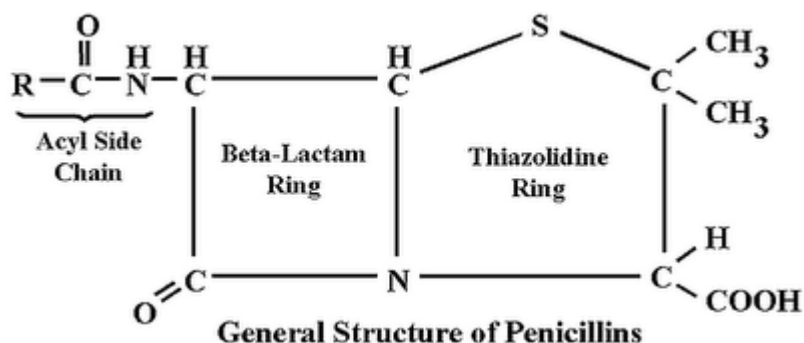


Module-26: Penicillin Fermentation

Introduction:

- The term antibiotic has been defined by Selman Waksman as being an organic compound produced by one microorganism that, at great dilutions, inhibits the growth of or kills another or even group of other harmful microorganisms.
- Antibiotics are available in various forms.
- For example, to treat bacterial infection on the surface, an antibiotic should be there in an ointment or cream form.
- But to treat internal infection it can be directly injected into the bloodstream which finally distributed throughout the body.
- Antibiotics are produced primarily by bacteria, Streptomyces, Nocardia and fungi.
- However, antibiotics produced by Streptomyces spp. Have found greatest commercial applications.
- On the basis of mode of action antibiotics are divided into various groups like,
 - i Antibiotics affecting cell wall
 - ii Antibiotics damaging cell membrane
 - iii Antibiotics interfering with protein synthesis
 - iv Antibiotics inhibiting nucleic acid synthesis
 - v Antibiotics blocking cell metabolism
- Fermentation is the process used for the large-scale production of an antibiotic.
- The first discovered natural antibiotic was **Penicillin**.
- Penicillin was obtained from multicellular fungi, “Penicillium molds”.
- Penicillin is a group of compounds having common basic nucleus, 6-amino penicillanic acid (6-APA).
- 6-APA contains ring like structure termed as a β -lactam ring.
- Penicillin are of two different types,
 - i Natural Penicillin
 - ii Semi-synthetic Penicillin
- **Natural penicillin** is directly harvested from the *penicillium mold*.
- **Semi-synthetic penicillin** consists of the basic Penicillin nucleus (6-APA), but with new side chain that change properties of natural penicillin.
- Semi synthetic penicillin includes Ampicillin, Methicillin, Penicillin V, etc.
- They are produced by modifying natural penicillin by removing the natural acyl group to leave 6-APA and then adding new acyl groups having different properties like,
 - i resistance to stomach acids so it can be taken orally
 - ii a degree of resistance to penicillinase (penicillin degrading enzyme)
 - iii an extended range of activity against some Gram-negative bacteria.



History

- In 1928, Sir Alexander Fleming made one of the most important contributions to the field of antibiotics.
- He first observed the antibiotic properties & therapeutic value of penicillin.
- In an experiment, he observed that air born contaminant, later shown to be *Penicillium notatum*, inhibited the growth of a culture of *S.aureus* on an agar plate.
- He called this material penicillin after the mold that had produced it.
- By that the progress of the first modern era antibiotic, penicillin gets started.
- In 1932, he published paper, which proposed a method for use of penicillin in treatment of infected wounds.
- But early samples of penicillin were not purified, and further refinements were needed.
- Howard Florey and associates discovered a new high yielding strain of *Penicillium* in the 1940s.
- This allowed extensive production of penicillin, which helped launch the modern antibiotics industry.
- Dr. Ernst B. Chain was there with Florey's team, who initiated extracting penicillin into a purified and powerful antibiotic.
- Later on, different scientists had work on in detail regarding different types of penicillin and their production on large scale.
- Penicillin is acting against many Gram positive bacteria, Nocardia, and Actinomycetes, but not against most Gram negative bacteria except at higher dosage level.
- It interferes with cell wall synthesis of actively growing sensitive organisms.
- It mainly inhibit the cross linking steps of peptidoglycan synthesis in the cell wall.
- The World War II had brought a demand for penicillin on a large scale for the treatment of burns and wounds.
- By the end of the war (late 1943), mass production of the penicillin had started by many drug manufacturing companies.
- In 1945 Fleming, Florey and Chain were awarded the Nobel Prize in Physiology and Medicine.

Penicillin Fermentation

Microorganisms

- Out of various species of the fungus *Penicillium* mainly two species are used in the fermentation.
- These are *P. notatum* & *P. chrysogenum*.
- Even from these two *P. chrysogenum* is high yielding strain and therefore most widely used as production strain.
- The production strain is improved by mutation with the help of X-rays or any other agents to give high yield.
- After strain improvement the production strain should be carefully maintained because *P. chrysogenum* is genetically unstable.
- Different preservation techniques are used like,
 1. A spore suspension may be mixed with a sterile, finely separated inert support like soil or sand and then desiccated.
 2. The spore suspension can be stored under liquid nitrogen (-196°C) i.e. in a frozen state.
 3. The spore suspension can be lyophilized in appropriate media.

