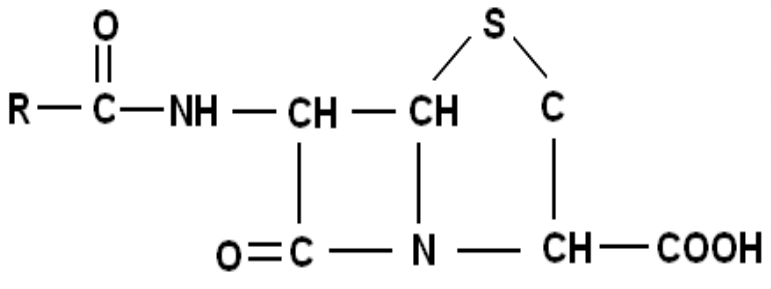
Penicillin Production

Penicillin fermentation

- Penicillin was the first antibiotic to be produced on a large scale and it is one of the best antibiotics available today.
- It was produced on large scale due to the impetus of World War II that protected many soldiers from battle field diseases.
- Penicillin was the first antibiotic discovered by Alexander Fleming in 1929 in an accidental incident. Fleming observed the production of Penicillin F from *Penicillium notatum*.
- This was further studied by Florey and Chain, who conducted trials for the production of penicillin from *P. notatum* by stationary mat culture technique in liquid broth.
- Florey, Chain and Fleming shared Nobel Prize for this vital discovery in 1942.
- *P. chrysogenum* was later found to provide greater yields as compared with *P. notatum*.
- The strains of Penicillin were mutated (strain improvement) for the overproduction of penicillin and no production of pigments as high-yielding strains were known to produce a pigment that gives yellow tint to final product due to water soluble pigment, chrysogenin. Thus present day strains of penicillin are high-yielding strains with no production of any pigments.

Structure of Penicillin and Precursors

- Penicillin is a generic name applied to a group of compounds having the same nucleus and approximately same antibiotic activity against sensitive microbes.
- Penicillins differ from each other in terms of "R" side chain group with a common nucleus.
- Fleming's original strain *P. notatum* strain when grown in his medium (where precursors were not utilized) produced Penicillin F (2-pentenyl penicillin).
- *P. chrysogenum* produced penicillin K with smaller amounts of penicillin F.
- Later, it was discovered that addition of corn steep liquor to production medium increases the yield of penicillin, particularly penicillin G as produced by *P. chrysogenum*. On further studies, it was noted that corn steep liquor contains phenylalanine and its breakdown products, phenethyl amine and phenylacetic acid. Thus it was clarified that addition of such compounds as precursors leads to the higher yields of penicillin G.
- Thus, synthesis of a particular type of penicillin by fungi may be directed by the addition of specific precursors.

