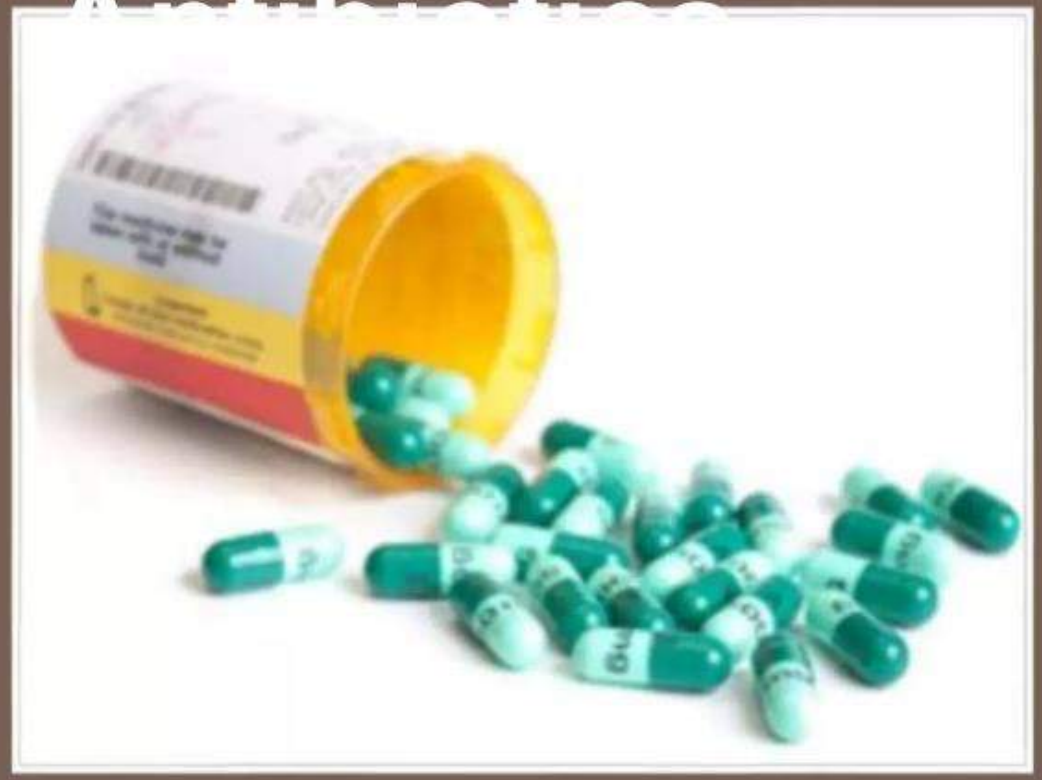
Production of Antibiotics



Antibiotics

- Compound that kill or inhibit the growth of other organisms.
- Most Antibiotics are produced by filamentous fungi or Actinomycetes.
- They are derived from special microorganisms or other living systems, and are produced on an industrial scale using a fermentation process.
- Today, over 10,000 antibiotic substances have been reported.

- Antibiotics are produced by fermentation.

Any large-scale microbial process occurring with or without air is called Fermentation.

- The process may take a few days to obtain an extractable amount of product.
- Antibiotic production is done by the batch process.

Properties of the Microorganism

- Properties of useful industrial microbe include:
- Grow rapidly on large scale in inexpensive medium.
- Produce desired product quickly.
- Should be Non-pathogenic.
- Amenable to genetic Modification.

