ANTIBIOTIC PRODUCT ION OF PENICILLIN:

Introduction:

Antibiotics are antimicrobial agents produced naturally by other microbes (usually fungi or bacteria). There are over 10 000 different antibiotics known, but only about 200 in commercial use, since most new antibiotics are no better than existing ones. There is a constant search for new antibiotics. Antibiotics are the most-prescribed drugs and are big business. Finding a new antibiotic and getting it on to the market is a very long process and can take 15 years. Antibiotics are produced on an industrial scale using a variety of fungi and bacteria.

The first antibiotic was discovered in 1896 by Ernest Duchesne and "rediscovered" by Alexander Flemming in 1928 from the filamentous fungus *Penicilium notatu*m. The antibiotic substance, named penicillin, was not purified until the 1940s (by Florey and Chain), just in time to be used at the end of the second world war. Penicillin was the first important commercial product produced by an aerobic, *submerged* fermentation. When penicillin was first made at the end of the second world war using the fungus *Penicilium notatum*, the process made 1 mg dm-3. Today, using a different species (*P. chrysogenum*) and a better extraction procedures the yield is 50 g dm-3. There is a constant search to improve the yield. From *P. chrysogenum (ancestral fungaus)*, high-yielding strains have been developed for the commercial production of penicillin. **(Table 1)**

Table 1: Some high-yielding strains of *Penicilium chrysogenum*

Designation of mould	**Strain History**	**Use**
Penicilium chrysogenum NRRL 1951.B25	Substrain of NRRL 1951. Yields in excess of 200 units/ml	Submerged culture production in industry during the latter half of 1944 and the first half of 1945.
P. chrysogenum Standford 25099	Substrain of NRRL 1951. B25 resulting from U.V. light irradiation.	Superior submerged culture production.
P. chrysogenum X-1612	Substrain of NRRL 1951. B25 resulting from x-rays irradiation. Yields in excess of 500 units/ml	Superior submerged culture production.
P. chrysogenum Q-176 (Wisconsin)	Substrain of *P. chrysogenum X-1612* resulting from treatment of conidia with U.V. light. Yields in excess of 761 units/ml	Submerged culture production.

It has been found that high-yielding strains of *Penicilium chrysogenum* are genetically unstable (in fact the higher the yield capacity, the more unstable is the strain). Therefore they are carefully maintained. Production strains are stored in a dormant form by any of the following standard preservation techniques:

i) A spore suspension may be mixed with a sterile, finely divided inert support (soil or sand is traditional) and desiccated.
ii) Spore suspension can be lyophilized in appropriate media, or
iii) Spore suspension can be stored under liquid nitrogen, i.e. in a frozen state.

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Inoculum Preparation: The aim is to develop a pure inoculums in sufficient volume and in the fast growing phase for the production stage fermentor. **Figure 1** shows a typical route by which inoculums is prepared. The time taken for each stage is measured in days, and it decreases as the sequence progress.

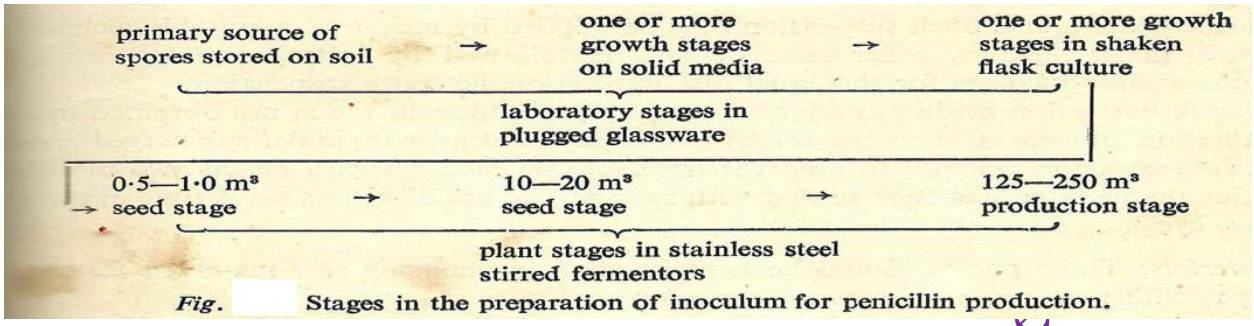

Fig. Stages in the preparation of inoculum for penicillin production.

