The Industrial Production of Penicillin

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Overview

- What is penicillin?
- History
- General structure of penicillins
- Fermenters
- Important aspects of fermentors
- Specific conditions for production
- Media consideration & formulation
- Production of penicillin
- Increase in yield
- References

Composition

- Corn steep liquor 8.5 % - improves yield as it is a good nitrogen source
- Sugar sources-
  - Glucose 1%
  - Ethanol 80%
  - Lactose 1%
- Phenyl acetic acid
- Probenecid
- Mineral sources-
  - Calcium carbonate 1%
  - Sodium hydrogen phosphate 0.4%
- Vegetable oil (prevents foaming due to agitation)

Organism

- Earlier, during the World War II the fungus known as *Peniccillium notatum* was used to develop the antibiotics
- Nowadays the fungus is called *penicillium chrysogenum*

What is Penicillin?

- First true naturally-occurring antibiotic ever discovered: a great medical breakthrough.

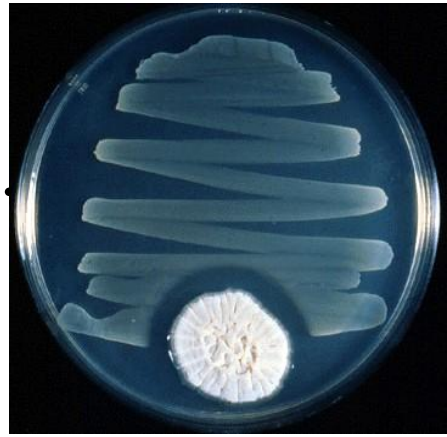
- Group of antibiotics produced by the *Penicillium* fungi.
  - It is a group of closely related compounds, not a single compound.
  - **Examples:** Amoxicillin, ampicillin, phenoxymethylpenicillin.

- Around 50 drugs that are penicillins.

History: Discovery & Production

A. Fleming

- 1928: Scottish biologist, Alexander Fleming discovered that the *Staphylococcus* culture he had mistakenly left growing in open was contaminated with a mold which had destroyed the bacteria.
- After isolating a sample and testing it, he found that it belonged to the *Penicillium* family.
- Later the mold was classified as *Penicillium notanum*.
- At first, it was difficult to convince people about its potential uses.



History: Discovery & Production

- But later (1939), using Fleming's work, two medical researchers, Howard Florey and Ernst Chain managed to purify penicillin in a powdered form.

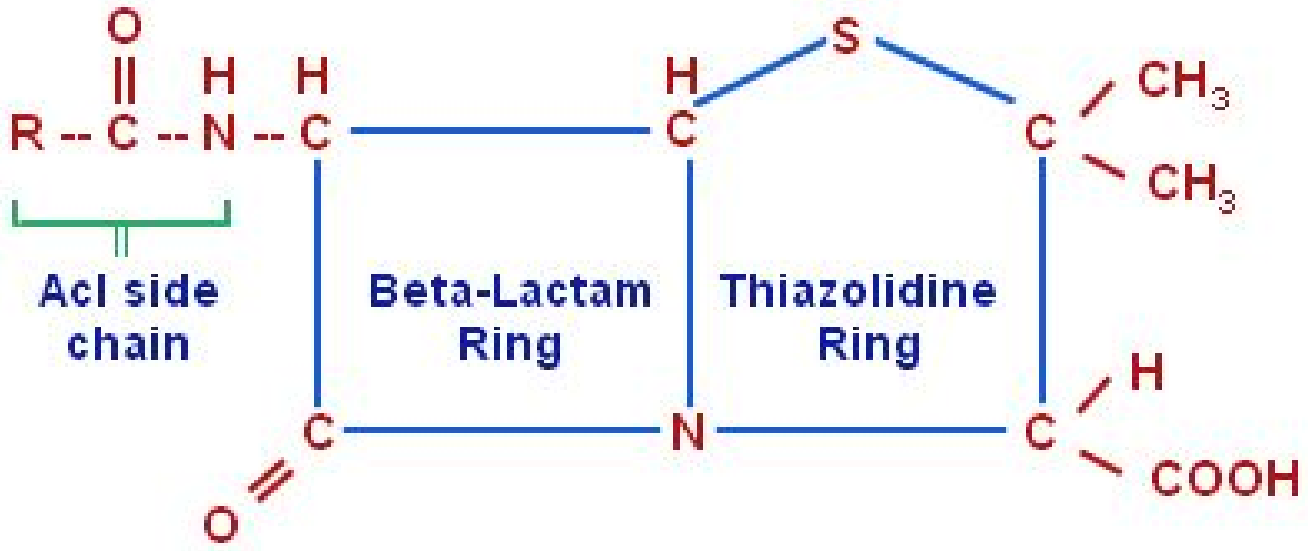
- 1941: They successfully treated a human.

