Antibiotics Production:

Antibiotics are substances produced by various species of microorganisms: bacteria, fungi, actinomycetes; to suppress the growth of other microorganisms and to destroy them (Bacteriostatic, Bactericidal). Today the term ATB extends to include synthetic antibacterial agents: sulfonamides and quinolones.

Production of antibiotics can be done by three methods.

1. Natural microbial production using fermentation technology.

2. Semi synthetic production (post production modification of natural antibiotics).

3. Synthetic production of antibiotics.

Although most antibiotics occur in nature, they are not normally available in the quantities necessary for large-scale production.

For this reason, a fermentation process was developed. It involves isolating a desired microorganism, fueling growth of the culture and refining and isolating the final antibiotic product. It is important that sterile conditions be maintained throughout the manufacturing process, because contamination by foreign microbes will ruin the fermentation.

The most important problem associated with infectious disease today is the rapid development of resistance to antibiotics.

The fermentation process requires the following:

1. A pure culture of the chosen organism, in sufficient quantity and in the correct physiological state.
2. Sterilized, carefully composed medium for growth of the organism.
3. A seed fermenter, a mini-model of production fermenter to develop inoculums to initiate the process in the main fermenter.
4. A production fermenter, the functional large model.
5. Equipment for:

   a) Drawing the culture medium in steady state.

   b) Cell separation.

   c) Collection of cell free supernatant.

   d) Product purification.

   e) Effluent treatment.

   Step 1 to 3 constitutes the **upstream** and step 5 constitutes the **downstream** of the fermentation process.

Raw Materials:

The compounds that make the fermentation broth are the primary raw materials required for antibiotic production. Typically, it contains a

1- Carbon source: like molasses, or soy meal, both of which are made up of lactose and glucose sugars.

2- Ammonia salt: is used as a nitrogen sources.

3- Trace elements: such as phosphorus, sulfur, magnesium, zinc, iron, and copper introduced through water soluble salts.

4- Anti-foaming agents such as lard oil, octadecanol, and silicones are used to prevent foaming during fermentation.

Penicilline:

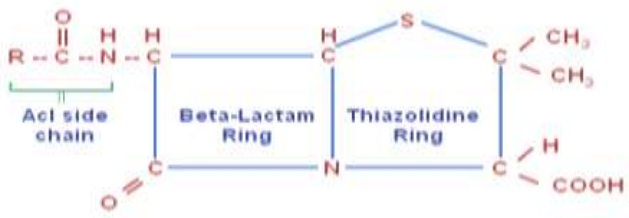
- Discovered by Alexander Fleming in 1928.
- In 1939, Howard Florey and Ernst Chain managed to purified Penicillin in powder form.
- In 1943, they produced Penicillin in large scale.
- Penicillin was first made at the end of the second world war using the fungus *Penicilium notatum* but now *penicillium chrysogenum* is used

General Structure:

Have β- Lactam functional group, thus belong to the β- Lactam antibiotic group.

How dose Pencillin work:

- Inhibits enzymes involved in synthesis of peptidoglycan for bacterial cell wall, causing cell lysis.
- Effective against actively growing G+ve bacteria, which have a thick cell wall.
- Narrow spectrum- little effect on Gram negative cells.
- Amoxicillin also effective against G-ve bacteria.
- Semisynthetic penicillins: made in laboratory by adding different side chains onto β-lactam ring ⇒ penicillinase resistant and broader spectrum of activity



General Structure of penicillins

Classification of penicillin:

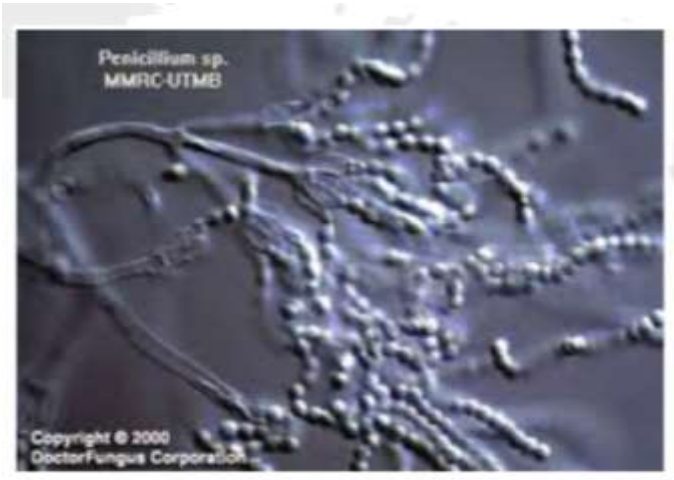
1. Natural Penicillin.
2. Penicillinase – Resistant penicillins
3. Aminopenicilins.
4. Extend spectrum penicillins.

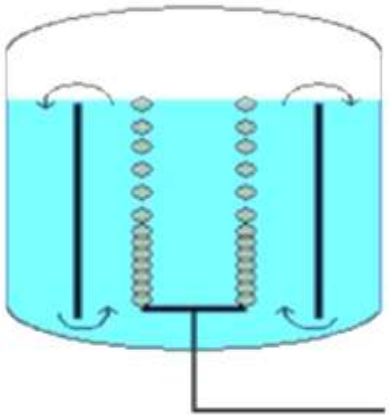
Categorized based on their ability to kill types of bacteria and how effective are they in doing so.

Specific condition for Penicillin production:

Most Penicillin form filamentous broths. This means they can be difficult to mix due to their viscosity. Also the increasing viscosity can hinder oxygen transfer.

Therefore, producing bubble columns (air lift reactors) which would distribute the oxygen equally and agitate the medium.



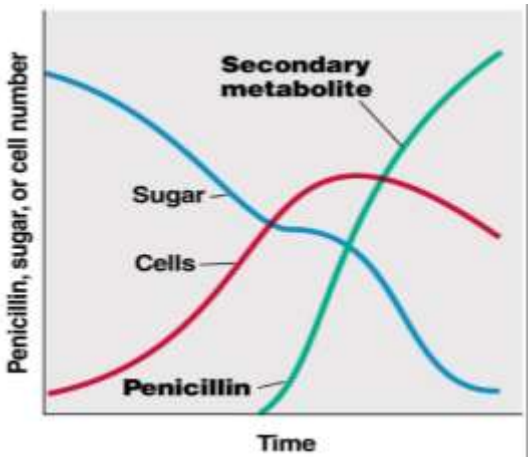


Media Formulation:

- pH 6.5
- Temperature 20 – 24 ^{o}C
- Oxygen
- Nitrogen: corn steep liquor 8.5%
- Glucose 1%
- 80% ethanol
- Phenylacetic acid
- Probenecid
- Lactose 1%
- Calcium Carbonate 1%
- Sodium hydrogen phosphate 0,4%
- Antifoaming agent: vegetable oil
- Microorganisms require C, H, O, S and N for cell growth and cell maintenance.
- Also require small amounts of trace elements such as Cu, Mn and Co (frequently depend on the water source) or growth factors such as vitamins or amino acids.
- Certain organisms such as *penicillium chrysogenum* that produce antibiotics, enzymes or other secondary metabolites frequently require precursors like purine/pyrimidine bases or organic acids to produce metabolites.

Production Method:

- Secondary metabolites are only produced in times of stress when resources are low and the organism must produce these compounds to kill off its competitors to allow it to survive.



Stages of production

1. Primary metabolism will be emphasized. Media for this stage will be focused on achieving maximum growth and biomass production.

2. Once the desired biomass has been achieved, starve (Limiting the amount of C and N available to the culture) the culture and induce the kind of stress conditions that trigger the production of the antibiotic.

* Use the fed – batch method to feed the culture. As stated above, this allows us to add the substrate to the reactor in small increments and to even change the substrate if we desire.