STUDY OF PRODUCTION OF SEVERAL COMPONENTS

PENICILLIN

- **Penicillin** (PCN) is a group of antibiotics, derived originally from common moulds known as Penicillium moulds.
- It is a β- lactam antibiotic, discovered by Alexander Fleming.
- Originally, Penicillin was discovered from isolated strain of *Penicillium notated*, but this species gave low yield of penicillin.
- The Fleming discovered *Penicillium chrysogenum,* that gave high yield of penicillin.
- The uses of Penicillin to treat infection like Respiratory tract infection, Pneumonia, Throat infection, Skin infection etc.
- **Production of Penicillin:** (Fig 1.13)

Inoculum:

1. Early strains of *P.chrysogenum* was isolated from mouldy spoiled fruits.
2. The isolated strains are allowed to get subjected to treatment with Mutagenic Agents like UV-radiation, X-ray and mechlorethamine (Nitrogen Mustard). [for the purpose development of **Highly productive mutant i.e Q-176 strain** of *P.chrysogenum* - which has the ability to produce maximum amount of Penicillin] .
3. Q-176 strain can produce more than 100units of penicillin/mL.

Cuture Media: (pH maintained at 7-7.5 and temperature -28 °C)

1. Carbon source- lactose, Glucose, Nitrogen source- Yeast extract
2. N_2 source- corn steep liquor solids.
3. Salt-calcium carbonate
4. Buffering agent- Potassium Hydrogen Phosphate.
5. Penicillin precursor - Phenyl acetic acid.
6. Oil- edible oil (cooling oil)

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PENICILLIN

Process: (Optimum pH 7-7.5 and temperature 28 °C)

1. The culture is kept for 20-30 hours for rapid growth.
2. The actual yield of penicillin is carried out within 48-96 hours.
3. The complete in fermentation is indicated by pH rise to 8.

Purification and Recovery: After completion of fermentation

- Discard the mycelium and other solid residue
- Performed the filtration , collected the filtrate and checked it pH.
- The pH of filtrate was maintained between 2-2.5 by adding Phosphoric acid/Sulphuric acid (to convert the resulting Penicillin into its anionic form).
- The resulting fermented broth is immediately extracted with solvent like Amyl acetate/Butyl acetate or Methyl isobutyl ketone by using Podbielniak-Counter Current Solvent Extractor.
- The organic solvent extract is added to KOH/NaOH to get aqueous form of Potassium/Sodium salts of Penicillin.
- Resulting aqueous solution was again acidified and re-extracted with Methyl isobutyl ketone
- (The Shifting of Aqueous to Organic solvent or vice-versa helps in ultimate purification). The resulting Solvent extract is finally subjected o back-extracted with KOH/NaOH to get aqueous medium.
- The Penicillin was crystallised , washed ,dried and preserved.

