اسس تقنيات احيائيه

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Biotechnology: Scope and importance

What is Biotechnology?

The term biotechnology was coined in 1917 by a Hungarian Engineer, Karl Ereky, to describe a process for large scale production of pigs. According to him all types of work are biotechnology by which products are produced from raw materials using living organisms.

- Biotechnology is defined by different organization in different ways. It has been broadly defined as "the development and utilization of biological processes" forms and systems for obtaining maximum benefits to man and other forms of life.
- Biotechnology is the science of applied biological process (1981) .
  * The application of biochemistry, biology, microbiology and chemical engineering to industrial process and products and on environment. (International Union of pure and Applied Chemistry (IUPAC, 1981).
  * Biotechnology is "the controlled use of biological agents such as microorganisms or cellular components for beneficial use (U.S. National Science Federation).

B-History of Biotechnology:

If we trace the origin of biotechnology, it is as human civilization. Development of biotechnology can be studied considering its growth that occurred in two phases:

1- The traditional (old) biotechnology

2- The new (moden) biotechnology.

1-THE TRADITIONAL BIOTECHNOLOGY

The traditional biotechnology is really the kitchen technology developed by using the fermenting bacteria.

Kitchen technology is as old as human civilization. During period (5000-7000 B.C), Sumerians and Babylonians were drinking the beer.

The traditional biotechnology refers to the conventional technology which have been used for many centuries. Beer, wine, cheese and many foods have been produced using traditional biotechnology which are based on the natural capabilities of microorganisms.

In Egypt and Palestine (about 1500 B.C.), the art of production of wine from crushed grapes.

Role of Microorganisms in Fermentation.

The causes of fermentation could be discovered after observing microorganisms using a microscope by Antony van Leeuwenhoek (1673-1723, Holland).

- The study of microbiology was started since the first report of Louis Pasteur (1857) on lactic acid fermentation from sugar.
- Pasteur suggested that high percentage of microbial population is killed by heating the juice at 62.8 °C (145 °F) which is now called as Pasteurization.
- Robert Koch (1881) gave the method for established relationship of a pathogen with a disease using the pathogenicity test, following this technique he proved that the anthrax disease is caused by *Bacillus anthracis*.

- Edward Buchner (1897) was the first to demonstrate enzymatically-mediated fermentation reaction; he showed that cell free yeast extract mixed with concentrated sugar solution produced the carbon dioxide (Co_2) and ethyl alcohol.
- Discovery of viruses and their role in disease was possible at 1884 by Charles Chamber land.
- Emil Von Behring (1890) injected the inactivated toxin into rabbit that induced to produce antitoxin in the blood. The antitoxin inactivated the toxin and protected against the disease.

2- MODERN BIOTECHNOLOGY:

The major feature of technology differentiates the modern biotechnology from the classical biotechnology is the capability of science to change the genetic material for getting new products for specific requirement through recombinant DNA technology.

- During World War I fermentation processes were developed which produced the acetone from starch.
- During World War II the antibiotic penicillin was discovered.
- After the discovery of double helix DNA by Watson and Crick (1953), Werner Arber (1971) discovered special enzyme in bacteria which he called the Restriction enzymes. These enzymes can cut the DNA strand and generate fragments.
- In 1973, S. Cohen and H. Boyer removed a specific gene from a bacterium and inserted into another bacterium using restriction enzymes. This discovery marked the start of recombinant DNA technology or genetic engineering.
- In 1978, a U.S company "Genetech" used genetic engineering technique to produce human insulin in *E.coli* (*Escherichia coli*)

Dolly - the first mammalian Clone

- In 1996, the first clone lamb "Dolly" was borne successfully by Scotland scientists.
- In 2001, the sequence of the Human Genome was published in Nature and science. Human Genome Project was completed by March 2003.
- Many biochemical companies such as Genetech Co. (USA) and Biogen (Switzerland) are producing of growth hormones, insulin, vaccines, immunogenic proteins, gene therapy, biofertilizers, Enzymes, antibiotics, acid and fuels.

The key difference between biology and biotechnology is their scale of operation. Usually the biologist works in the range of nanograms to milligrams but biotechnologists working on the production of vaccine may be satisfied with milligram –kilogram or tones (Smith 1996).

This led the evolution of biotechnology which is an outcome of integrated effort of biology with technology, the root of which lies in biological science (fig-1).

Following are some of the areas where biotechnology has done the best. (Table – 1).

1- Health care:

Biotechnology derived proteins and polypeptides (such as insulin) from the new class of potential drugs.

Since 1982, human insulin (Humulin) has been produced by microorganisms in fermenters, similarly hepatitis B vaccines are genetically engineered vaccines produced biotechnologically.

Some of the important products produced through genetically modified organisms are given in the table – 2.

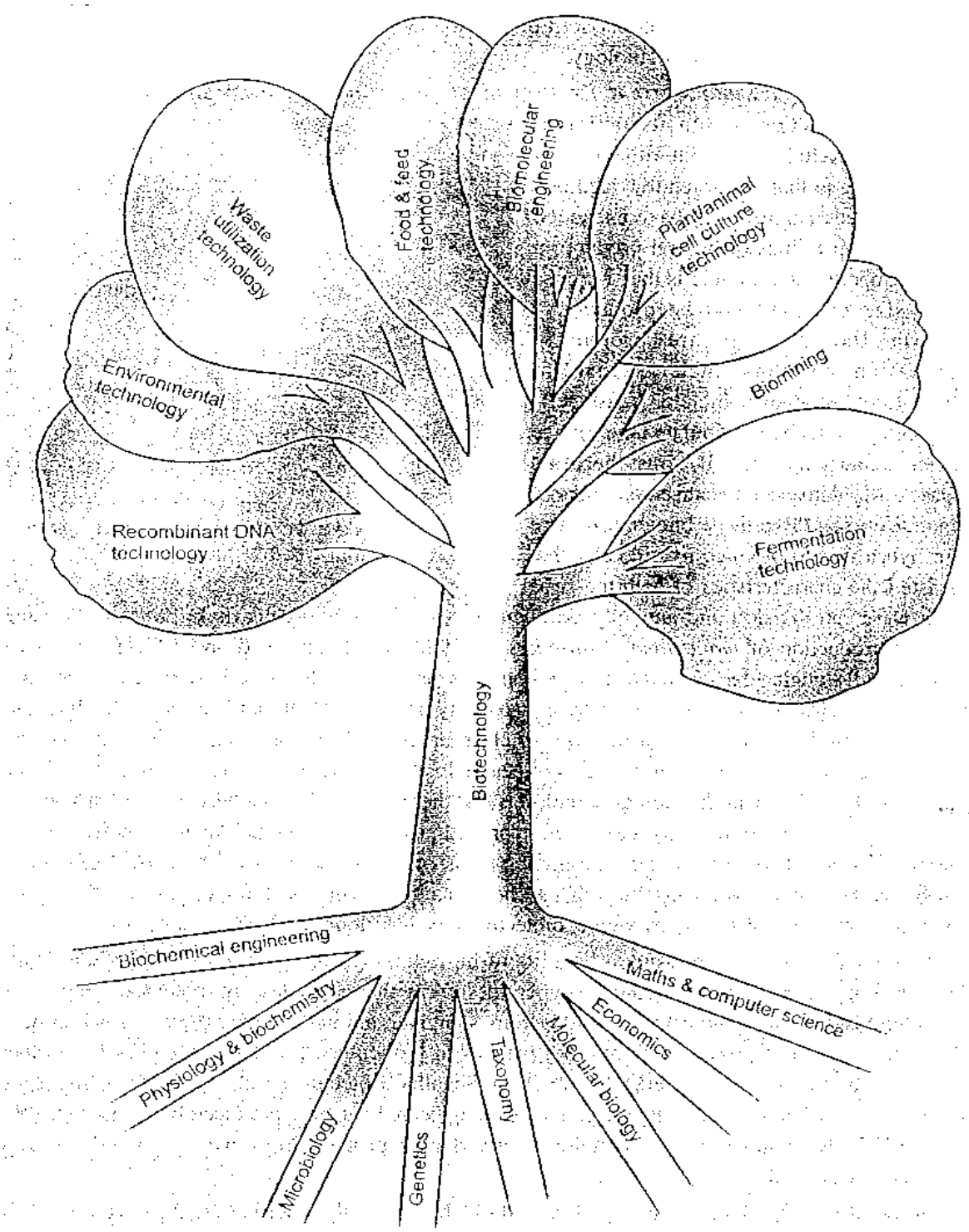

Fig. l. A Biotechnology tree.

Table – 1 Area of biotechnology.

Area of interest	Products
1. Recombinant DNA technology (genetic engineering)	Fine chemicals, enzymes, vaccines, growth Hormones, antibiotics, interferon.
2. Treatment and utilization of bio-materials (biomass)	Single cell protein, mycoprotein, alcohol and biofuels.
3. Plant and animal cell culture	Fine chemicals (alkaloids, essential oils, dyes, steroids), somatic embryos, encapsulated seeds, interferon, monoclonal. antibodie
4. Nitrogen fixation	Microbial inoculants (biofertilizers)
5. Biofuels (bioenergy)	Hydrogen (via photolysis;), alcohols (from biomass), methane (biogas produced from wastes and aquatic weeds).
6. Enzymes (biocatalysts) chemotherapy	Fine chemicals, food processing, biosensor
7. Fermentation	Acids, enzymes, alcohols, antibiotics, fine chemicals, vitamins, toxins (biopesticides).
8. Process engineering	Effluent, water recycling, product extraction, novel reactor' harvesting.

Table – 2: Example of some therapeutic products produced through recombinant DNA Technology

Products	Application
Interferon	Cancer and Viral infection
Human urokinase (tPA)	Plasminogen activator used in vascular disorder
Insulin	Treatment of diabetes
Human factor IV	Clotting factor for hemophilia
Lympholines	Auto-immune functioning
Serum albumin	In surgery
Attenuated pseudorabies virus antigen	Vaccine against rabies
Tissue plasminogen activator	In treatment of heart attack
Somatosatin	Treatment of Human growth disorder

2- Agriculture:

Biotechnology is making new ground in the food agriculture area.

Bovine somatotropin, BST (a hormone administrated to cows to increase milk production) is an example of biotechnology product.

- The Flavrsarv ^{Tm} tomato (produced by transgenic plants engineered to preserve flavor and quality.
- A transgenic "Golden rice" has been produced by introducing three genes for the production of vitamin A.
- Several insect resistant transgenic Bt plant have been produced by Bt gene of *Bacillus thuringiensis*.

3- **Human Genome project (HGP):** Almost the whole human genome has been sequenced and chromosome map has been developed in laboratories. Human chromosome mapping was completed by March 2003. There are about 33,000 functional genes in human. More than 97% genes are non-functional (that do not encoded for any polypeptide chain.)

4- **Environment:** The natural biodegradability of pollutants present in environment has increased with use of biotechnology.

Bioremediation: Is the use of microorganisms (such as *Bacillus, Candida* and *Trichoderma*) to detoxify pollutants present in soil or water.

5- **Genomics and proteomics:**

Genomic: computer-based study and designing of genome.

Proteomic: Study of proteins present on genome of organism using computer.

6- **Bioinformatics**: It is a new field of biotechnology linked with information technology.

Bioinformatics: may be defined as application of information sciences (mathematics, statistics and computer sciences) to increase the understanding of biology, biochemistry and biological data.

Culture Techniques

Requirements for growth

Like all living organisms , microorganisms require an energy source , usually in the form of an organic carbon compound , and a range of other nutrients for metabolism and cell growth . Microorganisms can be divided into two main groups according to the source of energy utilized. **Prototrophs** use light as an energy source , whereas **chemotrophs** use different chemical substances as their source of energy . Many microorganisms use organic substances as energy sources, although some are able to use inorganic substances. These are both different types of chemotrophs , but those which use organic substances as energy sources are known as **chemoorganotrophs** , whereas those which use inorganic substances are referred to as **chemolithotrophs** .

The nutrients which are required by microorganisms can be divided into two groups:

- **Macronutrients**, which are required in relatively large amounts
- **Micronutrients**, which are required in small quantities.

Macronuirients include carbon, hydrogen, oxygen, nitrogen, sulphur, magnesium and iron (Table : l). All nutrients have to be provided in a suitable form in the culture media in which the microorganisms are grown. Carbon is often provided in the form of organic substances, including glucose, organic acids, fatty acids or amino acids. Phototrophic microorganisms, such as ***Chlorella***, use carbon dioxide as their carbon source. Nitrogen is available to microorganisms either in organic substances, such as amino acids or nucleotide bases, or as inorganic substances including ammonia or nitrate ions. Many bacteria are able to use ammonia as their only source of nitrogen; the nitrogen fixing bacteria, such as ***Rhizobium***, use nitrogen gas.

Table : 1 Macronutrients required by microorganisms and the forms in which they are supplied in culture media

Elements	Forms in which the elements are supplied in culture media
Carbon (C)	Glucose , organic acid , yeast extract , peptone
Hydrogen (H)	Water , organic compound
Oxygen (O)	Water , oxgen gas , organic compounds
Nitrogen (N)	Nitrogen gas ammonium ions , nitrate ions , amino acid nucleotide bases
Phosphorus (P)	Inorganic phosphates
Sulphur (S)	Sulphates , sulphur – containing amino acids
Magnesium (Mg)	Magnesium salts such as magnesium sulphate
Sodium (Na)	Sodium chloride
Calcium (Ca)	Calcium chloride
Iron (Fe)	Iron salts such as iron sulphate
Potassium (K)	Potassium salts such as potassium chloride

Micronutrients (or trace elements) are metals and are essential for normal cell function. They are required in very small quantities and it is not normally necessary to add these separately to culture media as they will often be present in sufficient quantities in other ingredients. Micronutrients include copper, manganese, vanadium and zinc. These may function as enzyme activators or as constituents of enzyme molecules. Iron is sometimes considered to be a micronutrient, although it is required in larger quantities than the other metals.

In addition to these micronutrients, microorganisms may also require certain organic growth factors in very small amounts. Such factors include amino acids, vitamins, purines and pyrimidines. These compounds can be synthesized by the majority of microorganisms, but some microorganisms may require one or more of these to be present in their culture media.

All the nutrients required by microorganisms must be provided in the media in which they are grown. There are two main types of culture media used in

microbiology, defined and undefined (or complex) media. **Defined media** are made up using pure chemical substances, dissolved in distilled water so that the exact chemical composition is known. **Undefined media** contain mixtures of substances such as yeast extract, peptone, or casein hydrolysate, in which the exact composition is unknown. To illustrate these types of culture media, Table 2. shows examples of culture media for *Escherichia coli*.

Table 2.: Examples of culture media for *E. coli*

Defined culture medium for ***E. coli***	Un defined culture medium for ***E. coli***
K_2HPO_4	Glucose
KH_2PO_4	Yeast extract
$(NH_4)_2SO_4$	Peptone
$MgSO_4$	KH_2PO_4
$CaCl_2$	Distilled water
glucose	
Distilled water	

Environmental influences on growth

We have described the nutrients which are required by microorganisms for growth, but there are several other factors which have important influences on growth. These factors include **temperature**, availability of **oxygen**, **light** (for phototrophic microorganisms) and **pH.** Temperature is one of the most important factors. In general, as temperature increases, enzyme activity within the cells also increases and growth becomes faster. However, above a certain temperature, proteins - including enzymes will be denatured. Therefore, growth will increase up to a point above which enzymes become denatured and inactivated. Above this point, growth rate falls rapidly to zero. For every microorganism, there is a **minimum** temperature, below which there is no growth, an **optimum** temperature

where growth occurs at the most rapid rate and **a maximum** temperature above which growth will not occur.

