ANTIBIOTICS

- Antibiotics are chemical substances that can inhibit the growth of, and even destroy, harmful microorganisms. They are derived from special microorganisms or other living systems, and are produced on an industrial scale using a fermentation process.

- The first antibiotic was discovered in 1896 by Ernest-Duchesne and "rediscovered" by Alexander-Flemming in 1928 from the filamentous fungus Penicilium-notatum.

APPLICATION MODE

Antibiotics are used in many forms:

- For bacterial infections on the skin surface, eye, or ear, an antibiotic may be applied as an ointment or cream.

- If the infection is internal, the antibiotic can be swallowed or injected directly into the body. The antibiotic is delivered throughout the body by absorption into the bloodstream.

COMMERCIAL PRODUCTION OF ANTIBIOTICS

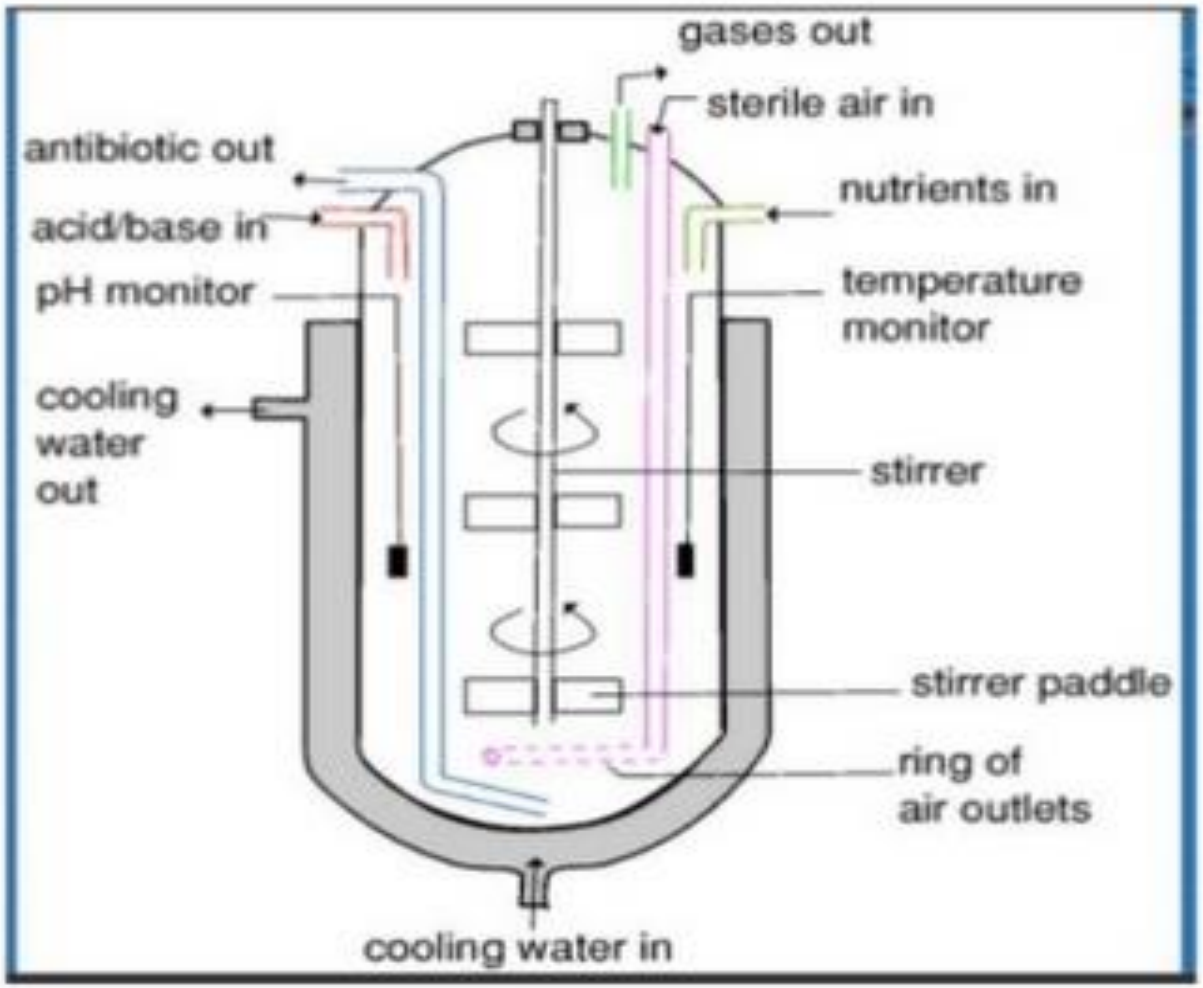
Inoculum:

- A small amount of material containing bacteria, viruses, or other microorganisms that is used to start a culture.
- A high yielding strain (Bacteria) is a prerequisite for antibiotic production.
- The Inoculum is prepared usually in the form of a spore suspension, which is transferred into the fermenter.

ANTIBIOTICS

Antibiotic	Productivity	Activity	Chemical Nature
Penicillin G	*Penicillium chrysogenum*	Gram⁺ bacteria	Peptide
Streptomycin	*Streptomyces griseus*	Gram⁺ and ⁻ bacteria and mycobacteria	Aminoglycoside
Gentamycin	*Micromonospora purpurea*	Gram⁺ bacteria	Aminoglycoside
Neomycin	*S. fradiae*	Gram⁺ and ⁻bacteria	Aminoglycoside
Tetracyclines	*Streptomyces* spp.	Gram⁺ and ⁻bacteria	Tetracycline
Cephalosporin	*Cephalosporium acremonium*	Gram⁺ and ⁻bacteria	Peptide
Kanamycin	*S. kanamyceticus*	Gram⁺ and ⁻bacteria, and mycobacteria	Aminoglycoside

BATCH FERMENTER



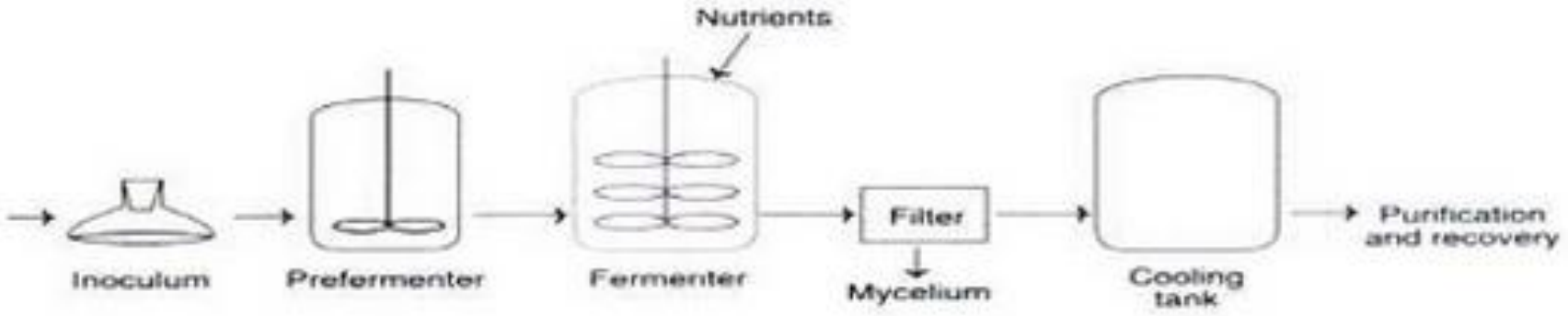
Fermenter / Bioreactor:

- Antibiotic are generally produced in stainless steel fermenters used in the batch or fed mode, see in figure below.
- Water cooling is often used to maintain the temperature between 24-26° C.
- Generally, the fermenter is maintained at above atmospheric pressure which reduces contamination risk and enhances O_2 supply.
- The final stage fermenter is preferably used for antibiotic production for the longest period.