Flowchart 1.2 Production of penicillin

STUDY OF PRODUCTION OF SEVERAL COMPONENTS

PENICILLIN

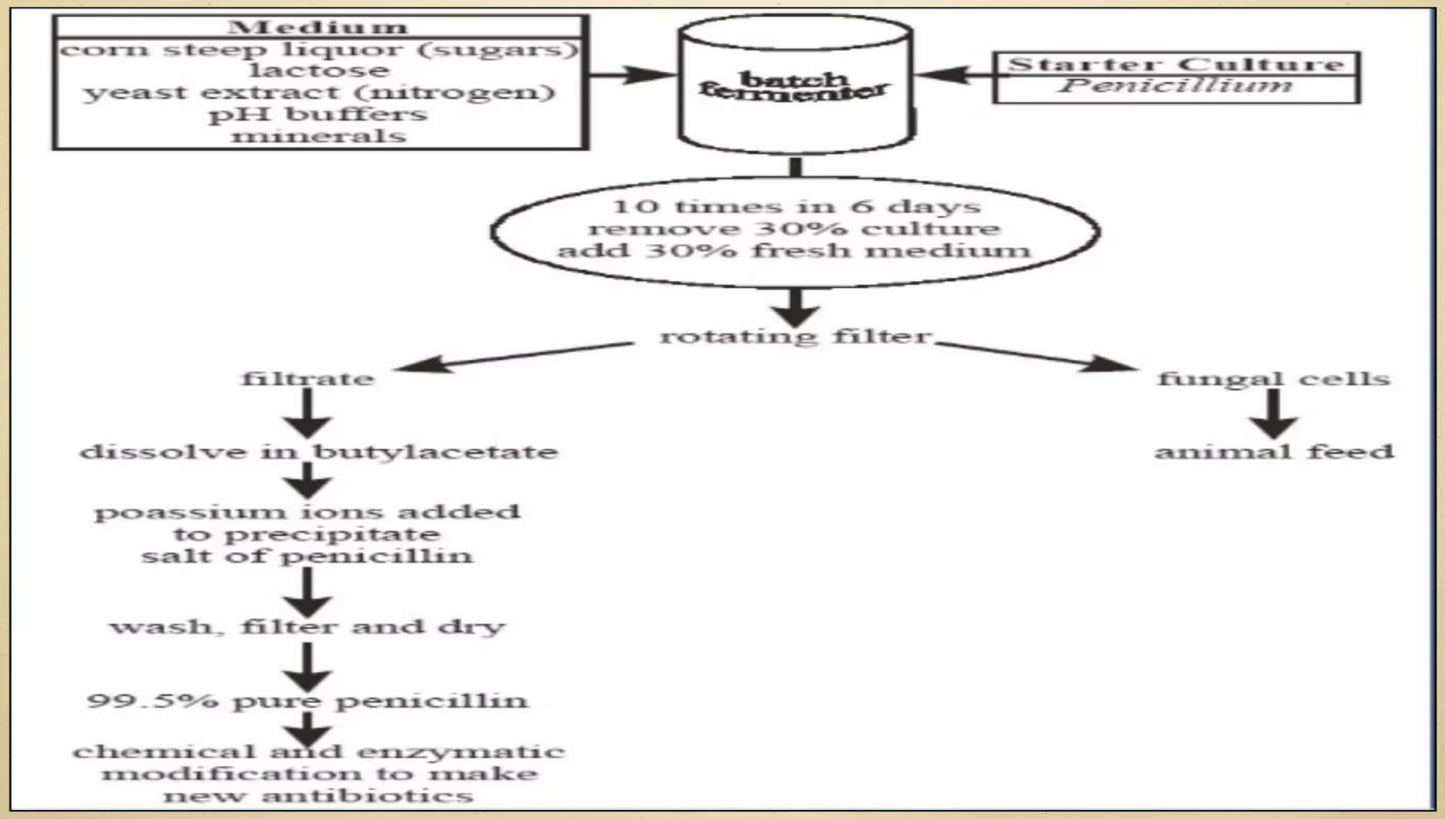

Fig 1.13 Flowchart representing the commercial production of Penicillin

STUDY OF PRODUCTION OF SEVERAL COMPONENTS

CITRIC ACID

- Citric is one of the most common acid found in primarily in several Fruits and Vegetables.
- It is used as food additive, Preservative, in cosmetics etc.
- It is a weak organic acid used as natural preservative , food and pharmaceutical industry.
- Citric avid was first isolated in 1783 by Swedish chemist **CARL WILHELM SCHEELE**, who crystallised it from lemon juice.
- Large number of micro-organisms including bacteria (*Bacillus licheniformis, Corynebacterium paraffins* etc), fungi (*Aspergillus nagger, Penicillium janthinelum* etc.) and yeasts(*Saccahromicopsis lipolytica, Hansenula anamosa etc*) have been employed to produce citric acid.
- Fermentation is the most economical and widely used way of citric acid synthesis.
- The industrial citric acid production can be carried in following Three ways:

SURFACE FERMENTATION

- Surface fermentation uses *Aspergillus niger* , on rice bran, or in liquid solution in flat aluminium or stainless steel pans.
- Special strains of *Aspergillus niger* which can produce citric acid despite of rice bran due the high content of trace metals, are used.

SUBMERGED FERMENTATION

- In this case , the strains are inoculated of about 15cm depth in fermentation tank.
- The culture is enhanced by giving aeration using air bubbles and kept it for 5 to 14 days.
- The citric acid is produced in the fermentation tank and is purified.

