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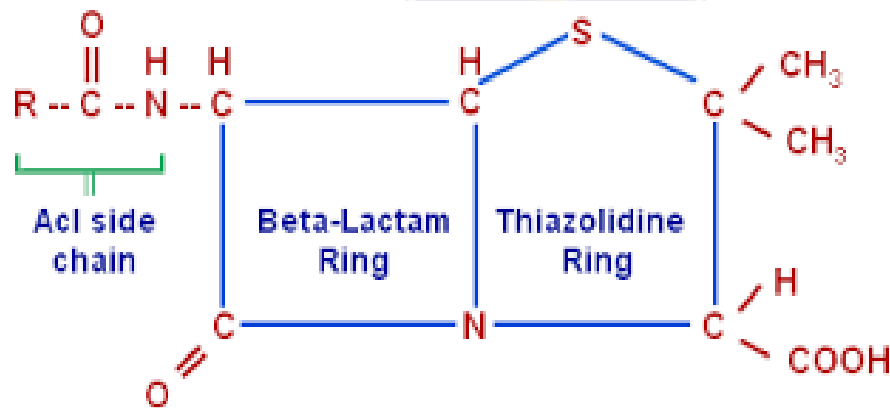
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FACULTY OF ENGINEERING &
TECHNOLOGY

Penicillin production commercially by fermentation biotechnology

Structure of Penicillin

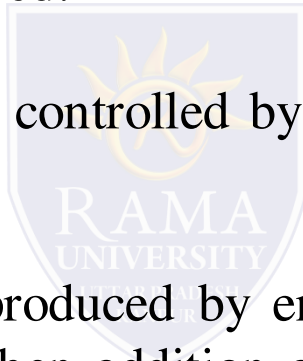
- The basic structure of penicillin consists of a thiazolidine ring condensed with a B-lactam ring.
- Natural penicillin is 6-amino-penicillanic acid (6APA).