- 1943: They produced penicillin on a large scale.
  - This helped immensely to treat casualties during t I t bacterial infections due to their wounds.


H. Florey


E. Chain

General Structure of Penicillins



Media Consideration

- The aim of the media is to:

1. provide all the elements required for the synthesis materials and the formation of the desired product.
2. provide favorable environment for the culture in question.
3. be cost effective.

Media Formulation

- pH 6.5
- Temperature 20-24 °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

The Industrial Production of Penicillin

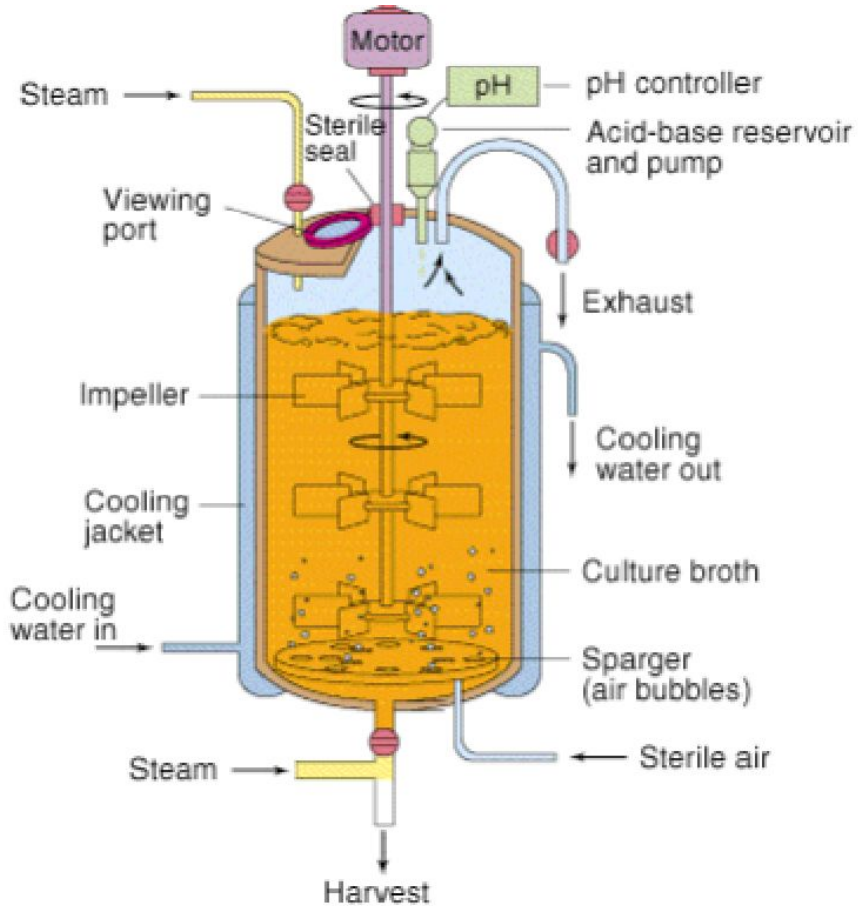
- **Upstream Processing**

Referring to processes before input to fermenter and encompasses any technology that leads to the synthesis of a product. It includes the exploration, development and production.

- **Downstream Processing**

Referring to processes done to purify the output of the fermenter until it reaches to the desired product, such as extraction and purification of a product from fermentation.

Fermentor Tank



Fermentor Properties

- Mass Transfer: good transfer of oxygen across the liquid interface-the Sparger delivers this oxygen efficiently.

- Heat Transfer: metabolism as a process tends to give off heat achieved through cooling jacket whereby cool water is passed through.

- Bulk Flow and Mixing: impellers, bubble columns or loop reactors.

- Batch, Fed-Batch and Continuous Culture: how nutrients and substrate will be delivered to a culture in a reactor.

- Steam: Used to keep the reactor running aseptically. (temperature/pressure of 121°C/15 psi for 15-30min)

Mode of Operation

- Batch: fixed amount of substrate is added at the beginning whereby the volume of nutrients remains the same throughout the process.