SOLID STATE FERMENTATION

- It is simplest method for citric acid production.(Koji process).
- Citric acid production reached a maximum(88g/kg dry matter)when fermentation is carried out with cassava having initial moisture of 62% at 26 °C for 120 hours.

STUDY OF PRODUCTION OF SEVERAL COMPONENTS

CITRIC ACID

- **Separation:**

-The biomass is separated by filtration

-The Liquid id transferred to recovery process.

-Separation of citric acid from the liquid precipitation.

-Calcium hydroxide is added to obtain Calcium citrate.

Tetra hydrate → **Wash the precipitate** → **Dissolved it with dilute sulphuric acid, yield citric acid and calcium sulphate precipitate.** → **Bleach and crystallisation** → **Anhydrous mono hydrate citric acid**

- **Purification:**

-Purification is a simple process of getting pure product i.e. Citric acid.

- Here purification occurs by => Precipitation & =>Filtration.

The reaction between Citric acid and calcium carbonate was allowed → **Filter and precipitate** → **Sulphuric acid is allowed to react with precipitate** → **Filter and precipitate** → **Purified citric acid**

STUDY OF PRODUCTION OF SEVERAL COMPONENTS

VITAMIN B_{12} (Cyanocobalamin)

- Vitamin B12 (Cyanocobalamin) is produces commercially by Streptomyces species i.e. from *Streptomyces oliveceus*.
- The fermentation is done in the medium composed of Glucose, Corn steep liquor,Cobalt chloride.
- **Best Method of production:** the latest method adopted these days for production is carried out by using the strains of Propionibacterium species- *Propionibacterium shermanii.* Or from pseudomonas species.
- The uses of it to treat neuronal disorders, to treat Megaloblastic anaemia (Vitamin B_{12} deficiency) etc.
- Three different types of medium are used in the production of Vitamin B12 from the species of *Propionibacterium shermanii.*

MAINTAINANCE MEDIUM

- Microbes are added to maintenance medium to develop culture.
- Composition -
  - Thytome
  - Yeast extract
  - Filtered tomato juice
  - Agar
- pH maintained at 7.2
- Incubation period 96hours
- Temperature 30 °C

SEED CULTURE MEDIUM

First stage medium
- Composition-
  - Thytone
  - Yeast extract
  - Filtered tomato juice
- Incubation period 48hours
- Temperature 30 °C without agitation.

Second stage medium
- Composition-
  - Glucose
  - Corn Steep Liquor
- pH maintained at 6.5
- Incubation period 24 hours
- Temperature 30 °C

MAIN CULTURE MEDIUM

- Composition -
  - Glucose
  - Corn steep liquor
  - Cobalt chloride
- pH maintained at 7.0
- Incubation period first 80hours without aeration but with slight introduction of N_2 with agitation.
- Temperature 30 °C

STUDY OF PRODUCTION OF SEVERAL COMPONENTS

VITAMIN B_{12} (Cyanocobalamin)

- General steps of production process :

S.olivaceus is allowed to grow at constant aeration at 27 °C in culture medium(Glucose as carbon source and cobalt chloride as cobalt source as precursor).

↓

Total fermentation process last upto 3-4 days or until such time when mycelium lysis takes place(before autolysis occur because a major portion of Vitamin produced ,remains within microbial cell until autolysis occur .

↓

After completing incubation, mycelium is collected before autolysis and destruction of vitamin occurs.

↓

Mycelium and the fermented broth are separated either by filtration or by centrifugation(dried form of mycelium can be used as vitamin B_{12} -enriched animal/poultry feed supplement)

↓

Now the filtrate (mycelium culture) and fermentation broth is acidified / treated with alcohol.

↓

Treated with sodium sulphite (to stabilise the vitamin)

↓

Heated the content by steam heated coils along with proper agitation in order to obtain the vitamin.

↓

The mixture is again filtered (to eliminate mycelial growth)

↓

The filtrate is concentrated and evaporated to dryness under vacuum (in certain cases it is further filtered and treated with acetone and ion-exchange resin to obtain ultra purified crystalline form of Vitamin B_{12}.