- Initial stages of fermentation are designed for considerable microbial growth; these are carried out in seed stage fermenters of smaller size.

- One or more seed stages may be used, depending on the process and the strain, to produce the maximum amount of biomass.

Flow Diagram



PENICILLIN G

- **What Is Penicillin?:**
  A class of antibiotics that comes from mold (fungi).
  Discovered by accident in 1928 by Alexander Fleming, is the first antibiotic called Penicillin.

- **Benzyl-penicillin**, also known as **penicillin G**, is an antibiotic used to treat a number of bacterial infections.

- **Penicillin** (**PCN** or **pen**) is a group of antibiotics which include penicillin G (intravenous use), penicillin V (use by mouth), procaine penicillin, and benzathine penicillin (intramuscular use).

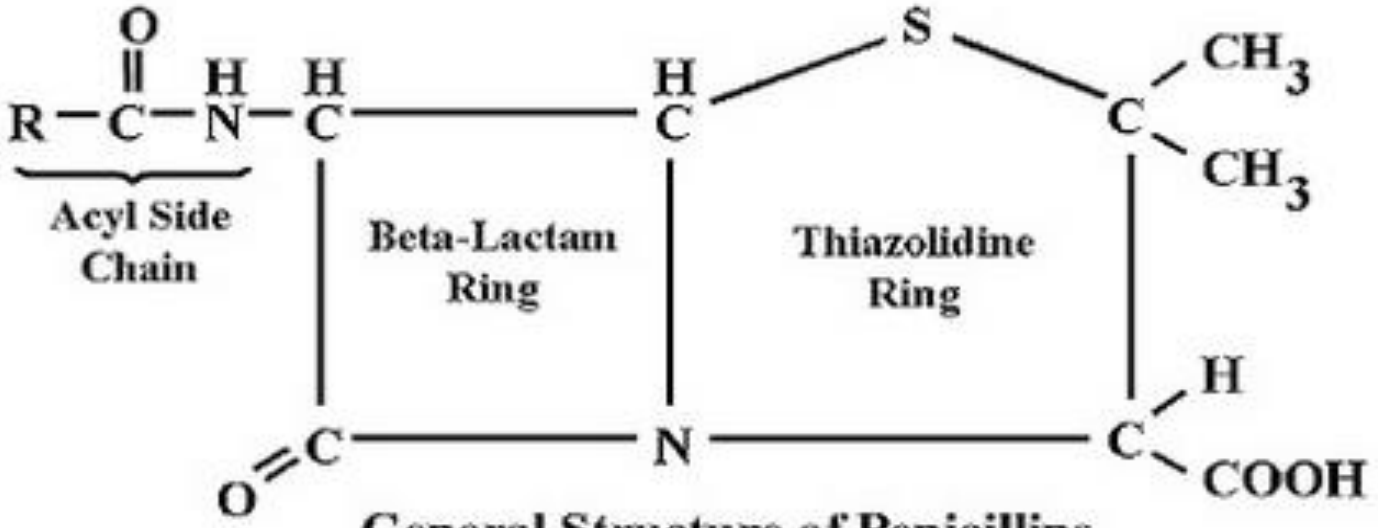
Introduction

We have all, at one point or the other, heard about **penicillin**. From its accidental discovery and massive use in World War II to the Nobel Prize for Medicine in 1945 by its rightful owners, penicillin has made its proof as the "Miracle Drug" that revolutionized the course of the medical industry.

Without further ado, let us take a look at an important aspect that made penicillin accessible at large; its **industrial production**.

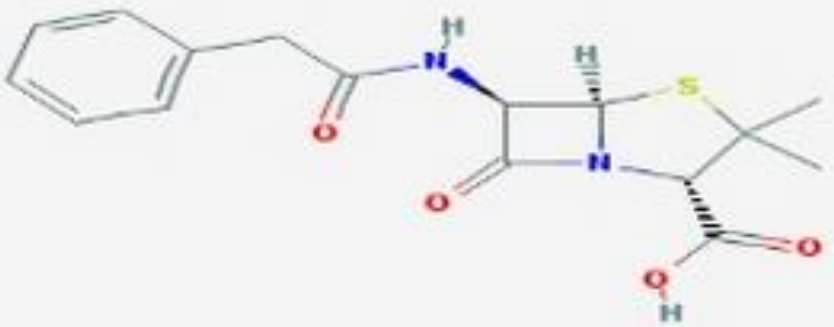
CHEMICAL STRUCTURE :

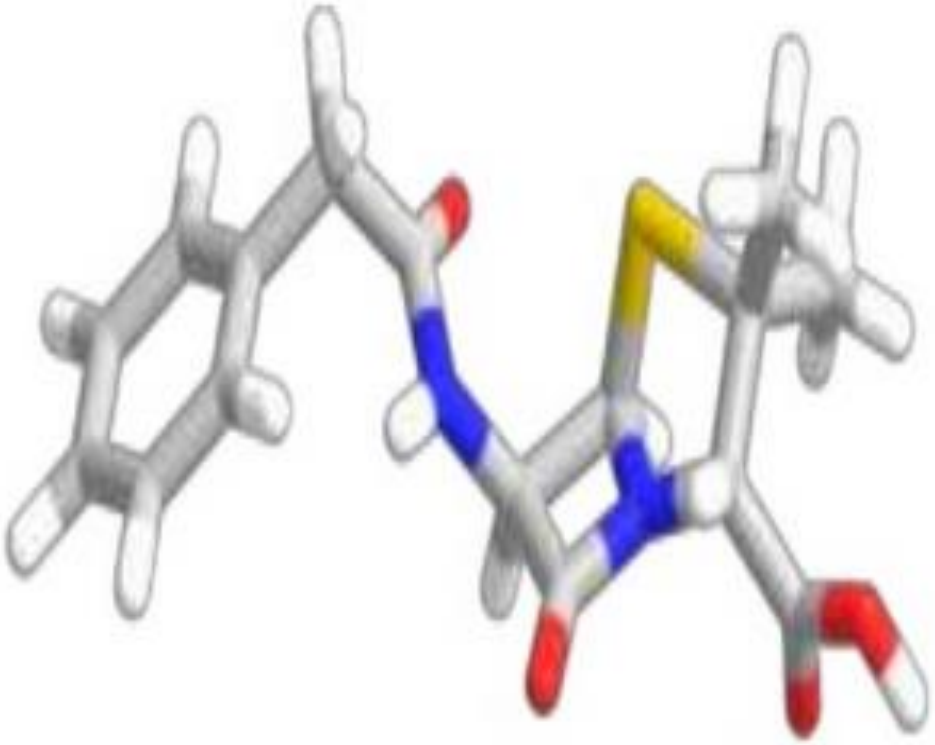
- The basic structure of penicillin is 6-aminopenicillanic acid(6-APA), consisting of a thiazolidine ring with a condensed B-lactam ring.