Products produced by Microorganisms

- Microbial cells
- Enzymes
- Antibodies
- Steroids
- Alkaloids
- Food additives
- Ethanol, citric acid etc

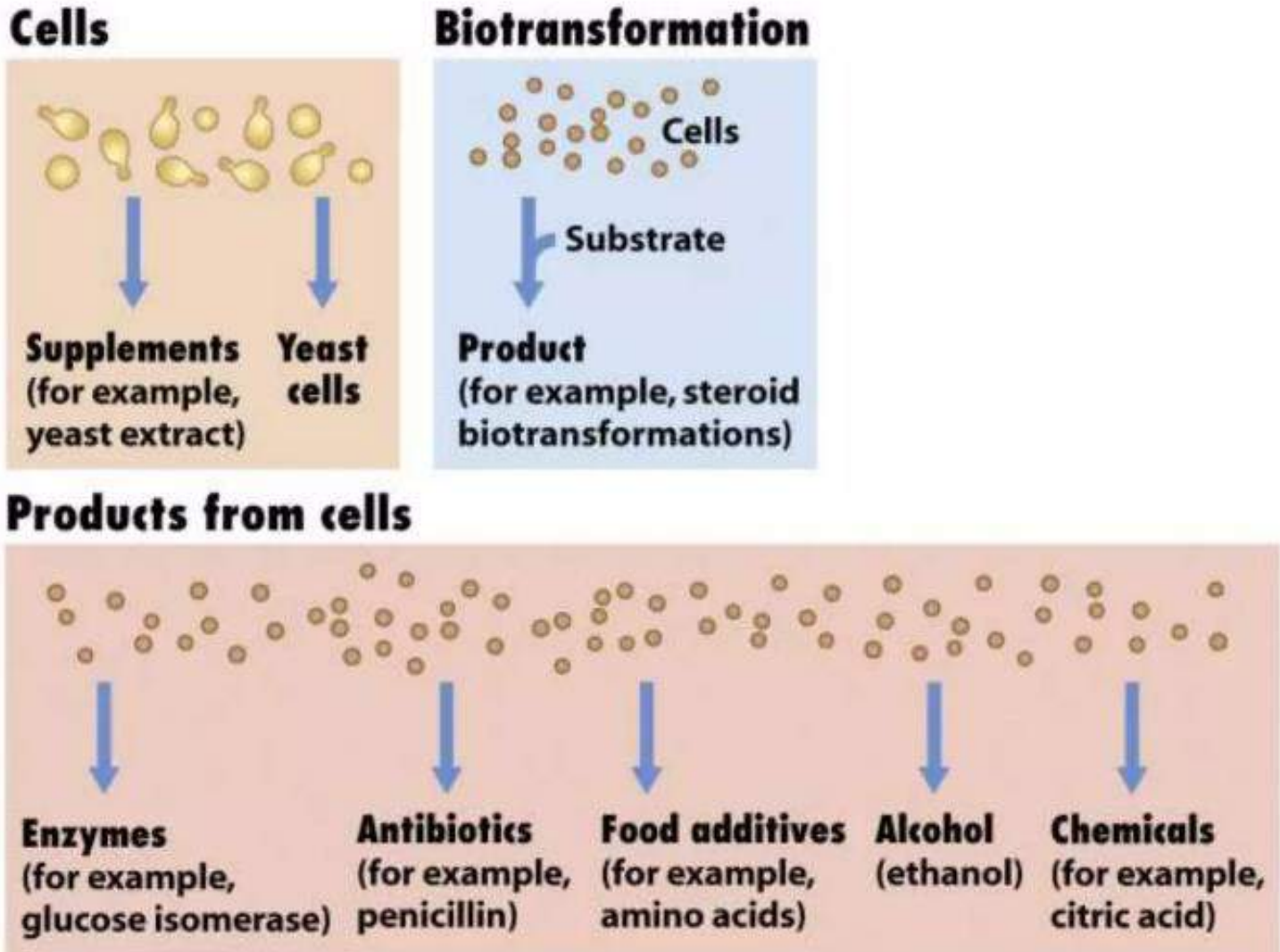


Figure 30-1 Brock Biology of Microorganisms 11/e

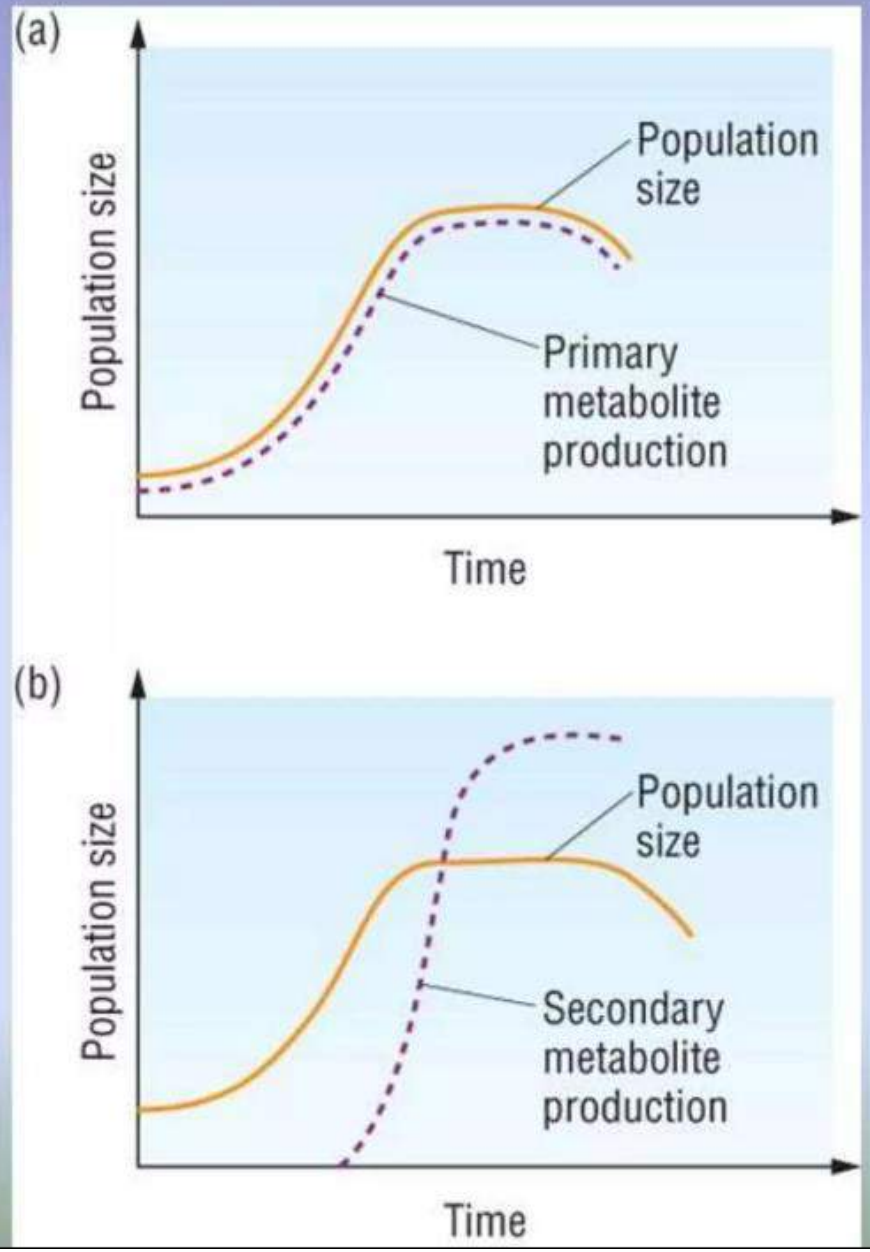
Primary Metabolites

- **Metabolites:** Metabolites are the intermediates and products of metabolism.
- **Primary Metabolites:** The metabolites which are required for the growth and maintenance of cellular functions are called primary metabolites.
- Primary metabolites are formed during the growth phase.
- Examples are amino acids, vitamins, carbohydrates, lipids, nucleic acids and enzymes.

Secondary Metabolites

- **Secondary Metabolites:** The metabolites which are not required for the growth and maintenance of cellular functions and are the end products of primary metabolites are called secondary metabolites.
- Secondary metabolites are formed near the stationary phase of growth.
- Drugs, toxins, steroids and polymeric substances like rubber are some of the examples of secondary metabolites.