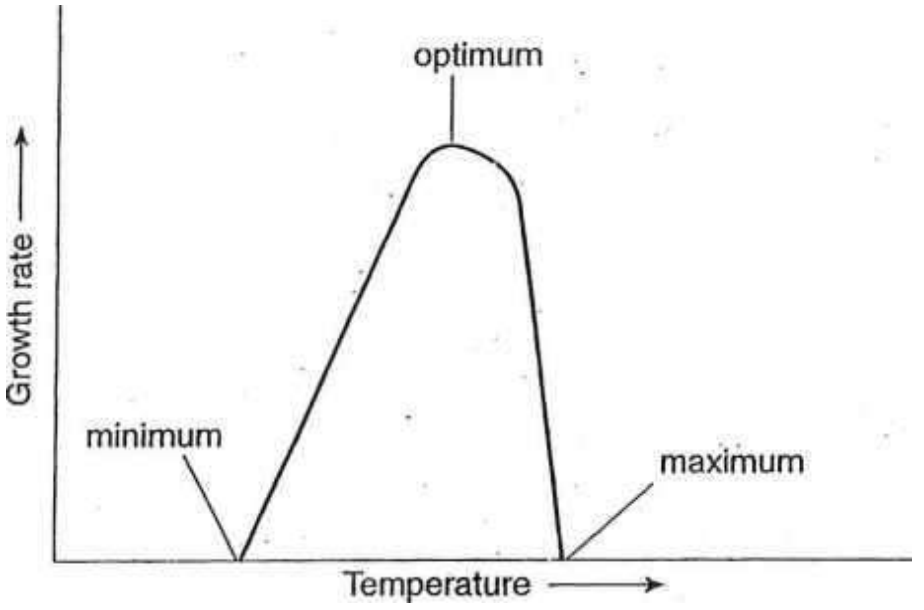

Figure : 1 The effect of temperature on the growth rate of a microorganism

The optimum temperature for microorganisms varies widely; some have optima between 5 and 10 °C, whereas at the other extreme, some have optima at 80 °C or above. Microorganisms can be divided into four groups on the basis of their temperature optima:

- **Psychrophiles**, which have low optima, e.g**.** ***Flavobacterium***, optimum **13 °C**
- **Mesophiles**, which have mid-range optima, e.g. ***Escherichia coli***, optimum **39 °C**
- **Thermophiles**, with high optima, e.g. ***Bacillus stearothermophilus***, optimum **60 °C**
- **Hyperthermophiles**, with very high optima, e.g. ***Thermococcus celer***, optimum **88 °C.**

Microorganisms also vary in their requirements for **oxygen**. Some grow only in the presence of oxygen, whereas others grow only in the absence of oxygen.

To the latter group of microorganisms, oxygen is actually toxic, probably because they are unable to remove toxic products of oxygen metabolism, such as **hydrogen**

peroxide. On the basis of their requirements for oxygen, microorganisms can be separated into several groups, as outlined below:

- **Obligate aerobes**, which will grow only in the presence of oxygen,

e.g. ***Micrococcus luteus***

- **Facultative aerobes**, which can grow in the absence of oxygen, but grow better if oxygen is supplied, e.g. ***Escherichia coli***
- **Microaerophilic aerobes**, which require oxygen at lower concentrations than atmospheric, e.g. ***Spirillum volutans***
- **Obligate anaerobes**, which will grow only in the absence of oxygen, e.g. ***Desulphovibrio***.

In small-scale culture, such as on agar in Petri dishes or in universal containers of broth media, oxygen diffuses directly from the air to the microorganisms. However, if aerobic microorganisms are grown on a larger scale, such as in a laboratory fermenter, it is necessary to aerate the culture, usually by bubbling sterile air through the medium. This supplies the microorganisms with oxygen, where otherwise . The rate of diffusion and poor solubility of oxygen in water would mean that insufficient oxygen was available. If it is necessary to culture obligate anaerobes, for example in a hospital laboratory, they are grown on agar plates placed in a special container referred to as an anaerobic jar, a container which can be filled with a mixture of hydrogen and carbon dioxide to replace the air. The jar contains a catalyst which will remove any residual oxygen, to ensure anaerobic conditions.

We have seen that microorganisms vary widely in their tolerance to environmental **temperatures** and **availability of oxygen**; they also have a wide range of environmental **pH** values at which they can grow. Most microorganisms have a pH **optimum** between 5 and 9, but a few species can grow at pH values outside this range. In general, fungi tend to be more tolerant of acid conditions than bacteria, with optima at pH 5 or below. These pH values refer to the extracellular environment, and although this may vary widely, the intracellular pH remains

nearly neutral. The pH of culture media is kept relatively constant by the use of buffer solutions, such as phosphate buffers. During the growth of a microorganism, the pH of the medium may change due to the production of acidic or alkaline products of metabolism and this may be regulated by the addition of appropriate sterile buffer solutions during the growth phase.

Figure:2 An anaerobic jar for the incubation of cultures under anaerobic conditions. Air in the jar is replaced with an oxygen-free gas mixture, such as hydrogen and carbon dioxide, or a chemical catalyst is placed in the jar which removes oxygen from the atmosphere

Growth of cultures

Under favorable conditions, the number of single-celled microorganisms will double at regular intervals. This is because each of the two daughter cells produced will have the same potential for growth as the original parental cell. **The time required for the number of cells to double is known as the mean doubling time**. Table : 3 shows how the number of cells will increase, starting with a single cell, assuming a doubling time of 20 minutes. The table shows both the arithmetic number of cells, and the number expressed as a logarithm to the base 10.

Table : 3 Increases in the numbers of bacterial cells with a doubling time of 20 minutes

Time /minutes	Number of divisions	Number of cells	Log_{10} number of cells
0	0		
20	1	2	0.3
40	2	4	0.62
60	3	8	0.9
80	4	16	1.2
100	5	32	1.5
120	6	64	1.8
140	7	128	2.1
160	8	256	2.4
180	9	512	2.7
200	10	1024	3.0

- Calculation the number of microbial cells after n generations (Generation time : The time taken for a bacterial population to double in number).

If the original inoculums is 10000(10^4) cell / cm^3 or ml

After 1 generation : $10000 \times 2 = 10000 \times 2^1 = 20000/cm^3$

After 2 generation : $10000 \times 2 \times 2 = 10000 \times 2^2 = 40000/cm^3$

After 3 generation : $10000 \times 2 \times 2 \times 2 = 10000 \times 2^3 = 80000/cm^3$

After n generation : $10000 \times 2 \times 2 \times 2 \times \ldots = 10000 \times 2^n$ cell/cm^3

Therefore = **$N = No \times 2^n$**

Where **No** = original number of cells

N = number of cells after **n** generations .

The growth rate is sometimes expressed as the time taken for the population to double, or the mean doubling time.(This is the reciprocal of the exponential growth rate, that is, 1/k. In the example above, then mean doubling time is 1/2.91, or approximately 21 minutes).

So far, we have considered cells only in the exponential phase of growth. Cell growth, with a limited supply of nutrients, does not continue indefinitely. **The growth of the population is normally limited** by either the exhaustion of one or more essential nutrients, or by the accumulation of toxic by-products of metabolism. Figure : 3 shows a complete growth curve for a microorganism. Four distinct phases of growth can be seen:

- **Lag phase**
- **Exponential (or logarithmic) phase**
- **Stationary phase**
- **Death phase.**

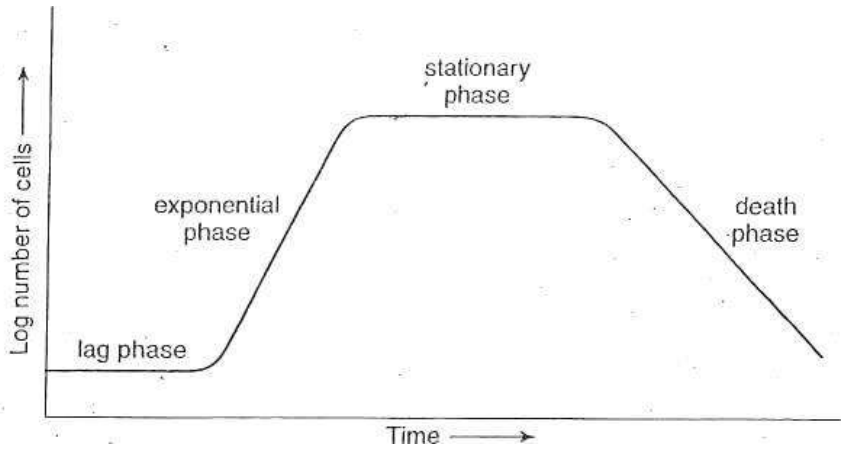

Figure:3 Typical growth curve for a bacterial culture

When fresh, sterile medium is inoculated with a culture of a microorganism, growth may begin immediately. There is a period of time in which the cells are synthesizing the enzymes required for the metabolism of nutrients present in the medium. This period of time is referred to as the **lag phase**, and can be seen as a period of adjustment to the culture conditions. A lag phase does not always occur. If, for example, cells which were already in the exponential phase were transferred to fresh, identical medium, exponential growth would continue at the same rate.

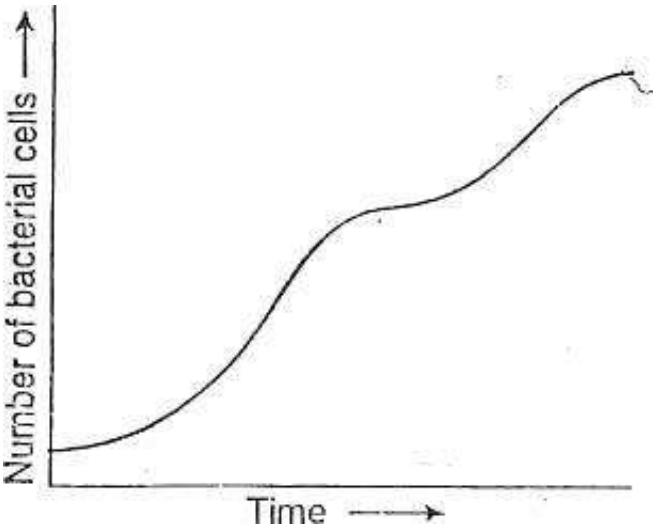

Figure : 4 Diauxic growth of E. coli in a medium containing glucose and lactose

We have already described **the exponential phase** as a period of constant growth in the size of the microbial population, in which both cell numbers and cell mass increase in parallel. The growth rate constant is affected by both genetic and environmental factors; it varies from one species to another, and is influenced by such factors as the concentration of nutrients in the growth medium, temperature and pH. The exponential phase is followed by the **stationary phase**, in which the overall growth rate is zero. During this phase, slow growth of some cells may occur, which is balanced by the death of others, so that the total number of viable cells remains constant. This phase is followed by the death phase in which the number of viable cells progressively decreases. **Death of cells** may be accompanied by cell lysis so that both the total cell number and the viable cell count decreases.

Diauxic growth is sometimes observed if a microorganism is grown in a medium containing two different carbon sources. **Diauxic growth** is characterized by two distinct phases of exponential growth, separated by a brief lag phase. For example, if *Escherichia coli* is grown in a medium containing both glucose and lactose, the glucose will be metabolized first. Glucose actually inhibits the synthesis of lactase (β-galactosidase) (referred to as catabolite repression) and only after the glucose has been used up will lactase be synthesized. Growth then resumes using lactose as an energy source.

Methods for measuring the growth of microorganisms

We have seen that the growth of a culture of unicellular microorganisms results from an increase in the number of cells, so growth of microbial populations can be measured by determining changes in either the number of cells, or the cell mass. The number of cells in a suspension can be determined by counting the number of cells present in an accurately determined, very small volume of culture medium. This is usually carried out using special microscope slides, known as counting chambers (Counting cells using a haemocytometer). These are slides which are ruled with a grid of squares of known area and are made so that when correctly filled, they contain a film of liquid of known depth. The volume of liquid overlying each square is therefore known. This method for determining cell numbers is referred to as a total cell count, which includes both viable and non-viable cells, as it is not normally possible to distinguish one from another using a microscope.

The number of cells can also be determined using a plate count (Counting cells using the pour plate dilution method). This method depends on the ability of each single, viable cell to grow in or on an agar medium and produce a visible colony. This method of counting is referred to as a viable count, as only those cells which are able to grow in the culture medium are detected. Appropriate dilutions of a bacterial culture are made and are used to inoculate a suitable medium. The number of viable cells present in the original' culture is then determined by counting the number of colonies which develop after incubation of the plates, and multiplying this number by the dilution factor. Two or three replicate plates of each dilution should be prepared, to reduce the sampling error. The greatest accuracy is obtained with relatively large numbers of colonies on each plate, but the practical limit is reached with between 30 and 300 colonies per plate.

The only direct method for determining cell mass is to measure the dry mass of cells in a known volume of culture medium. This is a suitable method for measuring the growth of a filamentous organism, where cell counting is inappropriate, but it is rarely used for unicellular bacteria, because of the relatively

insensitive method for weighing. It is difficult to weigh with an accuracy of less than 1 mg, but this represents the dry mass of between 1 and 5 x 10^9 bacteria. .

One useful approach for estimating the number of cells present in a suspension is to use an optical method, by determining the amount of light which is scattered by a cell suspension. A suspension of cells appears cloudy, or turbid, to the eye because the cells scatter light passing through the suspension. The cloudiness increases as the cell numbers increase and, within limits, the amount of light scattered by the cells is proportional to their numbers. A colorimeter is an instrument which can be used to measure the amount of light which is transmitted by a cell suspension. When a beam of light passes through a cell suspension, the reduction in the amount of light transmitted gives a measure of cell density. A colorimeter can be calibrated, by combining measurements of light transmitted with another method for- measuring cell growth, such as plate counting.

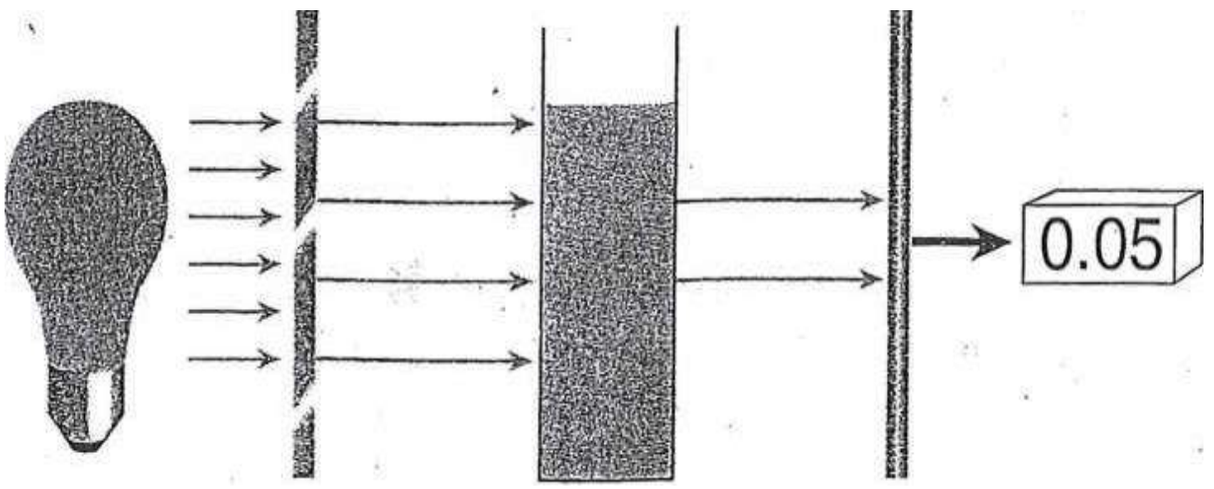

Figure:1 Principle of a colorimeter used to measure cell growth

Methods for culturing microorganisms

If we wish to study a single species of microorganism, it is often necessary to isolate it from a mixed culture of many different species. Microorganisms are

present in almost every habitat, soil and water are particularly rich sources, and in order to obtain a pure culture of a single species, it must be grown in a laboratory in suitable conditions, with all the necessary nutrients provided. It is also essential to avoid contaminating the culture with other, unwanted microorganisms. The medium used to grow the microorganism must be sterile, and it is essential to take precautions in handling the materials used for culture of the organism in order to avoid contamination.

Aseptic technique is the term used describe the proper handling of cultures, sterile apparatus and sterile media to prevent contamination. The method used to pour sterile agar medium into petri dishes.

Transfer of cultures from a mixed broth culture onto an agar plate, is usually carried out using a bacteriological **loop** which has been sterilized by heating until it is red hot using a Bunsen burner. If we wish to isolate a fungus from a mouldy tomato, a mounted needle can be used, sterilized in the same way as the loop. The needle is then used to transfer some of the fungal material, including spores, from the tomato to the centre of a Petri dish containing a suitable medium, such as malt extract agar.