General Structure of Penicillins

- Formula: $C_{16}H_{18}N_2O_4S$
- Mol. Wt: 334.39 g/mol





INDUSTRIAL PRODUCTION OF ANTIBIOTIC- PENICILLIN

- The industrial production of penicillin was broadly classified in to two processes namely,
- **Upstream processing**
- **Downstream processing**

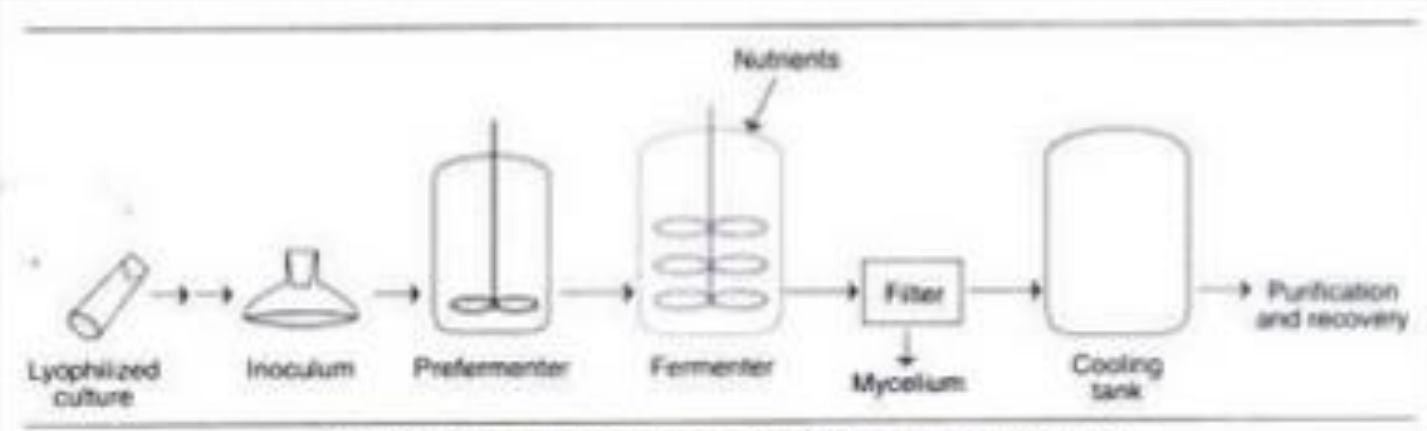


Fig. 25.3 : An outline of the flow chart for penicillin fermentation.

UPSTREAM PROCESSING

INOCULUM PREPARATION

- The medium is designed to provide the organism with all the nutrients that it requires.
- Inoculation method- submerged technique
- Spores -major source of inoculum

UPSTREAM PROCESSING

- Upstream processing encompasses any technology that leads to the synthesis of a product.
- Upstream includes the exploration, development and production.

DOWNSTREAM PROCESSING

- The extraction and purification of a biotechnological product from fermentation is referred to as downstream processing.

STAGES IN DOWNSTREAM PROCESSING

Removal of cells

- The first step in product recovery is the separation of whole cells and other insoluble ingredients from the culture broth by technique such as filtration and centrifugation.

PRODUCTION OF PENICILLIN G

RAW MATERIALS:

FUNGUS (MO): Penicillum chrysogenum

CARBON SOURCES: Lactose-concentration of 6%.
Others such as glucose & sucrose may be used.

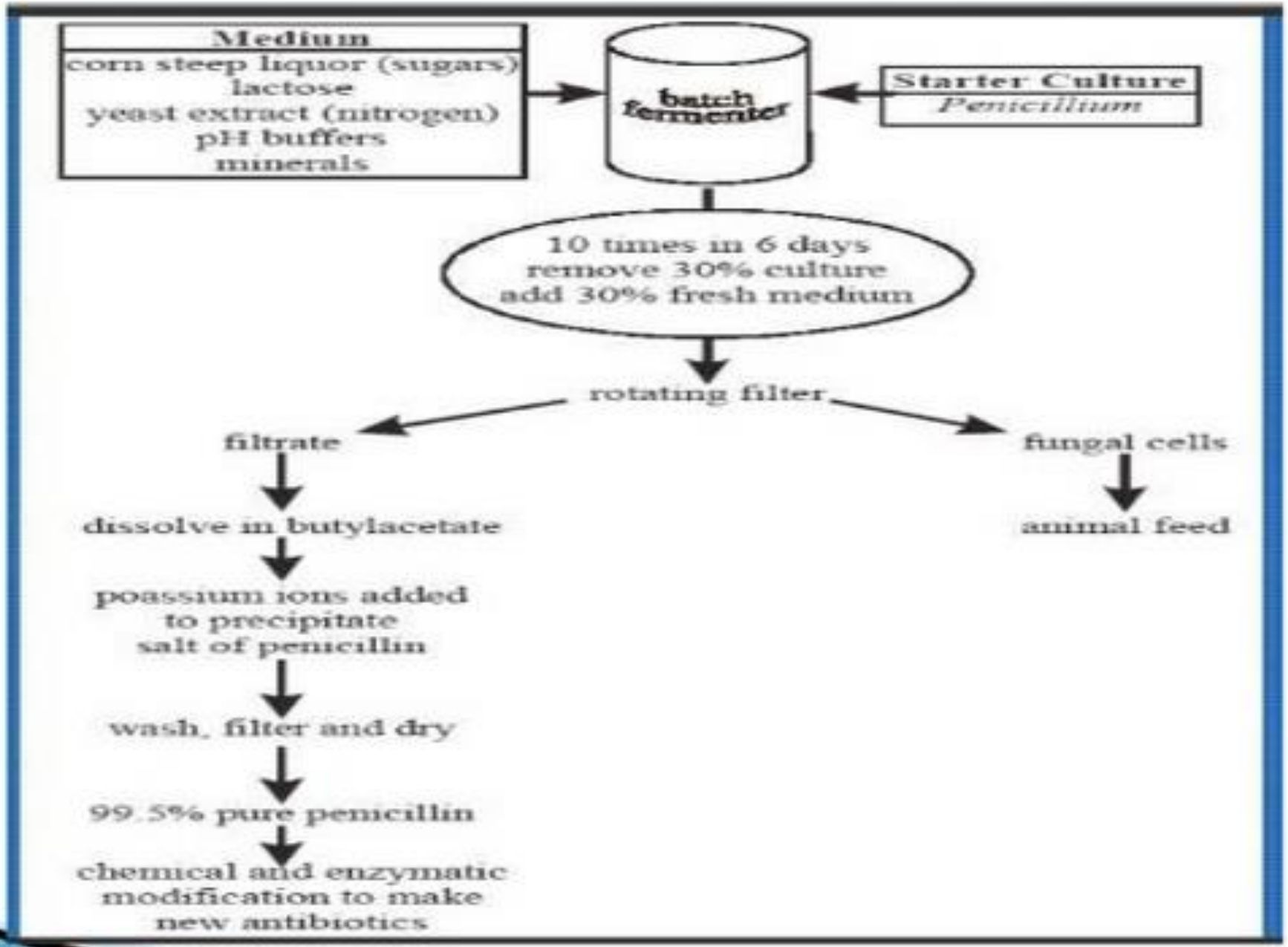
NITROGEN SOURCES: Corn steep liquor (CSL),
Ammonium sulphate and ammonium acetate, yeast extract etc

MINERAL SOURCES: Elements potassium, phosphorus, magnesium, sulphur, zinc and copper are essential for penicillin. e.g: Corn steep liquor