Primary metabolite and Secondary metabolite



Growth and product curves showing the production of (a) a primary metabolite and (b) a secondary metabolite

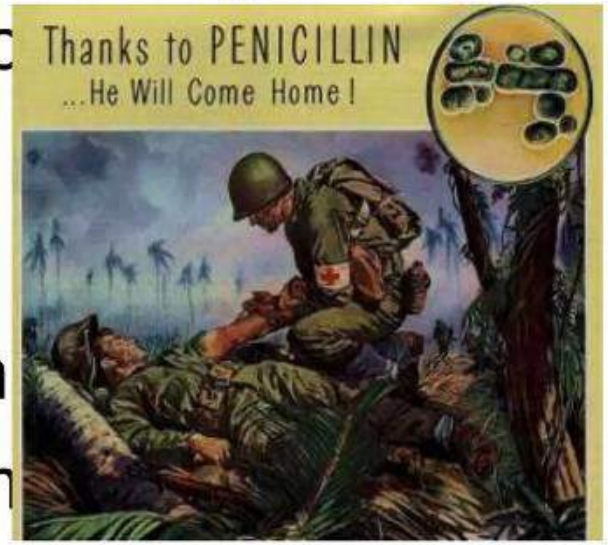
Primary metabolites are made during normal growth, eg proteins, enzymes, nucleic acids, ethanol, lactate etc. Their production rate follows the growth curve

Secondary metabolites are not produced during normal growth. Their production usually starts after the normal growth phase

Not all microbes produce secondary metabolites

Production of Antibiotics

- The mass production of antibiotics began during World War II with streptc and penicillin.



• Now most antibiotics are produced by staged fermentation in which strains of microorganisn producing high yields are grown under optimum conditions .

- Production of antibiotics can be done by 3 methods.
- 1. **Natural microbial production** using Fermentation technology.

  Example: Penicillin
- 2. **Semi synthetic production** (post production modification of natural antibiotics).
- **Example:** Ampicillin
- 3. **Synthetic production of antibiotics** made synthetically in the lab.
- **Example:** Quinoline

Fermentation Technology

- The source microorganism is grown in large containers (100,000–150,000 liters or more) containing a liquid growth medium.
- Oxygen concentration, temperature, pH and nutrient levels must be optimum.
- As antibiotics are secondary metabolites, the population size must be controlled very carefully to ensure that maximum yield is obtained before the cells die.

The fermentation process requires the following

1. A pure culture of the chosen organism, in sufficient quantity.
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 and
5. Equipment for:

a) Drawing the culture medium in steady state

b) Cell separation

c) Collection of cell free supernatant

d) Product purification and

e) Effluent treatment.

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

Strains used for production

- Species are often genetically modified to yield maximum amounts of antibiotics.
- **Mutation** is often used -introducing mutagens such as ultraviolet radiation, x-rays
- Selection and further reproduction of the higher yielding strains can raise yields by 20-fold or more.
- Another technique used to increase yields is **gene amplification**, where copies of genes coding for enzymes involved in the antibiotic production can be inserted back into a cell, via vectors such as plasmids.

Raw Materials

- The compounds that make the fermentation broth are the primary raw materials required for antibiotic production.
- The broth is an aqueous solution made up of all of the ingredients necessary for the proliferation of the microorganisms.
- Typically, it contains;
- **Carbon source:** molasses, or soy meal,acetic acid, alcohols, or hydrocarbons
- These materials are needed as a food source for the organisms.
- **Nitrogen Source :** Nitrogen is another necessary compound in the metabolic cycles of the organisms.

  ammonia salt is typically used.

