine.*
- *In Mutated case, where the microbes are HSD^- , dehydogenase enzyme is not functional so instead of threonine, lysine is formed.*

MICROORGANISMS USED

1. *Corynebacterium Glutamicum*
2. *Brevibacterium Flavum*

MEDIA

Glycerol, Cornsteep Liquor, Ammonium Sulphate and Calcium carbonate.

PHYSICAL REQUIREMENTS

1. *pH (7)*
2. *Temperature (28^0C)*
3. *Time (72 hours)*
4. *Aeration/Agitation is required*
5. *Stirred Fermentors are used*

PRODUCTION

Providing these conditions the microbes will yield lysine.

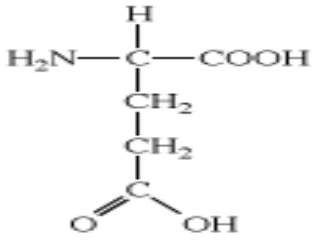
DOWNSTREAM PROCESSING

As the product is not present in the media so high temperature or antibiotics are provided for the cell cleavage resulting in the release of our product. It is then crystallized and made available in the market for commercial use.

GLUTAMIC ACID

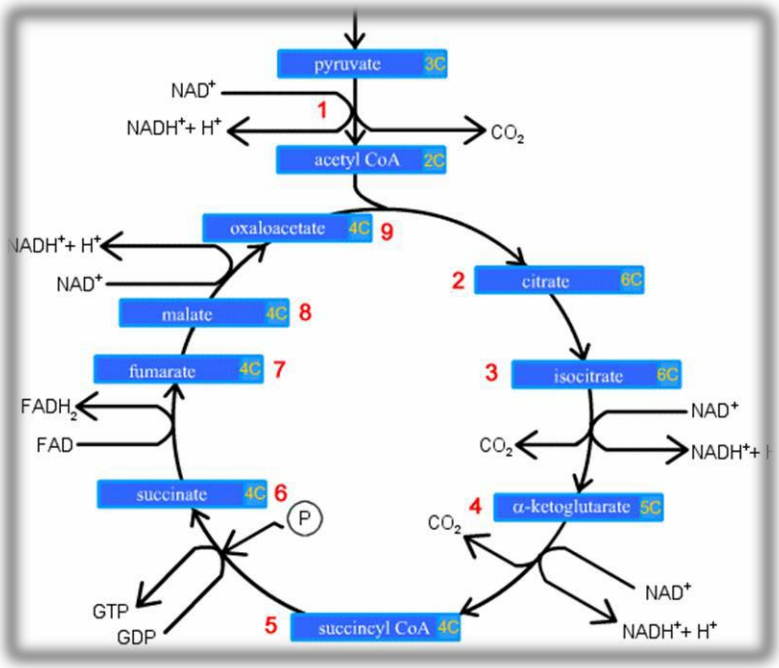
Glutamic acid *is one of the 20 proteinogenic amino acids, and its codons are GAA and GAG. It is a non essential amino acid. The carboxylate anions and salts of glutamic acid are known as glutamates. In neuroscience, glutamate is an important neurotransmitter that plays a key role in long-term potentiation and is important for learning and memory.*

STRUCTURE OF GLUTAMIC ACID



glutamic acid
(Glu, E; Glx or Z)

BIOSYNTHESIS OF GLUTAMIC ACID



Glutamic acid is a non essential amino acid which can be produced by human body. During the 4th step of Krebs Cycle which is the conversion of alpha-ketoglutarate to succinyl CoA by the action of alpha-ketoglutarate dehydrogenase, if the enzyme is defective, accumulation of alpha-ketoglutarae takes place. Hence the cycle gets stopped. During accumulation NH4 will combine with it forming glutamic acid. The defective cycle can again be started by oxaloacetate which is produced by the carboxylation of Pyruvate.

MICROORGANISM USED

1. *Micrococcus Glutamicus*

MEDIA

- *Glucose*
- *Ammonium acetate*
- *Molasses*
- *Potassium hydrogen phosphate*
- *Potassium sulphate.*

PHYSICAL REQUIREMENTS

2. *pH (8.5)* 2. *Temperature (35^0C)* 3. *Time (16 hours)*

4. *Aeration/Agitation is required* 5. *Stirred Fermentors are used*

PRODUCTION

Providing these conditions the microbes will yield Glutamate.

DOWNSTREAM PROCESSING

As the product is not present in the media so high temperature or antibiotics are provided for the cell cleavage resulting in the release of our product. It is then crystallized and made available in the market for commercial use. In industry or market, Glutamic acid is present in the form of Monosodium glutamate (MSG).