PRECURSOR:
Phenylacetic acid or Phenoxyacetic acid

PROCESS DESCRIPTION

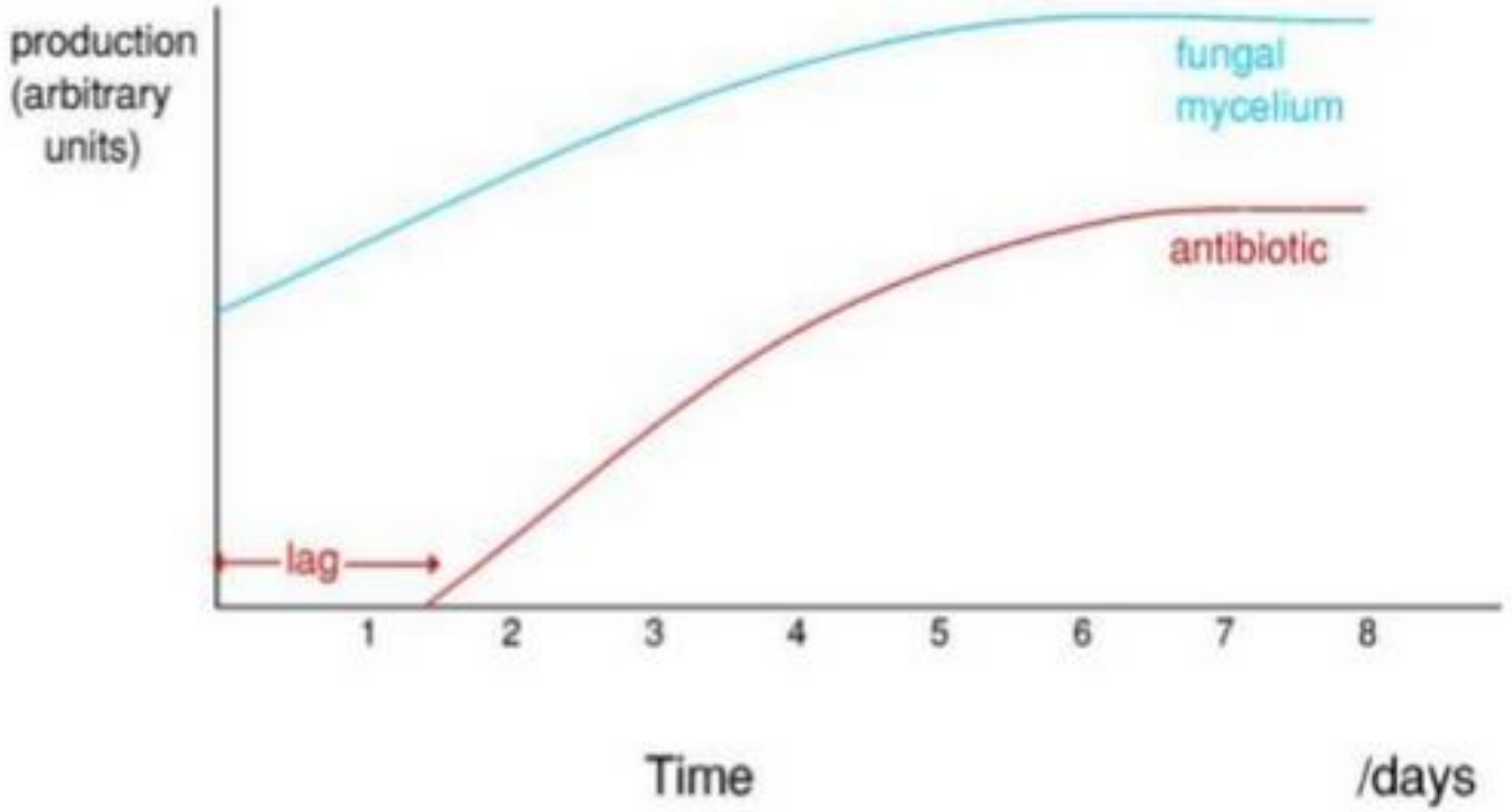
- The medium is inoculated with a suspension of conidia of Penicillium chrysogenum. The medium is constantly aerated and agitated, and the mould grows throughout as pellets.
- After about seven days, growth is complete, the pH rises to 8.0 or above, and penicillin production ceases.
- Downstream processing is relatively easy since penicillin is secreted into the medium.


Medium
corn steep liquor (sugars)
lactose
yeast extract (nitrogen)
pH buffers
minerals
batch fermenter
Starter Culture
Penicillium
10 times in 6 days
remove 30% culture
add 30% fresh medium
rotating filter
filtrate
fungal cells
dissolve in butylacetate
animal feed
poassium ions added
to precipitate
salt of penicillin
wash, filter and dry
99.5% pure pemicillin
chemical and enzymatic
modification to make
new antibiotics

- Removal of cells:

  The first step in product recovery is the separation of whole cells and other insoluble ingredients from the culture broth by technique such as filtration and centrifugation.

- The product needs to be very pure, since it being used as a therapeutic medical drug, so it is dissolved and then precipitated as a potassium salt to separate it from other substances in the medium.


production
(arbitrary
units)
fungal
mycelium
antibiotic
lag
1
2
3
4
5
6
7
8
Time
/days

The main stages of Penicillin production are:

1. A medium of corn steep liquor (a by product of starch manufacture), yeast extract and others substrates added to the fermenter.
2. After 40 hours, Penicillin begins to be secreted by the fungus
3. The mould mycellium (cell matter) is filtered from the harvested product.
4. Penicillin is extracted in the organic solvent: butylacetate, in which it dissolves.
5. Potassium salts are added and a penicillin precipitate is formed, this is washed and dried.

Isolation and purification

The first step is the recovery process is the removal of mycelium or cells by **filtration or centrifuging.**

Second step is to remove the antibiotic from the spent production medium by **solvent extraction, adsorption or precipitation.**

Additional **solvent extraction,distillation,sublimation, column chromatography or other methods accomplish purification.**

Semi-synthetic penicillins.

Semi synthetic such as penicillin such as Ampicillin, Methicillin, Oxocillin, Propicllin are prepared by chemical acylation of 6-aminopenicillanic acid.

INTRODUCTION

- Streptomycin is an bactericidal antibiotic drug belonging to class aminoglycosides.
- Used against TB
- Derived from actinobacterium Streptomyces griseus.
- Used against gram negative bacteria especially.
- Dihydrostrepomycin prepared by hydrogenation of streptomycin with platinum as catalyst & is commercially more successful.

STREPTOMYCIN

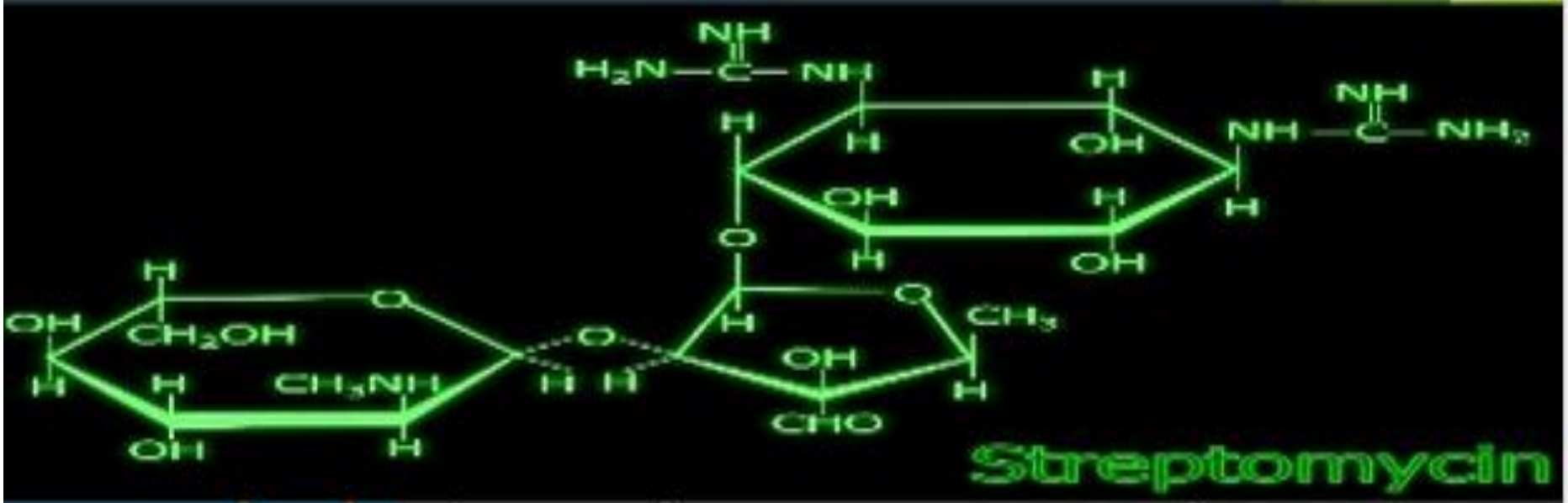
- Streptomycin is an aminoglycoside antibiotic produced by selected strains of Streptomyces griseus.
- The antibiotic works by inhibiting the synthesis of DNA and proteins.