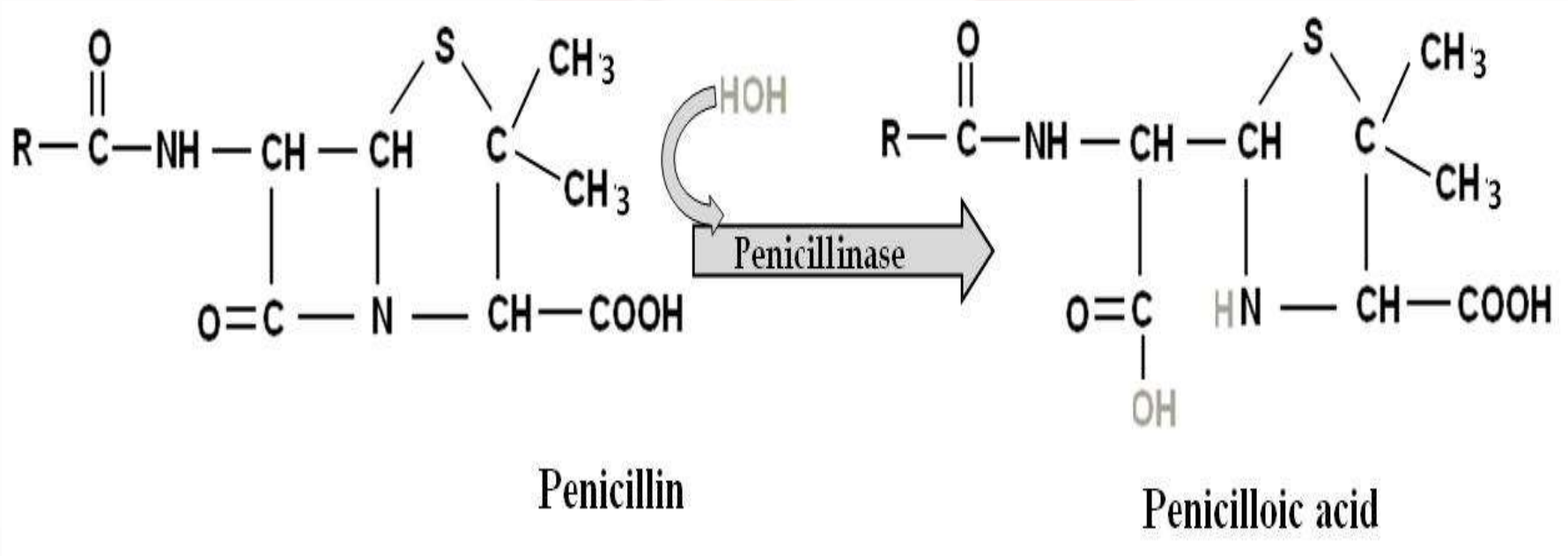


Properties of Penicillin

- ***Activity spectrum*** **-** active against many Gram positive bacteria, *Nocardia,* and *Actinomyces,* but not against most Gram negative bacteria (except at higher dosage levels).
- ***Mode of action:*** It interferes with peptidoglycan synthesis which is a principal constituent of bacterial cell wall and thus active against only growing cells.
- ***Toxicity:*** It is generally regarded as nontoxic to mammals, but certain allergic reactions may develop in a small percentage of individuals such as itching and rashes on skin.
- ***Types:*** There are several penicillins and all are closely related in structure and in activity against sensitive microorganisms.
- ***Physicochemical characters:*** It is soluble in water. It is very soluble in acetone, amyl alcohol acetate, cyclohexane, ethyl acetate, ethyl alcohol and ether. In benzene, chloroform and carbon tetrachloride, it is less soluble. It is unstable under number of conditions; the crystalline salts of penicillin, at low temperatures, are stable over a period of several months. Aqueous solutions of penicillin are unstable and must bee stored under refrigeration. Penicillin is most stable in the pH range of 6.0 to 6.5 and stable over the pH range of 5.5 to 7.5.

Action of Penicillinase

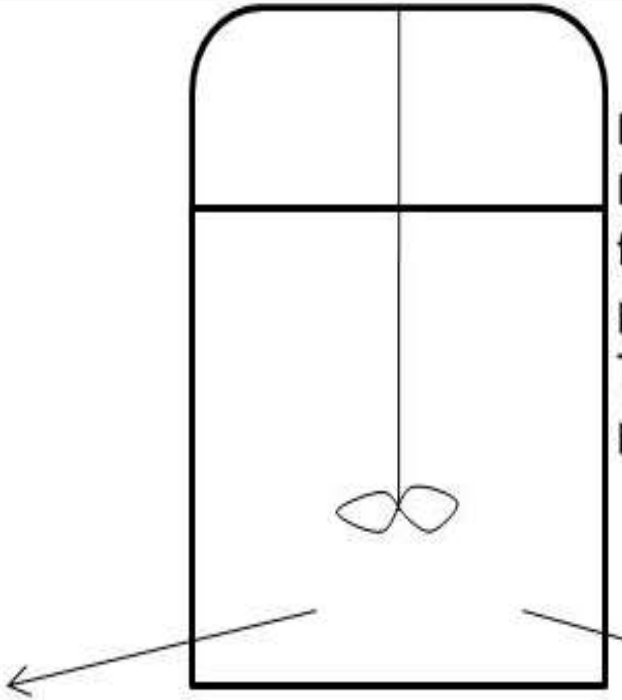
- Penicillinase is an extracellular enzyme, produced by members of coliform group of bacteria, by most *Bacillus* sp. and by some strains of *Staphylococcus*.
- This enzyme hydrolyses penicillin to penicilloic acid.
- Most penicillin resistant pathogenic strains of *S. aureus* contain this enzyme. This enzyme hydrolyzes penicillin to penicilloic acid. Thus, such microbes are the major threats to fermentation industry.



Semisynthetic penicillins

- These are synthesized by a combined process that includes enzymatic conversion of penicillin G to 6-APA (6-Aminopenicillanic acid) followed by its chemical conversion to desirable penicillin.
- This type of synthesis was possible only after the discovery of ***penicillin acylase*** enzyme required for the generation of 6-APA.
- This enzyme cleaves the side chain from penicillin as shown in figure. Various species of bacteria, *Nocardia*. *Streptomyces* and fungi including *P. chrysogenum* produce this enzyme extracellularly.
- Thus, the commercial production of this enzyme is possible. These discoveries made it possible to synthesize penicillin of interest i.e., semisynthetic penicillin.

