Industrial Production of Streptomycin

By- Dr. Ekta Khare

Department of Biosciences & Biotechnology

Streptomycin

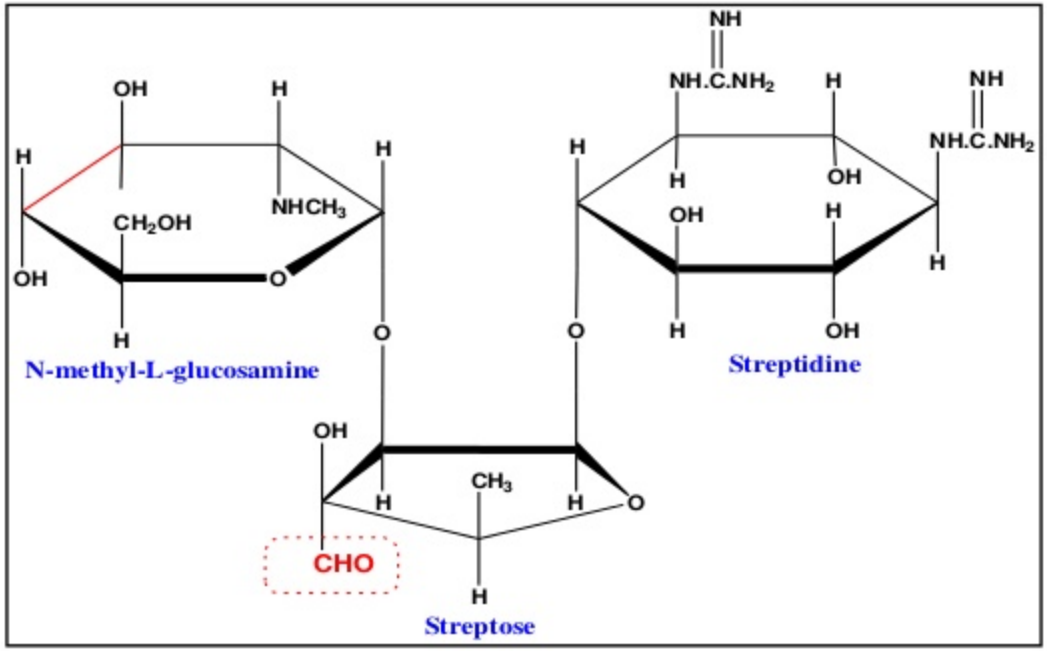
- Broad spectrum antibiotic
- Oligosaccharide antibiotic/ aminoglycoside family
- Discovered by Schatz, Bugie, and Waksman in 1944 from *Streptomyces griseus* isolated from soil

- Active against:
  - Gram (-) ve bacteria
  - Against tuberculosis bacterium, *Mycobacterium tuberculosis*.
  - However, it proved to be useful in the treatment of infections caused by Gram (+) ve specially resistant to penicillin.
  - It is also useful in the control of plant diseases caused by bacteria as it acts systemically in plants.
- One of the disadvantages of streptomycin is its neurotoxicity due to which hearing impairment and balance maintenance is lost in man due to prolonged streptomycin treatment at high dosage.
- Its reduction to dihydrostreptomycin results in the decreased toxicity. For this reason in recent times only dihydrostreptomycin is being produced.
- It is used mostly in conjunction with para aminosalicyclic acid or isoniazid (isonicotinic acid hydrazide) which minimizes resistance build up in sensitive microorganisms.

Structure

❖ Streptomycin is made up of 3 basic structural units called…

1. Streptidine(a diguanidino compound)
2. Streptose (a aldose sugar)
3. N-methyl-L-glucosamine.



- **Streptomycin is characterized chemically as an aminoglycoside antibiotic. It has the chemical formula $C_{21}H_{39}N_7O_{12}$**
- **Both guanido groups of streptidine are essential for the antibiotic activity. Removal of one group reduces activity up to 90%.**

Mechanism of action

- Streptomycin is a protein synthesis inhibitor. It binds to the small 16S rRNA of the 30S subunit of the bacterial ribosome, interfering with the binding of formyl-methionyl-tRNA to the 30S subunit.
- This leads to codon misreading, eventual inhibition of protein synthesis and ultimately death of microbial cells.

