syringes are removed and suitable broth medium added to the flask. The ends of the tubes are then covered with aluminium foil and the whole apparatus is sterilised by autoclaving. When in use, the fermenter may be kept at a constant temperature by standing it in a water bath at, say, 30 °C. Filter-sterilised air is supplied by means of an aquarium pump, and waste gases are vented through another filter.

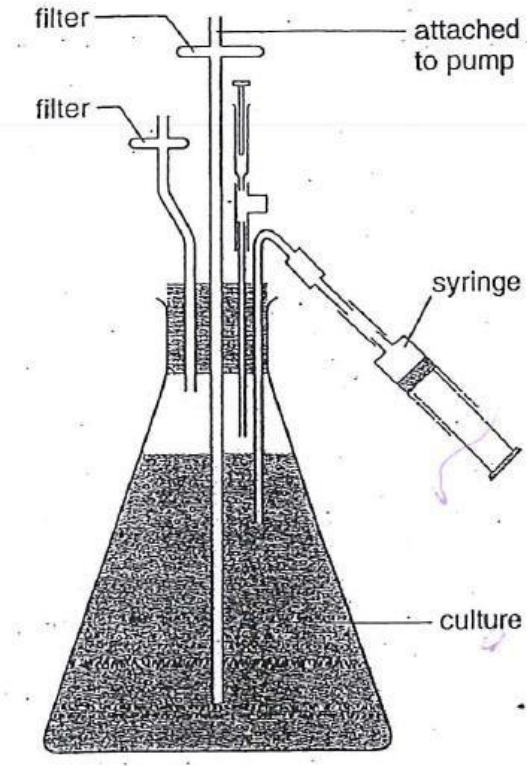


Figure: 4 A simple fermenter

The small syringe at the top of the apparatus is used to inoculate the sterile medium with a culture of the organism to be grown and samples may be removed at regular intervals using the syringe at the side. In this way, the growth of the organism may be monitored using a suitable counting technique, such as a haemocytometer, or by the pour plate dilution method. These are described in the Practical section. This apparatus could also be used for growing Chlorella in a mineral salts medium and keeping the fermenter illuminated using, for example, a Gro-lux fluorescent tube. Figure : 5 shows an industrial fermenter to illustrate how the simple fermenter is scaled up.

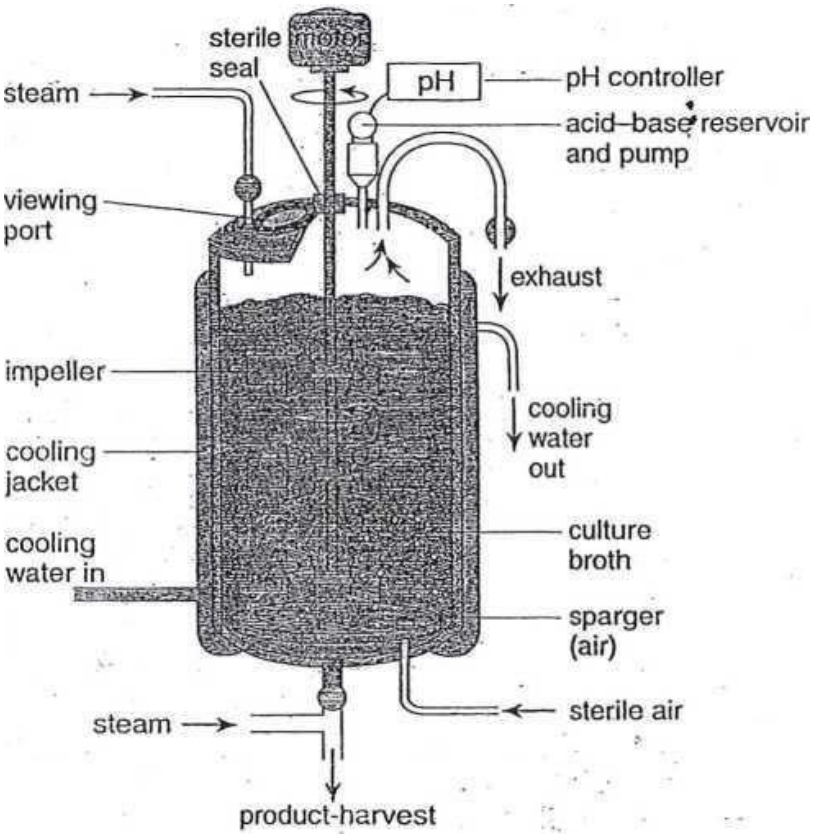


Figure: 5 Diagram of an industrial fermenter ,such as that used to produce the antibiotic penicillin