Microbiological media are formulated to contain all the nutrients required by particular microorganisms. Some species of microorganism are particularly fastidious in their requirements and their media, are therefore complex, containing a wide range of mineral salts, amino acids, purines, pyrimidines, vitamins and other organic growth factors. Other microorganisms will grow in relatively simple media containing an energy source, usually glucose, and a number of mineral salts. Different microorganisms therefore have different nutrient requirements and for the successful culture of a particular species it is necessary to provide all the essential nutrients, in the correct proportions, in the culture media.

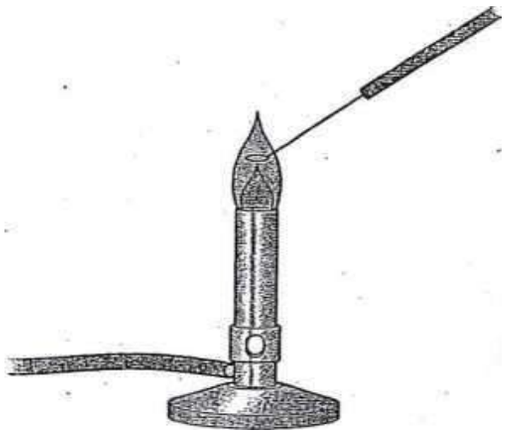

Figure : 2 Sterilizing a bacteriological loop. The loop should be held in the flame until it is red hot, then allowed to cool briefly before being used to transfer a culture aseptically. The loop must always be flamed again after use

Selective media contain substances which selectively inhibit the growth of certain microorganisms, whilst allowing others to grow. These media are particularly important in medical microbiology, as they are used to culture and isolate organisms from clinical specimens such as blood **and** urine. An example of a selective medium is **MacConkey agar**, which contains lactose and bile salts. This is used to isolate enteric bacteria, that is, bacteria which grow in the intestinal tract. Bacteria such as *Escherichia coli*, which are able to utilize lactose as an energy source, are able to grow, but the growth of other species of bacteria, including *Staphylococcus* spp. will be inhibited.

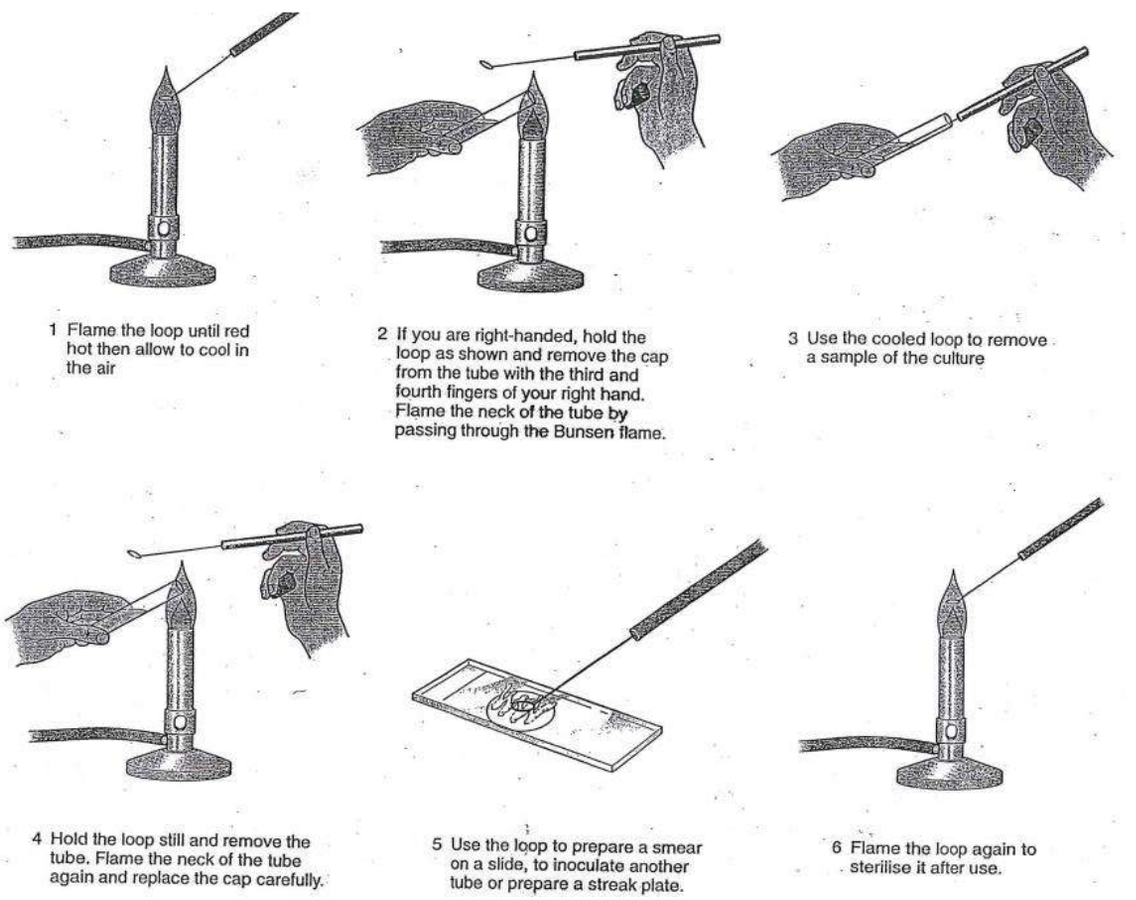

Figure:3 Using a bacteriological loop to carry out an aseptic transfer

Some microbiological media contain a coloured pH indicator substance, such as phenol red or bromocresol purple. These are known as indicator media (or differential media) and will show whether or not a change in pH has occurred as a result of the metabolism of the bacteria during growth. For example, if the bacteria produce **acids**, then a broth medium containing phenol red will change in colour from red to yellow. **Eosin-methylene blue** (EMB) agar is an example of a medium which is both selective and an indicator medium and is used to isolate Gram-negative enteric bacteria. EMB agar contains lactose and sucrose as energy sources, and the dyes eosin and methylene blue. Methylene blue inhibits the growth of. Gram-positive bacteria; eosin changes colour according to the pH of the medium, changing from colourless to black in acidic conditions. ***E. coli*** will grow in EMB agar to produce colonies which are black, with a greenish metallic sheen. ***Salmonella*** produces colonies which are translucent, or pink.

Although selective media are important in the isolation and identification of bacteria, many species can be successfully cultivated using nutrient broth, or nutrient agar. Broth media may be solidified by the addition of agar, a polysaccharide obtained from red algae. Agar is usually added at about 1.5 per cent by weight to the medium, and dissolved by boiling. On cooling to about 42 °C, the medium will set to produce a clear, firm gel.

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 unfavourable. 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.

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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 unfavourable. 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.

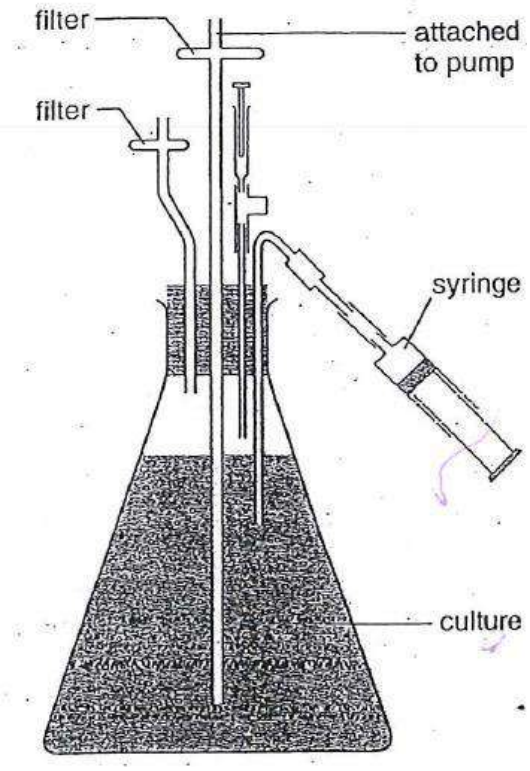


Figure: 4 A simple fermenter

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 Grolux fluorescent tube. Figure : 5 shows an industrial fermenter to illustrate how the simple fermenter is scaled up.

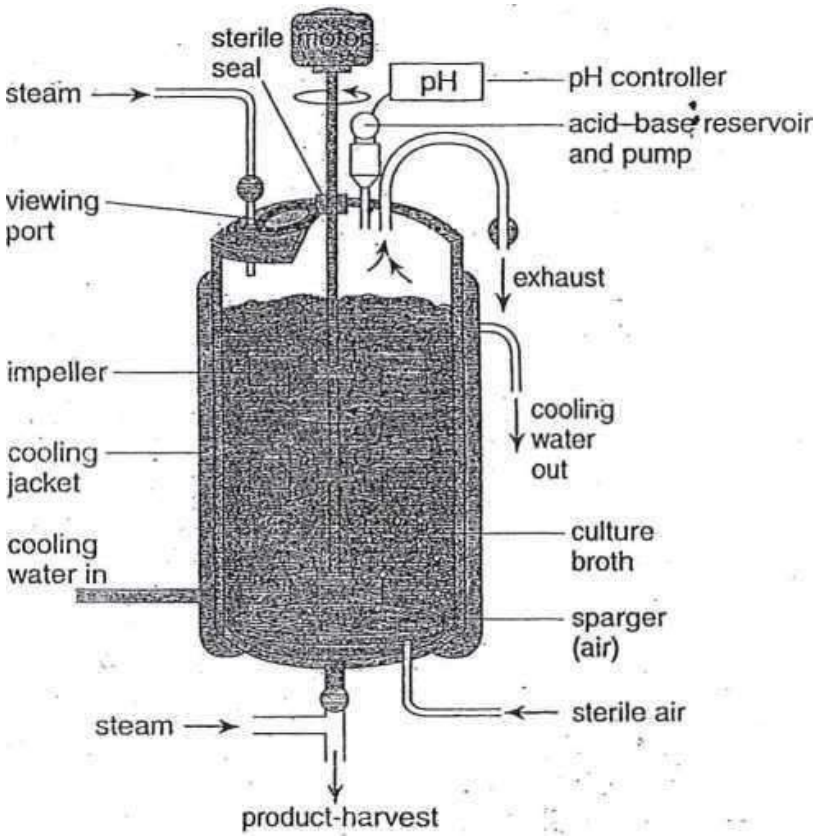


Figure: 5 Diagram of an industrial fermenter, such as that used to produce the antibiotic penicillin

Industrial fermenters are usually made of stainless steel, which can be 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.

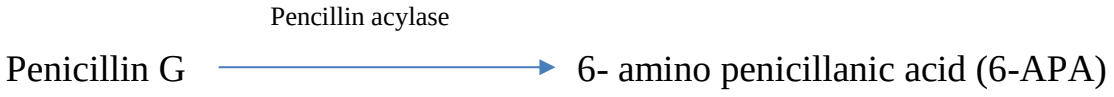
- Systems for monitoring the growth of the culture, controlling the **pH by the addition of buffers**, and for removing the products when growth is completed.

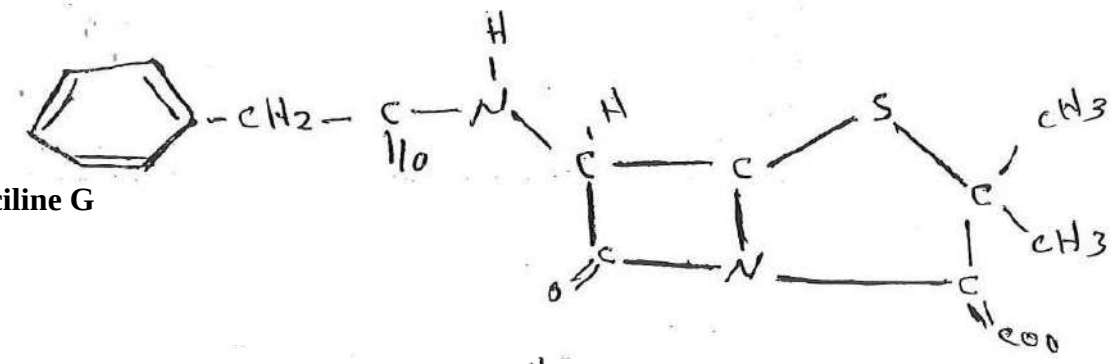
1) Contain N_2 sources & other growth factors
2) Which will be precipitation the enzyme from the solution
3) To separated the individual cells .

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 **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

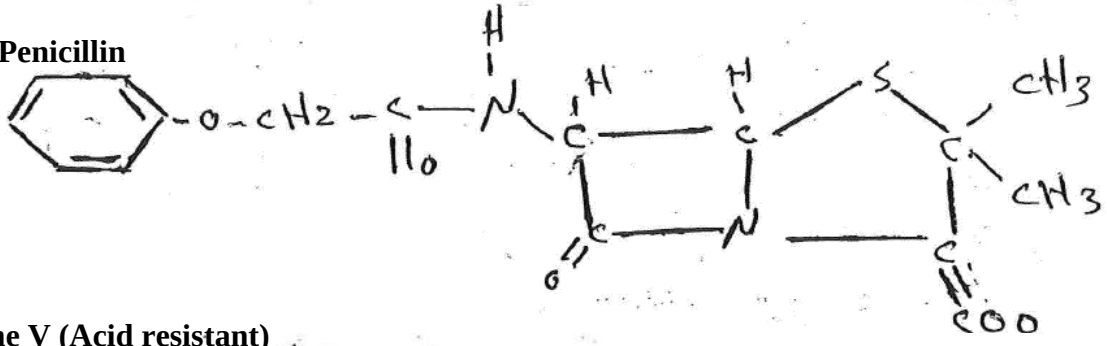
penicillin require aerobic conditions. Oxygen is supplied by means of filter-sterilised air pumped into the fermenter.

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.



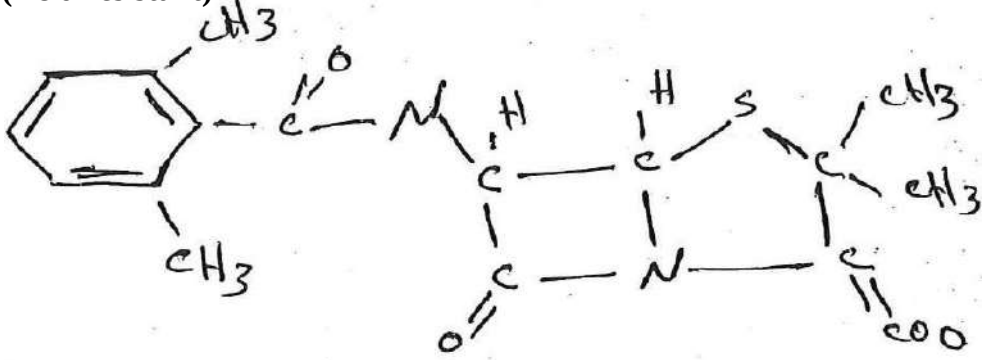


Peniciline G



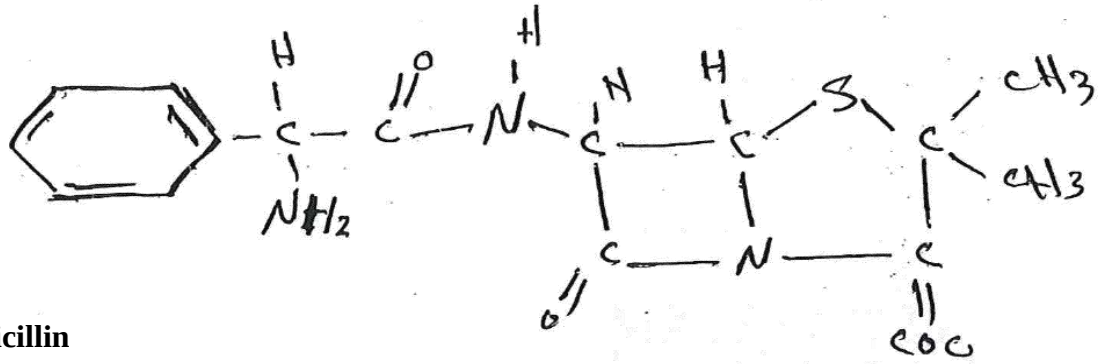
benzyle Penicillin

Peniciline V (Acid resistant)



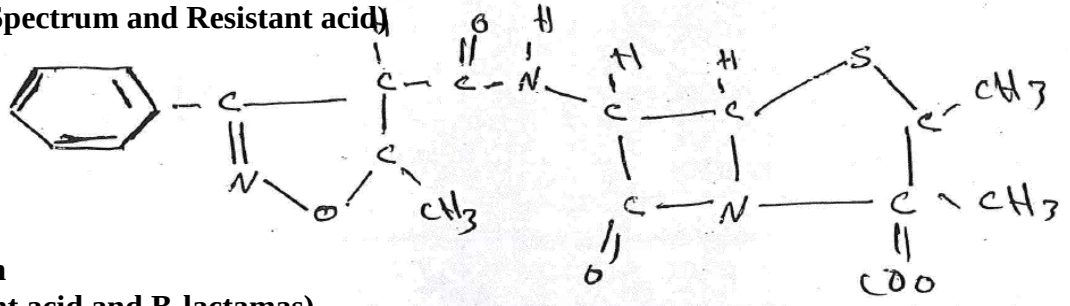
Methicillin

(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.