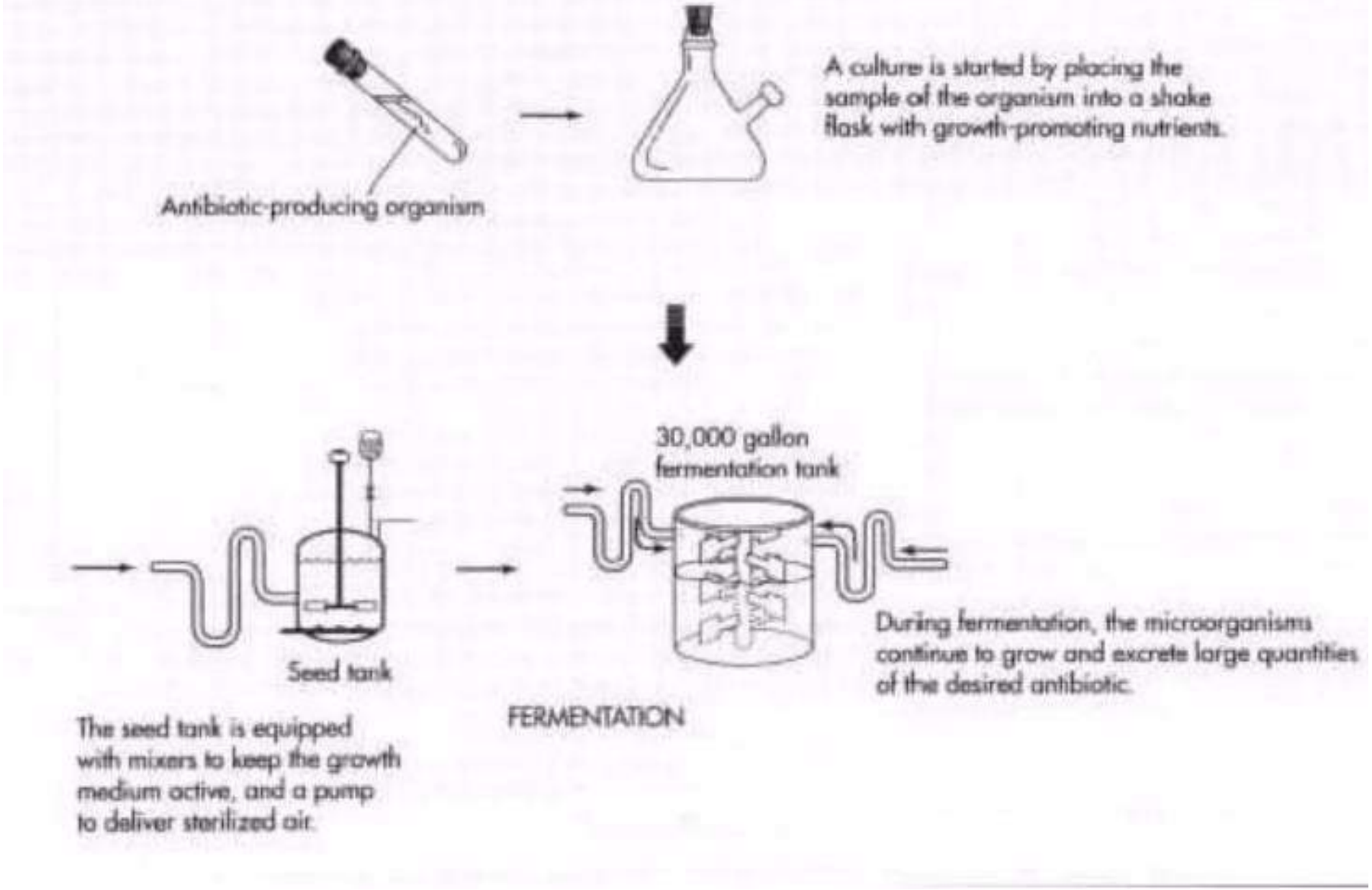
Steps in Production

- First the organism that makes the antibiotic must be identified.
- Desired microorganism must then be isolated.
- Then the organism must be grown on a scale large enough to allow the purification and chemical analysis of the antibiotic.
- The antibiotic tested against a wide variety of bacterial species.

It is important that sterile conditions be maintained throughout the manufacturing process, because contamination by foreign microbes will ruin the fermentation.

A) Starting a Culture

- Before the fermentation process the desired microbe must be isolated and its number must be increased by many times.
- A **starter culture** from a sample of previously isolated organisms is created in the lab.
- A sample of the organism is transferred to an agar-containing plate.
- The initial culture is then transferred to shake flask containing nutrients necessary for growth.
- A suspension is formed which is then transferred to seed tanks for further growth.