Industrial fermenters are usually made of stainless steel, which can be sterilised by passing steam, under pressure, through the whole equipment. Industrial fermenters have a number of important features including:

- a cooling jacket through which cold water is passed to remove excess heat produced by metabolic activities of the microorganisms. If the culture is not cooled in this way, the temperature would increase to a point at which enzymes would start to be denatured and the microorganisms killed.
- an efficient system for the aeration of the culture. This includes a sparger a device through which sterile air is pumped under a high pressure, breaking the stream of air into fine bubbles. An impeller is used to stir the contents of the fermenter. Stirring mixes air bubbles with the medium, helping oxygen to dissolve and, ensures the microorganisms are kept mixed with the medium. This ensures that access to nutrients is maintained.

- systems for monitoring the growth of the culture, controlling the pH by the addition of buffers, and for removing the products when growth is completed.

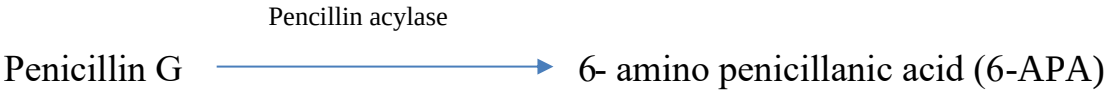
1) Contain N_2 sources & other growth factors
2) Which will be precipitation the enzyme from the solution
3) To separated the individual cells .

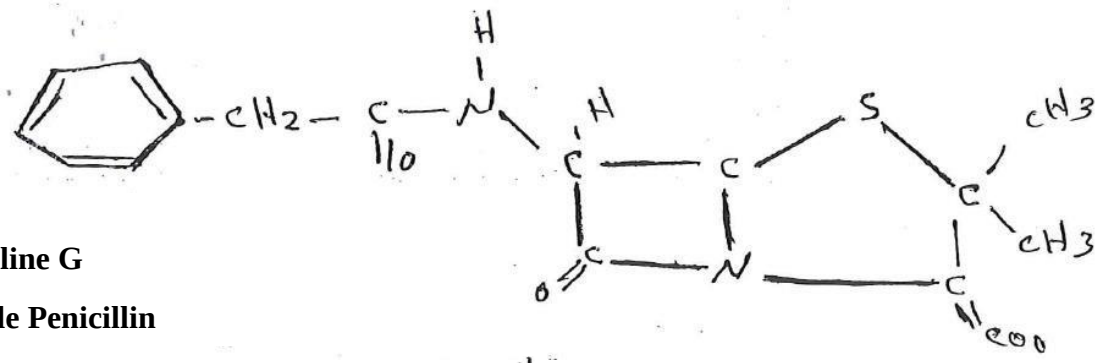
To illustrate the principle of an industrial fermenter, the production of the antibiotic penicillin can be used. The discovery of penicillin , Elexander Fleming. Fleming's original isolate was a strain of *Penicillium notatum*, which yielded about 20 units of penicillin per cm^3 when grown on the surface of a broth medium (1 million units of penicillin G = 0.6 g).

A search for natural variants of Penicillium led to the isolation of *P. crysogenum*, strain NRRL 1951, from a mouldy melon purchased at a market in Peoria, USA. The introduction of this strain, together with a change in culture methods, increased the yield of penicillin to 100 units per cm^3. Repeated steps of mutation and selection have led to the development of the strains of P. cbrysogenum used today, which produce penicillin at a concentration of about 30000 units per cm^3. Industrially, *P. crysogenum* is grown in large fermenters (with a capacity of up to 200 000 dm^3) similar to that shown in Figure5 . The fungus is grown initially in the laboratory on a small scale to produce an inoculum, which is used ultimately to inoculate the fermenter. *P. crysogenum* is grown in stages, from a solid medium, to flask culture in a broth medium, through to 'seed stages' of up to 100 m^3 in order to obtain a large enough inoculum to ensure rapid growth in the final fermenter. Many media for the production of penicillin contain corn steep liquor, a by-product of maize starch production. This contains the nitrogen source and other growth factors. The energy source is usually lactose. The production of penicillin is stimulated by the addition of phenylacetic acid, but the concentration is critical as it is toxic to the fungus. A supply of oxygen is

required, as the growth of *P crysogenum* and the production of penicillin require aerobic conditions. Oxygen is supplied by means of filter-sterilised air pumped into the fermenter.