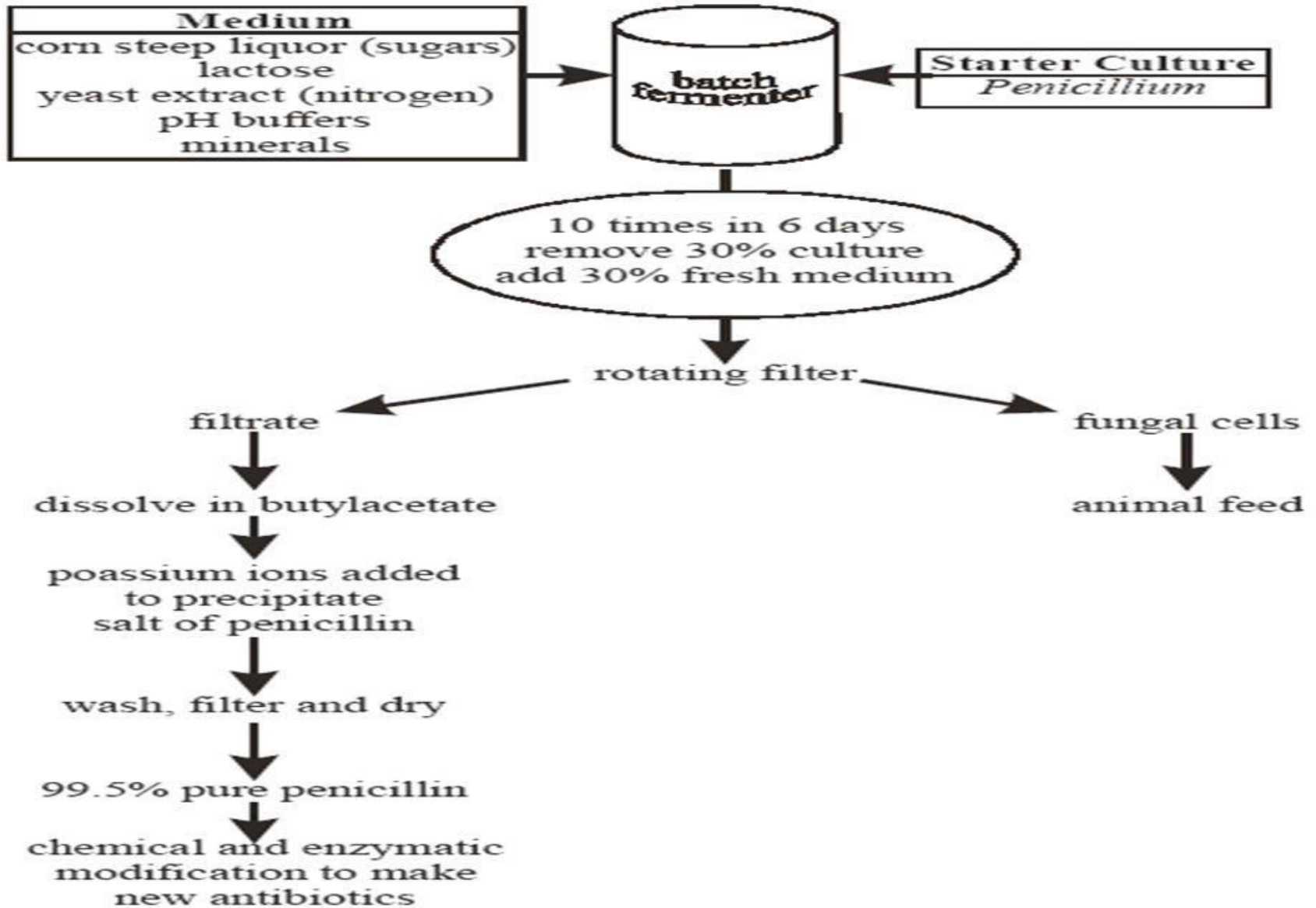


General Structure of Penicillins

Fermentation biotechnology for penicillin production:

- By fermentation technology penicillin is produced from *Penicillium* spp. If penicillin fermentation is carried out without addition of side chain precursor, the natural penicillins are produced.
- But fermentation can be better controlled by adding a side chain precursor to obtain derived penicillin.
- The synthetic penicillins are produced by enzymatic hydrolysis of 6APA by penicillin acylase enzyme and then addition of desired side chain by chemical means,
- Beta-lactum thiozolidine ring of penicillin is constructed from l-cystine and l-valine. These two amino acids when combined with L- α -aminoadipic acid (α -AAA) the tripeptide is formed which undergoes two step cyclization process to give isopenicillin.





Regulation of penicillin production:

- The amino acid lysine is synthesized from a pathway that involves L- α -AAA, so that penicillin and lysine share a common but branched biosynthetic pathway.
- Higher concentration of lysine causes feed back inhibition of homocitrate synthase, an enzyme involved in α -AAA synthesis. Either lysine level should keep low or α -AAA level should added during fermentation.
- Penicillin biosynthesis is affected by Po_4 —concentration and also shows a distinct catabolic repression by glucose. Therefore, either slowly metabolizable sugars such as lactose is used or fed continuously with glucose with small dose.

Penicillin Production process:

- Penicillin production is previously achieved by surface process ie. Solid state fermentation and surface liquid fermentation. Now a days a commercial production is carried out by fed batch process
- Inoculum (Organism): *Penicillium chrysogenum* (improved strain)

(i.) Inoculum preparation:

- For inoculum preparation, spore from heavily sporulated working stocks are suspended in water or non-toxic wetting agents (sodium sulfonate 1: 10000)
- These spores are then added to flask containing wheat bran and nutrient solution for heavy sporulation
- Incubate for 5-7 days at 24°C
- Spores are then transferred to seed tank and incubated for 24-48 hours at 24°C with aeration and agitation for sufficient mycelial growth
- These mycelia can be used for production fermenter

(ii) Production fermentation:

Method: fed-batch or batch

Substrate: glucose, phenoxyacetic acid (fed component used for production of side chain), Corn steep liquor, Additional nitrogen source ie, soyameal, yeast extract, Lactic acid, inorganic ions, growth factors

Fermenter: stirred tank or air lift tank

pH: set at 5.5 to 6.0 which increased upto 7-7.5 (optimum) due to liberation of NH₃ gas and consumption of lactic acid. If pH is 8 or more, CaCO₃ or MgCO₃ or phosphate buffer is added

temperature: 25-27 C

aeration: 0.5-1 vvm (initially more, latter less O₂)

agitation: 120-150 rpm)

time: 3-5 days

antiform: edible oil (0.25%)

(iii.) Product recovery:

- Harvest broth from fermenter tank by filtration (rotary vacuum filtration)
- Chill to 5-10 C (because penicillin is highly reactive and destroyed by alkali and enzyme)
- Acidify filtrate to pH 2.0-2.5 with H₂SO₄ (to convert penicillin to its anionic form)
- Extract penicillin from aqueous filtrate into butyl acetate or amyl acetate (at this very low pH as soon as possible in centrifugal counter current extractor)
- discard aqueous fraction
- allow the organic solvent to pass through charcoal to remove impurities and extract penicillin from butylacetate to 2% aqueous phosphate buffer at pH 7.5
- acidify the aq. Fraction to pH 2-2.5 with mineral acid and re-extract penicillin into fresh butylacetate (it concentrated upto 80-100 times)
- add potassium acetate to the solvent extract in a crystallization tank to crystallize as potassium salt
- recover crystal in filter centrifuge
- sterilization
- further processing
- packaging

Application of penicillin:

Clinical Uses

Naturally effective antibiotics against gram + bacteria

Used for treatment of bacterial endocarditis