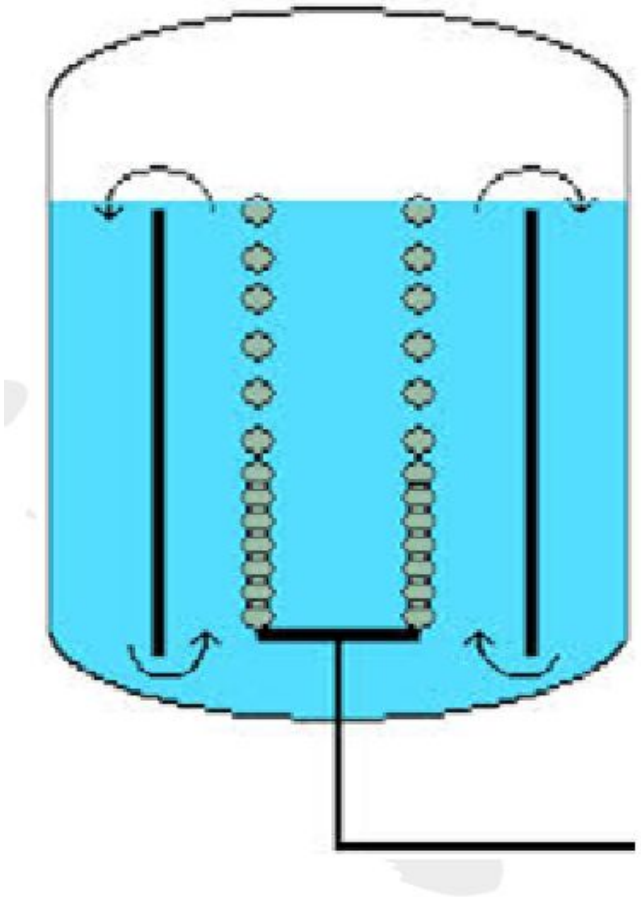
- Fed Batch: substrate is added in small increments at various times in the fermentation and consequently volume increases.

- Continuous: substrate is constantly added to the reactor while an equal amount of fermented medium is removed. Volume again remain the same but constantly renewed with fresh ones.

Specific Conditions for Penicillin Production

- Most penicillins form filamentous broths. This means they can be difficult to mix due to their high viscosity. Also the increasing viscosity of the broth can hinder oxygen transfer.

- A solution for the viscosity and the filamentous growth of penicillium species could be bubble columns (air lift reactors) which would distribute the oxygen equally and also to agitate the medium.



Some Important Aspects

- Mass Transfer: good transfer of oxygen across the liquid interface-the Sparger delivers this oxygen efficiently.
- Heat Transfer: metabolism as a process tends to give off heat achieved through cooling jacket whereby cool water is passed through.
- Bulk Flow and Mixing: impellers, bubble columns or loop reactors.
- Batch, Fed-Batch and Continuous Culture: how nutrients and substrate will be delivered to a culture in a reactor.
- Steam: Used to keep the reactor running aseptically. (temperature/pressure of 121°C/15 psi for 15-30min).