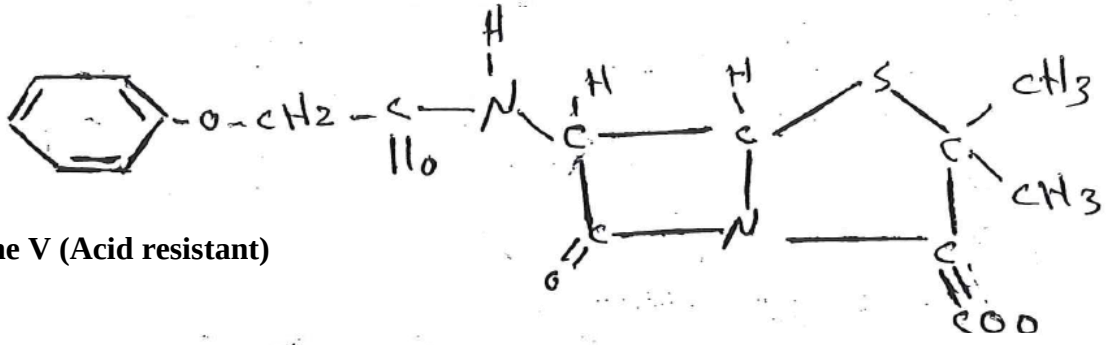
Penicillin is excreted into the medium and so is in solution with various other substances. The process of extraction, purification and subsequent chemical modification of penicillin, referred to as downstream processing, involves solvent extraction. The penicillin is extracted, firstly by filtration, which separates fungal material from the medium, then by using solvent extraction to isolate the penicillin. The pH is first reduced to 2.0 to 2.5 and the penicillin is extracted into an organic solvent such as amyl acetate. Penicillin is then re-extracted back into an aqueous buffer at pH 7.5, concentrated, and then crystallised. Penicillin produced in this way is known as penicillin G, which may be converted to, semi-synthetic penicillins, as a means of overcoming the problems of penicillin-resistant strains of bacteria. Penicillin G is fust converted into 6-amino penicillanic acid (6-APA) using the enzyme penicillin acylase. 6-APA is then chemically modified by adding various chemical side groups, to produce a range of substances known collectively as semi-synthetic penicillins, such as amoxycillin, ampicillin and methicillin. The structures of penicillin G and some examples of semi-synthetic penicillins are shown in Figure 6.