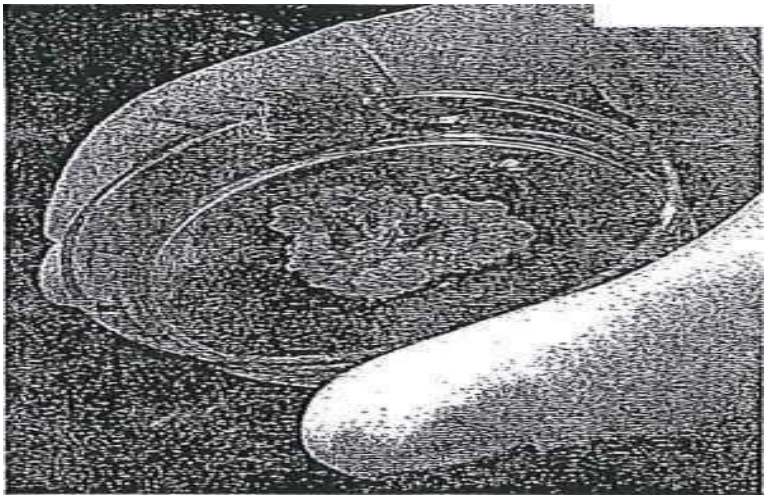


Figure :7 Plant tissue culture on a sterile agar medium

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 virضus-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.

Use of Microorganism in Biotechnology

Mycoprotein:

Fungi have a high protein content and grow rapidly, so offer considerable potential as a source of protein in human diet or as a supplements of animal feeds. They can grow on a wide range of substrates, including waste materials from industrial or other processes. Some edible large fungi are already well-known for their eating qualities- these include the common edible mushroom (*Agaricus biosporus*), other oyster mushroom (*Psalliota* sp.) and truffles (*Tuber melanosporum*) .During the 1950s to 1970s, there was active research into ways of utilizing microorganisms as a source of food, to produce Single Cell Protein (SCP). The term SCP is used to describe protein derived from microbial cells such as (yeasts, other fungi, algae and bacteria), though the microorganism producing the protein is not necessarily 'single celled'. The whole organism is harvested and consumed, rather than using the products of their fermentation or other processing. Exploitation of SCP production offers a way of increasing the available protein for consumption by humans and by livestock, and could be valuable particularly in areas where the land infertile or the climate inhospitable. While SCP production may have potential for feeding the ever increasing world population, in practice only a few schemes have proved to be commercially successful-the most successful for human consumption being mycoprotein, marketed under the name of Quorn.

Mycoprotein is obtained from the growth of the fungus *Fusarium graminareum*. Glucose syrup is used as the carbon source, gaseous ammonia supplied the nitrogen and salts are added. Wheat or maize starch is used as a source of glucose, though other starchy crops can be used. Choline is added to encourage growth of long hyphae and biotin (A vitamin) is also required. The *Fusarium graminareum* is grown in 1300 liter continuous culture fermenter at 30°C and pH 6. The ammonia gas helps to maintain the pH and oxygen gas is supplied to keep conditions aerobic.

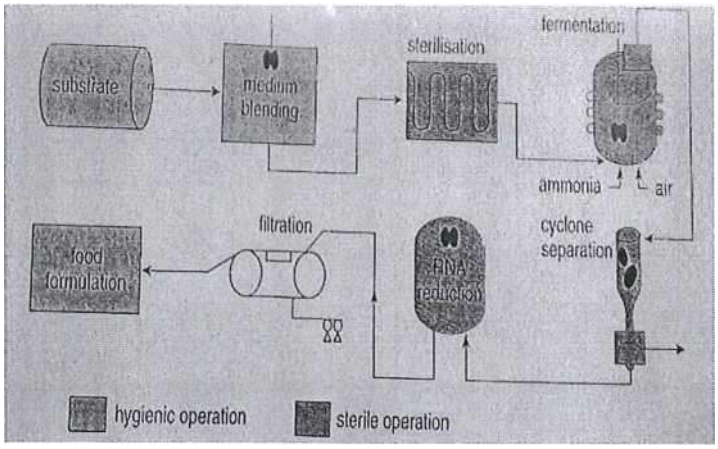
Agaricus biosporus (edible mushrooms)

The fast growth rate of microorganisms leads to a high RNA content which is unsuitable for consumption by human and other animals. In humans, excess nucleic acids are converted to uric acid which is not excreted by the kidneys, resulting in the accumulation of uric acid crystals in the joints giving gout-like symptoms. In the normal production of mycoprotein, after fermentation the RNA content is around 10 percent which is too high, but this can be reduced to about 2 percent by using thermal shock and the action of ribonuclease. After RNA reduction, the mycelium is harvested continuously on horizontal filter bed and the filter cake which is recovered can be stored at 18°C for long periods.

The harvested mycoprotein is a mat interwoven fungal hyphae which can then be formulated into a range of food products. Its filamentous nature gives it a texture and 'bite' similar to that of meat. Mycoprotein itself tastes bland but can be flavoured to resemble chicken and is added to pies, burgers and cold slicing meats. Its composition compared to that of lean beef it's given in table 1.

Table (1): Comparison of Mycoprotein and Beef

Feature	Mycoprotein	Beef
Protein	44.3%	68.2%
Dietary Fiber	18.3%	0.0%
Fat	13.0%	30.2%



Other SCP products are represented by *Fusarium gramminarium* (filamentous fungi), *Candida lipotytica* (yeast), *Spirulina* sp. (blue green bacterium) and *Methylophilus methylotrophus* (bacterium). Ultimately, the success depends on the economics of microbial production compared with protein production from animals and plants in conventional agriculture and horticulture table 2. listed some of the perceived advantage of SCP production and some of the disadvantage that have come to light with industrial schemes already attempted.

Table (2): Some Advantage and Disadvantage of Single Cell Protein (SCP)

Advantage of SCP	Disadvantage of SCP
• Fast growth rate, high yield in relative short time.	• May be deficient in certain amino acid, such as methionine or other sulphur containing amino acids, which are essential for humans or other animals.
• Production throughout the year regardless of season	• Microbial cell walls indigestible by humans and non-ruminant mammals.
• Range of substrates can be utilized, including waste materials from industrial prosesses.	• The high RNA content in microbial cells unsuitable for humans because they lack the enzyme which would break it down
• High protein content compared with some other sources such as (soya bean or fish meal).	• Concern that toxins may persist in the growth medium when using waste from industrial process.

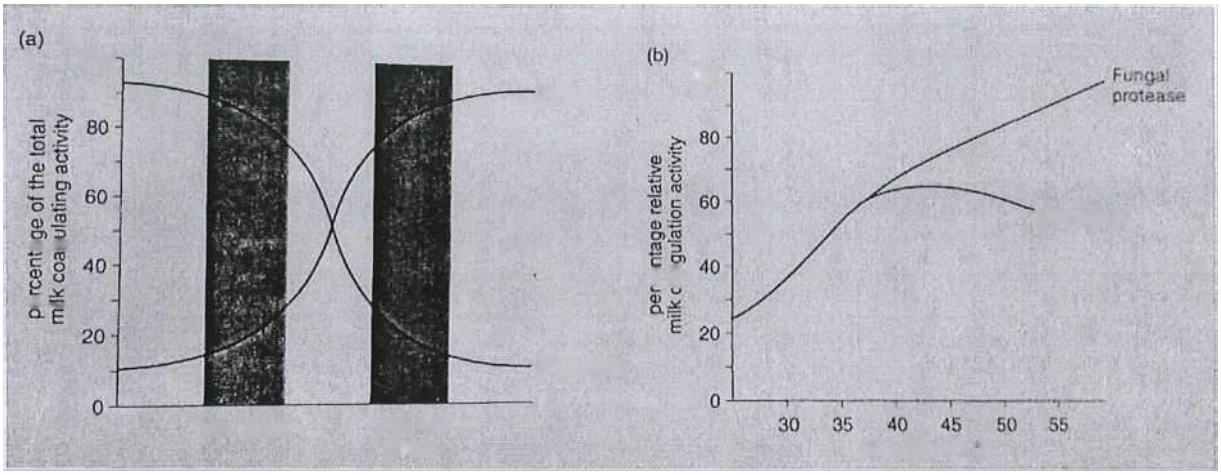
Production of Chymosin (Microbial Rennin):

In cheese making, chymosin (rennin) is the main enzyme involved in the coagulation of casein, the protein in milk. Traditionally, the source of chymosin was rennet, an extract form the abomasum (stomach) of young claves, or sometimes from kids or lambs. In the 16th century, rennet was prepared by cutting strips of the stomach of young claves and steeping these in warm milk or brine to extract the rennet. By the late 19th century the first industrial preparation of calf rennet was establish by a Danish chemist. Calves destined for consumption as veal were used, so they were not sacrificed specially for the extraction of the enzyme. More recently, in 1960s, because of changing eating patterns, there was concern that there would be a world- wide shortage of rennet for commercial cheese production. This led to pressure to find alternative source of rennet and to develop substitutes to keep up with the demand.

Bovine rennet from adult cattle can be used as an alternative to calf chymosin, but the bovine extract contains a higher proportion of pepsin and gives a lower

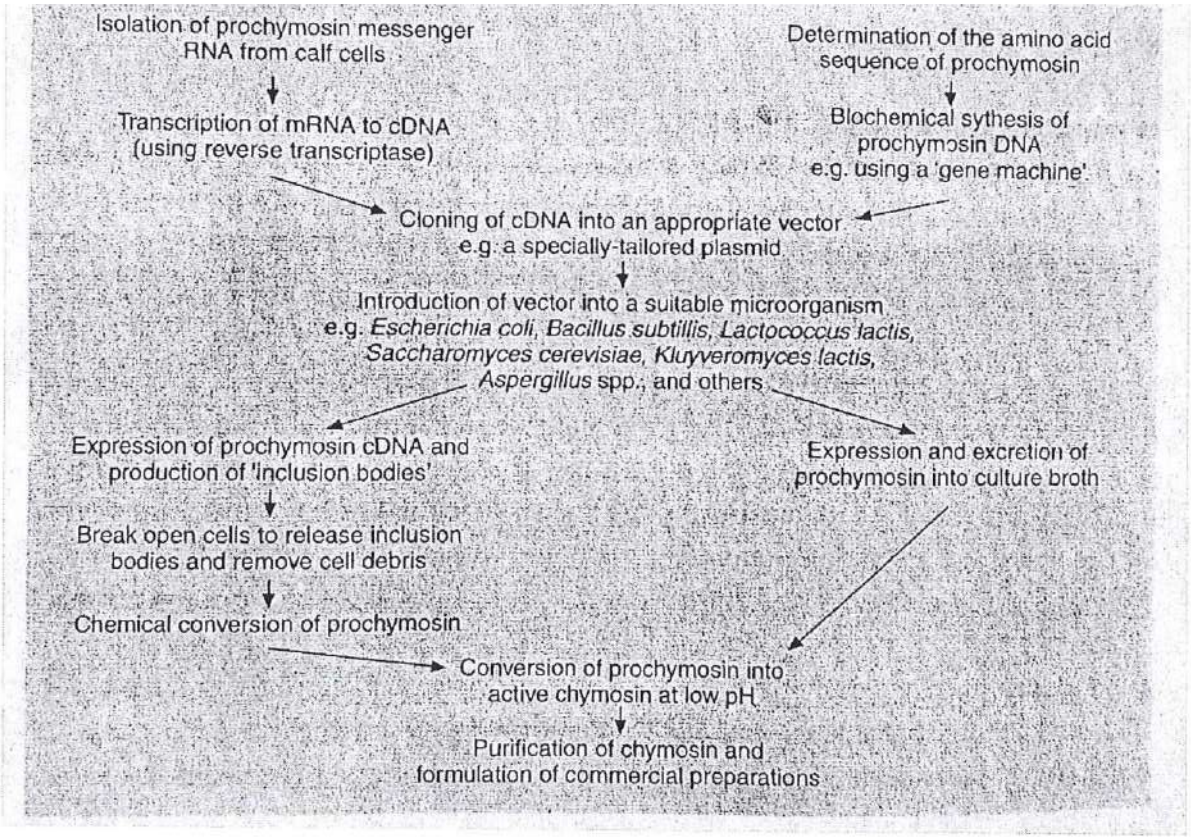
yield of cheese. Certain fungi produce proteases which can clot milk proteins. Fungal enzymes are now used in more than one third of cheese produces world-wide. Three fungal used for production of the enzymes are *Mucor miehei, M. pusillus* and *Endothia parasitica*. Compared with calf chymosin, the fungal enzymes are more stable, but this can be a disadvantage in cheese which have a long maturing stage (e.g. Cheddar cheese) because degradation of the milk protein continues. To counteract this, these enzyme can be destabilized, using oxidizing agents, so that they behave in a way similar to the more successful calf chymosin. Fungal enzymes are used widely in production of cheese for vegetarians.

DNA technology has provided further substitutes for calf rennet. The first microorganism capable of making chymosin were produced in 1981, using *Escherichia coli*. Now chymosin were produces from genetically modified yeasts, including *Kluyveromyces lactis* and *Saccharomyces cerevisiae*. Precisely the some DNA code as in the calf is incorporated into the microorganism, so the enzyme produced is identical to that from claves. Expert tasters can detect no differences between the cheeses produced using chymosin from genetically modified organism and that from extracted calf rennet. The enzymes actually have fewer impurities and their behavior is more predictable. At first there was resistance to accepting cheese made with the involvement of genetically modified organism (GMOs). Before being release for general consumption, there was rigorous tasting of the products. The enzyme used for cheese produced in this way have been proved by the relevant regulatory bodies and by the vegetarian society. Such cheese is on sale in several countries, including the UK.



(a) Changing in relative proportions of chymosin and pepsin in rennet from young calf to adult cow, showing relative chymosin to claves and higher pepsin in adult bovine rennet;

(b) Difference in behavior of calf rennet and fungal protease- influence of temperature



Stage in the production of calf chymosin by genetically modified microorganism. Prochymosin is an inactive precursor of chymosin

Industrial uses of enzymes

Cellulases :

- Function of cellulases :

$$\text{Cellulose} \xrightarrow[\text{(Break down)}]{\text{cellulase}} \text{shorter chains} \longrightarrow \text{cellobiose + B-glucose}$$

- Commercial Sources : *Aspergillus, Trichoderma , Penicilium* .

- **Advantages of cellulases:**

1) Production of fermentable sugars in brewer's mashes .

2) Clarification of orange and lemon juices .

3) Improving the release of colours from fruit skins .

4) To clear the haze from beer and to tenderize green beans.

* Saccharification : The processing of waste materials such as straw , sugar cane bagasse , saw dust and news paper to produce sugars from the cellulose .

Pectinases :

- Function of pectinases :

$$\text{Pectins} \xrightarrow[\text{(degradation)}]{\text{pectinases}} \text{shorter mdecules (galacturonic acid) + other compounds + sugars}$$

- Commercial sources : *Aspergillus , Penicillium*

-Industrial uses of Pectinases :

1) Extraction and clarification of fruit juices .

2) Pectinases are added to crushed fruit such as apples and grapes to increase the yield of juice extracted and improve colour .