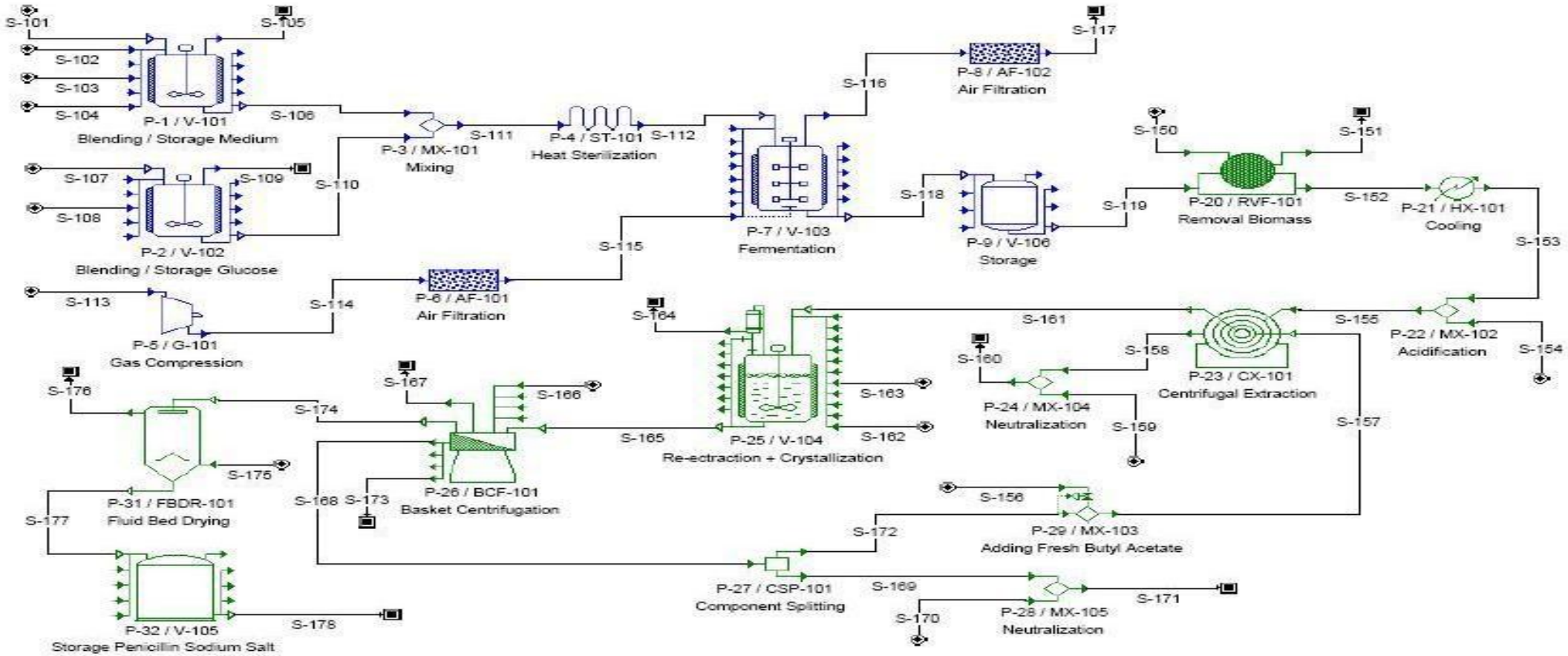
Deep-Tank fermentation

- The process is not an active growth
- It is growth of penicillium in stress conditions
- pH=6.5
- Temperature=20^0C to 24^0C

Reactor medium composition

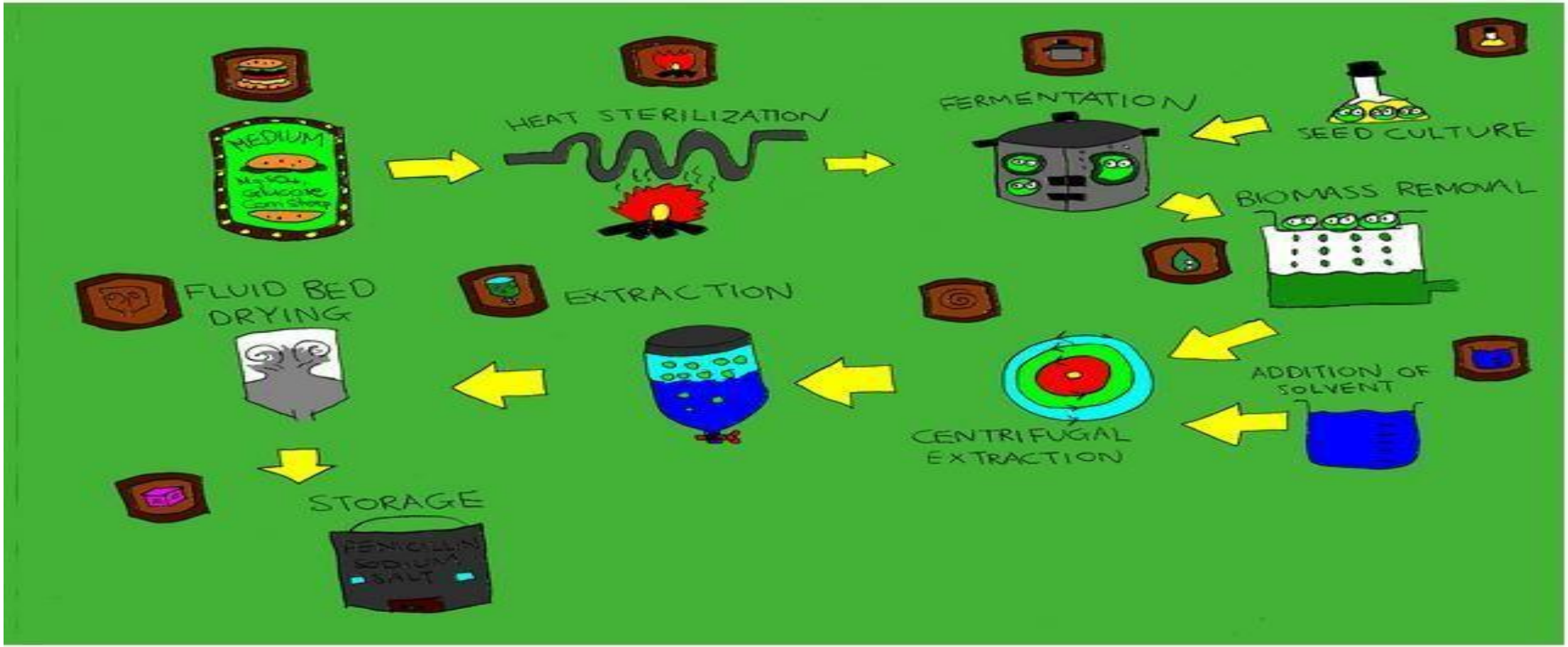
- Broadly reactor contain 3 components-
  - Organisms or cells
  - Carbohydrates or sugar as fuel
  - Penicillin as a result of conversion
  - Nutrient sources of carbon, nitrogen and minerals
  - Sufficient aeration for oxygen