CHEMISTRY

- It is basic in nature, with solubility in water at the rate of 20 g/L.
- It is stable to pH changes.
- It can withstand boiling temperature.
- Being a base, streptomycin is usually produced as salt, normally of HCl and sulfate.
- One unit of streptomycin is equal to 1 µg of free base.
- Streptomycin is composed of 3 subunits: (i) aminocyclitol (= streptidine), (ii) L-streptose, and (iii) Nmethyl- L-glucosamine.

CHEMICAL COMPOSITION

- Chemically, it contains 3 sugars derived from glucose with C, N, O & H elements.
- Chemical formula - $C_{21}H_{39}N_7O_{12}$



MEDIUM

- Medium is a nutritive substance in which cultures are grown for scientific purposes.

- The culture medium for streptomycin consists of -

1. Carbon source : starch, dextrin, glucose, glycerol & other economically available material.
2. Nitrogen source : natural agricultural by-products, soybean meal, corn steep liquor, cotton seed flour, casein hydrolyte, or yeast & its extract. Inorganic N salts like ammonium sulphate & ammonium nitrates are also used.
3. Animal oils, vegetable oils and mineral oils are also used.

- INOCULUM - ###### of S.griseus spores maintained in soil stocks or lyophilized in carrier are inoculated into sporulation medium which builds up mycelial inoculum.

FERMENTATION PROCESS

- Spores of S.griseus are inoculated into a medium to establish a culture with high mycelial biomass for introduction into inoculum tank, using inoculum to initiate the fermentation process.

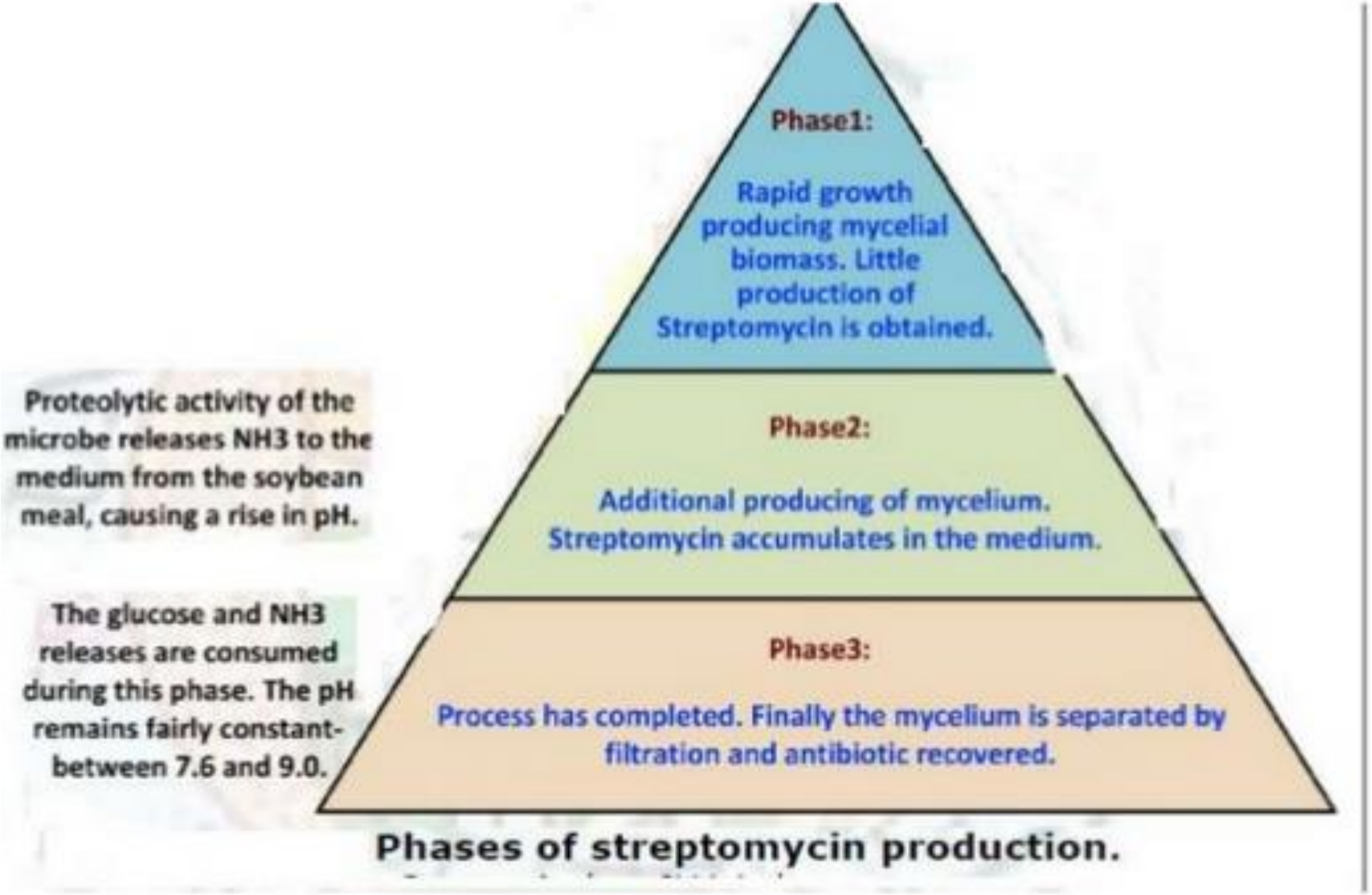
- Yield in production vessel responds to high aeration & agitation conditions. Other conditions involve-

  Temperature range 25-30°C

  pH range 7-8

  Time 5-7 days

- The fermentation process for production of Streptomycin involves 3 phases.



Phases of streptomycin production.

PHASE 1

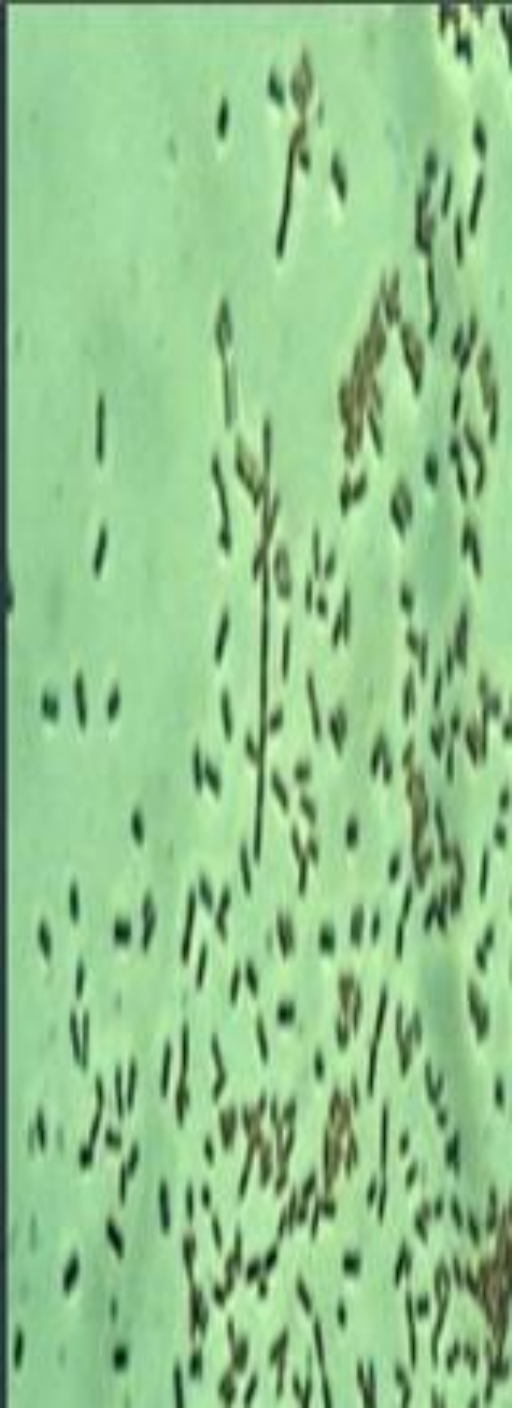
- Initial fermentation phase and there is little production of streptomycin.
- Rapid growth with production of mycelial biomass.
- Proteolytic enzymatic activity of S.griseus releases NH_3 from soya meal, raising the pH to 7.5
- Characterized by release of ammonia.
- Carbon nutrients of soya meal are utilized for growth.
- Glucose is slowly utilized with slight production of Streptomycin.