3) They act by removing the pectin around charged protein particles , which then clump together and settle out of the liquid .

4) Pectinases can also be used to prevent jelling when fruit juices are concentrated .

Amylases :

- Function of Amylases :

Starch or glycogen $\xrightarrow[\text{Amylases}]{\text{Hydrolyse}}$ Dextrins or maltose .

- Commercial sources : *Bacillus* spp. (Bacteria), *Aspergillus* spp, *Rhizopus* spp. , *Streptomyces* spp.

- Industrial uses of Amylases *:*

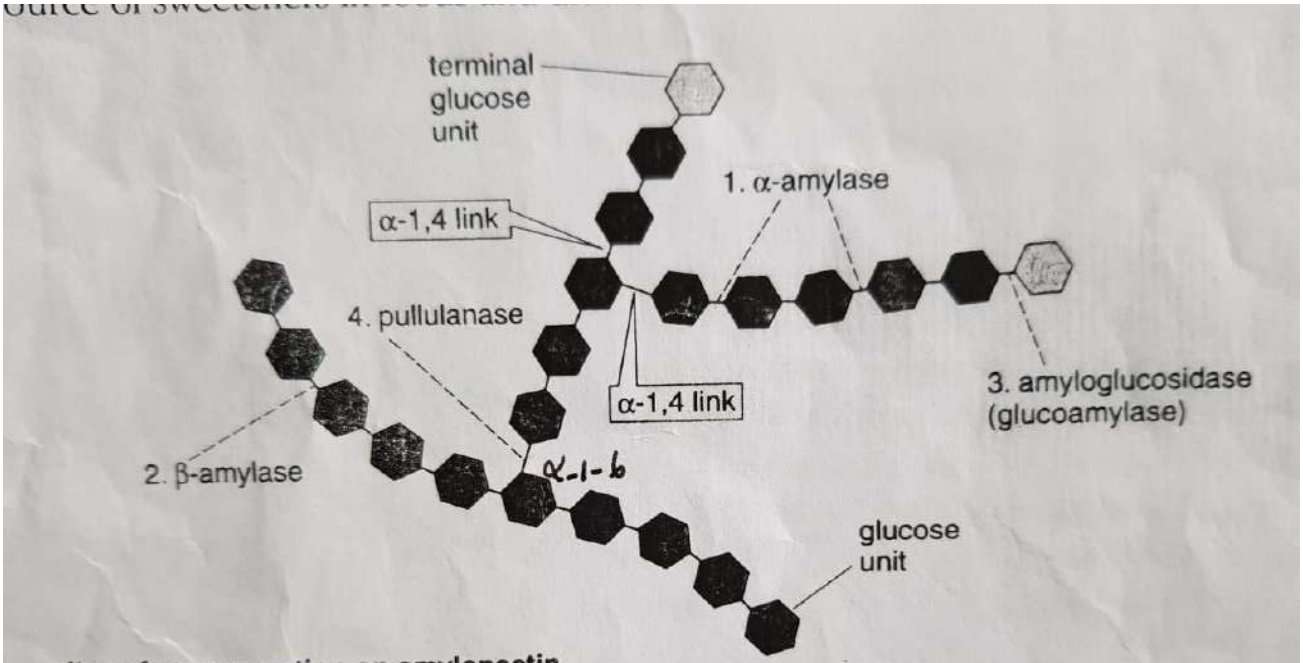
1) Amylases are used to clarify fruit juices by removing starch

2) In bread making and brewing, addition of amylases can yield more sugars from starch in flour.

3) Production of sweet glucose syrups from starch which are used in food industry .

* glucose $\xrightarrow{\text{Glucose isomerase}}$ Fructose (which is sweeter than both sucrose and glucose)

* High fructose corn syrups (HFCS) derived from hydrolyzing corn (maize) starch . (HFCS) used as source of sweeteners in food and drinks in the USA.



sites of enzyme action on amylopectin

1. α-amylase – hydrolyses internal α-1,4 glucosidic links, forming dextrins, maltose, glucose
2. β-amylase – hydrolyses alternate α-1,4 glucosidic links, forming maltose
3. amyloglucosidase – hydrolyses terminal α-1,4 glucosidic links, forming glucose
4. pullulanase – hydrolyses α-1,6 glucosidic links (at branching points), forming dextrins

Figure 3.5 Activities of amylases, a group of enzymes which act on glucosidic bonds and break down complex carbohydrates, such as starch and glycogen, to simpler residues

Proteases : or Proteinases and peptidases .

- Function of proteases :

Hydrolyse peptide

Protein ⟶ small peptide or Amino acids.

Bonds by proteases

- Commercial sources : *Aspergillus* , *Mucor* , *Rhizopus* (Fungi) *Bacillius* spp. (Bacteria).

- Industrial uses :

1) Enzymes are used in cheese making as a substitute for rennet to help clot milk .

2) Clarification of fruit juices and beer by removing the protein haze .

3) Thinning egg white so it can be filtered before drying .

4) Tenderisation of meet .

5) Digestion of fish livers to allow better extraction of fish oil .

6) Used in biological detergents industry for breaking down of protein stains .

*** Lactases : (B-galactosidases)**

- Function of lactases .

Lactases

Lactose ⟶ glucose + galactose

(Disaccharide)

- Commercial sources : *Aspergillus* spp. (fungi) , *Kluyveromyces* spp. (yeast)

Advantages of Lactases

1) The lactase used for hydrolyzing the lactose in milk to make it suitable for people who are unable to digest lactose .

2) Lactase is used to increase sweetness in ice cream and to produce a sweet syrup from whey .

3) Lactase is used in ice cream manufacture to remove lactose which crystallizes at low temperatures (Sandy texture).

***Lipase**

- Function of Lipases :

Lipases

Lipid (glyceride) ⟶ Fatty acids + glycerol

- Advantages of Lipases

1) Enzyme is used to enhance the ripening of certain cheeses .

2) To help break down fatty materials in waste .

3) Lipases are used in biological detergents industry for breaking down of Lipid stains .

Antibiotic Production

- In 1929, Alexander Fleming discovered penicillin almost accidentally , and the medical use came during the 1939-1945.
- Antibiotic: This term was used to describe substances produced by microorganisms that could be used to kill or inhibit growth of certain other microbes .
- Microbicidal : The substances was used to kill the other microbes.
- Microbistatic : The substances was used to inhibit or retard growth of other microorganisms .
- The antibiotic depend on :

a) The concentration of antibiotics .

b) Type of bacteria : Gram positive (G+) bacteria are more sensitive to antibiotics than Gram negative (G-) bacteria .

The effectiveness of an antibiotic is described as :

- Broad spectrum : when it acts on a wide range of (g+) and (g-) bacteria
- Narrow – spectrum antibiotics: are more specific , these can be useful medically because they target a limited range of microbes .

The mechanism of action of antibiotics :

1) Interference with cell – wall synthesis , (in bacteria) .
2) Interference with membrane function , (in fungi) .
3) Protein synthesis .
4) Nucleic acid synthesis .

Commercial Sources :

Penicillium notatum, Penicillium chrysogenum .

Antibiotic resistance: can be defined as the acquired ability of microorganisms to resist the effects of an antibiotic to which it is normally susceptible.

- Some organisms are naturally resistant, whereas others may developed and acquired by genetic mutation (conjugation) transduction or transformation in bacteria.

An antibiotic sensitivity test. The agar plate has been streaked with a culture of *E. coli* and sensitivity discs, impregnated with antibiotics, placed on the agar. After incubation, clear inhibition zones are seen around discs which contain antibiotics to which the organism is sensitive. No inhibition zone is seen around the disc containing methicillin, showing resistance to this antibiotic

Microbes for biological control

Microbial pathogens can thus be exploited as biological control agents, and success has been achieved using bacteria, fungi and viruses. The term microbial insecticide is used when the microorganism is used to control insects, and the term mycoherbicide when pathogenic fungi are used to control weeds. While only a few mycoherbicides are currently being used commercially, it is an area where further research may be successful in providing alternatives to chemical herbicides for weed control.

A well established example of a microbial insecticide is *Bacillus thuringiensis.* This bacterium produces a glycoprotein, known as Bt, which is toxic to a variety of insects, such as butterflies, moths and beetles, but not to animals and humans. When ingested by the insect larvae, the toxin leads to paralysis or degeneration of the gut. The insect is usually killed within a few hours.

Maize and resistance to pests

Maize is used for boil human and animal food and may be processed to provide flour, oil, syrups or other food ingredients. The European Corn Borer (ECB) is an insect pest which destroys around 4 percent of maize crops on a global scale and up to 20 percent in some regions. The pest causes damage by boring through the stem and ear of the maize plant, which then falls over.

Genetically modified maize has been produced which shows resistance to pests. The pest-resistant maize produces a lethal protein when attacked by the insect. The gene for this toxic protein comes from *Bacillus thuringiensis*, which is already used widely in biological control. This protein is toxic to a variety of insects but not to animals or humans.

Rice and resistance to disease

Rice is a very important crop on a global scale, but suffers from the rice stripe virus (RSV). Transgenic rice, produced by introducing the gene for the virus protein coat into the rice plant genome, shows noticeably increased resistance to the rice stripe virus.

Microbes for biological control

- Microbial insecticide : is used when the microorganisms is used to control insects :
- Myco herbicide : pathogenic fungi are used to control weeds .
- Commercial source : *Fusarium oxysporum*

Mycoherbicides have been proposed as an eradication tool to supplement the current methods of herbicide spraying, mechanical removal, and manual destruction of illicit-drug crops. Mycoherbicides are developed from plant pathogenic fungi that occur naturally in the environment.

Microbial mining:

Some bacteria are useful in extracting metals from low-grade ores. This is because they are chemoautotrophic. Which means they derive their energy from inorganic chemicals. Bacteria of the genus Thiobacillus are used commercially to

extract copper and uranium from otherwise uneconomic reserves. Cobalt, lead and nickel may also be extracted in this way in the near future. The extraction process may require extremes of environmental conditions, such as pH and temperature. Genetic engineering is being used to confer acid and heat resistance on these organisms.

Thiobacillus ferrioxidens, can oxidise insoluble chalcopyrites or ($CuFeS_2$) and convert it to the soluble salt Copper Sulphate. Sulphoric acid is produced during the process, yet the organism is able to flourish in these highly acidic conditions Copper can be extracted by reacting the copper Sulphate with iron. This method is responsible for about 25% of the copper produced in the USA.

$$CuFeS_2 + 2Fe_2(SO_4)_3 + 2H_2O + 3O_2 \longrightarrow CuSO_4 + 5FeSO_4 + 2H_2SO_4 + \text{energy}$$

$$CuSO_4 + Fe \longrightarrow Cu + FeSO_4$$

Uranium and copper are not the only metals which can be mined using bacteria *(Thiobacillus ferrioxidans*). In Brazilian, Australian and South Africa gold mines, microorganisms are used to treat the raw ore before final processing with cyanide to extract the metal.

Genetics and Biotechnology

Genetic Engineering:

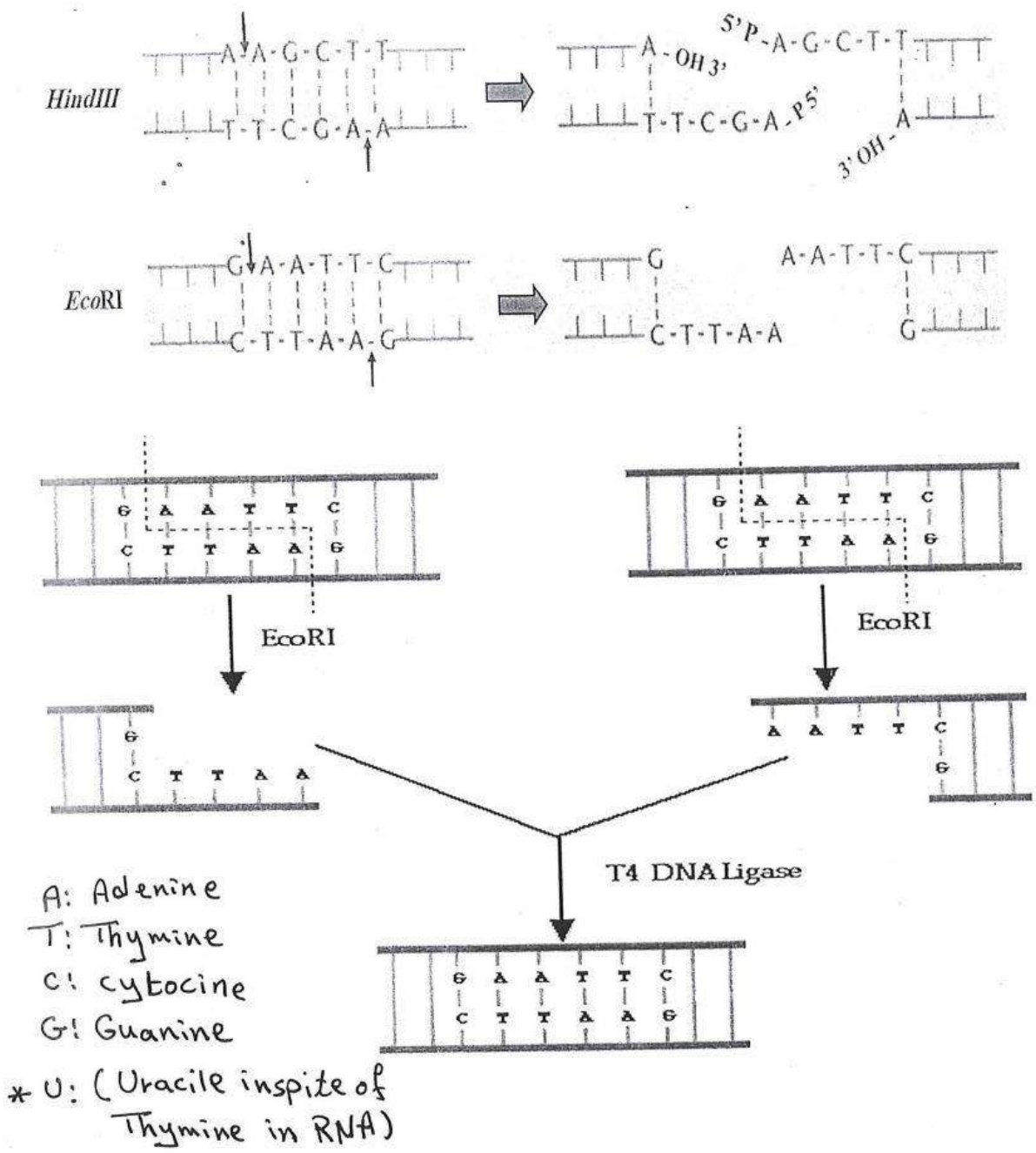
The gene is the basic unit of heredity: apiece of deoxyribonucleic acid DNA (consist of Nucleotides) that codes for the production of single proteins. These proteins are usually enzymes, and because enzymes control the formation of many different substances, genes are able to exert a direct influence on the characterization of organisms. A single gene which codes for a particular characteristic in this way can be passed on from generation to generation.

Genetic engineering or modification is a means of introducing new genes into cells or modifying existing genes. The resulting cells will, therefore have new characteristics. Organisms which have been genetically engineering are often referred to as (GMO_s) [genetically modified organisms].

In the early 1970_s researchers found restriction enzymes in bacterial cells. These enzymes are part of the natural defense mechanism of bacteria and are released when any foreign nucleic acid enters the bacteria cell. For instance, when a virus invades a bacterium, it injects its own nucleic acid, either DNA (Deoxyribose + Nitrogen base + Phosphate group) or RNA (Ribose + Nitrogen base + Phosphate group), which "reprogrammes" the bacterial cells and uses the bacterial ribosomes to manufacture new virus particles. To defend itself, the bacterium produce restriction enzymes that cut the viral DNA or RNA into small fragments, which cannot reprogramme the cell.

Genetic engineers have found these restriction enzymes useful for a number of reasons. Restriction enzymes are very specific and each recognizes and cuts only one particular nucleotide sequence in the DNA.

Another useful property of restriction enzymes in that some of them has the ability to produce a staggered cut. This means that fragments produced from a double strand of DNA have single-stranded "sticking ends" protruding (figure).



