***Commercial production of penicillin***:

-Antimicrobial agents: Inhibitory properties against microorganisms (antibiotics and synthetic) with minimal effects on mammalian cells.

-Antibiotic: Substance produced by a microorganism that inhibits the growth (bacteriostatic) or kills other microorganisms (bactericidal, viricidal, fungicidal).

-Semi-synthetic: Antibiotics chemically altered to improve properties.

-Selective toxicity: A drug that kills harmful microbes without damaging the host.

***What Is Penicillin?***

Penicillin is a fungal secondary metabolite, which is used as an antibiotic. Discovered in 1896 by Ernest Duchesne and "rediscovered" by Alexander Flemming in 1928 from the filamentous fungus *Penicilium notatum*. The first generation penicillin products from these fungi were benzyl penicillin (penicillin G) and penicillins V, X, F and K. The antibiotic substance, named penicillin, was not purified until the 1940s (by Florey and Chain), just in time to be used at the end of the Second World War. Penicillin was the first important commercial product produced by an aerobic, submerged fermentation. When penicillin was first made at the end of the WWII using the fungus *Penicilium notatum*, the process made 1 mg\ $dm^3$.Today, using a different species (*P. chrysogenum*) and better extraction procedures the yield is 60 g\ $dm^3$.

***How Penicillin Works?***

All penicillin like antibiotics inhibit synthesis of peptidoglycan (Penicillin binds to cell wall of bacteria, prevents peptide chains from linking, and lyses it) an essential part of the cell wall, they do not interfere with the synthesis of other intracellular components. These antibiotics do not affect human cells because human cells do not have cell walls.

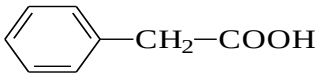
Spectrum activity: Penicillins are active against Gram positive bacteria .Some members (e.g. amoxicillin) are also effective against Gram negative bacteria but not *Pseudomonas aeruginosa*

***The production process of penicillin:***

1. ***Strain of organism used in penicillin fermentation:***

Penicillin production is usually via a batch, and a fed batch process is normally used to prolong the stationary period and so increase production. Carried out aseptically in stirred tank fermentors of 40000–200000L capacity. These processes are maintained at 25–27°C and pH 6.5–7.7 by the automatic addition of H2SO4 or NaOH as necessary, the specific conditions depending upon the *Penicillium* strain used. The *P. notatum* was first used for penicillin mass production but fermentation yields were poor. A yield was found to be increased with *P. chrysogenum* by the addition of precursors.

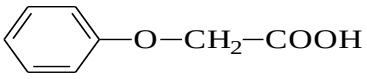
Precursors of the appropriate side-chain are added to the fermentation. Thus if benzyl penicillin is desired, phenyl acetic acid is added. Phenyl acetic acid is nowadays added continuously as too high an amount inhibits the development of the fungus. High yielding strains of *P. chrysogenum* resistant to the precursors have therefore been developed. But if penicillin V is desired, phenoxy acetic acid is added.



phenyl acetic acid

(Penicillin G)

- obtained from corn steep liquor



phenoxy acetic acid

(Penicillin V)

- first oral penicillin

2. ***Fermentation for penicillin production:***

Inoculum development is usually initiated by adding lyophilized spores or a frozen culture to a small fermentor at a concentration of $5x10^3$ spores /ml, developed through vessels of increasing size to a final 5-10% of the fermentation tank. The medium for penicillin production now usually has as

carbohydrate source glucose, beet molasses or lactose. The nitrogen is supplied by corn steep liquor. Cotton seed, peanut, linseed or soybean meals have been used as alternate nitrogen sources. Calcium carbonate or phosphates may be added as a buffer. Sulfur compounds are sometimes added for additional yields since penicillin contains sulfur.The practice nowadays is to add the carbohydrate source intermittently, i.e. using fed-batch fermentation. Lactose is more slowly utilized and need not be added intermittently. Glucose suppresses secondary metabolism and excess of it therefore limits penicillin production.

***Penicillin fermentation can be divided into three phases.***

-The first phase **(trophophase)** during which rapid growth occurs lasts for about 30-40 hours during which mycelia are produced.

-The second phase **(idiophase)** lasts for five to seven days; growth is reduced and penicillin is produced.

-The **third phase**, carbon and nitrogen sources are depleted, antibiotic production ceases, the mycelia lyse releasing ammonia and the pH rises.

### *3. Extraction of penicillin after fermentation:*

At the end of the fermentation the broth is transferred to a settling tank. Penicillin is highly reactive and is easily destroyed by alkali conditions (pH 7.5-8.0) or by enzymes. It is therefore cooled rapidly to 5-10°C. A reduction of the pH to 6 with mineral acids, sometimes accompanied by cooling helps also to preserve the antibiotic. The fermentation broth contains a large number of other materials and the method used for the separation of penicillin from them is based on the solubility, adsorption and ionic properties of penicillin.

The fermentation broth is filtered with a rotary vacuum filter to remove mycelia and other solids and the resulting broth is adjusted to about pH 2 using a mineral acid. It is then extracted with a smaller volume of an organic solvent such as amyl acetate or butyl acetate, keeping it at this very low pH for as short a time as possible. The aqueous phase is separated from the organic solvent usually by centrifugation.

