UNIT 15 BIOTECHNOLOGY

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15.1 INTRODUCTION

So far in this course we have described the various types of biomolecules present in living organisms, their chemistry and involvement in metabolism and generation of energy and principles of molecular biology. In this unit, we will show you how this basic knowledge of biochemistry can be deliberately exploited in a number of ways. This exploitation is increasing rapidly and forms the basis of a current revolution in industry that has come to be known as biotechnology. It is expected to transform the ways in which we produce many vital commodities, including food, chemicals and energy. The success of biotechnology owes much to an increasing knowledge of genetics - which enables us to manipulate the genes of organism to our own ends, the science of genetic engineering.

In this unit first we will give a broad overview of biotechnology, its origin, current status and potential and then examine in more detail some major aeas of biotechnology.

Objectives :

After reading this unit you should be able to :

- explain and describe the meaning of biotechnology and its importance,
- explain the basis of

(i) genetic engineering,

(ii) enzyme engineering and

(iii) describe fermentation technology.

15.2 WHAT IS BIOTECHNOLOGY ?

Man has been trying too understand,reproduce and modify the natural facts and phenomena of the mother nature since his existence on Earth. The knowledge gathered by observation, experimentation, systematic and critical testing is referred to as science. Technology, on the other hand is referred to as the useful application of science in industry or elsewhere and it plays an important role in daily life. When a technology is dependent on living cells or their constituents, it is referred to as biotechnology.

The biotechnological processes were started long ago. These include many traditional processes such as brewing, baking, wine making, cheese making and production and preservation of food materials. However, the newly acquired biological knowledge, specially

the ability to manipulate genes, i.e., genetic engineering, has widened the scope of biotechnology tremendously.

Biotechnology has been defined in many forms (Table 15.1). Essentially it is the use of microbial, animal or plant cells or enzymes to synthesise, breakdown or transform materials. From this particular definition one can make out that biotechnology is an interdisciplinary science and requires integration of several disciplines like biology, biochemistry, microbiology and chemisal engineering in a way that optimise the exploitation of their potential [Fig. 15.1].

Table 15.1 : Some selected definitions of biotechnology

1.	The integrated use of biochemistry, microbiology and engineering sciences in order to achieve industrial applications of the capabilities of microorganisms, cultured tissue cells and parts thereof.
2.	A technology using biological phenomena for copying and manufacturing various kinds of useful substances.
3.	Biotechnology is the use of living organisms and their components in agriculture, food and other industrial processes.
4.	The science of the production processes based on the action of microorganisms and their active components and of production processes involving the use of cells and tssues from higher organisms.
5.	The application of biological organisms, systems or processes to manufacturing and service industries.