PHASE 2

- Little production of mycelia.

- Glucose added to the medium & the NH_3
- released from soya meal are consumed.

- pH remains fairly constant ranging
- between 7.6 to 8.



PHASE 3

- Final phase of fermentation.
- Depletion of carbohydrates from medium.
- Streptomycin production ceases & bacterial cells begin to lyse.
- Ammonia from lysed cells increase the pH.

RECOVERY & PURIFICATION

- Mycelium is separated from broth by filteration & streptomycin is recovered.

- Recovery process - broth is acidified, filtered & neutralized. Then its subjected to column containing cation exchange resin to adsorb Streptomycin from the broth & column is washed with water & streptomycin eluted with HCl before concentration in vacuo almost to dryness.

CONTINUED. .

- The streptomycin is dissolved in methanol & filtered.
- Acetone is used in filterate to precipitate the antibiotic.
- Percipitate is washed with acetone & dried in vacuo.
- Purification is done by dissolving in methanol to form pure S. chloride complex. Further by, adsorbing it onto activated charcoal & eluting with acid alcohol.

USES

- In the treatment of tuberculosis, urinary tract infection, systemic infection by Gram positive bacteria
- for bacteria that have gained resistance to penicillin.
- Non-medical uses include preparation of selective media
- in cloning experiments
- as laboratory standard for quantitative analysis of streptomycin.

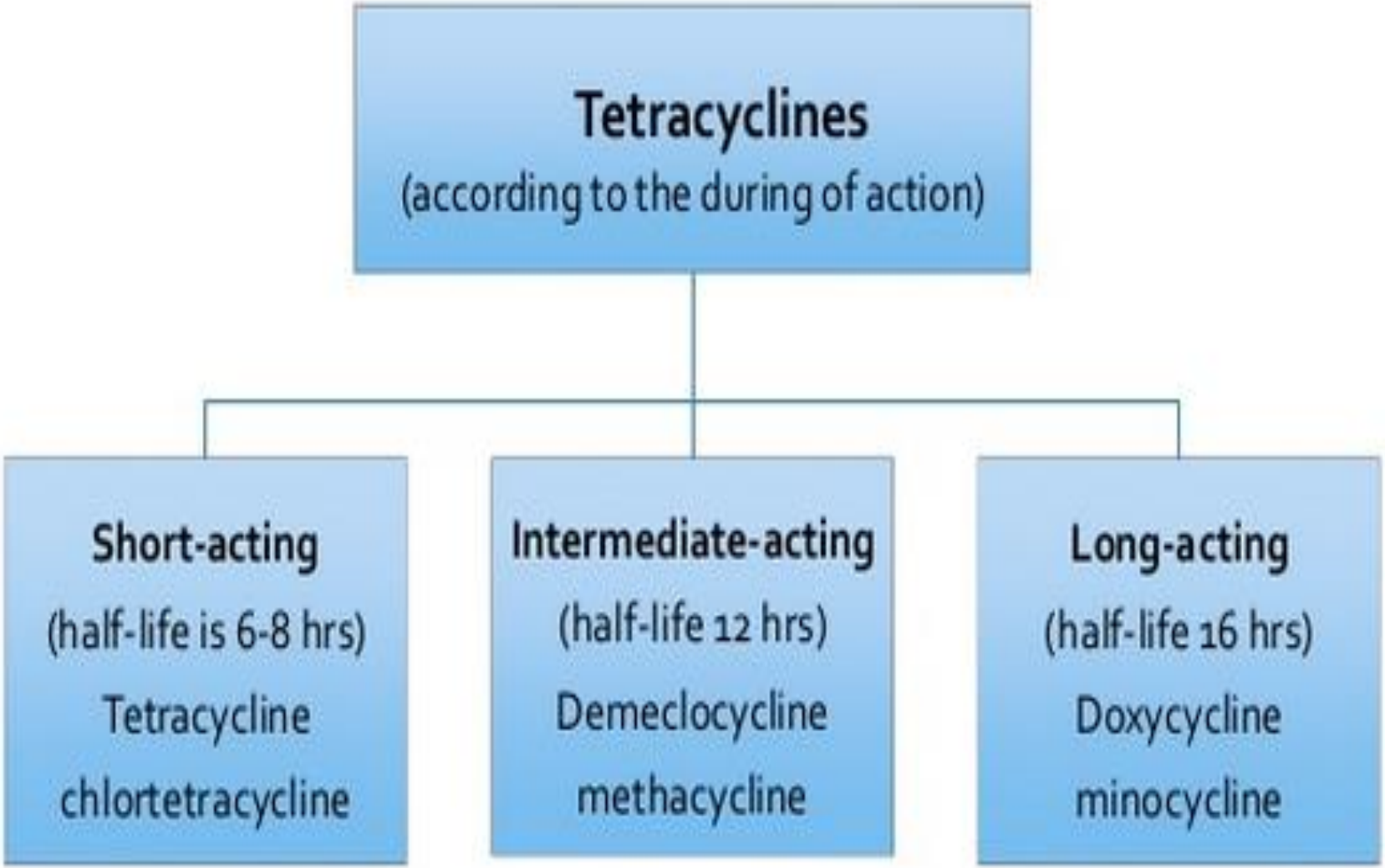
LIMITATIONS

- The antibiotic exerts a neurotoxic reaction upon prolonged use.
- It can lead to hearing loss and loss of balance (that is, it is ototoxic).
- Streptomycin may sometimes damage kidney also.
- The drug may lead to development of streptomycin-resistant forms. It is therefore advisable to use the drug along with p-aminosalicylic acid or isoniazid.
- Dihydrostreptomycin has lesser side effects than streptomycin.

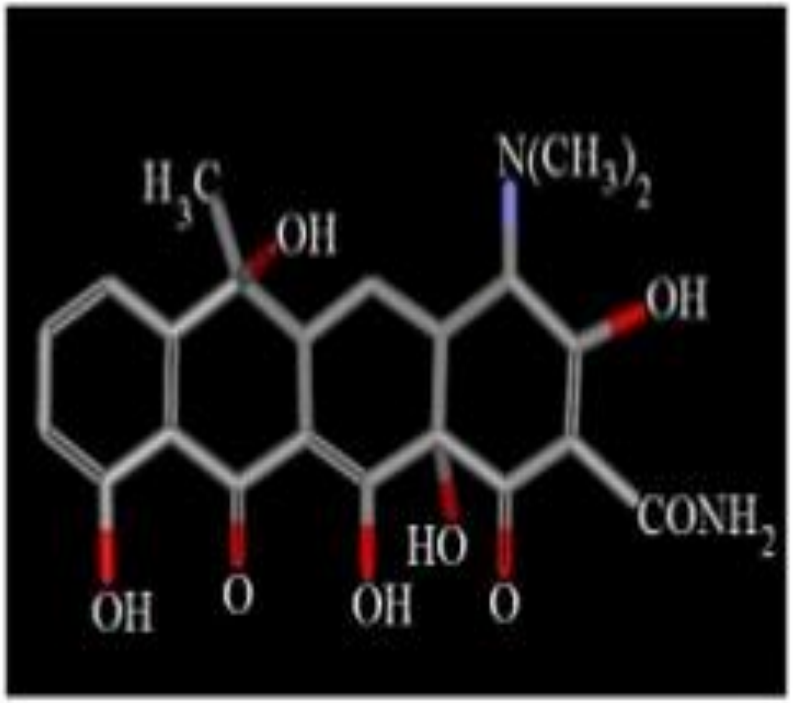
INTRODUCTION

- Tetracycline are broad spectrum antibiotics, which are chemical substances produced by a microorganism that are able to kill other microorganisms without being toxic to the person, animal or plant.
- Tetracyclines were derived from *Streptomyces* bacteria.
- **Tetracyclines** were discovered as natural products by Benjamin Minge Duggar in 1945 and first prescribed in 1948.