The medium is designed to provide the organism with all the nutrients that it requires. Many sporulation media have been developed to obtain large number of spores. For example, Moyer and Coghill (1946) suggested the following sporulation medium:

Component	*g./ltr.*
Glycerol	7·5
Cane molasses (edible quality as commonly sold at retail)	7·5
Corn-steep liquor	2·5
$MgSO_4 . 7H_2O$	0·050
KH_2PO_4	0·060
Peptone	5·00
NaCl	4·00
Fe-tartrate	0·005
$CuSO_4 . 5H_2O$	0·004
Agar	2·50
Distilled water to make	1·0 litre

The flasks containing the seeded medium are incubated on a rotary shaker to effect aeration ...ion.

Sporulation medium Moyer and Coghill (1946)

The above medium may be solidified by making the total amount of agar at least 15-25g per litre (in presence of adequate oxygen, at 24˚C). The volume of culture increases approximately ten-fold with each successive stage in progression. The mould *P. chrysogenum*, grows in a filamentous form.

Inoculation Method: Any one of the following inoculation method may be used to inoculate the fermentation medium in the submerged culture production of penicillin:

i. Dry spore may be used to seed the fermentation medium

ii. Inoculation of fermentation medium may be acocomplished by a suspension of un-germinated mould spores. A non-toxic wetting agent (e.g. 1 : 10,000 sodium lauryl sulphonate) in sterile water is used for the preparing a more uniform suspension than can be obtained with sterile water alone. Tion and aeration of the fermentation medium for the equal distribution of the spore suspension. or

iii. The fermentation medium may be seeded by pellet inoculants which are obtained e germination of spores, with the formation of mycelial growth, under submerged conditions.

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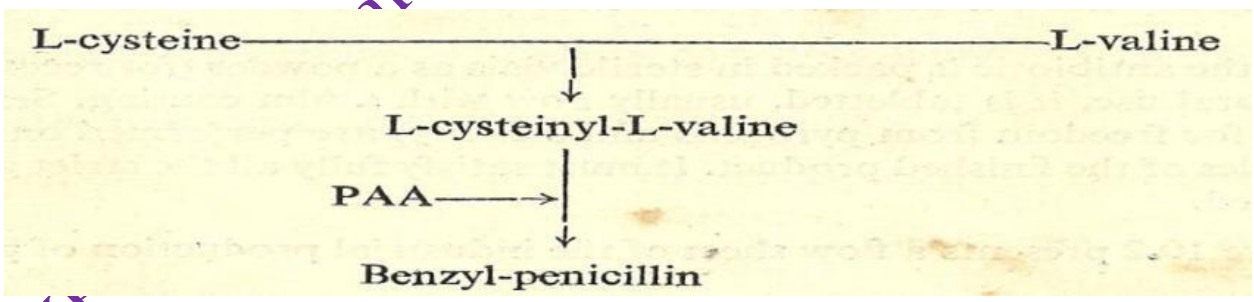
Raw material: Three points should be kept in mind in choosing raw materials for the manufacture of penicillin:

i. An abundant growth of mycelium
ii. Maximum accumulation of penicillin and
iii. Ease of extraction and purification of the antibiotic.

Carbon sources: Lactose acts as very satisfactory carbon compound (@ 6%), other sugars (glucose and sucrose) may be used as substitute for slow feeding rates.
Nitrogen sources: Ammonium sulphate, ammonium acetate, ammonium lactate or other materials can be used as nitrogenous compounds. Nitrogen is often supplied as ammonia gas. Corn-steep liquor also supplies cheap and readily available nitrogen.
Mineral sources: Elements namely K, Mg, P, S, Zn & Cu are required for penicillin production. Some of these are supplied by corn-steep liquor. These minerals are supplied in the form of potassium dihydrogen phosphate magnesium sulphate, iron sulphate copper sulphate etc. Corn- steep liquor contain sufficient amount of potassium dihydrogen phosphate and Magnesium sulphate ($MgSO_4.7H_2O$). However, small amount of these salts may be incorporated in the fermentation medium.
***Corn- steep liquor* (CSL) :** The used steep water results from the steeping of the corn during manufacture of starch, gluten and other corn products. This by-product is subjected to concentration to approximately 50% solids and this concentration is called Corn- steep liquor. The presence of CSL in fermentation medium gives higher yields of penicillin G. According to Foster et al., (1946), stimulatory effect of CSL were due, in part to argenine, histidine and glutamic acid and possibly to phenyl acetic acid derivatives.
***Precursors*:** The formation of desired penicillin can be stimulated by addition of phenyl acetic acid derivatives. For example, *P.* chrysogenum synthesizes large quantities of penicillin G, if phenyl acetic acid ($C_6H_5.CH_2.COOH$) is present in fermentation medium. Phenyl acetic acid (PAA) supplies the side chain of penicillin G. Fortunately; CSL is a source of PAA derivatives. High levels of PAA are however, toxic to the mould and so all of it cannot be added at a time. The feed aims at providing reserve of PAA in the medium at all times, without approaching toxic limits.