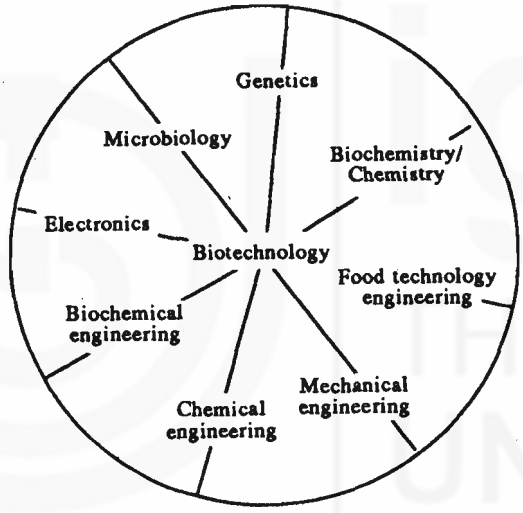

Fig. 15.1 : The interdisciplinary nature of biotechnology

Biotechnology is expected to form characteristic base of the industry of the twenty first century, as those based upon physics and chemistry did in the twentieth century. This arises from recent advances in our understanding of biochemistry, microbiology, genetics and molecular biology, parallel in technical advances in engineering which has led to a worldwide search for harvesting economic benefits from them. With the realisation regarding the limited supply of nonrenewable resources, there is a global search for cheaper, more secure, environmentally friendly resources, where biotechnology could play a major role. Realising its immense scope the governments of most of the developed and developing countries have allocated large sum of money to support research and industrial development in this field. More than 300 companies specialising in biotechnology have sprung up in the last decade. The main areas of application of this technology are genetic engineering, enzyme engineering, fermentation technology, waste technology, environmental technology and renewable resources technology. A representative diagram showing different disciplines (roots nurturing biotechnological processes and their applications are shown in Fig. 15.2). In the present unit you will learn about some of these applications.

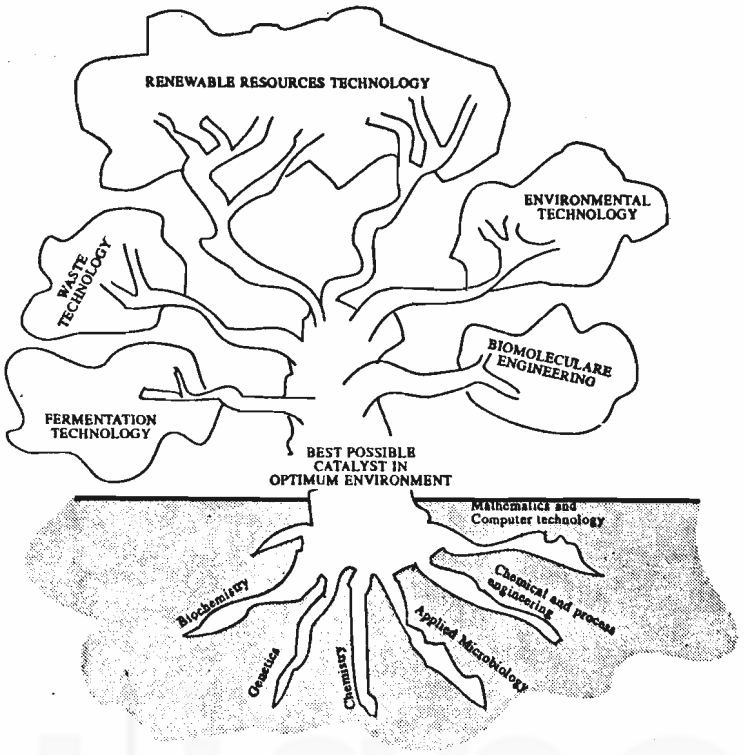

Fig 15.2 : Biotechnology tree

15.3 GENETIC ENGINEERING

You may recall that DNAs are the molecules of heredity which control the production of specific proteins in living cells. The genetic information is stored and transmitted from the parent cell to its progeny in the form of sequences of bases in DNA. Any alteration in the structure, i.e., base sequence of cellular DNA will, therefore, alter the characteristics and capabilities of the cell. These changes may be in the form of addition, deletion or alteration of some bases or large segments of bases. This takes place in nature when a cell undergoes mutation, but not in normal cell division. It is now possible to carry out suh alterations in the laboratory and thus incorporate desired changes in the genetic make-up of a living cell. This is achieved by the **recombinant DNA technique,** also called **gene cloning or genetic engineering.** For example, a complete "foreign" responsible for the synthesis of a particular protein may be incorporated into a micro-organism, such as bacateria or yeast, which does not normally have it. The altered bacterial or yeast cell will now synthesise the desired protein although it was not doing so earlier. This technique is finding immenses applications in producing some modified strains of *E. coli* which synthesise clinically important polypeptides and proteins, e.g., insulin, sometostatin and interferon.