Process Flow Diagram: Penicillin



http://penicillin.wikispaces.com/Home

Simplified Flow Chart



★ Medium for penicillin

1. The *Penicillium chrysogenum* usually contain its carbon source which is found in corn steep liquor and glucose.
2. A medium of corn steep liquor and glucose are added to the fermenter. Medium also consists of salts such as $MgSO_4$, K_3PO_4 and sodium nitrates. They provide the essential ions required for the fungus metabolic activity.

★ Heat sterilization

3. Medium is sterilized at high heat and high pressure, usually through a holding tube or sterilized together with the fermenter.

4. The pressurized steam is used and the medium is heated to 121°C at 30 psi or twice the atm. pressure. High temp. and short time conditions are used to minimize degradation of certain components of media

★ Fermentation

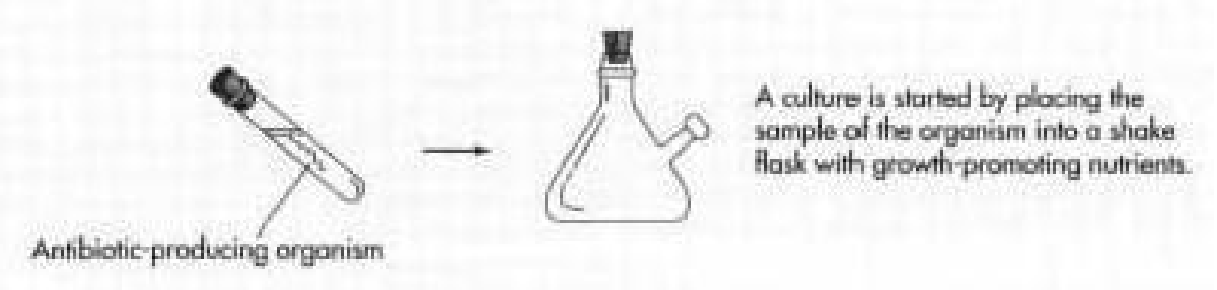
5.It is done in a fed-batch mode as glucose must not be added in high amounts at the beginning of growth (which will result in low yield of penicillin production as excessive glucose inhibit penicillin production).

6.The fermentation conditions for the Penicillium mold, usually requires temperatures at 20-24°C while pH conditions are kept at 6.5

7.The pressure in the bioreactor is much higher than the atmospheric pressure (1.02atm). This is to prevent contamination from occurring as it prevents external contaminants from entering.