- Streptomycin is an antibiotic that inhibits both Gram-positive and Gram-negative bacteria, and is therefore a useful broad-spectrum antibiotic

Fermentation Process of Streptomycin

- Industrially streptomycin is produced by submerged culture method.
- Hocken hull (1963) recommended the medium consisting of:
  - glucose (2.5%)
  - soyabean meal (4.0%)
  - distillers dry soluble (0.5%)
  - sodium chloride (0.25%)
  - pH 7.3-7.5
- Spores of *S. griseus* maintained as soil stocks or lyophilized in a carrier such as sterile skimmed milk, is employed as stock culture.
- The spores from these stock cultures are then transferred to a sporulation medium to provide enough sporulated growth to initiate liquid culture build-up of mycelial inoculum in flasks or inoculum tanks.
- After sufficient mycelial growth, it is fed to production fermenter.
- Sterilized liquid medium is fed to the production fermenter. Appropriate volume of inoculum (4-5%) is introduced into it.
- The optimum fermentation temperature is in the range of 25 to 30°C
- The optimum pH range is between 7.0 and 8.0. High rate of streptomycin production, however, occurs in the pH range of 7.6 to 8.0.

Fermentation Kinetics

The process of fermentation is highly aerobic and lasts approximately for 5 to 7 days and passes through 3 phases:

(a) The First Phase:

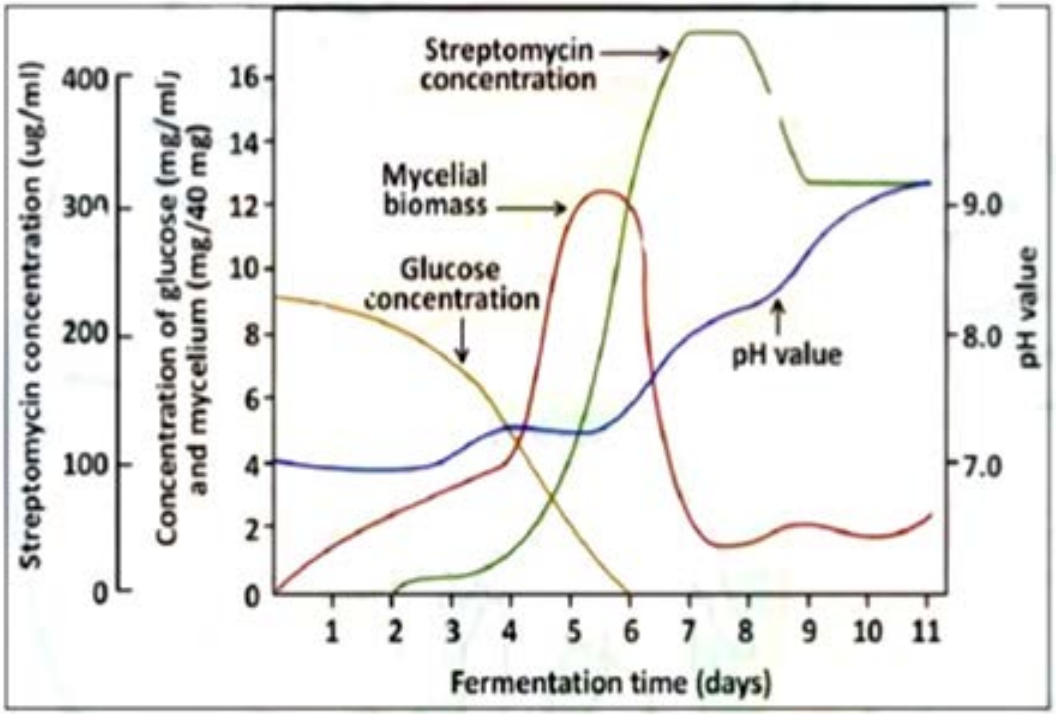
- It takes about 24 hours to 48 hours.
- Rapid growth and formation of abundant mycelium occurs during this phase.
- The pH rises to 8.0 due to release of ammonia into medium, due to proteolytic activity of *S. griseus*.
- Glucose is utilized slowly and little production of streptomycin is witnessed.

(b) The Second Phase:

- It lasts for 2 days.
- Streptomycin production takes place at a rapid rate without increase in the mycelial growth.
- The ammonia released in the first phase is utilized, which results in the decrease of pH to 7.6-8.0.
- Glucose and oxygen are required in large quantity during this phase.