CLASSIFICATION OF TETRACYCLINE

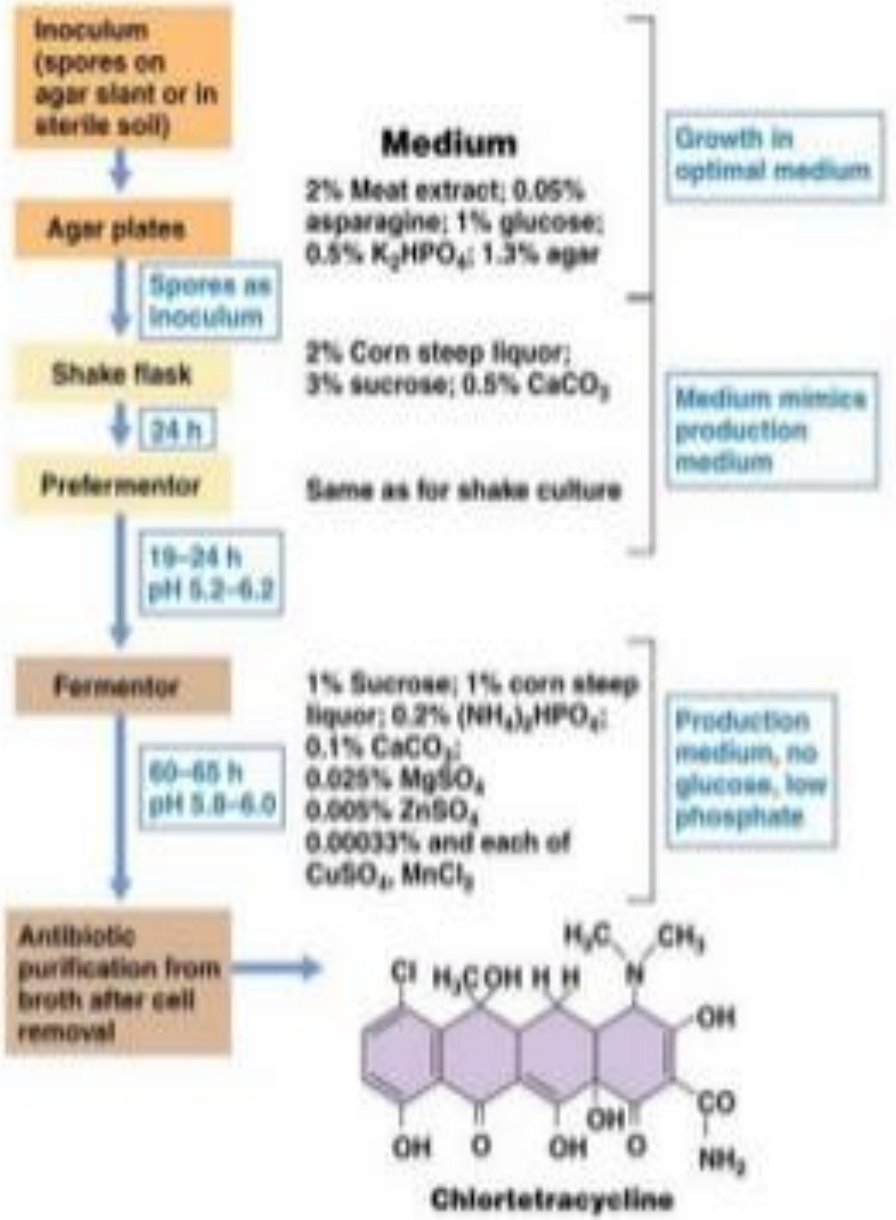


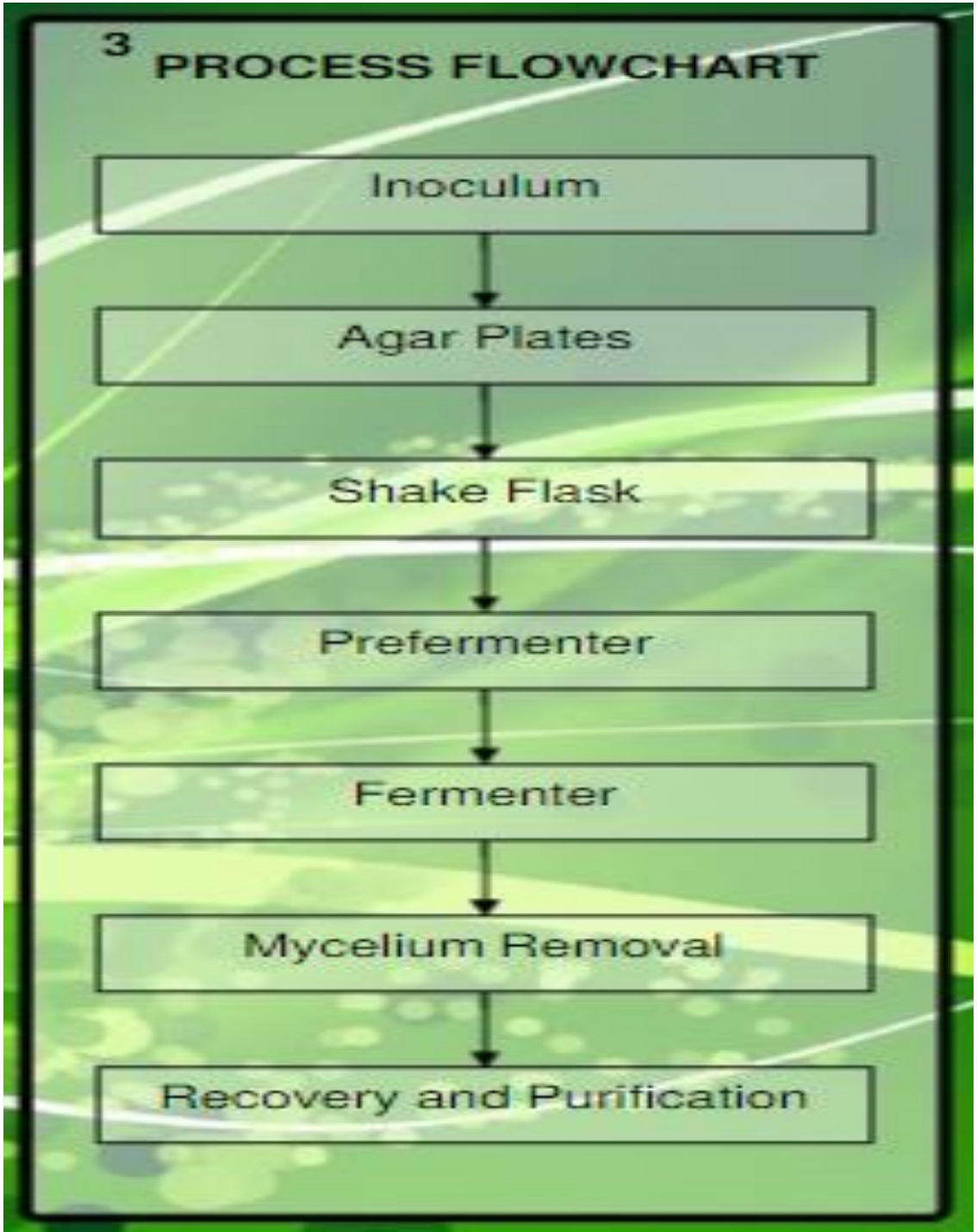
STRUCTURE OF TETRACYCLINE



- Amphoteric compounds, forming salts with acid or base.
- Zwitter ions in neutral solution
- They are yellow in colour