★ Seed Culture

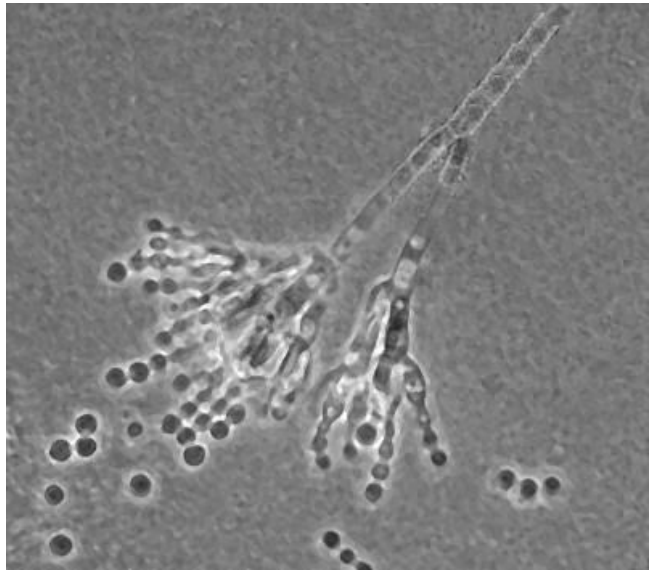
- 9. The seed culture is developed first in the lab by the addition of *Penicillium chrysogenum* spores into a liquid medium. When it has grown to the acceptable amount, it is inoculated into the fermenter.



- 10. The medium is constantly aerated and agitated. Carbon and nitrogen are added springly alongside precursor molecules for penicillin fed-batch style. Typical parameters such as pH, temperature, stirrer speed and dissolved oxygen concentration, are observed.

★ Seed Culture

11. After about 40 hours, penicillin begins to be secreted by the fungus.
12. After about 7 days, growth is completed, the pH rises to 8.0 or above and penicillin production ceases.



★ Removal of biomass

13. Filtration is carried out as bio separation is required to remove the biomass from the culture (removing the fungus and other impurities away from the medium, which contains the penicillin).

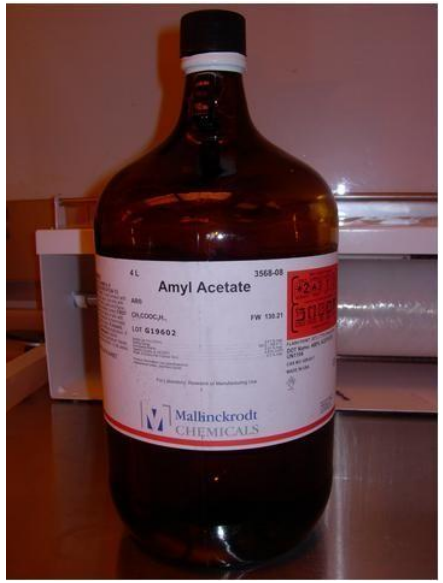
14. A R otary vacuum filter is commonly employed for filtration as it is able to run in continuous mode in any large scale operations.

15. After filtration, phosphoric acid, a non-oxidising age
decrease pH from 8.0 to 6.5 so as to prevent loss of ac

★ Addition of solvent

16. Organic solvents such as amyl acetate/butyl acetate are added to dissolve the penicillin present in the filtrate.

17. At this point, penicillin is present in the solution and any other solids will be considered as waste (can be used as fertilizers and animal feed).



★ Centrifugal Extraction

18. Centrifugation is done to separate the solid waste from the liquid component which contains the penicillin.

19. Usually a disk centrifuge is used at this point.

20. The supernatant will then be transferred further in process to continue with extraction.

- Disk centr… common … large …

…ost …or

★ Extraction

21. A series of extraction processes are carried upon the dissolved penicillin, to obtain a better purity of the penicillin product.

22. The acetate solution is first mixed with a phosphate buffer, followed by a chloroform solution, and mixed again with a phosphate buffer and finally in an ether solution.

23. Penicillin is present in high concentration in the ether solution and it will be mixed with a solution of sodium bicarbonate to obtain the penicillin-sodium salt, which allow penicillin to be stored in a stable powder form at rtp.

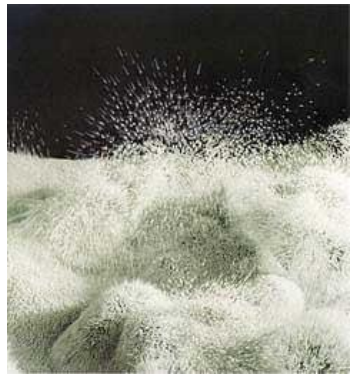
★ Extraction

- 24. The penicillin-sodium salt is obtained from the liquid material by basket centrifugation, in which solids are easily removed.

★ Fluid bed drying

- 25. Drying is necessary to remove any remaining moisture present in the powdered penicillin salt.
- 26. In fluid bed drying, hot gas is pumped from the base of the chamber containing the powdered salt inside a vacuum chamber.
- 27. Moisture is removed this way, and this result in a much drier form of penicillin.