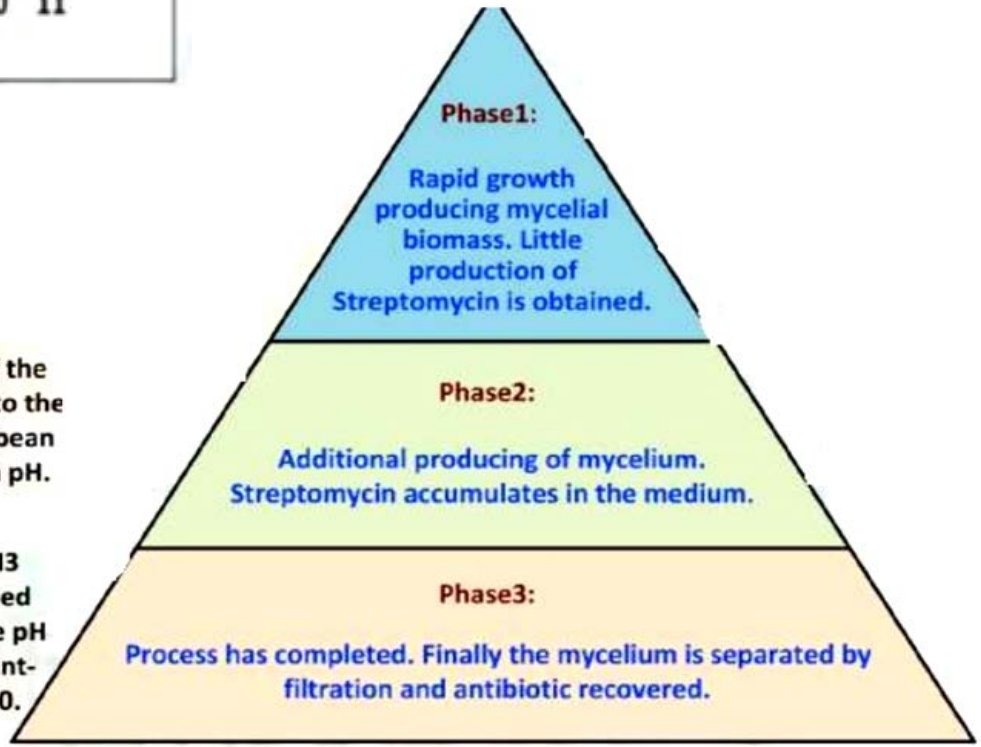
(c) Third Phase:

- Cells undergo lysis, releasing ammonia and increase in the pH, which falls again after a period of continuous streptomycin production.
- Requirement of oxygen decreases and the contents of the medium including sugar get exhausted.
- Finally streptomycin production ceases.
- A yield of 1200 micrograms per milliliter of streptomycin is obtained.



Proteolytic activity of the microbe releases NH3 to the medium from the soybean meal, causing a rise in pH.

The glucose and NH3 releases are consumed during this phase. The pH remains fairly constant- between 7.6 and 9.0.