The steps in gene cloning or genetic engineering are shown in Fig.15.3. A specific gene from a donor species, say one coding for a desired human protein, is to be inserted into the genetic make-up of a bacterium, say *E. coli.* The desired DNA segment is identified, out from the donor chromosome and isolated. It is then combined with a vector or "carrier" DNA which may, for example, may be a bacterial plasmid. The latter are circular DNA molecules which are normal constituents of the bacterial cell. Combination of the desired gene with the plasmid gives the recombinant DNA which is then incorporated into the bacterium. Only the basic principles are illustrated here. For detailed account of the enzymes involved and conditions required for vrious steps, some advanced textbook of biochemistry may be consulted.

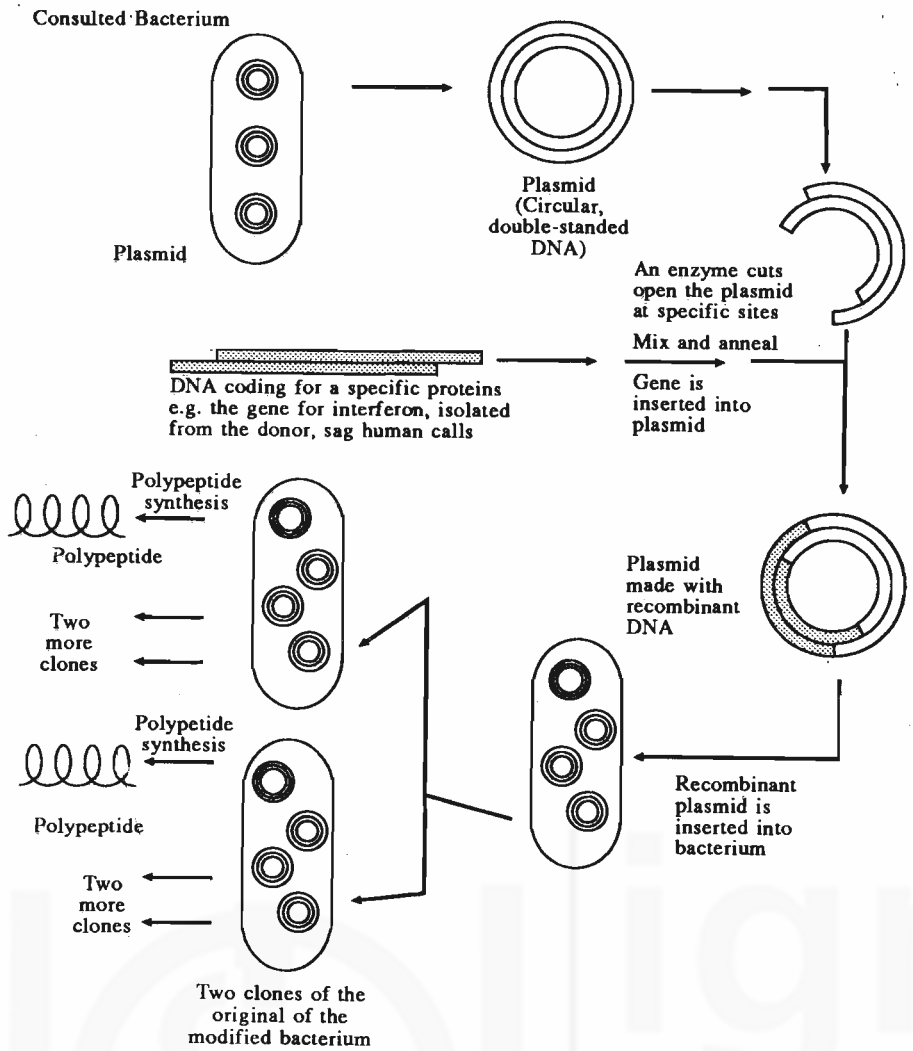

Fig. 15.3 : Recombinant DNA is made by inserting a desired DNA segment, coding for some protein not made by the bacteria, into the circular DNA of the bacteria plasmid.