USES OF GLUMATIC ACID

- *Glutamic acid increases brain function and mental activity. It also attaches itself to nitrogen atoms in the brain and detoxifies the brain of ammonia. This action is the only way the brain can be detoxified from ammonia.*
- *Glutamic acid also helps with the transportation of potassium across the blood-brain barrier.*
- *Glutamic acid is also used in the body to balance the alkaline level, and is a building block for RNA and DNA. It fuels intestinal cells, and is used by white blood cells which are important for immune function.*
- *It can be used as flavor enhancer (Chinese salt).*

Chapters #8 A quick glance at Vitamins

VITAMINS

A **vitamin** is an organic compound required as a nutrient in tiny amounts by an organism. In other words, an organic chemical compound (or related set of compounds) is called a vitamin when it cannot be synthesized in sufficient quantities by an organism, and must be obtained from the diet. Thus, the term is conditional both on the circumstances and on the particular organism. For example, ascorbic acid (vitamin C) is a vitamin for humans, but not for most other animals, and biotin and vitamin D are required in the human diet only in certain circumstances. By convention, the term vitamin does not include other essential nutrients such as dietary minerals, essential fatty acids, or essential amino acids (which are needed in larger amounts than vitamins), nor does it encompass the large number of other nutrients that promote health but are otherwise required less often. Thirteen vitamins are universally recognized at present. Vitamins are classified by their biological and chemical activity, not their structure.

VITAMIN B (COBALAMINE)

Vitamin B_{12}, also called cobalamin, is a water soluble vitamin with a key role in the normal functioning of the brain and nervous system, and for the formation of blood. It is one of the eight B vitamins. It cannot be synthesized by human body. It is normally involved in the metabolism of every cell of the human body, especially affecting DNA synthesis and regulation, but also fatty acid synthesis and energy production. It is the largest and most structurally complicated vitamin and can be produced industrially only through bacterial fermentation-synthesis.

COMMERCIAL PRODUCTION

Neither plants nor animals are independently capable of constructing vitamin B12. Only bacteria have the enzymes required for its synthesis. The total synthesis of B12 was reported by Robert Burns Woodward and Albert Eschenmoser in 1972, and remains one of the classic feats of organic synthesis.

MICROORGANISMS USED

1. Streptomeces Olivaceus
2. Pseudomonas Denitrificans

MEDIA

Three types of media are used for synthesis of vitamin B_{12}

1. Media A

Sugar Beet molasses

Yeast Extract

$MgSO_4$, $MnSO_4$, $ZnSO_4$

Agar (used for inoculum development)

2. Media B

The composition is same as that of media A with exception of Agar. Inoculum formed in media A is then introduced in media B where its mass number is increased.

3. Media C

Sugar beet nolasses

Yeast extract

$MgSO_4$, $MnSO_4$, $ZnSO_4$

5-6 Dimethyl Benzimidazole

This media is utilized in the fermentors. Increased mass of media is added in media C.

PHYSICAL REQUIREMENTS

1. *Time (90 hours)* 2. *Temperature (29^0C)* 3. *Aeration is required*

PRODUCTION

The production involves two steps:

1. *5-deoxyadenosyl cobinamide is formed*
2. *It is then converted to 5-deoxyadenosyl Cobalamine (Vitamin B_{12})*

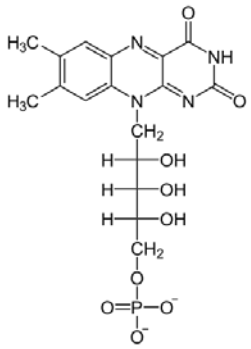
FUNCTIONS

- *Vitamin B12 is involved in a number of key body processes, but is especially key to red blood cell production, nervous system function, sperm production, normal growth.*
- *It helps in the proper functioning of the immune system.*
- *It has also been shown to improve memory and concentration, help prevent cancer and also protect against allergies and toxins (especially the cyanide found in cigarette smoke and some foods).*
- *Vitamin B12 is also closely involved in the production of melatonin, which controls the release of many hormones in the body and is involved with the sleep/wake cycle.*

VITAMIN B_2 (RIBOFLAVIN)

Vitamin B_2, also called Riboflavin, is an easily absorbed micronutrient with a key role in maintaining health in humans and animals. It is the central component of the cofactors FAD and FMN, and is therefore required by all flavoproteins. As such, vitamin B_2 is required for a wide variety of cellular processes. It plays a key role in energy metabolism, and for the metabolism of fats, ketone bodies, carbohydrates, and proteins. Milk, cheese, leafy green vegetables, liver, kidneys, legumes, tomatoes, yeast, mushrooms, and almonds are good sources of vitamin B_2, but exposure to light destroys riboflavin.