Antibiotic-producing organism
A culture is started by placing the sample of the organism into a shake flask with growth-promoting nutrients.
30,000 gallon fermentation tank
Seed tank
The seed tank is equipped with mixers to keep the growth medium active, and a pump to deliver sterilized air.
FERMENTATION
During fermentation, the microorganisms continue to grow and excrete large quantities of the desired antibiotic.

- The **seed tanks** are steel tanks designed to provide an ideal environment for growing microorganisms.
- The seed tanks are equipped with mixers, which mix the growth medium with microbes, and a pump to deliver sterilized, filtered air.

- After about 24-28 hours, the material in the seed tanks is transferred to the primary fermentation tank.

B) Fermentation

- The fermentation tank is a larger version of the seed tank, which is able to hold about 30,000 gallons.
- Microorganisms are allowed to grow and multiply.

• During this process, they excrete large quantities of the desired antibiotic.

• The tanks are cooled to keep the temperature between 73-81° F (23-27.2 ° C).

• It is constantly agitated, and a continuous stream of sterilized air is pumped into it.

- Anti- foaming agents are periodically added.

• Since pH control is vital for optimal growth, acids or bases are added to the tank as necessary.

C) Isolation & Purification

- After 3-5days, the maximum amount of antibiotic will have been produced.

- The isolation process can begin.

- The isolation depend on the specific antibiotic produced, the fermentation broth is processed by various purification methods.

ISOLATION, PURIFICATION, AND REFINING

Once the antibiotic is isolated from the fermentation broth and purified using either the ion-exchange or solvent extraction method, a purified powder form of the antibiotic is produced.

PACKAGING AND SHIPPING

Water soluble Antibiotics

- Antibiotic compounds that are water soluble, an ion-exchange method is used for purification.
- The compound is first separated from the waste organic materials in the broth.
- Then sent through equipment, which separates the other water-soluble compounds from the desired one.

Oil soluble Antibiotics

- Solvent extraction method is used for the isolation of oil soluble or organic antibiotics.
- The broth is treated with organic solvents such as butyl acetate or methyl isobutyl ketone, which can dissolve the antibiotic.
- The dissolved antibiotic is then recovered using various organic chemical means.
- At the end of this step a purified powdered form of the antibiotic is obtained which can be further refined into different product types.

Refining/Packaging

- Antibiotic products can take on many different forms. They can be sold in solutions for intravenous bags or syringes, in pill or gel capsule form, or powders, which are incorporated into topical ointments.
- Various refining steps may be taken after the initial isolation.

- For **intravenous bags**, the crystalline antibiotic can be dissolved in a solution, put in the bag, which is then hermetically sealed.

- For **gel capsules**, the powdered antibiotic is physically filled into the bottom half of a capsule then the top half is mechanically put in place.
- When used in **topical ointments**, the antibiotic is mixed into the ointment

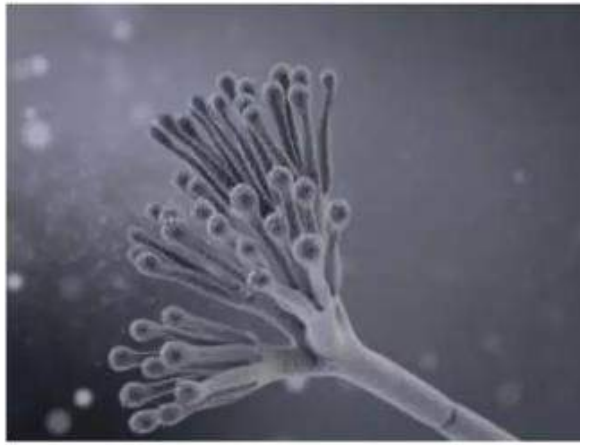
Antibiotics packaging

Antibiotics packaging

Industrial production of Penicillin

Penicillin was the first important commercial product produced by an aerobic, submerged fermentation.

Penicillium Chrysogenum specie is used.