Once the vector with the donor gene is inside the cell, it starts replicating as the latter begins to grow and divide. At the same time, the newly incorporated gene is expressed, i.e., the protein coded for by it is synthesised by the transformed cell. The replicated DNA passes from the parent to the daughter cell and gives rise to clones. Thus, an organism having novel genetic make up, the new chemical capabilities comes into being. This technique may help produce compounds of great clinical or industrial importance, e.g., human insulin. Normally, *E. coli* cells do not synthesise insulin or a related protein, but these may be transformed by insertion of human gene for insulin. In a commercial process, such transformed cells as being used to produce human insulin.

15.3.1 Some Applications of Genetic Engineering

The immediate and foremost requirements of every human being are good food and good health. It is a matter of deep sorrow and regret that in many parts of the world, people do not have access to the minimum medical care and so people die, some times due to non-availability of essential medicines. In our country, under the guideline of WHO, the government has undertaken an ambitious programme promising health for all by the year 2000 A.D. The success of such a programme lies in the availability of the more effective drugs at affordable prices. Genetic engineering is likely to make substantial contribution in this field. A number of compounds of clinical importance, such as proteins, hormones, etc. have been prepared successfully by this technology.

15.3.2 Interferon Production

Interferons are glycoproteins produced by virus infected vertebrate cells. They bind to other cells and make them virus resistant. This resistance is not limited to the originally infecting virus only, but have much wider specificity. Three families of interferons are known, called α, β, and γ interferons. Till recently, the only known natural sources of interferons were

only animals. However, the human leucocyte interferon gene has now been attached to that of alcohol dehydrogenase of yeast. The recombinant gene has been cloned in Babker's yeast, *Saccharomyces cerevisiae*. The transformed yeast cells produce interferon attached to alcohol dehydrogenase molecule. These are subsequently separated and isolated.

15.3.3 Insulin Production

It is an animal protein (Fig. 15.4) secreted by pancreas that controls level of sugar in the blood. It has been marketed as an antidiabetic drug since 1922. The source was of course animal pancreas. However the way of production was very cruel and expensive as large number of animals to be killed for producing a few grams of insulin. Moreover the cattle

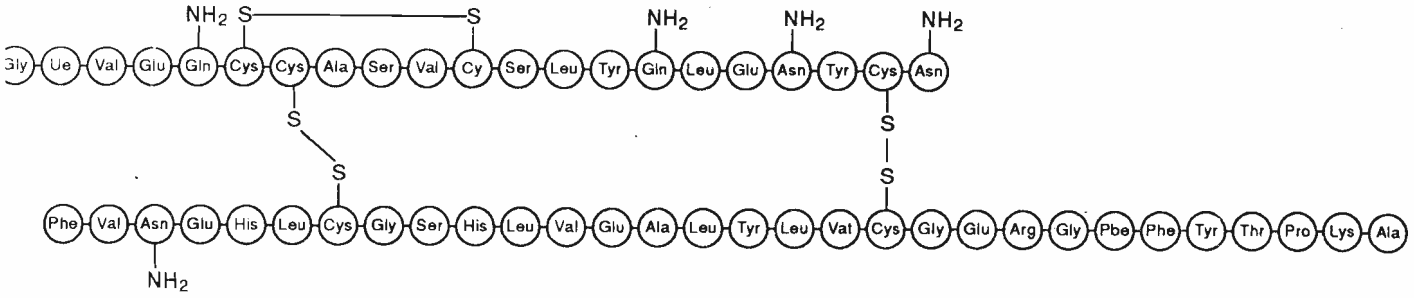

Fig. 15.4 : Insulin

and bovine insulins are somewhat different than that of humans in sequence of certain aminoacids, and for this reason are not effective in controlling deterioration of kidneys and the retina in the diabetic patients. The chemical method of synthesising insulin is difficult and very-very expensive. However with the advent of genetic engineering it has been possible to prepare and link a synthetic gene, responsible for synthesis of insulin to a plasmid of *E. coli*. After gene expression and translation of mRNA into protein, insulin can be obtained. The process has already been scaled up for industrial preparation. This has resulted in higher production and consequently lower costs which are affordable by most patients.