STRUCTURE OF VITAMIN B_2



COMMERCIAL PRODUCTION

Neither plants nor animals are independently capable of constructing vitamin B12. Only bacteria have the enzymes required for its synthesis. The total synthesis of B12 was reported by Robert Burns Woodward and Albert Eschenmoser in 1972, and remains one of the classic feats of organic synthesis.

MICROORGANISM USED

1. *Ashbya Gossypii (Fungus)*

MEDIA

- *Cornsteep Liquor*
- *Peptone*
  - *Soyabean Oil*
  - *Yeast extract*

PHYSICAL REQUIREMENTS

2. *Time (4-5 days)* *2. Temperature (26-28^{0}C)* *3. Limited supply of Oxygen*

PRODUCTION

Production involves three phases:

1. ***Phase I:*** *Mycelial Growth producing more pyruvate.*
2. ***Phase II:*** *Due to acidic pH, mycelia are converted into spores.*
3. ***Phase III:*** *Intracellular production of vitamin B2.*

DOWNSTREAM PROCESSING

1. ***Filtration****: Mycelial cells are removed from the media.*
2. ***Crystallization****: Crystals of Vitamin B2 are obtained.*

FUNCTIONS

- *Essential for converting carbohydrate into energy*
- *Essential for normal tissue respiration*
- *Necessary for healthy mucous membranes.*

Chapter #9 **Let us study Enzymes**

ENZYMES

Enzymes are proteins that catalyze chemical reactions. In enzymatic reactions, the molecules at the beginning of the process, called substrates, are converted into different molecules, called products. Almost all chemical reactions in a biological cell need enzymes in order to occur at rates sufficient for life.

The top-level classification of enzymes is

- ***EC 1 Oxidoreductases**: catalyze oxidation/reduction reactions*
- ***EC 2 Transferases**: transfer a functional group (e.g. a methyl or phosphate group)*
- ***EC 3 Hydrolases**: catalyze the hydrolysis of various bonds*
- ***EC 4 Lyases**: cleave various bonds by means other than hydrolysis and oxidation*
- ***EC 5 Isomerases**: catalyze isomerization changes within a single molecule*
- ***EC 6 Ligases**: join two molecules with covalent bonds.*

PECTINASE

Pectinase is an enzyme which causes the breakdown of pectin, a polysaccharide substrate that is found in the cell walls of plants. One of the most studied and widely used commercial pectinases is polygalacturonase. It is useful because pectin is the jelly-like matrix which helps cement plant cells together and in which other cell wall components, such as cellulose fibrils, is embedded. Therefore pectinase enzymes are commonly used in processes involving the degradation of plant materials, such as speeding up the extraction of fruit juice from fruit. They can be extracted from fungi such as Aspergillus niger. The fungus produces these enzymes to break down the middle lamella in plants so that it can extract nutrients from the plant tissues and insert fungal hyphae. If pectinase is boiled it is denatured (unfolded) making it harder to connect with the pectin at the active site, and produce as much juice.

COMMERCIAL PRODUCTION

Microorganisms used for the commercial production of pectinase are

3. *Aspergillus Niger* 2. *Aspergillus Wentii* 3. *Penicillium Species*

MEDIA

The media contains Nitrogen Source, Pectin like compounds, Yeast Extract and Peptone.

PHYSICAL CONDITIONS

1. *Time (60-80 hours)* 2. *pH (3-4)* 3. *Temperature (37^0C)*

PROCEDURE

The enzymes are produced for the transcription of mRNA. Pectin like compounds are added to the media as substrates for microorganisms so that microbes produce enzyme pectinase. Inoculum is introduced in the media present in fermentors.

DOWNSTREAM PROCESSING

The Mycelia are dried and crushed. Extraction is done with the help of H_2O. The product is then purified and isolated by chromatography or Ion exchange method.

USES

- *They are used to clarify and filter the fruit juices.*
- *Pectinases have also been used in wine production since the 1960s. The function of Pectinase in brewing is twofold, first it helps breakdown the plant (typically fruit) material and so helps the extraction of flavours from the mash.*
- *The presence of pectin in finished wine causes a haze or slight cloudiness, Pectinase is used to break this down and so clear the wine.*
- *Pectinases are also used for retting.*

INVERTASE

Invertase is an enzyme that catalyzes the hydrolysis (breakdown) of sucrose .The resulting mixture of fructose and glucose is called inverted sugar syrup. Related to invertases are sucrases. Invertases and sucrases hydrolyze sucrose to give the same mixture of glucose and fructose. Invertases cleave the O-C(fructose) bond, whereas the sucrases cleave the O-C(glucose) bond. For industrial use, invertase is usually derived from yeast. It is also synthesized by bees, which use it to make honey from nectar.