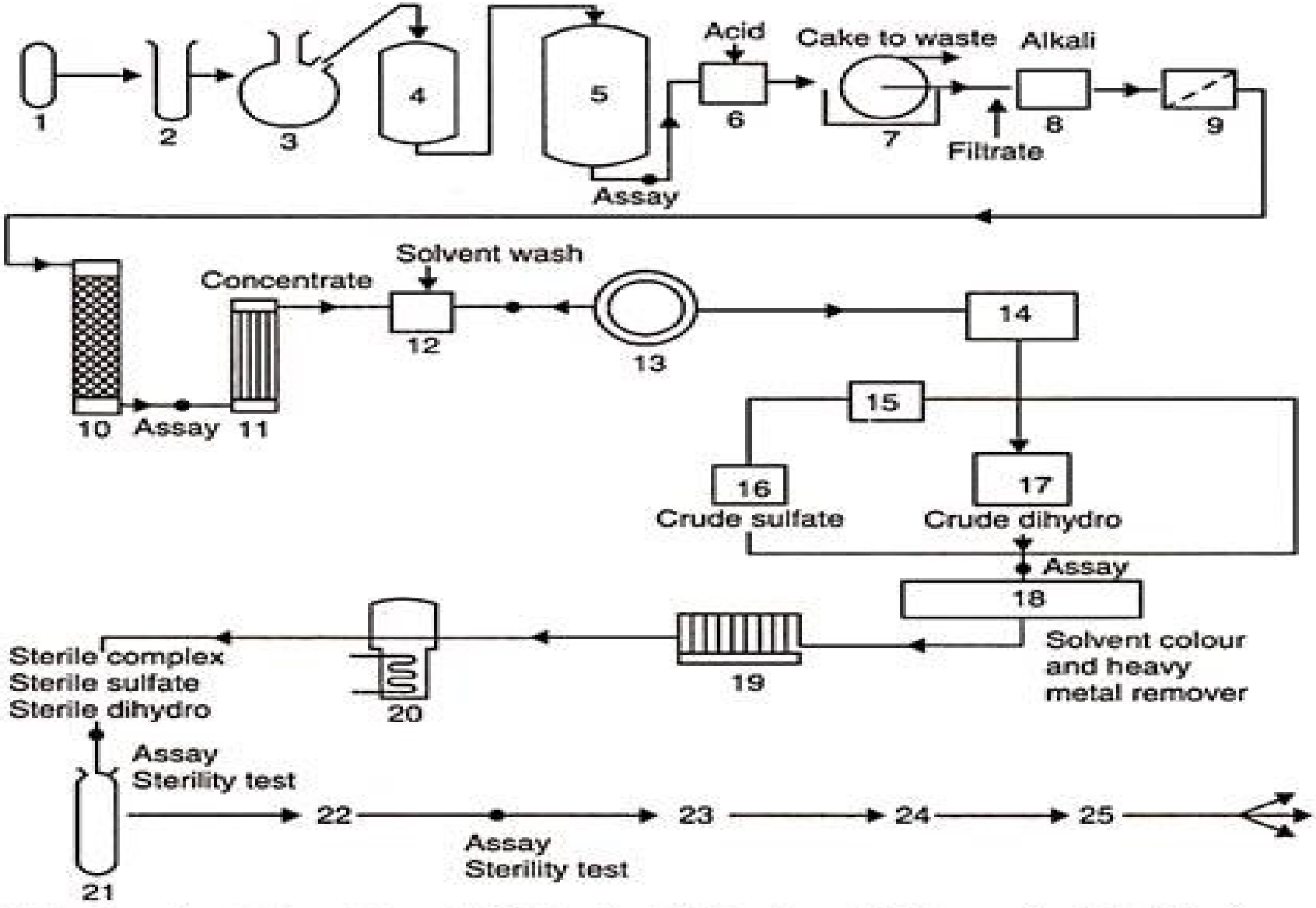
Phases of streptomycin production.

Harvest and Recovery

- After completion of fermentation the mycelium is separated from the broth by filtration. Streptomycin is recovered by several methods.
- **But the one which is generally employed is described below:**
- The fermentation broth is acidified, filtered and neutralized.
- It is then passed through a column containing a cation exchange resin to adsorb the streptomycin from the broth.
- The column is then washed with water and the antibiotic is eluted with hydrochloric acid or cyclohexanol or phosphoric acid.
- It is then concentrated at about 60°C under vacuum.
- The streptomycin is then dissolved in methanol and filtered and acetone is added to the filtrate to precipitate the antibiotic.
- The precipitate is again washed with acetone and vacuum dried. It is purified further by dissolving in methanol.
- The streptomycin in pure form is extracted as calcium chloride complex.

Byproduct Vitamin B_{12}:

- Vitamin B_{12} is produced as a byproduct which will not affect adversely the yield of streptomycin.



1. Master culture 2. Agar slopes 3. Shaker flask 4. Seed vessel 5. Fermentor 6. Acidification
7. Filtration 8. Neutralization 9. Filter clarification 10. Ion-exchange reagent 11. Evaporator
12. Crystallization 13. Vacuum oven 14. Calcium chloride crude complex 15. Calcium chloride removal
16. Crystallization 17. Catalytic hydrogenation 18. Finishing 19. Seitz filter 20. Freeze drying
21. Vial filling machine 22. Capping 23. Labelling 24. Packing 25. Despatch

Fig. 6.12: Flow sheet of streptomycin production by submerged-method

Questions

- Write a short note on streptomycin.

  Ans. Discovery, disadvantage, structure in brief, mode of action, broad spectrum
- Write in detail industrial production of streptomycin.
- Briefly discuss the fermentation kinetics of streptomycin.
- Short note on down stream processing of streptomycin production.