15.3.4 Production of Some Other Hormones

Hormones are compounds secreted by endocrine glands and regulate many vital functions by interaction with target-cells or organs elsewhere in the system. Some important hormones and their triggering functions are listed in Table 15.2. The importance of hormones and the possible adverse effects caused by their deficiency. However, synthesis of hormones is not an easy task and required a number of synthetic steps and the costs are exceedingly high. Recombinant DNA technique or gene cloning is one of the possible solution to this problem. Two hormones, namely, somatostatin, a hypothalamic hormone and somatotropin, human growth hormone have been successfully synthesised by the recombinant-DNA technique using transformed *E. coli* cells.

Table 15.2 : Different Hormones, with their Regulatory Functions

Metatonin	Regulates cardiac rhythms
Somatoliberin	Stimulates somatotropin secretion
Somatostatin	Decrease gastric secretion inhibits somatotropin secretion
Lipotropin	Fatty acid release from adipocytes
Somatotropin	General anabolic effects, stimulates release of growth factors
Thyroxine and Triiodothyronine	General stimulation of many cellular reactions
Gastrin	Gastric acid and pepsin secretion.
Secretin	Regulates pancreas secretion
Insulin	Glucose uptake, lipogenesis, general anabolic effects
Glucagon	Glycogenolysis, release of lipid
Estrogen	Maintenance of pregnancy
Androgen	Maturation of function of secondary sex organs
Relaxin	Muscle tone
Epinephrine	Smooth muscle contraction heart function, glycogenolysis and lipid release

For small peptide hormones, the required genes, i.e., DNAs, are chemically synthesised and attached to a bacterial gene, e.g., that of β-galactosidese. The combined DNA is then attached to the vector, plasmid, and finally inserted into the bacterial cell. The hormone is synthesised as a short tail at one end of the enzyme molecule and is obtained by specific peptide cleavage (Fig. 15.5).