The penicillin nucleus, to which the 6-amino-penicillic acid (6-APA) side-chain is attached, believed to be synthesized from the amino acid L-cysteine and valine, as shown in the **following diagram:**



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A typical medium reported by Jackson (1958) has the following chemical composition:

Component	%
Corn-steep liquor solids	3·5
Lactose	3·5
Glucose	1·0
Calcium Carbonate	1·0
Potassium dihydrogen phosphate (KH_2PO_4)	0·4
Edible Oil	0·25
Penicillin precursor with slow Feeding rate	

Jackson's medium (1958)

Extraction and Purification: This operation is carried out in three different steps:

a) **Removal of mycelium:** At harvest, the penicillin is in solution exocellularly, together with a range of other metabolites and medium components. The first step is to remove the mycelium by filtration. A rotary vacuum filter is employed for the filtration of fermented production medium. This stage is carried out under such conditions as would avoid contamination of the filtrate with penicillinase producing micro-organisms which may cause serious or total loss of an antibiotic.

b) **Countercurrent solvent extraction of penicillin:** The next stage is to extract the penicillin. Countercurrent solvent extraction is the generally accepted process. The pH of filtrate (from the stage (a)) is adjusted here is 2-2.5 with the help of phosphoric or sulphuric acids. In aqueous solutions, at such low pH values there is high partition coefficient in favour of certain organic solvents (e.g. amyl acetate, butyl acetate or methyl iso-butyl ketone). This step has to be carried out quickly, for penicillin is very unstable at low pH values. Podbielniak Countercurrent solvent extractor is used for this purpose. The antibiotic is then extracted back into aqueous buffer at pH 7-7.5, the partition coefficient now being strongly in favour of the aqueous phase. The resulting aqueous solution is again acidified and re-extracted with an organic solvent. These shifts between the water and the solvent help in the purification of penicillin. The spent solvent is recovered by distillation for reuse.

c) **Treatment of Crude Extract:** Penicillin is manufactured as various salts according to its intended use (e.g. for administration to human and/or animals).The treatment of the crude penicillin extract varies according to the objective, but involves the formation of an appropriate penicillin salt (usually sodium penicillin). The solvent extract (as secured in the stage (b)) is carefully extracted back with aqueous sodium hydroxide. This is followed probably by charcoal treatment to eliminate pyrogens (i.e. fever producing substances), and by sterilization (i.e. by the use of Seitz filter to remove any bacteria). Pure metal salts of penicillin can be safely sterilized by dry heat, if desired. Thereafter, the aqueous solution of penicillin is subjected to crystallization.

For parental use, the antibiotic is packed in sterile vials as a powder (for reconstitution before use) or suspension. For oral use, it is tabletted, usually now with a thin film coating. Searching tests (e.g. for potency, for purity, for freedom from pyrogens and sterility) are performed on an appropriate number of random samples of the finished product. It must be satisfy fully the strict government standards before being marketed. **Flow Sheet: (Figure 2)** presents a flow sheet of the industrial production of penicillin G.