COMMERCIAL PRODUCTION

Microorganism used for the commercial production of Invertase is

Saccharomyces Cerevisiae (Baker's Yeast)

MEDIA

The media contains Sucrose and Ammonium salts like NH_4Cl & NH_4SO_4 .

PHYSICAL CONDITIONS

a. Time (8 hours) 2. pH (4.5) 3. Temperature (28-30^{0}C)

DOWNSTREAM PROCESSING

The Mycelia are dried and crushed. Extraction is done with the help of H_2O. The product is then purified and isolated by chromatography or Ion exchange method.

USES

- Used for production of Ice cream
- In candies as a sweetener
- It prevents crystal formation in ice
- In artificial honey.

PROTEASE

A protease is any enzyme that conducts proteolysis. It begins protein catabolism by hydrolysis of the peptide bonds. These peptide bonds link amino acids together in the polypeptide chain forming the protein. Proteases are currently classified into six broad groups:

- Serine proteases
- Threonine proteases
- Cysteine proteases
- Aspartate proteases
- Metalloproteases
- Glutamic acid proteases

COMMERCIAL PRODUCTION

Microorganisms used for the commercial production of pectinase are

1. Pseudomonas Species 2. Clostridium Species 3. Penicillium Species

MEDIA

The media contains Proteins as substrates, carbohydrates and ammonium salts.

PHYSICAL CONDITIONS

2. Time (3-5 days) 2. Temperature (37^0C)

DOWNSTREAM PROCESSING

The Media is filtered and dried. Crushing takes place. The product is then purified and isolated by chromatography or Ion exchange method.

USES

- They are used for the tenderization of meat.
- Used in leather industries to remove hairs from skin.
- Papain is a protease found in papaya fruit that is used during the chilling process of beer-making.
- Most laundry and dish detergents contain proteases to break down proteins in stains and to eliminate food residue from dishes.

Chapter #10 Genetic Engineering…Last but not the least

GENETIC ENGINEERING

Genetic engineering, also called genetic modification, is the direct human manipulation of an organism's genome using modern DNA technology. It involves the introduction of foreign DNA or synthetic genes into the organism of interest. The introduction of new DNA does not require the use of classical genetic methods; however traditional breeding methods are typically used for the propagation of recombinant organisms. An organism that is generated through the introduction of recombinant DNA is considered to be a genetically modified organism. The first organisms genetically engineered were bacteria in 1973 and then mice in 1974. Insulin producing bacteria were commercialized in 1982 and genetically modified food has been sold since 1994.

HORMONES

A hormone is a chemical released by a cell or a gland in one part of the body that sends out messages that affect cells in other parts of the organism. Only a small amount of hormone is required to alter cell metabolism. In essence, it is a chemical messenger that transports a signal from one cell to another. All multicellular organisms produce hormones; plant hormones are also called phytohormones. Hormones in animals are often transported in the blood. Cells respond to a hormone when they express a specific receptor for that hormone. The hormone binds to the receptor protein, resulting in the activation of a signal transduction mechanism that ultimately leads to cell type specific responses. Endocrine hormone molecules are secreted (released) directly into the bloodstream, whereas exocrine hormones are secreted directly into a duct, and, from the duct, they flow either into the bloodstream or from cell to cell by diffusion in a process known as paracrine signalling.

INSULIN

Insulin is a hormone central to regulating carbohydrate and fat metabolism in the body. It consists of 51 amino acids. Insulin causes cells in the liver, muscle, and fat tissue to take up glucose from the blood, storing it as glycogen in the liver and muscle. Insulin stops the use of fat as an energy source by inhibiting the release of glucagon. With the exception of the metabolic disorder diabetes mellitus and Metabolic syndrome, insulin is provided within the body in a constant proportion to remove excess glucose from the blood, which otherwise would be toxic. When blood glucose levels fall below a certain level, the body begins to use stored sugar as an energy source through glycogenolysis, which breaks down the glycogen stored in the liver and muscles into glucose which can then be utilized as an energy source.

In humans Insulin is formed in islets of langerhans in pancreas. But in patiens with diabetes type I the process cannot take place due to destruction of pancreatic cells by autoimmunity. Insulin is a polypeptide consisting of 51 amino acids. The polypeptide is made up of 2 peptides namely:

1. *Peptide A (21 amino acids)*
2. *Peptide B (30 amino acids)*

After post translational modifications the insulin polypeptide is formed.