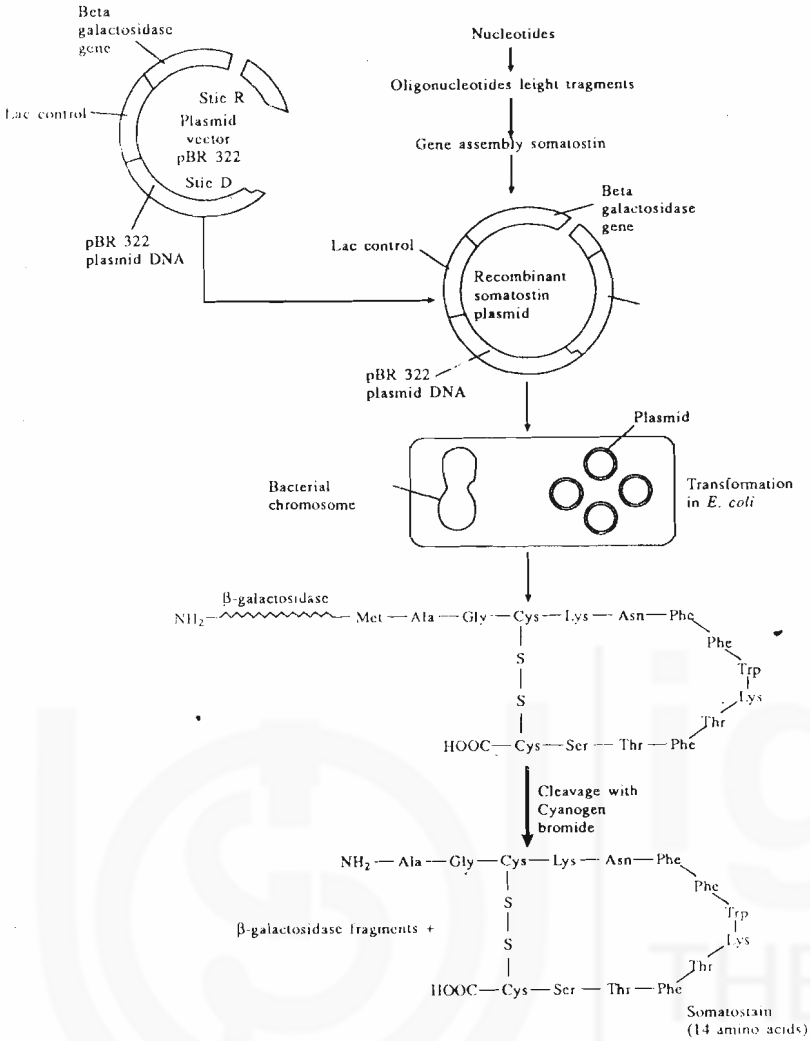

Fig. 15.5 : Production of Somatostatin by Recombinant DNA Technology.

These examples illustrate the usefulness and the potential of genetic engineering for making human life more comfortable and safe. However, with any branch of science, it is not free from hazards and dangers. The constitution of recombinant-DNA and their insertion into microorganisms could create novel organisms which might inadvertently be released from the laboratory and become a biohazard to humans or environment. This risk can be minimised by enforcing strict safety codes for working in the laboratory with pathogenic microorganisms.

SAQ 1

Fill in the blanks :

a) Circular DNAs are known as .....

b) Gene cloning essentially involves alteration in .....

c) Deficiency of insulin causes .....

d) Interferons are .....

SAQ 2

Mark True (T) or False (F) against each statement given below :

a) Gene cloning is a natural process (T/F)

b) Recombinant DNA leads to microorganisms with modified genetics (T/F)

c) Insulin is produced by genetic engineering (T/F)

d) Interferons are DNA (T/F)

e) Interferon may cause cancer (T/F)

15.4 ENZYME TECHNOLOGY

Enzymes are biological catalysts and are protein in nature (Unit 6). They may be isolated from the living cells without loss of catalytic activity. This capability of enzymes to catalyse chemical reactions in and outside a living cell has led to a branch of science, now popularly called enzyme technology. This is a broad term and includes isolation, purification of enzymes and their applications.

As catalysts the enzymes exhibit a high degree of reaction and substrate specificity, including stereospecificity. Consequently, there are practically no side reactions. Further, a single stereo-isomer may be produced by an enzyme-catalysed reaction where the usual chemical process gives rise to a racemic mixture of enantiomers. This property of the enzymes is particularly useful in the production of pharmaceuticals, where only an enantiomer may exhibit the desired drug action and the other may either be inactive or may even be harmful to the system. For example, the second enantiomer may be an antagonist of the drug.

Like fats and lipids, proteins are also responsible for some of the 'dirt'. This dirt can't be removed by soaps and detergents. This problem has led to the development of detergent that also contain enzymes that catalyse the 'digestion' of the proteins. Such enzyme containing detergents are quite popular now a days.

In the physiological systems, from which the enzymes are isolated, they act always in aqueous medium. They retain this activity outside the cell also. It has recently been shown that some enzymes are also catalytically active even when dispersed in water immiscible organic solvents. This observation has widened the scope of application of enzymes.

Crude enzyme preparations have been in use for many years, e.g., the use of malt extract in brewing industry and of rennin in cheese manufacture. Availability of pure enzymes on a large scale (gram quantities) became possible in the later half of the present century. Now, more and more pure enzymes are being used.

At present most of the worldwide enzyme production is for their applications in three sectors, namely, in the conversion of starch into sugar (40 %), as additives in detergent preparations (30 %) and in dairy industry (10 %). The worldwide production of enzymes has increased significantly in the recent past due to the use of enzymes in detergent industry. The enzymes improve their washing quality. In starch industry too, enzymes such as amylase and amylobiosidase have substituted the acids completely in manufacuture of dextrose.