The organic solvent containing the penicillin is then typically passed through charcoal to remove impurities, after which it is back extracted with a 2% phosphate buffer at pH 7.5. The buffer solution containing the penicillin is then acidified once again with mineral acid (phosphoric acid) and the penicillin

is again extracted into an organic solvent (e.g. amyl acetate). The product is transferred into smaller and smaller volumes of the organic solvent with each successive extraction process and in this way; the penicillin becomes concentrated several times over, up to 80-100 times. The penicillin may be converted to a stable salt form in one of several ways which employ the fact that penicillin is an acid:

**(a)** It can be reacted with calcium carbonate slurry to give the calcium salt which may be filtered, lyophilized or spray dried.

**(b)** It may be reacted with sodium or potassium buffers to give the salts of these metals which can also be freeze or spray dried.

**(c)** It may be precipitated with an organic base such as triethylamine.

When benzyl penicillin is administered intramuscularly it is given either as the sodium (or potassium) salt or as procaine penicillin. The former gives high blood levels but it quickly excreted. Procaine penicillin gives lower blood levels, but it lasts longer in the body because it is only slowly removed from the blood. It is produced by dissolvingsodium or penicillin in procaine hydrochloride.

***Penicillin Recovery :*** There are ten steps in the recovery of Penicillin:

1. Broth Filtration
2. Filtrate Cooled
3. Further Filtration
4. Extraction of Penicillin with Solvent
5. Carbon Treatment
6. Transfer back to Aqueous Phase
7. Solvent Recovery
8. Crystallization
9. Crystal Washing
10. Drying of Crystals

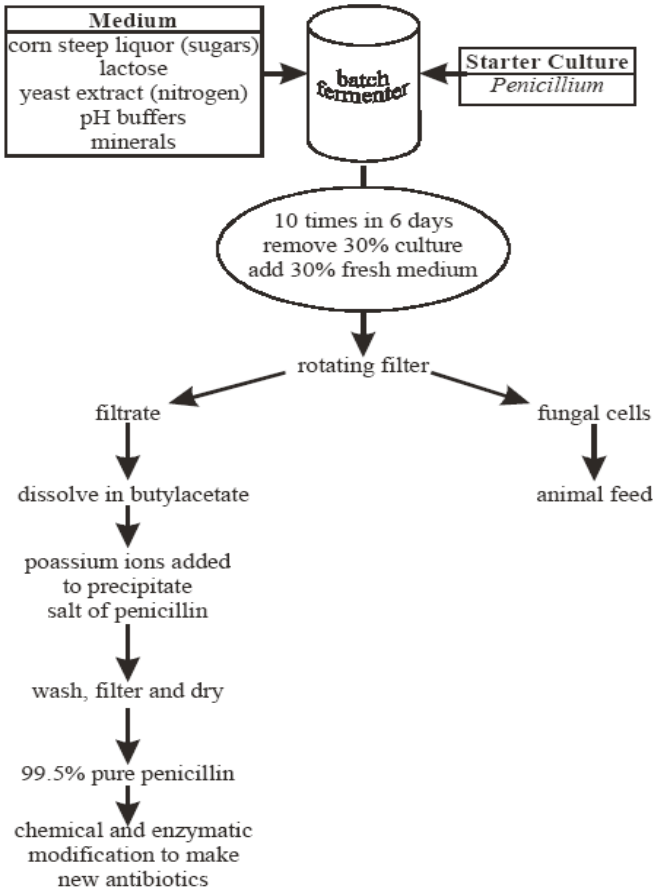



***Fig (1): steps in the recovery of Penicillin***

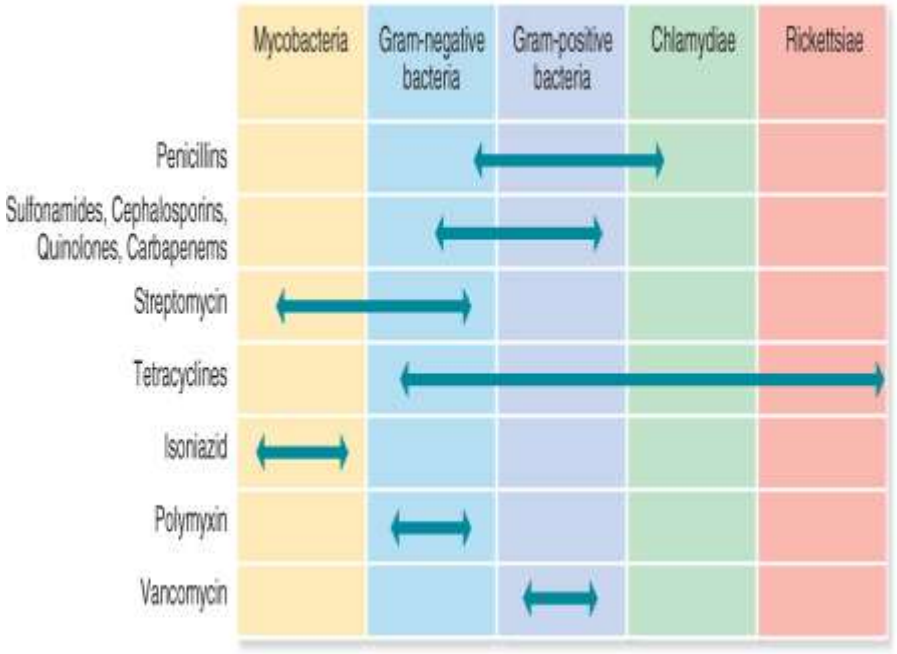



***Fig (2): Spectrum antimicrobial activity***