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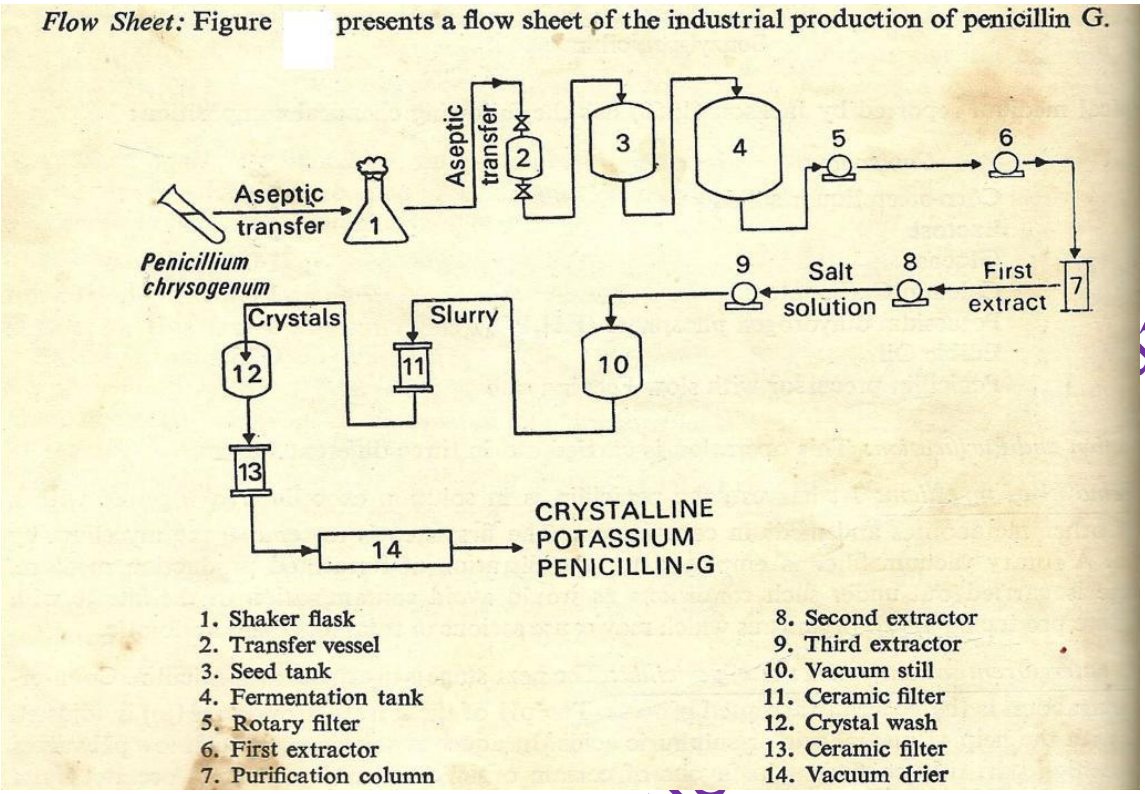

Figure 2

REMEMBER: 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. It requires a batch fermenter, and a fed batch process is normally used to prolong the stationary period and so increase production. Downstream processing is relatively easy since penicillin is secreted into the medium (to kill other cells), 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, so it is dissolved and then precipitated as a potassium salt to separate it from other substances in the medium. The resulting penicillin (called penicillin G) can be chemically and enzymatically modified to make a variety of penicillins with slightly different properties. These semi-synthetic penicillins include penicillin V, penicillin O, ampicillin and amoxycillin.

Downstream processing: Products in a fermenter are impure and dilute so need to be purified by downstream processing. This usually involves filtration to separate the microbial cells from the liquid medium, followed by chemical purification and concentration of the product. Downstream processing can account for 50% of the cost of a process.} **Production process**

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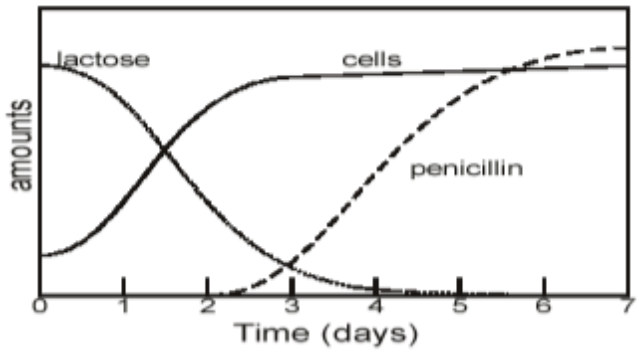

