Fermenters:

Use of fermenters

Microorganisms may be grown on a large scale for the purposes of producing a wide range of useful products including antibiotics, enzymes, food additives and ethanol.

Fermenters are vessels used for the growth of microorganisms in liquid media. These vary in size from small scale laboratory fermenters containing perhaps 250 cm^3 of medium, to very large-scale industrial fermenters containing up to 500 000 dm^3. The majority of microorganisms grown are aerobic and it is therefore essential to ensure an adequate supply of oxygen to maintain aerobic conditions.

Two main systems for culturing microorganisms are used, referred to as **batch culture and continuous** culture. **In batch** culture, growth of the microorganism occurs in a fixed volume of medium and, apart from oxygen, substances are not normally added to the medium during culture. The organism typically goes through the usual phases of growth, that is, lag, exponential and stationary. The organism continues to grow in the medium until conditions become unfavorable. In **continuous culture**, fresh, sterile medium is added to the fermenter at a constant rate and spent medium, together with cells, is removed at the same rate. **The number of cells and the composition** of the medium in the fermenter therefore remains constant. Continuous culture can, theoretically, run indefinitely but, apart from the production of Quorn™ mycoprotein, few industrial cultures are maintained continuously.

To illustrate the principle of a fermenter, Figure 4 shows a simple fermenter which is suitable for use in a school laboratory.

This fermenter could be used to grow an organism such as yeast (*Saccbaromyces cerevisiae*) under controlled conditions. Before use, the syringes are removed and suitable broth medium added to the flask. The ends of the tubes are then covered with aluminum foil and the whole apparatus is sterilized 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-sterilized air is supplied by means of an aquarium pump, and waste gases are vented through another filter.

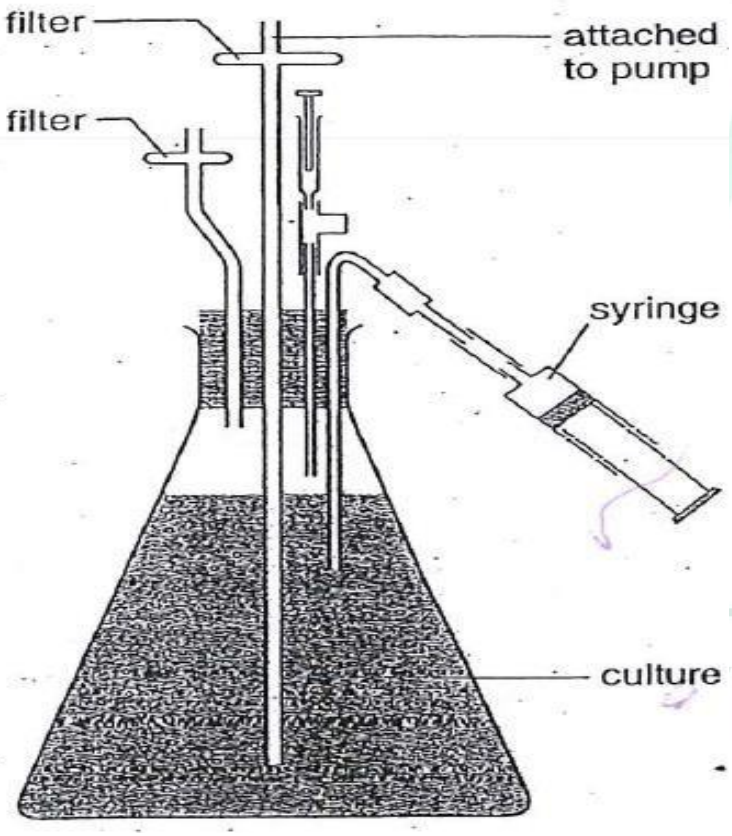

Figure: A simple fermenter

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 salt medium and keeping the fermenter illuminated using, for example, a Grolux fluorescent tube. Figure below shows an industrial fermenter to illustrate how the simple fermenter is scaled up.

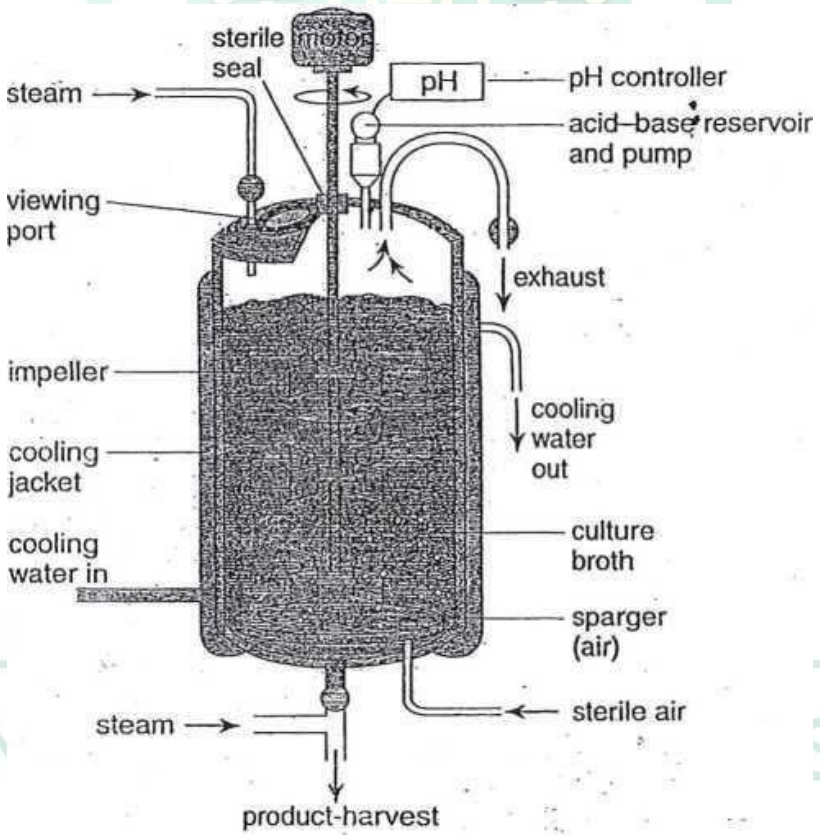

Figure: Diagram of an industrial fermenter, such as that used to produce the antibiotic penicillin

Industrial fermenters are usually made of stainless steel, which can be sterilized 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. crysogenum* 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 $\mathbf{cm^3}$ 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 first 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.