PRODUCTION OF INSULIN BY GENETIC ENGINEERING

Synthesizing human insulin is a multi-step biochemical process that depends on basic recombinant DNA techniques and an understanding of the insulin gene. The insulin gene codes for the protein insulin.

The insulin gene consists of two separate chains of amino acids, an A above a B chain, that are held together with bonds. The insulin A chain consists of 21 amino acids and the B chain has 30. Before becoming an active insulin protein, insulin is first produced as preproinsulin. This is one single long protein chain with the A and B chains not yet separated, a section in the middle linking the chains together and a signal sequence at one end telling the protein when to start secreting outside the cell. After pre-proinsulin, the chain evolves into proinsulin, still a single chain but without the signaling sequence. Then comes the active protein insulin, the protein without the section linking the A and B chains.

We need the two mini-genes: one that produces the A chain and one for the B chain. Since the exact DNA sequence of each chain is known, we synthesize each mini-gene's DNA in an amino acid sequencing machine.

These two DNA molecules are then inserted into plasmids. The recombinant, newly formed, plasmids are mixed up with the bacterial cells. Plasmids enter the bacteria in a process called transfection.

The bacteria synthesizing the insulin then undergo a fermentation process. They are grown at optimal temperatures in large tanks in manufacturing plants. The millions of bacteria replicate roughly every 20 minutes through cell mitosis, and each expresses the insulin gene.

After multiplying, the cells are taken out of the tanks and broken open to extract the DNA. The bacterium's DNA is then treated with cyanogen bromide, a reagent that splits protein chains at the methionine residues. This separates the insulin chains from the rest of the DNA. The two chains are then mixed together and joined by disulfide bonds through the reduction-reoxidation reaction.

The batch is then placed in a centrifuge. The DNA mixture is then purified so that only the insulin chains remain. We can purify the mixture through several chromatography, or separation, techniques that exploit differences in the molecule's charge, size, and affinity to water.

SOMATOTROPIN (STH)

Somatotropin is a peptide hormone that stimulates growth, cell reproduction and regeneration in humans and other animals. It is a 191-amino acid, single-chain polypeptide that is synthesized, stored, and secreted by the somatotroph cells within the lateral wings of the anterior pituitary gland. Its deficiency causes stunted growth and mental abnormality in children.

PRODUCTION BY GENETIC ENGINEERING

The mRNA from the gene responsible for Somatotropin is taken. cDNA of that mRNA is formed and converted to dsDNA. This double stranded DNA is then inserted into a vector most commonly E-coli. After that process similar to insulin production is carried out and E-coli is allowed to produce multiple copies. This dsDNA is then isolated by the action of restriction enzymes and a fragment of 25-191 amino acids is formed. The remaining 24 amino acids chain are cut from the DNA fragment and multiples copies are formed from it. Using DNA ligase, both these sequences are combined together and inserted in a vector. The E-coli then produce the product through rapid multiplication. The product is the isolated and purified.

VACCINES

A vaccine is a biological chemical that improves immunity to a particular disease. A vaccine typically contains an agent that resembles a disease-causing microorganism, and is often made from weakened or killed forms of the microbe. The term vaccine derives from Edward Jenner's 1796 use of cow pox, to inoculate humans, providing them protection against smallpox.

KILLED VACCINES

A vaccine prepared from dead microorganisms, generally used to provide immunization from organisms that are too virulent to be used in the living attenuated state. The immune system reacts to the presence of the pathogen in the same manner, whether the organism is alive or dead. These are those vaccines whose epitope remains alive. E.g. Hepatitis B virus is killed by means of formaldehyde, so that they cannot cause disease. Epitope is present on the surface of pathogen. Pathogen is killed but epitope remains alive. When the pathogen is introduced, the body will produce antibodies against it. Antibodies which are specific for this epitope are produced by B-cells. Two types of cells are produced by antibodies. Plasma cells which will kill pathogen and Memory cells will store the information of pathogen. When infection is caused, body can produce immune response against it.

LIVE ATTENUATED VACCINES

Some vaccines contain live, attenuated microorganisms. Many of these are live viruses that have been cultivated under conditions that disable their virulent properties. Examples include the viral diseases yellow fever, measles, rubella, and mumps. Now-a-days recombinant vaccines are used which can cause the epitope protein. There are three proteins present in the serum of hepatitis B patients. These are Viral surface antigen, viral core antigen and E-antigen. Viral surface antigen is used as attenuated vaccine. It is a glycosylated protein. Yeast is used for its expression because E-coli cannot express this system. The DNA is isolated and inserted into vector (yeast). Viral surface antigen is produced which can be used as attenuated vaccine.

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