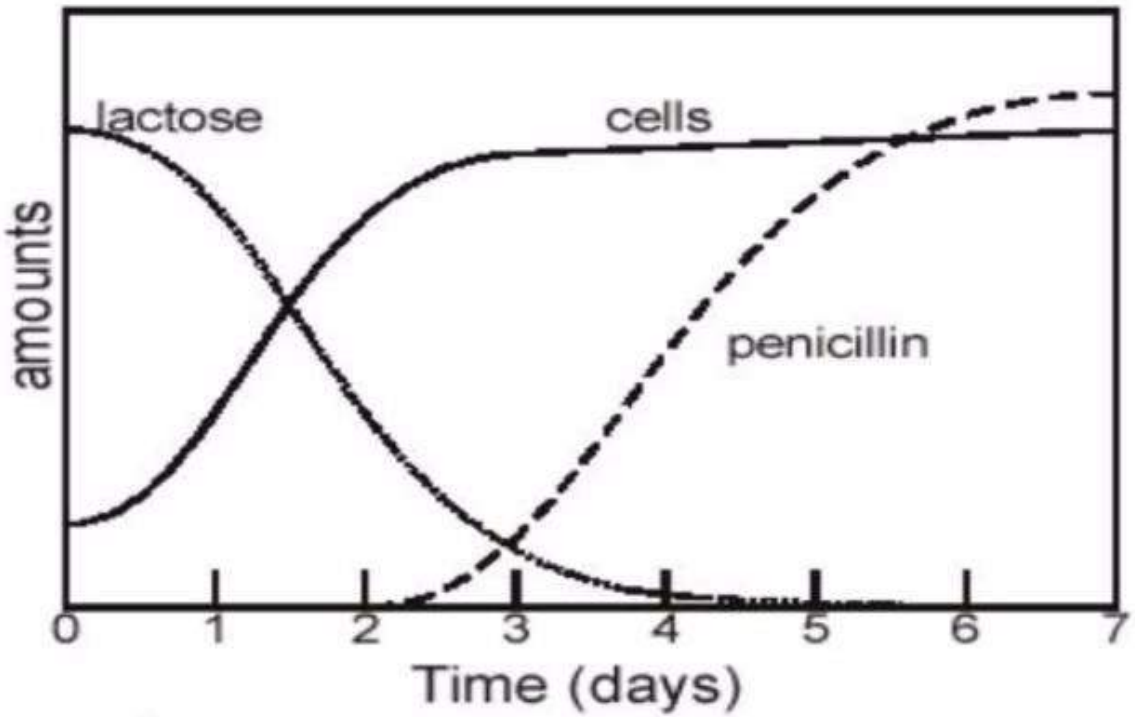
Production

- Penicillin is produced by the fungus Penicillium chrysogenum which requires lactose, other sugars, and a source of nitrogen (in this case a yeast extract) in the medium to grow well.
- Like all antibiotics, penicillin is a **secondary metabolite**, so is only produced in the stationary phase.