Tetracycline Production




3 PROCESS FLOWCHART
Inoculum
Agar Plates
Shake Flask
Prefermenter
Fermenter
Mycelium Removal
Recovery and Purification

4 PRODUCTION PROCESS

The ingredients like corn steep liquor, soy flour or peanut meal is added to the fermentation medium as the suppliers of carbon and nitrogen. The addition of glucose or starch also helps in the good growth of the microorganisms and the production of antibiotic. The optimum temperature for the growth is 27-30°C, pH 6.5-7.5 and aeration 0.8-1.0 vvm. The duration of fermentation is around 4 days. To recover the antibiotic, the culture broth is filtered at the end of fermentation process to remove the mycelium. The filtrate is treated with n-butanol or methylisobutylketone in acidic or alkaline condition for extracting the antibiotic. The process of adsorption to activated charcoal is done to remove other impurities. After the elution of tetracycline, it is crystallized.[3]

- The unit '**vvm**' is used for bioreactor culture. The first 'v' stands for volume of air (e.g. liter) ; the second 'v' stands for per unit of medium (e.g. liter); 'm' stands for per unit of time (e.g. minute). For example, 2 **vvm** (l/l/m) means in 1 minute time there is 2 liter of air passing through 1 liter of medium.

USES OF TETRACYCLINE

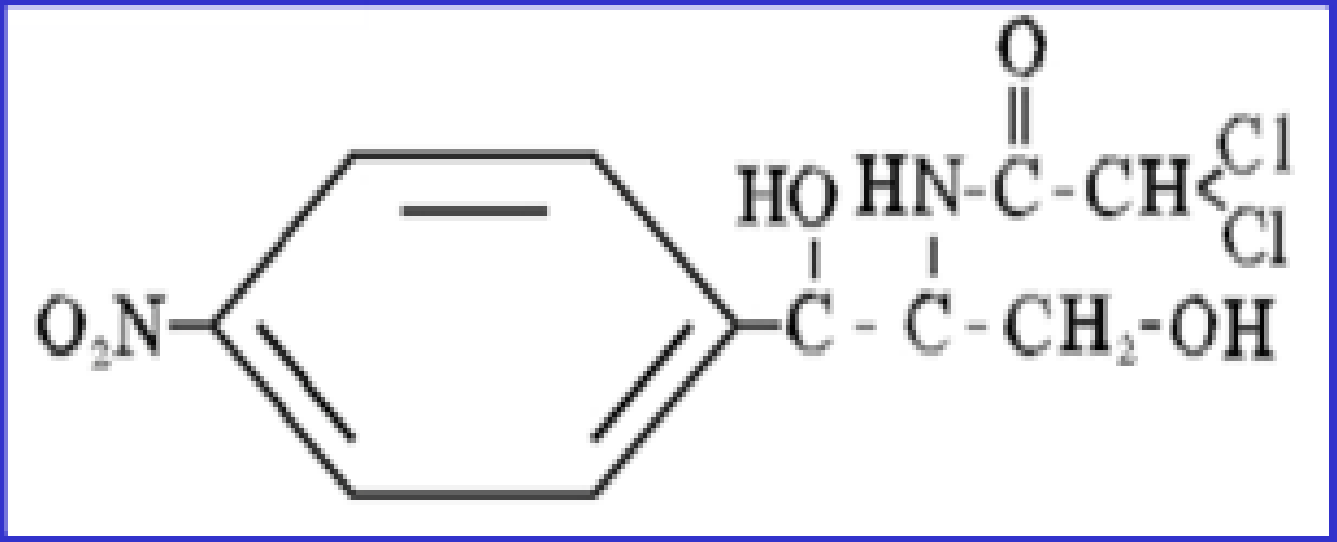
- Antibacterial resistance
- Non antibacterial resistance like inflammation
- Tissue destructive disease like antifibrilogenics
- Parkinson and other neurodegeneration disease
- Antiviral and anti cancer
- Upper/lower respiratory tract infections
- Skin and soft tissue infections
- Relapsing fever
- Cholera
- Urinary tract infection
- anthrax

STORAGE

- Keep at room temperature 65-85 °F (18-29 °C)
- Keep away from heat, moisture and light.

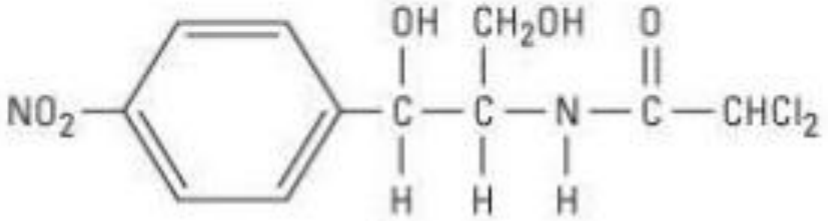
Chloramphenicol

- Structure:



CHLORAMPHENICOL

- Chloramphenicol was initially obtained from *Streptomyces venezuelae* in 1947. It was soon synthesized chemically and the commercial product now is all synthetic.
- It is a yellowish white crystalline solid, aqueous solution is quite stable, stands boiling, but needs protection from light. It has a nitrobenzene substitution, which is probably responsible for the antibacterial activity and its intensely bitter taste.



Chloramphenicol

- It is soluble in alcohol but poorly soluble in water. Chloramphenicol succinate , which is used for parentral administration is highly water soluble. It is hydrolyzed invivo with liberation of free chloramphenicol

PRODUCTION :

REQUIRMENTS:

MEDIUM:

18 liter of fermentation medium is used and sterilized at 120^{o}C for 1hr.

CARBON SOURCE:

Glycerol(1%) is commonly used. Sometimes molasses and dried distillers grain slops are also used.

NITROGEN SOURCE:

Proteinaceous meterials like yeast fermentation solubles and hogstomach. Trypton is the best source of nitrogen.

MINERAL NUTRIENT:

Sodium chloride, zinc and iron in traces.

pH:

It is adjusted to 7.5 with NaOH.

PRODUCTION:

Inoculum is inoculated in the fermenter.

Maximum cell mass is obtained in 3-4 days at 25C.

Antibiotic level varies from 200-300mg/L.

High yield is obtained in 10-15 days of fermentation

ISOLATION AND PURIFICATION:

The product is extracted from the clarified broth.

Filtegold at slightly acidic pH or slightly alkaline pH with ethyl-or amyl acetate.

Extract is concentrated , diluted with kerosene.

Lipids with petroleum ether are removed from evaporated residue.

Crude product is decilorized by passing through short coloum of charcoal or alumina.

Purified product is dissolved in hot water,decolorized,clarified and induced tocrystallization.

The product is recrystallized.

,chloramphenicol should not be used for colds, flu, other virus infections, **sore** throats or other minor infections, or to prevent infections. Chloramphenicol should only be used for serious infections in which other medicines do not work.

Chloramphenicol is used to treat serious **infections** in different parts of the body. It is sometimes given with other **antibiotics**. However, chloramphenicol should not be used for colds, flu, other virus **infections**, **sore** throats or other minor **infections**, or to prevent **infections**.

. Chloramphenicol is an antibiotic useful for the treatment of a number of **bacterial infections**. This includes as an eye ointment to treat conjunctivitis. By mouth or by injection into a vein, it is used to treat **meningitis**, plague, **cholera**, and **typhoid fever**.