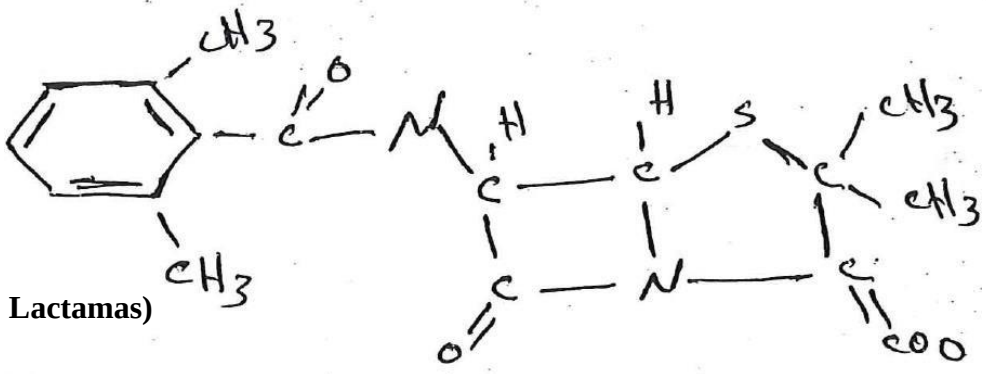


Peniciline G

benzyle Penicillin

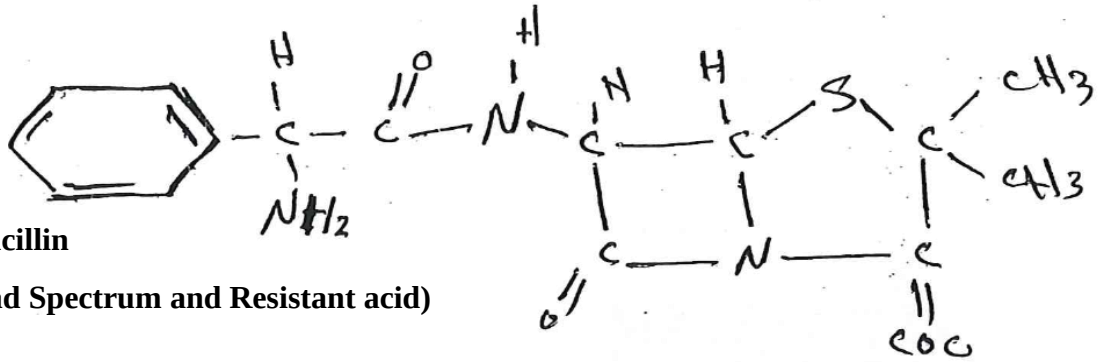


Peniciline V (Acid resistant)



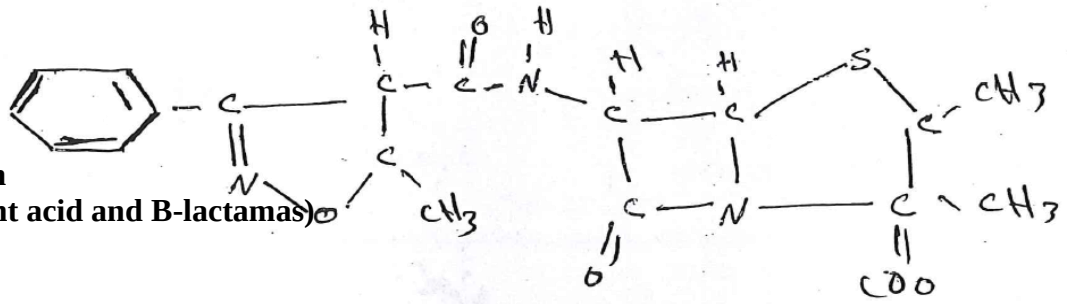
Methicillin

(Resistant to Lactamas)



Ampicillin

(Broad Spectrum and Resistant acid)



Oxacillin

(Resistant acid and B-lactamas)

Figure :6 The structure of Pencillin G and some examples of semi-synthetic penicillins

Antibiotics belong to a group of chemical substances referred to as secondary metabolites. These are substances which are produced by microorganisms, towards the end of the growth phase and into the stationary phase. The synthesis of secondary metabolites is very dependent on the culture conditions, particularly the composition of the medium. It appears that they are not essential for the growth and reproduction of the microorganism, and often accumulate in the growth medium in relatively high concentrations. In order to maximize the production of penicillin, nutrients such as nitrogen sources may be added to the medium towards the end of the growth phase — this is referred to as fed-batch culture.

Similar techniques for the large scale culture of microorganisms can be used for the production of enzymes, such as a-amylase by the *bacterium Bacillus licheniformis*. Many enzymes used in industry are extracellular and are excreted by the microorganisms into the culture medium.

Extracellular enzymes can be extracted from the medium by a process of filtration, to remove the microorganisms, then reverse osmosis is used to separate the enzyme from other components of the medium. The extraction of intracellular enzymes is more complex and involves cell disruption, followed by purification of the enzyme. Cells are disrupted to release the enzymes, by treatment with detergents, or lysozyme (an enzyme which digests some bacterial cell walls), or by mechanical methods. After removal of cell debris, the enzyme may be purified and concentrated using, for example, ammonium sulphate solution which will precipitate the enzyme from solution.

Plant and animal cell culture

The principles involved in the culture of microorganisms can be applied to the culture of cells and tissues obtained from plants and animals.‘‘Essentially, this involves the culture of suitable cells under aseptic conditions, in complex media which have been specially formulated for this purpose. The maintenanc of strict aseptic conditions is essential in cell and tissue culture, as any contaminating microorganisms are likely to grow very much

faster than the plant or animal tissue.