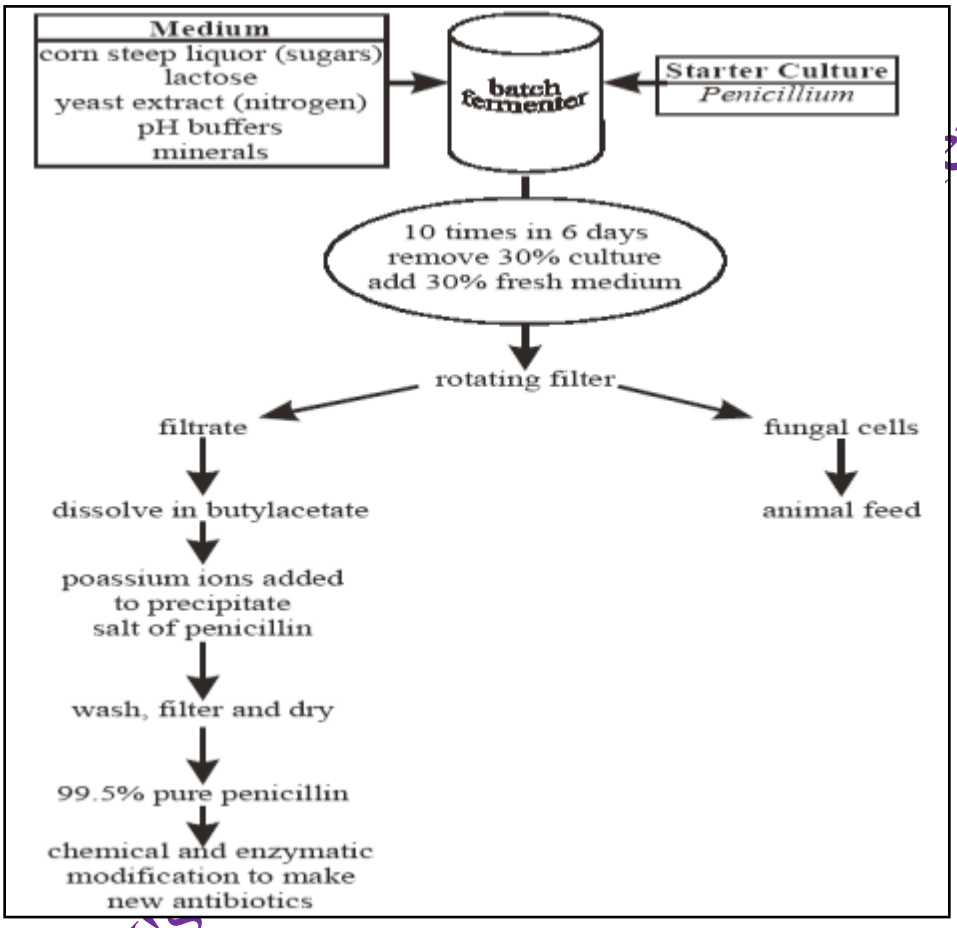

Production process

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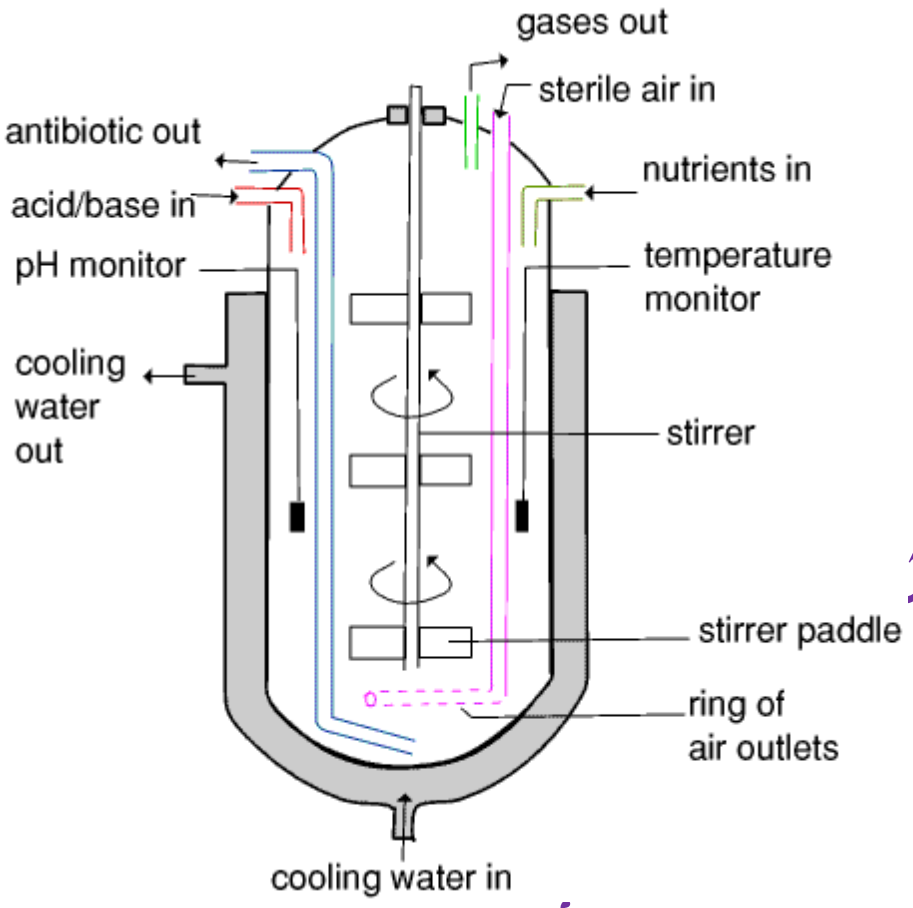

FERMENTOR

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