- What sort of fermenter does it require?
- A **batch Fermentor.**

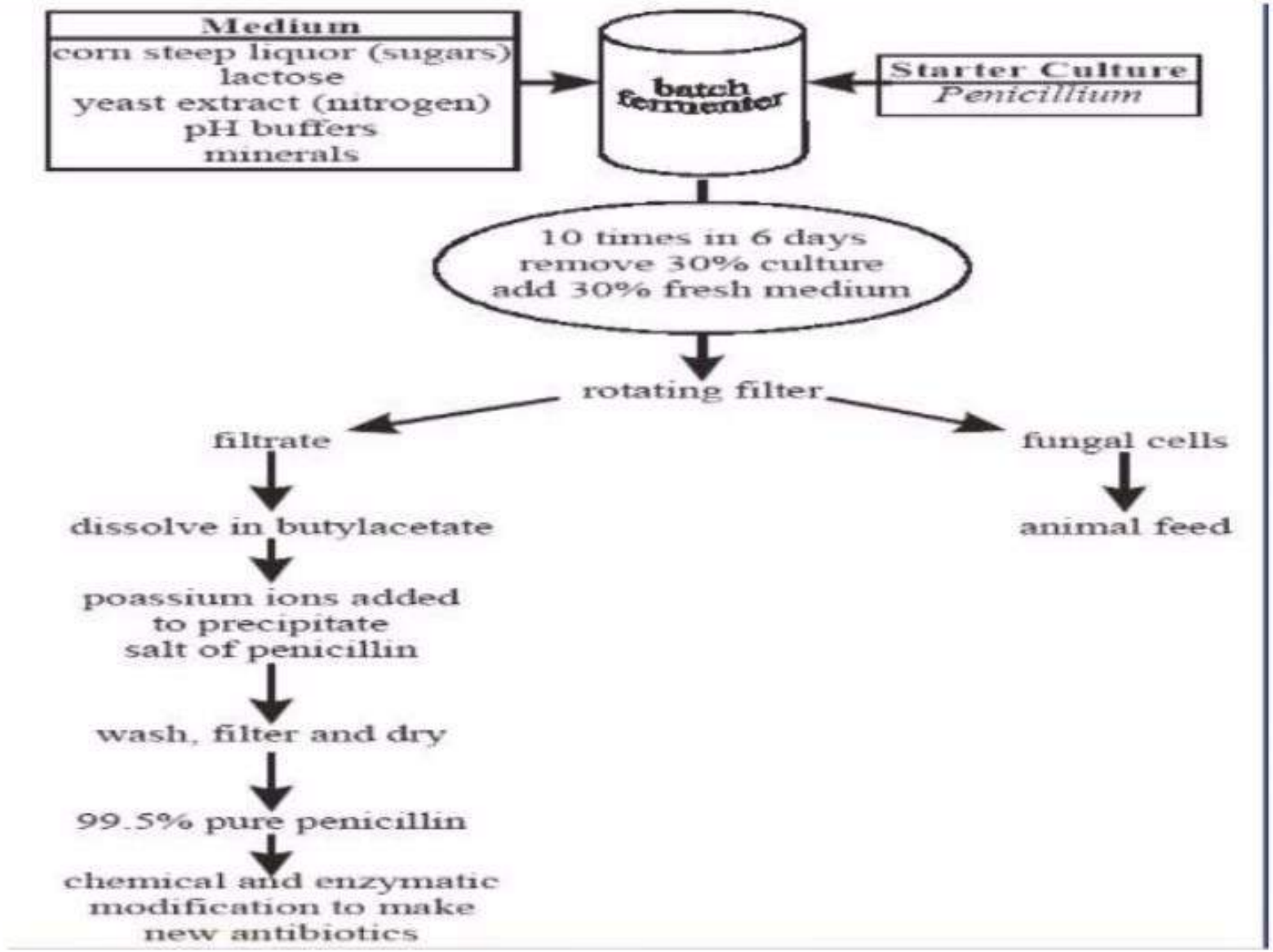
- A fed batch process is normally used of a growth limiting nutrient to prolong the stationary period and so increase production.

Colony growth and penicillin Production



Purification

- Downstream processing is relatively easy since penicillin is secreted into the medium.
- So there is no need to break open the fungal cells.
- However, the product needs to be very pure, since it being used as a therapeutic medical drug.
- It is dissolved and then precipitated by potassium salt to separate it from other substances in the medium.


Medium
corn steep liquor (sugars)
lactose
yeast extract (nitrogen)
pH buffers
minerals
batch fermenter
Starter Culture
Penicillium
10 times in 6 days
remove 30% culture
add 30% fresh medium
rotating filter
filtrate
fungal cells
dissolve in butylacetate
animal feed
poassium ions added
to precipitate
salt of penicillin
wash, filter and dry
99.5% pure penicillin
chemical and enzymatic
modification to make
new antibiotics

Products

- The resulting penicillin (called penicillin G) can be chemically and enzymatically modified to make a variety of penicillin with slightly different properties.

- These **semi-synthetic penicillin** include penicillin V, penicillin O, ampicillin and amoxicillin.