Where :

A : Adenine T : Thymine C : Cytocine G : Guanine

* U : (Uracile in spite of thymine in RNA)

Figure : How "sticky ends" are produced

These single-stranded ends have a sequence of bases [purines (A and G) and pyrimidines (T, C and U)] that can recognize and pair with one another. For example, the restriction enzyme (EcoRl from *Escherichia coli* RY13) recognizes the sequence GAATTC and cuts the DNA between G and A so that the fragments have the sequence A ATT on one end and TTAA on the other. The restriction enzyme Hind III from *Haemophilus influenza*. Rd, recognizes the sequence AAGCTT and cuts the DNA between the two A bases so that AGCT is on one fragment and TCGA on the other. If the fragments are brought together, provided that the conditions are right, they will join together again and can be resealed by using another enzyme called a DNA ligase. This allows DNA from different sources to be combined, forming recombinant DNA. This will happen provided that the DNA fragments are cut originally by the same restriction enzyme.

Mutations:

Microorganisms used in biotechnological processes were originally isolated from the natural environment but have subsequently been modified by the industrial geneticist into superior organisms for specific productivity. The success of strain selection and improvement programmes practiced by all biologically-based industries (e-g-brewing and antibiotics, etc.) is a direct result of the close cooperation between the technologist and geneticist . All properties of organisms depend on the sum of their genes. There are two types of genes. Structural and regulatory genes. Structural genes encode for amino acid sequences of protein which, as enzymes. In contrast, the regulatory genes control the expression of the structural genes by determining the rate of production of their protein products. In biotechnological processes the aim is primarily to obtain better organisms by using screening and selection techniques for optimize the conditions for production.

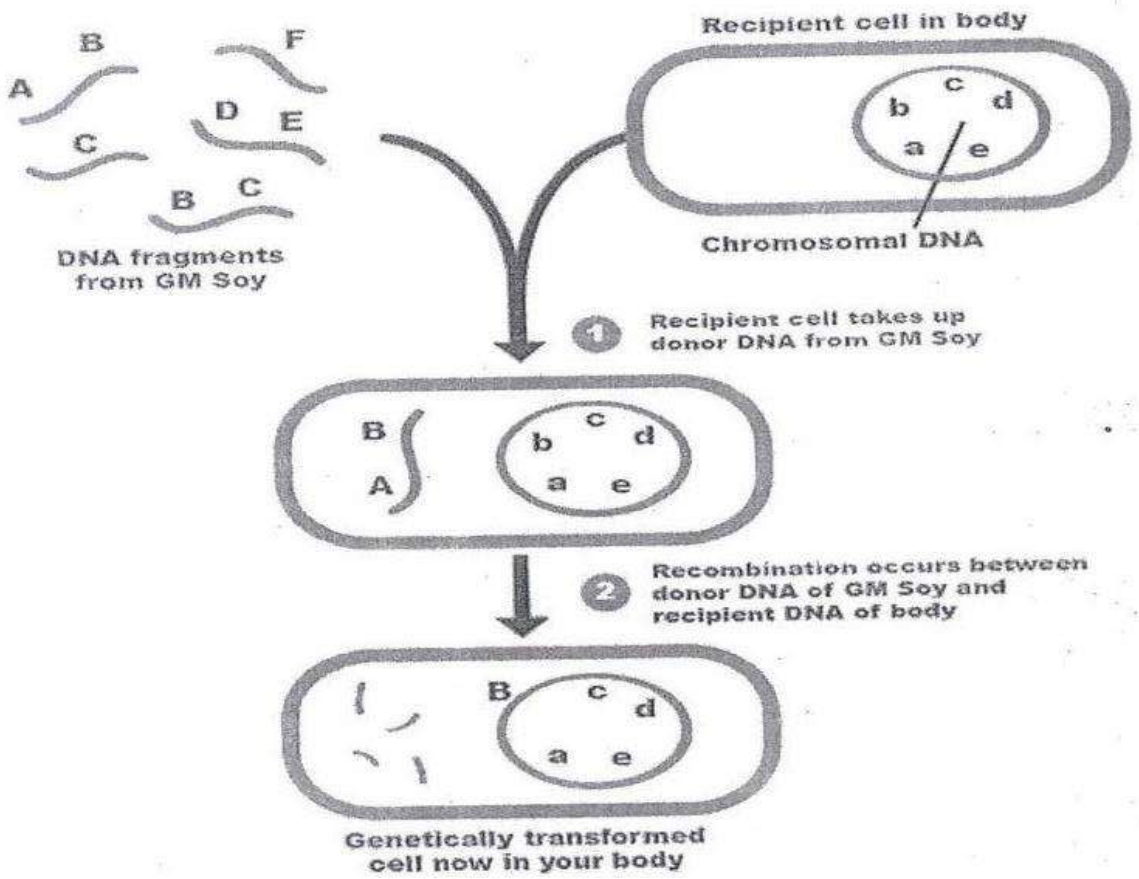
In most industrial genetics the basis for changing the organisms genome has been by mutation using physical mutants such as X-rays, U.V-rays and thymine starvation or chemical mutants such as Mitomycin c, Methyl methane sulphonate, 5-Bromo uracil, Ethidium bromide and Nitrosoguandin. These methods of mutation depend on the type and dose of mutants and the time of mutation. However, such methods normally lead only to the loss of undesired characters or increased production due to loss of control functions.

Natural Para-sexual modes of gene (DNA) transfer:

Three natural processes are known to transfer of genetic material (DNA) from one organism to another.

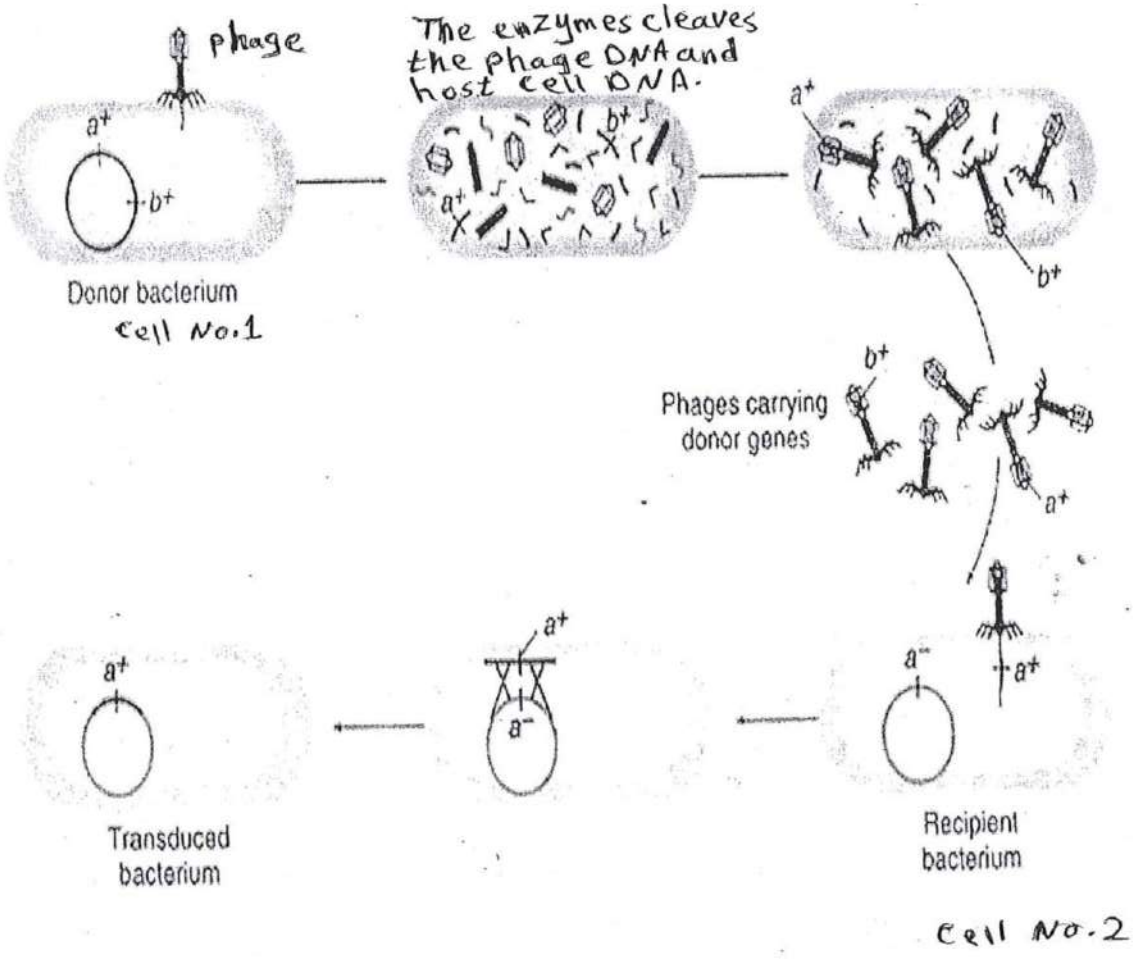
1- Transformation:

The direct uptake of DNA by microorganism from its environment or the method is to transfer the extracted DNA from one cell into a suitable host organism (Figure). The exact method of transformation depends on the host (recipient cell). *E.coli* is made competent (capable of taking up purified DNA by treating it with cold shock $CaCl_2$), heat shock many other bacteria including *Streptomyces* spp., can only be transformed if converted to protoplast by removing their cell walls.



2- Transduction:

The DNA is transferred from one organisms to another by way of carrier or vector system, this para-sexual method means of gene transfer or transduction is a process by which bacterial DNA fragments are introduced into natural bacteriophage and the random fragments of bacterial DNA (No.1) are introduced into fresh cells (No.2) by infection with the phages carrying the DNA fragments of bacteria. (No.1)

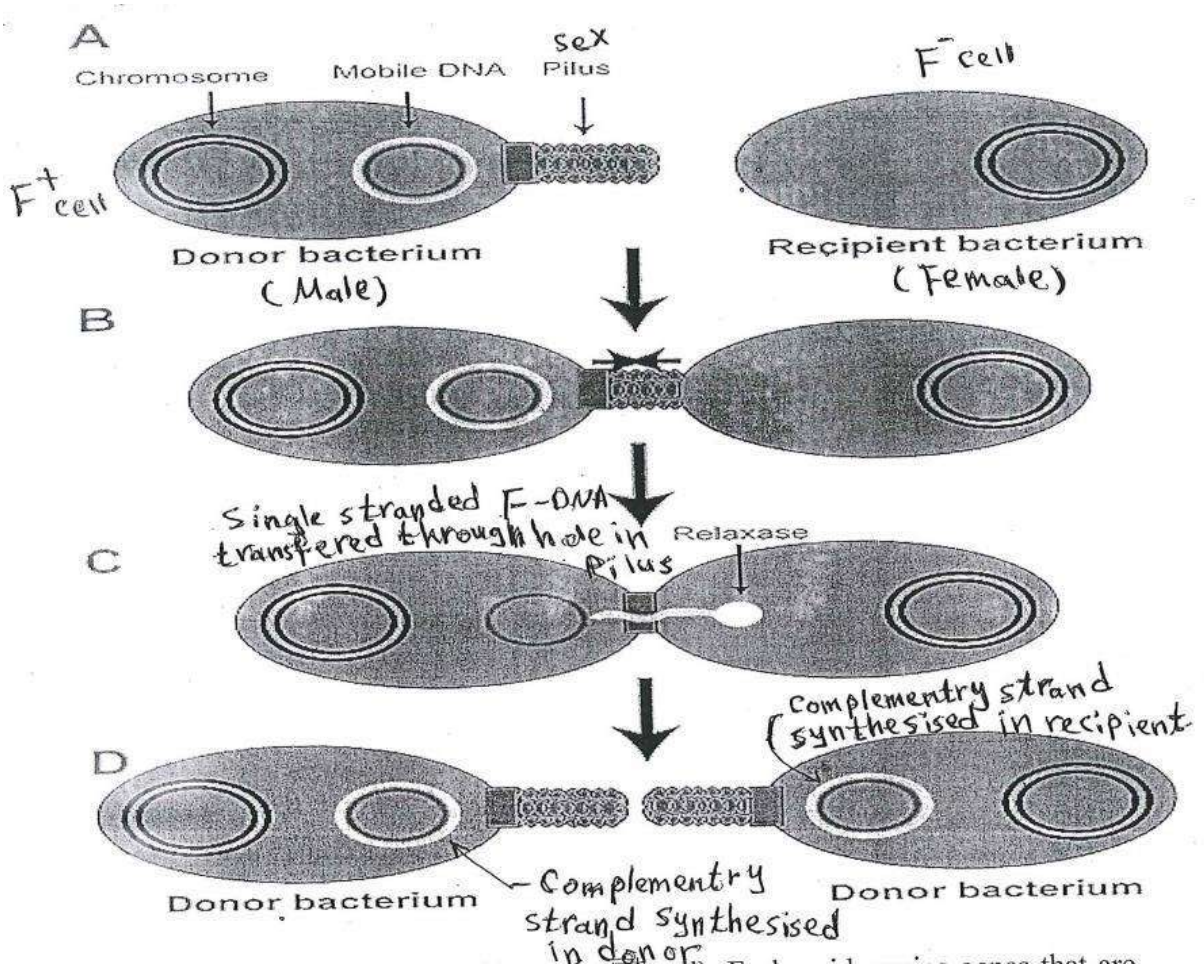


3- Conjugation:

The conjugation method is essentially a sexual process in which there is donor called male, and a recipient called female.

One of the natural plasmide of bacteria (F^+ cell) . F plasmid-carried genes that are capable to transfer to another cell (F^- cell) through sex pilus. A cell with a free F- plasmid is called an F^+ cell; a cell without one is called an F^-cell. F-plasmid acts as a sex factor in bacteria such as *E.coli.*

This method used to development many type of bacteria such as *E.coli, shigella, salmonella* and *pseudomonas species*. These natural processes are essential and provide in nature means of mixing the gene pool to allow new genes to enter an organism. Sexual conjugation is the main natural method for transferring nucleus information.



<u>Protoplast fusion</u>:

Plant and most microbial cells are characterized by having a distinct outer wall which gives the shape characteristic to the cell or organism and in some time this cell wall prevent the transformation of DNA between the cells. For some years now it has been possible, using special techniques, in particular hydrolytic enzymes such as proteases and cellulases, to remove the cell wall and produce the protoplasts. These protoplasts are extremely fragile but can be maintained in isolation for variable periods of time. Isolated protoplast cannot propagate themselves but requiring first to regenerate a cell wall be for regaining reproductive capacity. The fusion of protoplast can be enhanced by treatment with the chemical polyethylene glycol (PEG), which under optimum conditions can lead to extremely high frequencies of recombinant formation which can be

increased still further by ultraviolet irradiation of the parental protoplast preparation. Protoplast fusion can used in *Bacillus* sp. and Streptomyces sp. and can also occur with plant or human or animal cell types.

Plasmids:

Many of the properties of bacteria which make them interesting for the biotechnologist are encoded by genes on bacterial plasmids. Plasmids are circular DNA molecules which can be stably inherited in bacterial cells without being linked to the main bacterial chromosome. Plasmids are also used in genetic engineering to clone genes of interest. Plasmids exist in bacterial cells in the form of double stranded circular DNA molecules (closed circules) which are twisted to form super coils.