Fluid bed drying tube



Powdered penicillin being blown by hot air

★ Storage

The White Penicillin-Sodium salt

- 28. Penicillin is stored in containers and kept in a dried environment.

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

30. These can be semi-synthetic penicillins, such as; Penicillin V, Penicillin O, ampicillin and amoxycillin.

Brunton LL, Parker K, Blumenthal D, et al. The goodman and gilman's manual of pharmacological therapeutics. McGraw-Hill Professional, 2007.

Penicillins

Class	Drugs	Drug of Choice)	Toxicity
Penicillin	Penicillin G Aqueous penicillin G Procaine penicillin G Benzathine penicillin G Penicillin V	Strep. pyogenesStep. agalactiaeC. perfringens(Bacilli)	Hypersensitivity reaction Hemolytic anemia
Aminopenicillins	Ampicillin Amoxicillin	Above + ↑ Gram-negative: E. faecalis E. Coli	Above
Penicillinase-resistant-penicillins	Methicillin Nafcillin Oxacillin Cloxacillin Dicloxacillin	Above + PCNase-producingStaph. Aureus	Above + Interstitial nephritis
Antipseudomonal Penicillins	Carbenicillin Ticarcillin	Above + Pseudomonas aeruginosa	Above

Increasing Yield

1. Adding corn syrup
2. Selection of strain
3. Mutation and Selection
4. Sexual reproduction

Increasing Yield

- 1. Corn syrup
- Allow maximal growth of the culture at the expense of product (antibiotic) formation. This is because growth and antibiotic production are inversely proportional.
- More secondary metabolites during stress phase.

Increasing Yield

- 2. Selection of strain
  - Selection of the best strain depends on the production rate of the secondary metabolite (penicillin).
- *Penicillium notatum* (1 mg/dm^3) and *Penicillium chrysogenum* (50 mg/dm^3).
- Strains are grown on cultures in laboratories and those with best yield is determined.

Increasing Yield

•3. Mutation and Selection

- This is based on trial and chance.
- Several strains of Penicillium are cultured.
- Ethyl methanesulphonate (EMS), near-ultraviolet light
- in the presence of 8-methoxypsoralen (8MOP) are used as mutagens.
- Combination of the mutagens leads to a more positive result.
- Strains are exposed to the mutagens at different intensities and proportions.
- Each time the best strains are selected and are further exposed.
- Statistical tests are done to determine the strains with highest yields.
- Dry conidia is stored in silica gel at 4°C at each stage.

Increasing Yield

4. Sexual Reproduction

- It was assumed that thepenicillin-producing *Penicillium chrysogenum* only reproduced asexually through spores. Fungus also has a sexual cycle (Jan.8 2013)

- The progenies possess a combination of genes from both mating partners and thus have new properties, both at the molecular level, as wellin their phenotypes.

- Specific environmental conditions; in the dark under oxygen deprivation conditions in a nutrient medium supplemented with the vitamin biotin.