Flowchart 1.3 Production of Vitamin B_{12}

STUDY OF PRODUCTION OF SEVERAL COMPONENTS

GLUTAMIC ACID

- Glutamic acid is an α- amino acid that is used in biosynthesis of proteins.
- It is non-essential in humans i.e. the body can synthesise on its own.
- Amino acids are important as Nutrients(food), flavouring and starting material for pharmaceutical cosmetics and other chemicals.
- Amino acids always play an important role in biology of life , biochemistry and industrial applications.
- Large scale chemical and microbial production processes have been commercialised for the production of number of essential and non-essential amino acids.
- L-glutamic acid is one of the major amino acids that is present in a wide variety of foods. It is mainly used as a food additive and flavour enhancer in the form of sodium salt. *Corynebacterium glutamicum* is one of the major organisms widely used for glutamic acid production.
- In biotechnological processes, Corynebacterium species are used for economic production of glutamic acid by submerged fermentation .
- Glucose is one of the major carbon sources for production of glutamic acid.
- Glutamic acid was produced with various kinds of raw materials using sub-merged fermentation of palm waste hydrolysate , cassava starch , sugar cane bagasse (dry pulpy fibrous residue) , date waste.
- The manufacturing of Glutamic acid by fermentation comprises - Fermentation, isolation and purification are as follows:

STUDY OF PRODUCTION OF SEVERAL COMPONENTS

GLUTAMIC ACID

Sugar cane is taken and squeezed to make syrup and sterilised.

↓

The heated sterilised raw material and other requirements are put it into the Fermentor.

↓

The micro-organism *Corynebacterium glutamicum* which produces glutamic acid is added to the fermentation broth.

↓

The micro-organism reacts with sugar to produce glutamic acid

↓

The Fermentation broth is acidified and glutamic acid is crystallised.

↓

After the Fermentation process, specific method is require to separate and purify the amino acid product, which include- centrifugation, filtration, crystallisation , ion exchange, evaporation etc.

↓

The glutamic acid crystal is added to the sodium hydroxide solution and converted into MonoSodium Glutamate (MSG)

↓

MonoSodium Glutamate (MSG) is more soluble In water , and is cleaned by using active carbon.

↓

The cleaned MonoSodium Glutamate (MSG) is concentrated by heating and the monosodium glutamate crystal is formed.

↓

The monosodium glutamate crystal produced are dried in a closed system under hot air

↓

The crystals are packed and ready for use.

Flowchart 1.4 Production of Glutamic acid

STUDY OF PRODUCTION OF SEVERAL COMPONENTS

GRISEOFULVIN

- Griseofulvin is an Anti fungal drug .
- Its was first isolated from *Penicillin griseofulvin* as secondary metabolite in 1939.
- It is used for the treatment of Ringworm infection on body.
- The process includes several process are as follows:
- **Preparation of fermentation medium: Czapek dox medium**

-Composition Glucose, Sodium Nitrate, Potassium Hydrogen Phosphate, Magnesium sulphate.

- Steps included in manufacturing process are:

STUDY OF PRODUCTION OF SEVERAL COMPONENTS

GRISEOFULVIN

Fermentation: Adjusted the pH of medium between 6-7.2 and added into the fermenter.

Added the suspension of fungus which is obtained from Rapper steep agar (Czapek dox medium+Corn steep+Agar)

The mixture is allowed to ferment for 14days at 24 °C

Pre-treatment of fermentation broth: After the fermentation , the broth is heated at 60 °C for 20-30min or at 80 °C for 5-10min.(heating offers suitable flow rate for filtration process or improvement in separation features of broth)

Filtration: the Broth is filtered big using rotatory vacuum filter

Solid residue form cake

Washed with water

Immediately dewatered by blowing air over it

Used as animal feed supplements

Solid cake can immersed into the medium and filtrate can be gain collected

Filterate

Extraction: Filtrate is extracted with cold Acetone which is highly efficient solvent to extract Griseofulvin - 75-96%

De-coloration of extract: by Calcium hydroxide at more the pH 10

Isolation and separation : Impurities are removed by washing with Hexane

Precipitation and purification: the separated product is added with alkaline water(ammonia+water/ alkali metal carbonates/alkali metalhydoxideso at pH 8.5) which allows precipitation of Griseofulvin .

Precipitate are purified by menthol followed by drying and collection of final product

Flowchart 1.5 Production of Griseofulvin