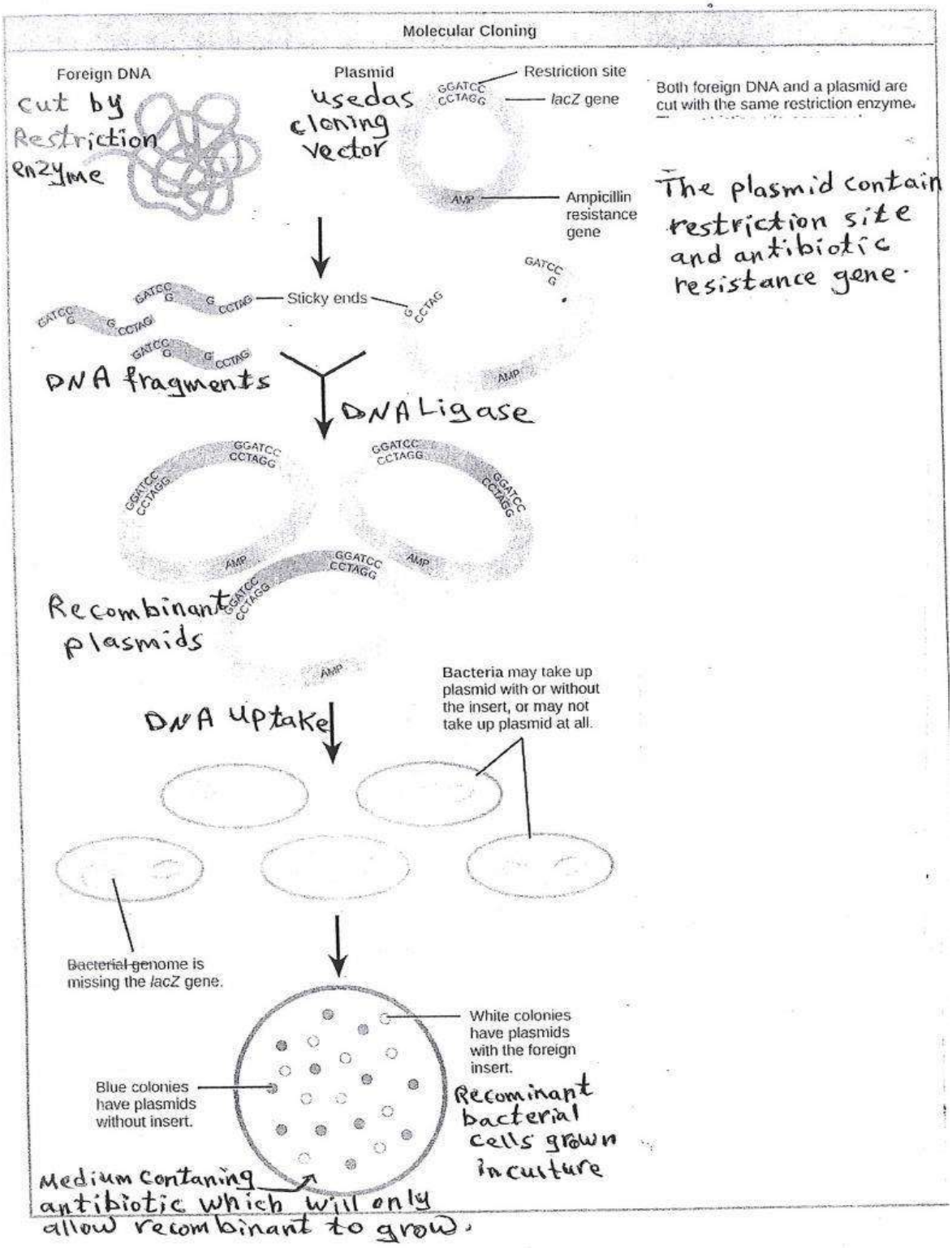
Plasmid genes which are particularly interesting to the biotechnologist include those coding for nitrogen fixation, the degradation of organic compounds and virulence factors of pathogenic bacteria.

Gene cloning (recombinant DNA technology):

The reproduction of foreign genes in bacteria or other cells is called gene cloning, the foreign gene has first to be inserted into the DNA of a culture of bacterial or other cells. The recombinant DNA is replicated as the cells divide and produces many copies of the gene (Fig.).

Plasmids are circular strands of DNA which are found in many bacteria, and are separate from bacterial chromosome. They are often used to insert foreign genes into cells. Viruses also used. When genetic engineering techniques are applied to microbial cells, not all the cells will contain recombinant DNA, it is important that the genetic engineer can select out those cells which have been successfully transformed (i.e which contain the recombinant DNA) . This is usually done by adding a "marker gene" as well as the gene that is required. These marker genes are often for antibiotic resistance such as tetracycline resistance.

The genetic engineer can then select the transformed organism by growing them on a medium containing tetracycline, so that all the untransformed bacteria will die.



(Figure): Gene cloning or Recombinant DNA technology.

Bio-separation ((Downstream processing)) for Biotechnology-Products purification:

Large amounts of biochemical products can be made by different types of cells such as animal cells, plant cells and microbial cells. After these bio-chemicals are made, they must be separated and purified. These separations are difficult and frequently cost more than the initial manufacture of the bio-chemicals.

Cell Disruption:

Bio-separation usually begin with the separation of biomass from broth by using the filtration or centrifugation method. In many cases, the desired product is in the broth (extracellular product).

Antibiotics are commonly in the broth; so extracellular enzymes, many polysaccharides, and most amino acid. In all these cases the separated broth can be treated to isolate and purify the product. The biomass is discarded or sold as a byproduct. In some cases, the products of interest, are not in the broth but are in the biomass. In many cases the product, such as lipids, antibiotics, enzymes are trapped in the biomass: it is (intracellular products) releasing this trapped material usually involves rupturing the cell wall. The methods of cell rupture have largely been developed in biochemistry. These methods, listed in table:

After the insoluble are removed, the second step in a bioseparation is usually product, the second methods used in isolation or purification product.

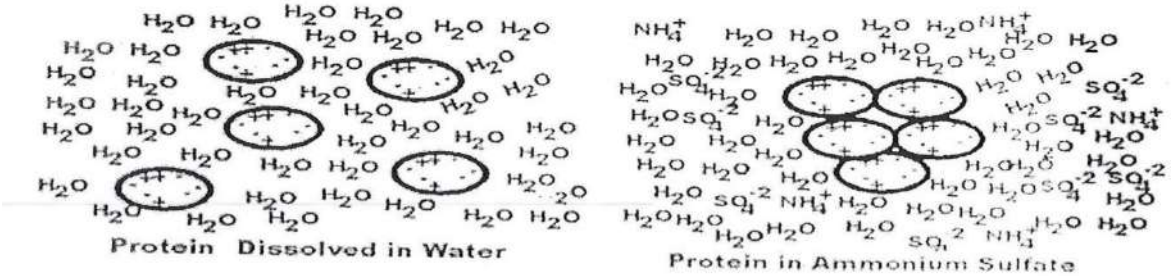
Table: Cell disintegration (rupture) technology.

Method	Technique	Principle	Examples
Chemical	Osmotic shock	Osmotic rupture of membrane	Rupture of red blood cells
	Enzyme digestion	Cell wall digestion	Bacteria treated with egg lysozyme
	Solubilization	Detergents solubilize cell membrane	Bile salts acting on *E.coli*
	Lipid dissolution	Organic solvent dissolves in cell wall	Toluene disruption of yeast
	Alkali treatment"	Saponification of lipids solubilizes membrane	
Mechanical	Homogenization	Cells forced through small hole are broken	Large scale treatment of cell suspension
	Ultrasonication	Cells broken with ultrasonic cavitation	Cell suspension at least on small scale

Precipitation by Ammonium sulfate $(NH_4)_2SO_4$:

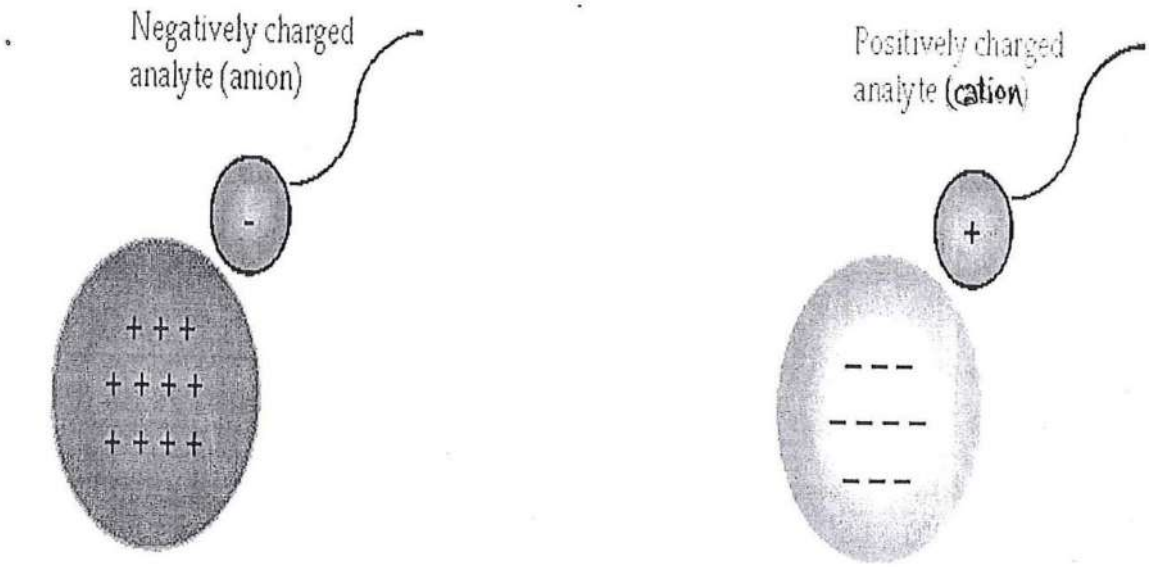
Ammonium sulfate is a good salt to precipitate the protein (product), also this method called salting out. The principle of this method depend on the formation of the complex between the protein molecule and salt, and this complex is insoluble and cause precipitation.

Ammonium sulfate precipitation



Ion exchange chromatography:

Ion exchange chromatography can be broadly defined as the separation of compounds on an insoluble matrix containing labile ions capable of exchanging with ions in the surrounding media, and this method depend on the charge of the protein molecule (product). Ion exchange materials are either anionic exchanger such as DEAE-cellulose (Diethyl amino ethyl cellulose) or cationic exchanger such as CMC (Carboxy methyl cellulose).



anion exchanger stationary phase particle cation exchanger stationary phase particle

Gel filtration chromatography:

Molecules of product can also be separated on the basis of differences in their size [molecular weight (M.W.)] by passing them down a column containing swollen particles of a gel such as Sephadex G 100, G200 or Sepharose 2B, 4B and 6B, or biogel. The large molecules leaving the column first and the smaller ones last. Fig.

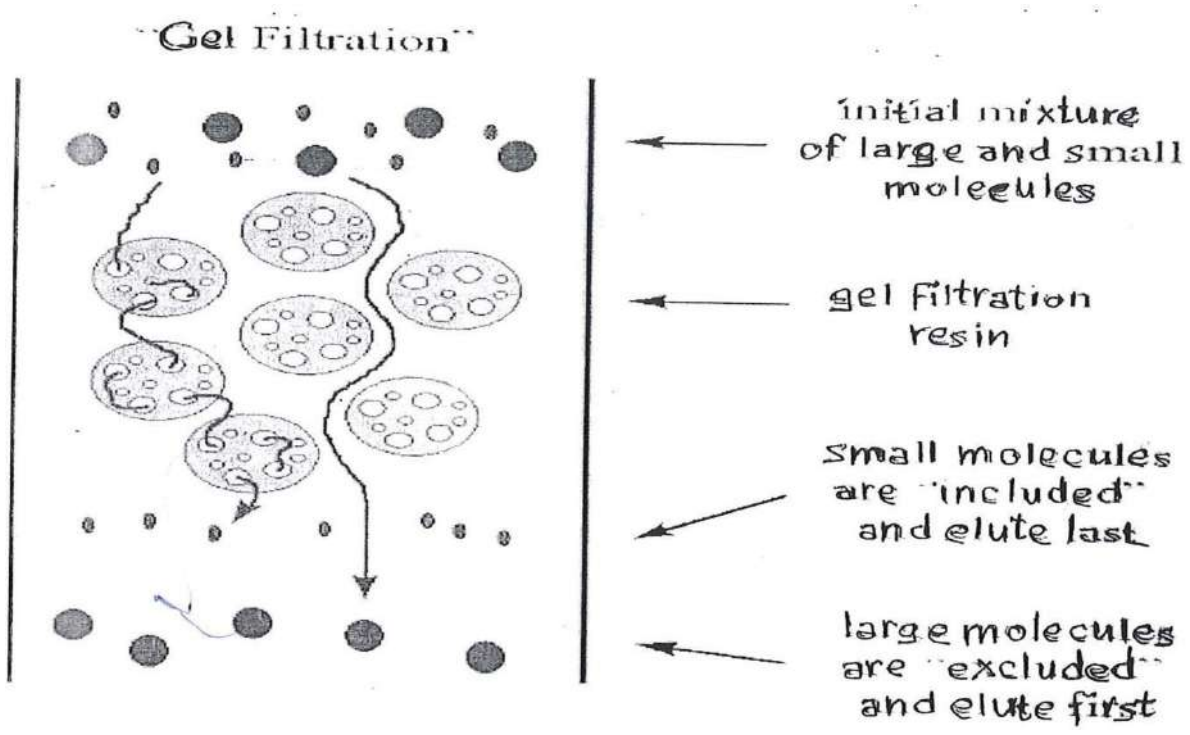
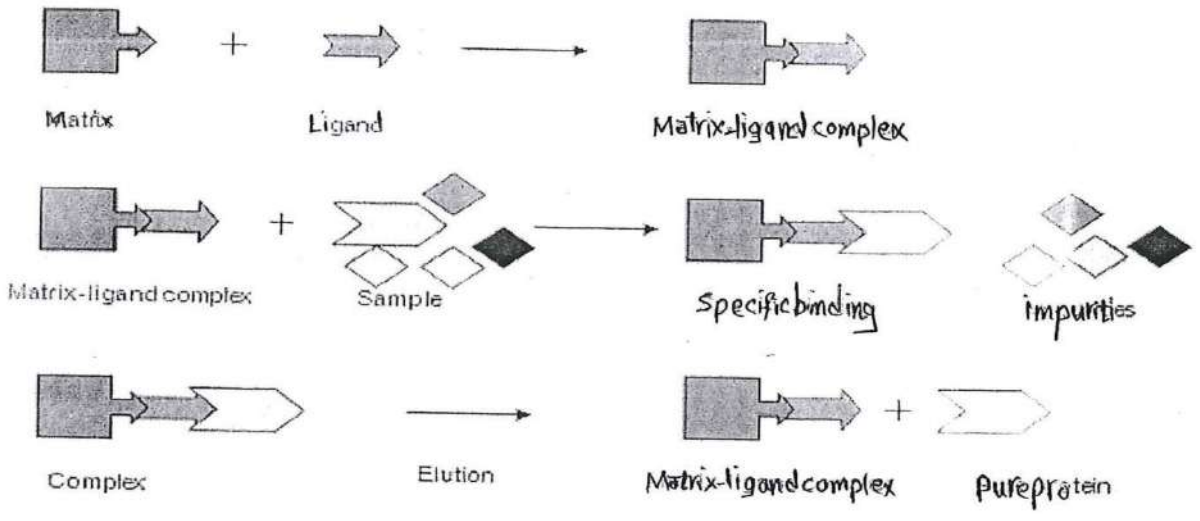


Figure: Gel filtration chromatography

Affinity chromatography:

This method depends on specific chemical interactions between the product (solute) and resin of column. Affinity adsorption is much more selective between the product and ligand (in column) Fig.

The support is bound to the ligand by covalent or ionic bound ligands do react selectively.



Immobilized enzyme

Recently, methods have been developed where enzymes are attached to insoluble materials that act as a support for the enzyme. The enzyme can then be help in place during the reaction, removed after words and used again. This is called immobilization of the enzyme. Sometimes entire microbial cells are immobilized.

Immobilized whole cells are useful because, as it is not necessary to stant with a pure enzyme, the process is cheaper and quicker. Whole cells are immobilized in the same way as purified enzymes. They are being used increasingly for complex cultures, such as waste treatment, nitrogen fixation, the synthesis of steroids, semi synthetic antibiotic and other medical products. There are different methods for immobilizing enzyme. They can be: (Fig.)

1- Adsorbed onto an insoluble matrix, such as collagen, (a)

2- Held inside a gel, such as silica gel. (b)

3- Held within a semi-permeable membrane, (c)

4- Trapped in a microcapsule, such as polyacrylamide or alginate beads, (d)

5- This processes all involve a physical bonding of the enzyme. They are easy to carry out and generally result in low enzyme activity. Alternative enzyme can be chemically bounded to the support medium, (e). Where enzyme activity is high. Although preparing enzyme in this way is difficult.