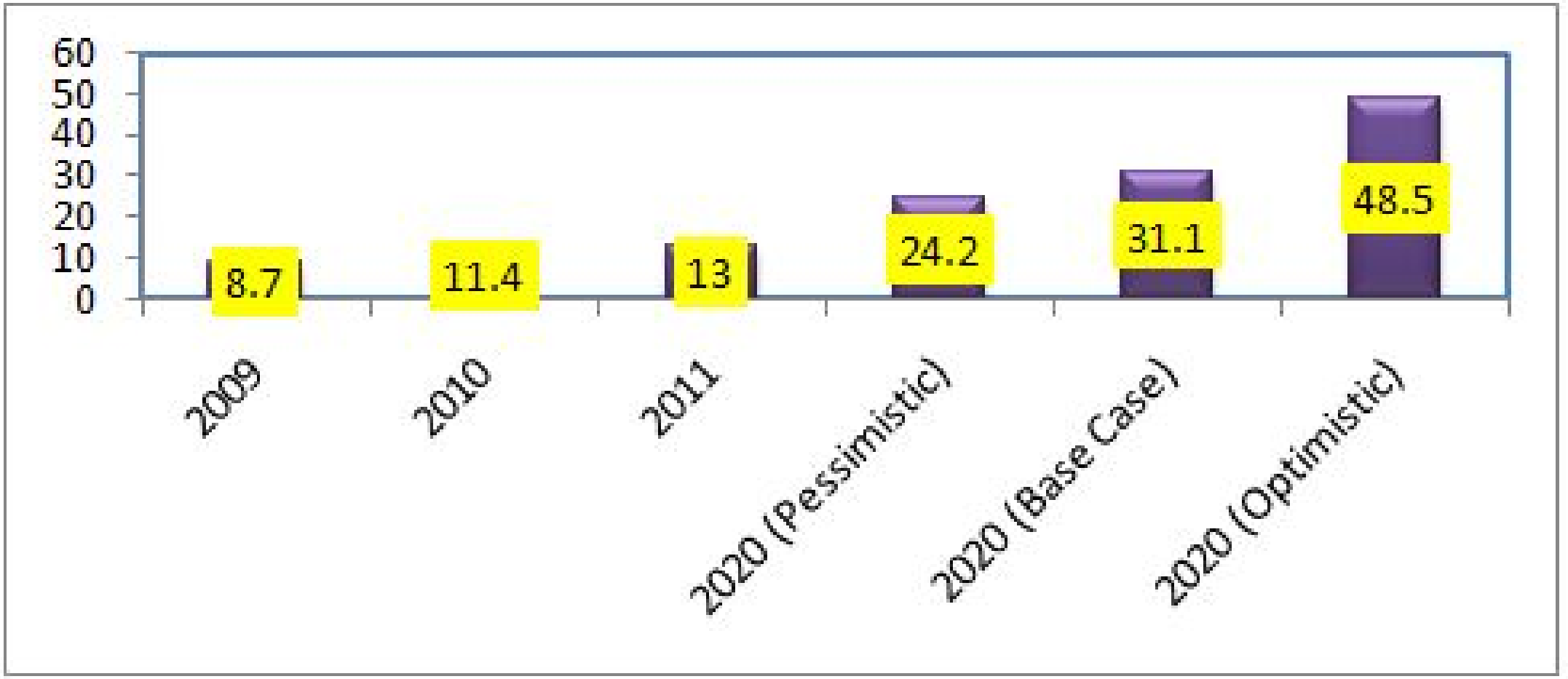
Future Scope

Overview of the industry

- The Pharmaceutical industry in India is the world's third-largest in terms of volume and 14th in terms of value.
- In terms of the global market, India currently holds a modest 1-2% share, but it has been growing at approximately 14% per year during the last four year.
- Many Indian Companies in 2013 showing a sales growth of >20%

Pharma in India

- ***Strengths-*** Low cost of innovation, manufacturing and operations, low cost of skilled manpower.
- ***Weaknesses-*** Stringent pricing regulations, presence of more unorganized players versus the organized ones.
- ***Opportunities-*** Opening of the health insurance sector and increase in per capita income.
- ***Threats-*** Other low-cost countries such as China and Israel affecting outsourcing demand for Indian pharmaceutical products. Entry of foreign players



Indian pharmaceutical market by 2020 (US $billion)

Issues

- Between 2012-2018, the “patent cliff" will wipe an estimated $148 billion off pharmaceuticals industry revenues due to many drugs coming out of patents
- Rising cost of R&D, the cost of bringing a molecule to market globally is estimated to be from $800 million to $4 billion.
- On average, out of every 10,000 molecules been developed; only one or two are likely to reach the market.
- Increasing government pressure, with harsher price controls and taxes.
- European Medical Agency (EMA) and the US Food and Drug Administration (USFDA) are focusing on risk management; thereby putting pressures on profitability of pharmaceutical companies.

Scope for the Industry in India

- Microbiology
  - The most important contribution of microbiology to the pharmaceutical industry is the development of antibiotics.
  - All antibiotics were originally the products of microbial metabolism, however the recent genetic manipulations have enabled the production of more enhanced drugs.
  - The production of vaccines against bacterial diseases usually requires the growth of large amounts of bacteria.
  - Steroids can also be obtained from microorganisms.
  - Apart from Drugs and product development, microbiology also helps in quality control of a pharma lab.

Pollution by Pharmaceutical Industry

- Water Pollution – because of spillage , drains and generation of effluent water , sewage water for uncontrolled pH , and treating it ineffectively . MOST dangerous for aquatic living organisms or their livelihood.
- Air Pollution – releases of gaseous , fumes , dust , particulates to atmosphere , poor air quality ,
- Land /Soil Pollution – Spillage , chemicals , oils , lubricants leakages to land /soil – seepage causation of non fertility to soil /lands , dumping or disposal of incinerated sludge unauthorized
- Noise Pollution – persistent vibration in operating machines, use of power generators and air compressors .

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THANK YOU!