Inoculum preparation

- Here the chief purpose is to develop a pure inoculum in an adequate amount and in the fast growing phase for the production stage fermenter.
- To do so various sequential steps are necessary like,
 1. A starter culture which is available in cold-stored form is transferred to an agar-containing plate to allow growth.
 2. After getting growth on solid media, one or two growth stages should allowed in shaken flask cultures to create a suspension, which can be transferred to seed tanks for further growth.
 3. The seed tanks are made up of stainless steel which is designed to provide an ideal environment to production strain. They contain all the nutrients including Growth factors like vitamins & amino acids. The seed tanks are equipped with agitators, which allow continuous mixing of growth medium, and a pump to deliver sterilized, filtered air.
 4. After about 24-28 hours, the content of the seed tanks is transferred to the primary fermentation tanks.
 5. The main fermentation tank is a larger version of the seed tank, which contains same growth media and also provides proper growth promoting environment. During this process, they excrete huge amounts of the desired antibiotic.
 6. All the bio parameters like temperature, pH, aeration, agitation etc. should be properly maintained.

Bio parameters

- PH: near 6.5
- Temperature: 26°C to 28°C
- Aeration: a continuous stream of sterilized air is pumped into it.
- Agitation: have baffles which allow constant agitation.

Raw Materials

- Raw materials are primary requirement to design the fermentation broth for antibiotic production.
- Fermentation broth contains all the necessary elements required for the proliferation of the microorganisms.
- Generally, it contains a carbon source, nitrogen source, mineral source, precursors and antifoam agents if necessary.

Carbon Source

- Lactose acts as a very satisfactory carbon compound if it is used in a concentration of 6%.
- Other carbohydrates like glucose & sucrose may be used but it has to provide with slow feeding rate.
- Complex as well as cheap sources like molasses, or soy meal can also be used which are made up of lactose and glucose sugars.
- These materials are desired as a food source for the organisms.

Nitrogen Source

- Another essential compound for metabolism of organisms is nitrogen.
- Ammonium salts such as ammonium sulfate, ammonium acetate, ammonium lactate or ammonia gas are used for this reason.
- Sometime corn steep liquor may be used.

Mineral Source

- Additionally, some minerals are necessary for the proper growth of these organisms. are included.
- These elements include phosphorus, sulfur, magnesium, zinc, iron, and copper which generally added in the form of water soluble salts.

Precursors

- Various types of precursors are added into production medium to produce specific type of penicillin.
- The most important naturally occurring penicillin is penicillin-G.

- But depending upon the precursors added, the type of penicillin going to produced can be changed.
- For example, if phenyl acetic acid is provided then only penicillin-G will be produced but if hydroxy phenyl acetic acid is provided then penicillin-X will be produced.
- Phenoxy acetic acid is provided as precursor for penicillin-V production.
- When corn steep liquor is provided as nitrogen source, it also provides phenyl acetic acid derivatives; therefore it is widely used in the production of penicillin-G.

Anti-foam agents

- Anti-foaming agents such as lard oil, octadecanol and silicones are used to prevent foaming during fermentation.
- Following three points should be kept in mind before choosing raw materials for manufacture of penicillin,
 1. An abundant growth of mycelium
 2. Maximum accumulation of penicillin
 3. Ease of extraction and purification of antibiotics.

Production medium

Components	Percent (%)
Lactose:	3.5 to 6
Calcium Carbonate:	1.0
Cornsteep Liquor:	3.5
Glucose:	1.0
Phenyl acetic acid:	0.5
Sodium hydrogen phosphate:	0.4
Antifoaming Agent: Edible oil:	0.25

- In inoculum medium lactose is generally absent because it induces penicillin production and retarding the growth of production strain.

Recovery

- The recovery of penicillin is carried out in three successive stages:
 1. Removal of mycelium
 2. Counter current solvent extraction of penicillin
 3. Treatment of crude extracts
- At harvest the fermentation broth is filtered on a rotatory vacuum filter to remove the mycelium and other solids.

- Phosphoric or sulfuric acids are added to lower the pH (2 to 2.5) in order to transform the penicillin to the anionic form.
- Then the broth is directly extracted in a Podbielniak Counter Current Solvent Extractor with an organic solvent such as methyl isobutyl ketone, amyl acetate or butyl acetate.
- The filtration is carried out under such conditions which avoid contamination of the filtrate with penicillinase producing organisms which otherwise may allow serious or full loss of an antibiotic.
- This step has to be carried out rapidly because penicillin is very unstable at low pH values.
- Penicillin is then again extracted into water from the organic solvent by adding an adequate amount of potassium or sodium hydroxide to form a salt of the penicillin.
- The resulting aqueous solution is again acidified & re-extracted with methyl isobutyl ketone.
- This shifts between water and solvent help in purification of the penicillin.
- The solvent extract is carefully back extracted with NaOH and from this aqueous solution; various procedures are utilized to cause the penicillin to crystalize as sodium or potassium penicillinate.
- The resulting crystalline penicillin salts are then washed and dried.
- Final product must pass rigorous government standards.
- Spent solvents resulting from the above procedure are recovered for re use.
- Sometimes the crude extract of penicillin is passed out from charcoal treatment to eliminate pyrogens; even sterilization can also be done.
- Sterile vials are used for packaging of an antibiotic either as a powder or suspension.
- For oral use it is tableted usually with a film coating.
- It must satisfy all the criteria of the government standards before being marketed.

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