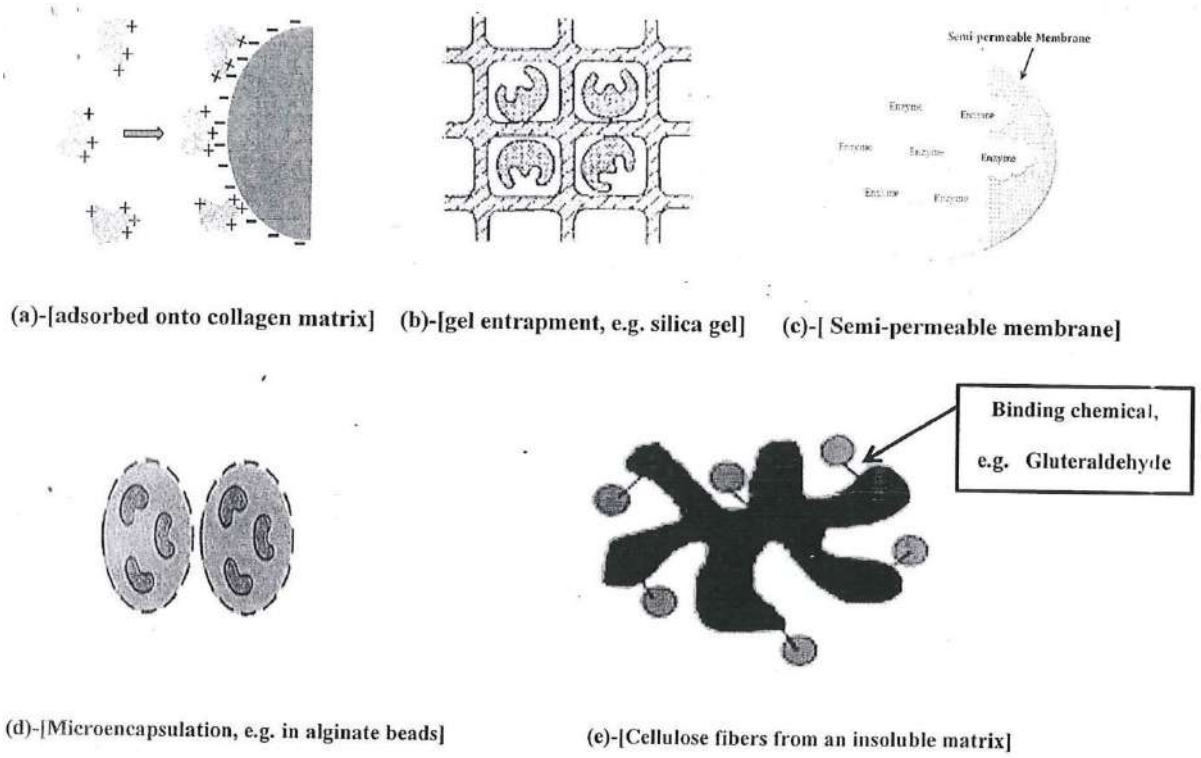


Figure: Immobilization methods of enzymes.

<u>The advantages of using immobilized enzymes are:</u>

1 - Enzymes can be reused again, which is particularly useful when the enzyme is expensive or difficult to produce.

2- The product will not be contaminated by the enzyme, because the enzyme is held in a matrix.

3- The matrix protects the enzyme with a physical condition, so that it is more stable at extremes of temperature and pH.

4- These properties make immobilized enzymes very suitable for continues culture.

5- Immobilized enzymes can be controlled more accurately.

6- Immobilized whole cells mean that several enzymes can participate in the process.

Immobilized enzyme technology is still developing quickly and there are likely to be many new applications for immobilized enzymes in industry, medicine and waste treatment.

The production of lactose free milk :

In many parts of the world, milk is an important part of the adult human diet. It contains the disaccharide sugar lactose which is digested to the monosaccharaides glucose and galactose by the enzyme lactase, present in the small intestine, there are many adults who lack this enzyme. These people are said be lactose intolerant. If they drink even a small amount of milk, they suffer severe abdominal cramps, wind and diarrhea.

Because the lactose does not get digested in the small intestine, it passes through to the colon where bacteria feed on it and produce fatty acids, methane, Co_2 and hydrogen. However immobilized lactase can be used to break down the lactose in milk making it suitable for lactose in tolerant people to drink.

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 unfavourable. 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.

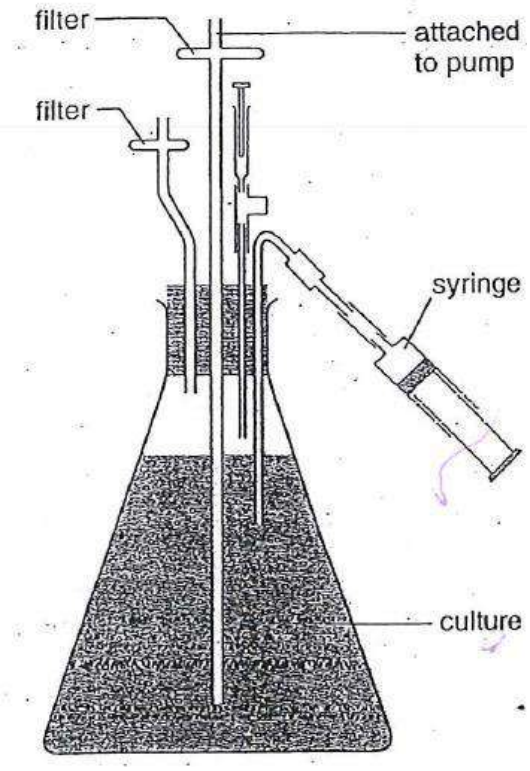


Figure: 4 A simple fermenter

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 Grolux fluorescent tube. Figure : 5 shows an industrial fermenter to illustrate how the simple fermenter is scaled up.

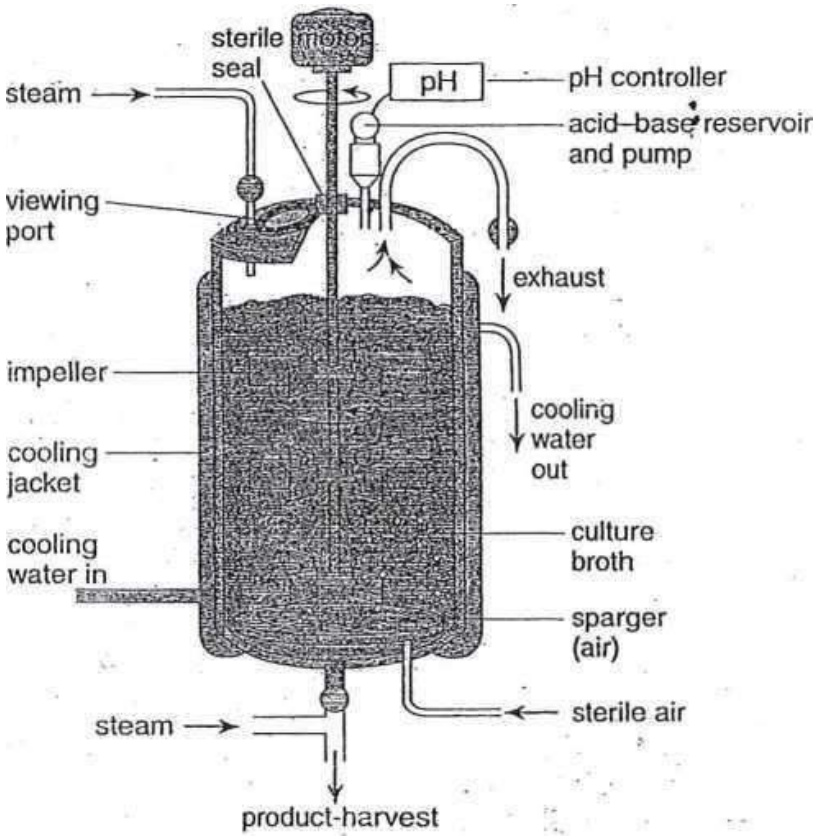


Figure: 5 Diagram of an industrial fermenter, such as that used to produce the antibiotic penicillin

Industrial fermenters are usually made of stainless steel, which can be 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.

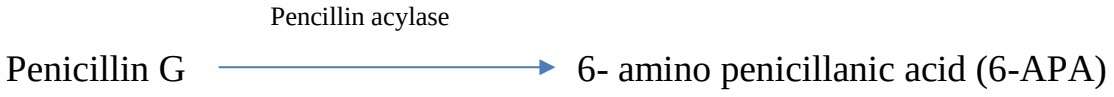
- Systems for monitoring the growth of the culture, controlling the **pH by the addition of buffers**, and for removing the products when growth is completed.

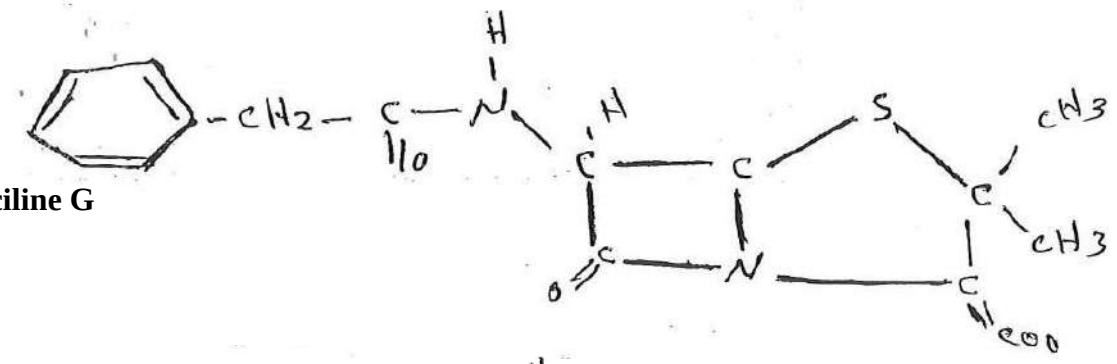
1) Contain N_2 sources & other growth factors
2) Which will be precipitation the enzyme from the solution
3) To separated the individual cells .

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 **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

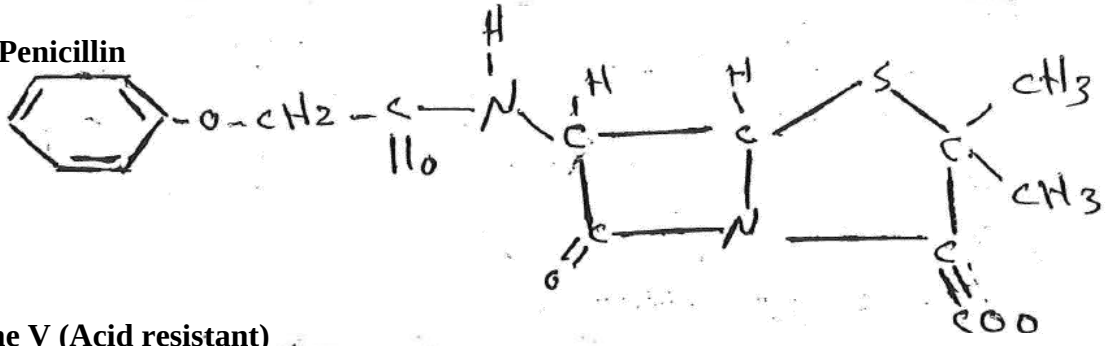
penicillin require aerobic conditions. Oxygen is supplied by means of filter-sterilised air pumped into the fermenter.

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.



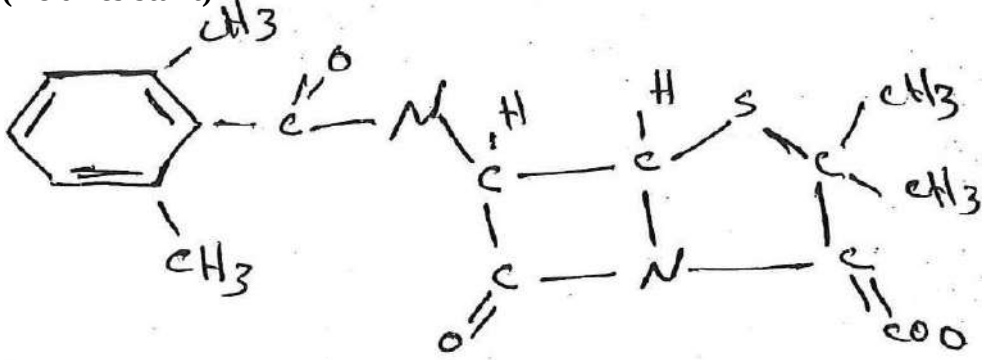


Peniciline G



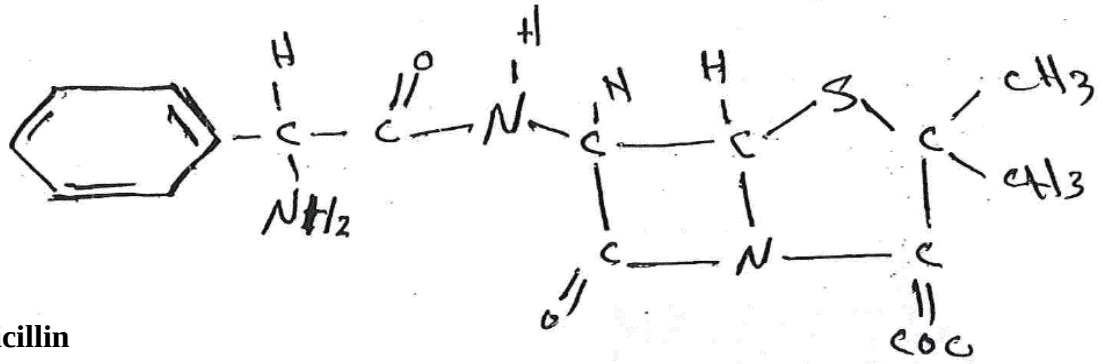
benzyle Penicillin

Peniciline V (Acid resistant)



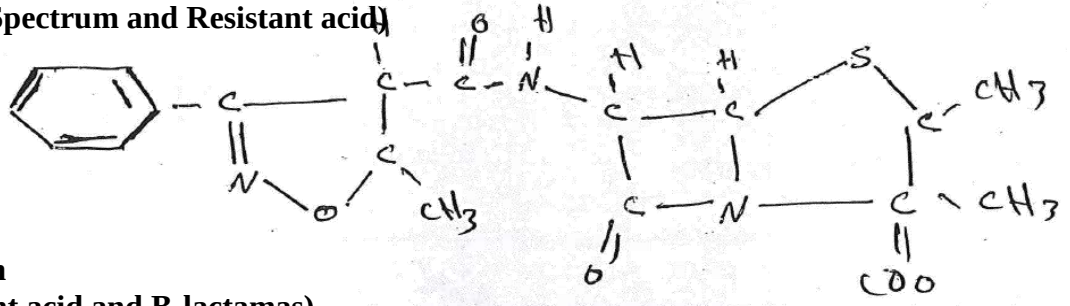
Methicillin

(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.

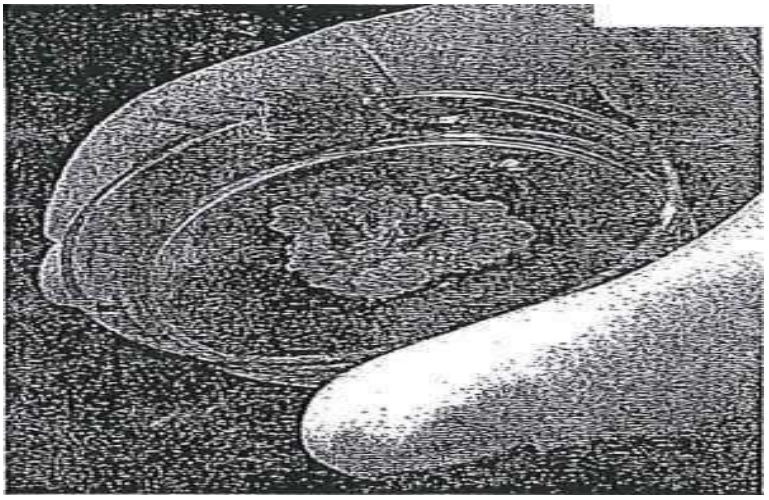


Figure :7 Plant tissue culture on a sterile agar medium

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 virضus-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.