Production

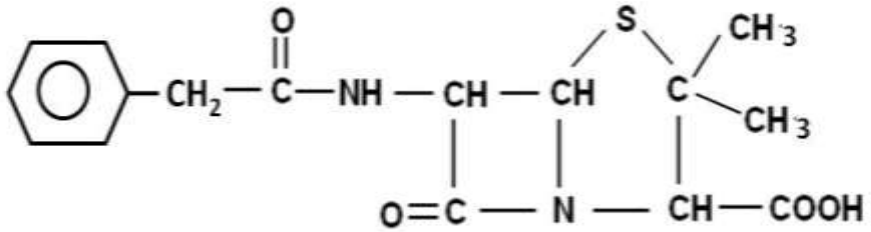


Phenylacetic acid
reused as precursor in
penicillin G production

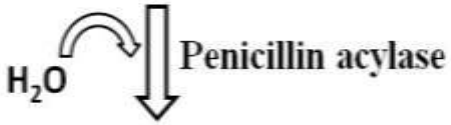
Recovery of 6-APA
pH is lowered to 4.3
Precipitation of 6-APA

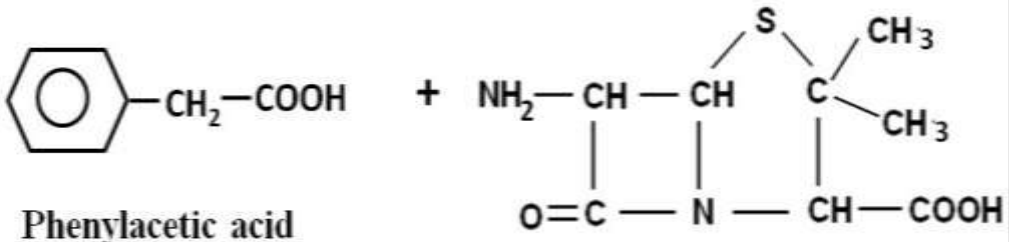
Production

(A)



Penicillin G (Benzyl Penicillin)

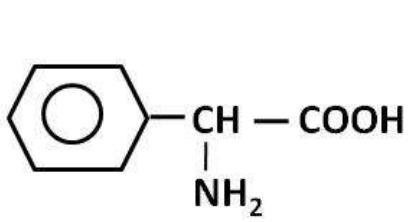




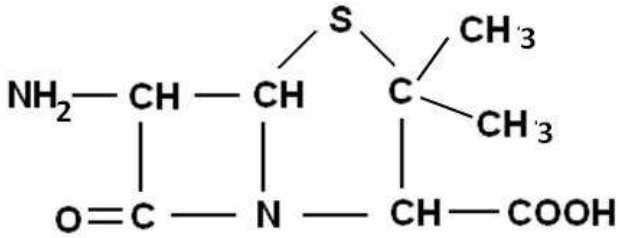
Phenylacetic acid + 6-Aminopenicillanic acid

Reused as precursor

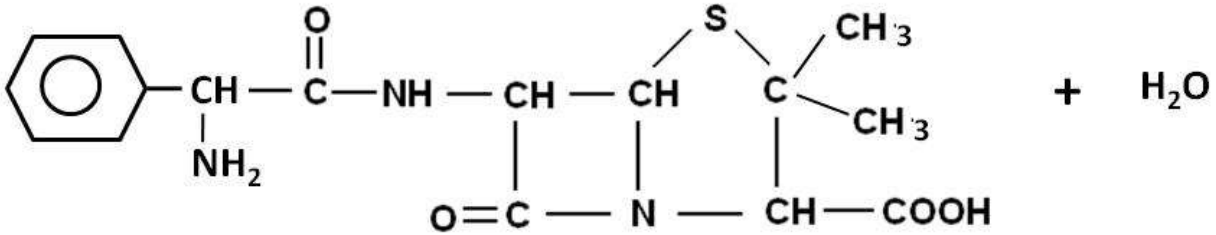
(B)