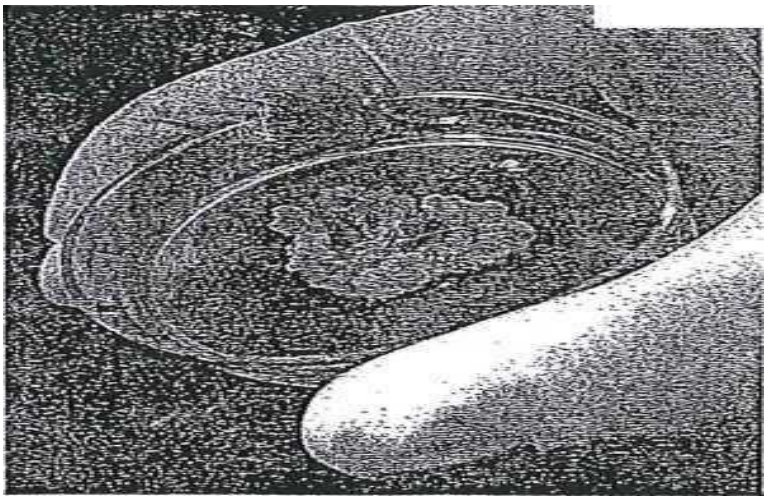


Figure :7 Plant tissue culture on a sterile agar medium

Plant tissue culture involves the growth of isolated cells or tissues in controlled, aseptic conditions. It is possible to use plant tissue culture to regenerate whole plants, a technique referred to as micropropagation. One of the uses of this technique is to propagate rare, or endangered, species which are difficult to propagate using conventional methods of plant breeding. Micropropagation is also used to produce ornamental plants, including pot plants, cut flowers and orchids on a large scale for commercial purposes. The techniques of plant tissue culture are also used to eliminate pathogens from infected plants, for example in the production of virus-free plants, such as carnations and potatoes. There are a number of different types of plant tissue culture, including:

- Embryo culture, cultures of isolated plant embryos
- Organ cultures, cultures of isolated organs including root tips, stem tips, leaf buds and immature fruits
- Callus cultures, which arise from the disorganized growth of cells derived from segments of plant organs, such as roots.

The isolated part of the plant used for culture is referred to as the explant, which can be almost any part of the plant. The tissue used as the explant is grown in culture media containing a variety of mineral nutrients, plant growth regulators such as auxins and cytokinins, sucrose, and amino acids.

A callus culture may be grown by removing tissue from a suitable plant organ such as a carrot. This must be surface sterilised by placing it in a suitable chemical disinfectant such as 20 per cent sodium hypochlorite solution. The carrot is then washed with sterile distilled water and, using sterile instruments and aseptic technique, a segment of tissue removed from the cambium. This is then transferred to a flask containing sterile culture medium and incubated at 25 °C. The explant will grow to form a mass of pells known as a callus, which has a distinctive crumbly appearance. The callus can. be maintained indefinitely by sub-culturing the tissue onto fresh medium every 4 to 6 weeks, or the callus can be transferred to a medium containing a different balance of plant growth regulators and can be. induced to form structures known as embryoids, from which complete, plants can be regenerated. This method has a number of important commercial applications, such as the rapid propagation of agricultural crop plants.

Animal cells which are cultured can be derived from explants of the four basic tissue types, epithelial, connective, nervous or muscular tissues. Some .of these cells, such as lymphocytes (derived from connective tissue), can be grown in a suspension culture, similar to bacteria in a liquid medium. Most normal mammalian cells, however, grow attached to a surface and form a single layer of cells referred to as a monolayer. Tissues removed from an animal are usually treated with a proteolytic enzyme, such as trypsin, to separate individual cells. The cells are then washed in sterile saline solutions and transferred to a suitable sterile container, such as a plastic flask, containing

A culture medium. The cells settle on the bottom of the flask, attach, and begin to divide to form a monolayer. The cells can be removed, by treatment with trypsin, and used to inoculate fresh medium. In this way, the growth of some cells can be maintained indefinitely, whereas some cells have a finite capacity for growth.

Media used for animal cell culture are usually very complex and contain a range of amino acids, glucose, vitamins and other enzyme cofactors, inorganic ions and buffers to maintain the pH. Serum may also be added to the media to provide essential growth factors. Antibiotics, such as penicillin and streptomycin, are sometimes added to the media to inhibit the growth of bacteria which may accidentally contaminate the cultures.