Phenylglycine +



6-Aminopenicillanic acid



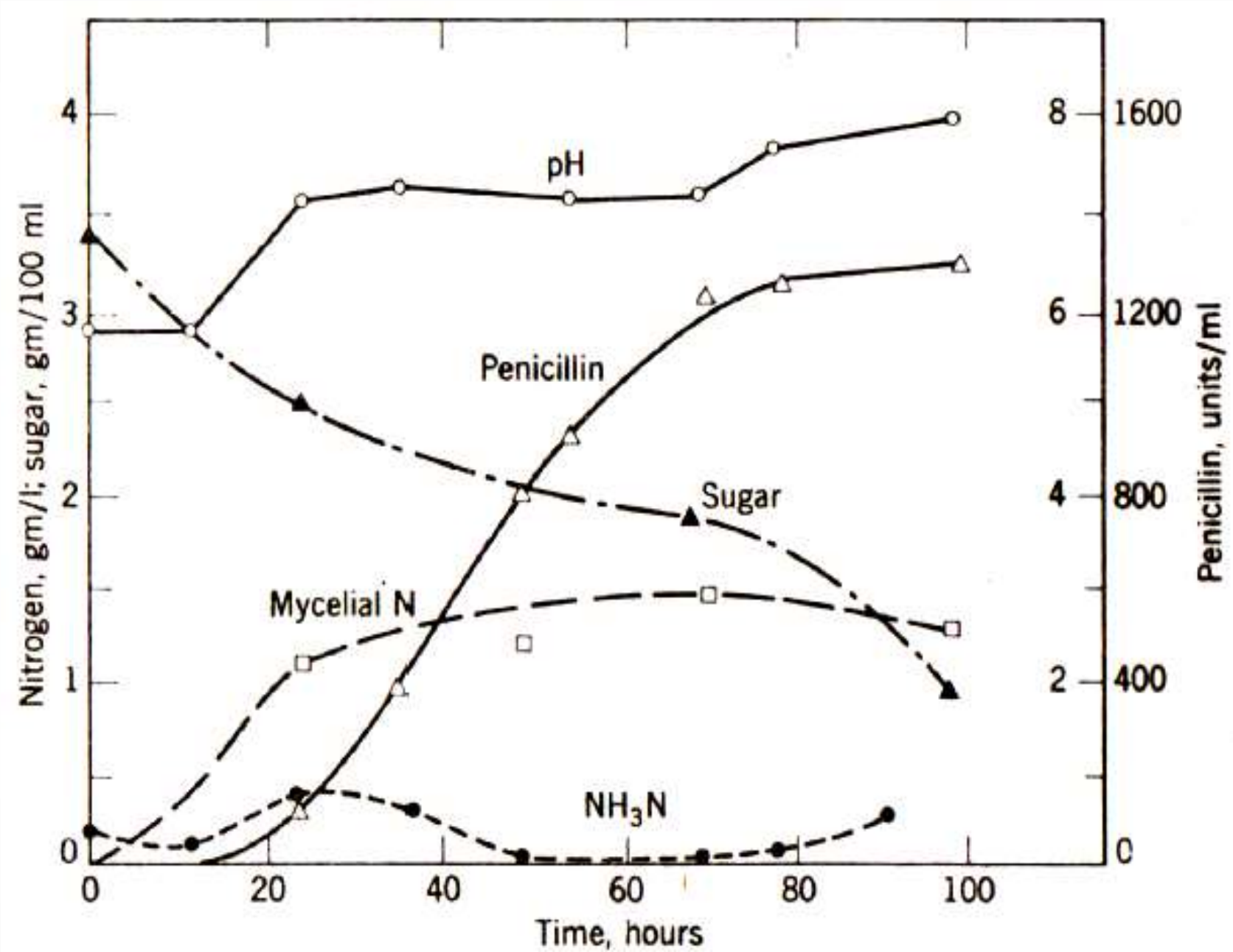
Ampicillin + H_2O

(A broad spectrum Semisynthetic penicillin)

Penicillin production

- Initially the process of production of penicillin was by stationary mat culture. But this technique was associated with several drawbacks such as poor aeration, contamination by penicillinase producing bacteria, requirement of large amount of labors and equipments, and poor yield.
- This technique was further improved as replacement mat culture technique where mycelial mat used in the first batch was reused by placing it in the fresh medium in the trays.
- *Inoculum development* - Inoculum production is done by inoculation of spores from working stocks are suspended in water with surface active agents such as sodium lauryl sulfonate. These spores are then inoculated in to flasks or bottles of wheat bran nutrient solution followed by incubation at 24^{o}C for five to seven days for heavy sporulation.
- *Production* - The production tanks are then inoculated with 10% by volume of inoculum into the tank. The actual penicillin fermentation is incubated at approximately 25 to 26^{o}C for approximately three to five days. The exact composition of production medium actually used in the industry is a trade secret. However, most of these media have similar composition. A typical medium used contains corn steep liquor solids, 3.5%; lactose, 3.5%; glucose, 1%; calcium carbonate, 1 %; potassium phosphate, 0.4%; edible oil, 0.25% and penicillin precursor. The pH of the medium is 5.5 to 6.

Course of fermentation



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