Odour less — Strong amber like smell

Labadan

Sweet — Tasteless

Asparagine

Smalls like lemon — Smells like orange

Limons

Fig. 15.6 : Enantiomers of some industrial products.

In pharmaceutical industry, enzymes are specially useful because of their ability to differentiate between different stereo-isomers. At present about 1850 drugs are in the market out of which 528 can exist as enantiomers. Thus, there is a need for production of these drugs in pure enantiomeric form. Enzymes can play an important role in this field as they can help in preparing pure enantiomers starting from the corresponding prochiral material or by resolution of the racemic mixtures. Already in the production of many drugs and pharmaceuticals, enzymes are being used in crucial steps to achieve desired level of selectivity and efficiency. In addition to pharmaceuticals, there are other industrial products, such as food additives and perfumes, where one enantiomer is useful and the other is not or may be harmful. Labadane, asparagine, limonene, etc. are examples of such compounds (Fig. 15.6), where use of enzymes can play significant role in their synthesis.

15.4.1 Production of Enzymes

Enzymes are invariably present in all living organisms and may be isolated from animal, plant and microbial sources. The use of microbial enzymes has been increasing because of several advantages. Some of them are :

1) The unethical cruelty involved in production of animal enzymes can be avoided if microbial enzymes are used.

2) Micro-organisms can be grown in the laboratory/industry under rigorously controlled conditions, thus ensuring uniform enzyme yields.

3) With the advent of gene cloning techniques the possibility of creating microorganisms with modified genetics is available and hence a different kind of enzyme can be produced.

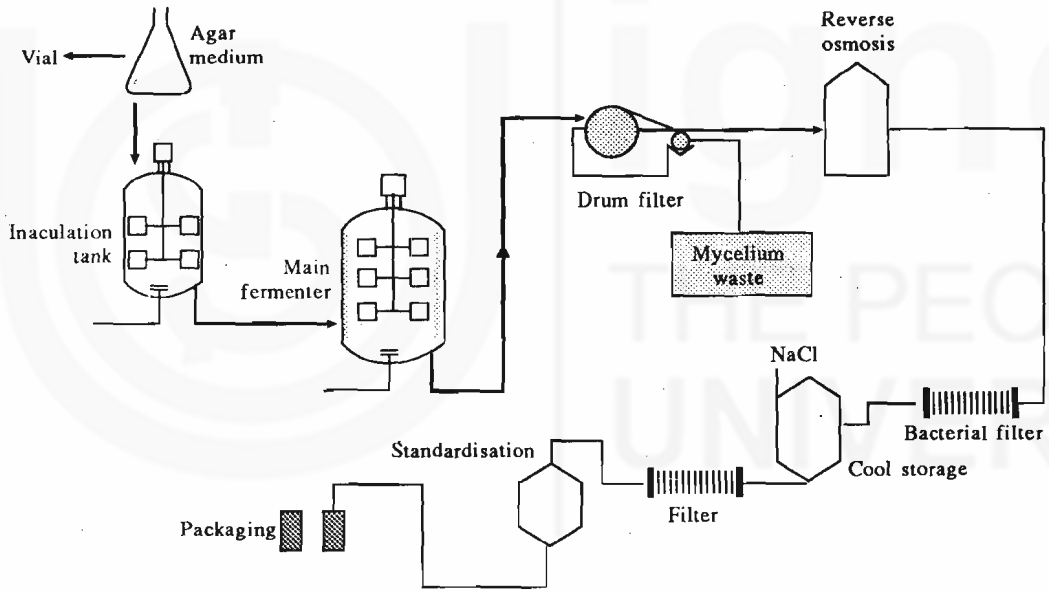

Fig. 15.7 :Flow line diagram showing variouos stages in the production of a liquid enzyme preparation.

The industrial production of enzymes from microorganisms is carried by the method of fermentation wherein the microorganisms is placed in a fermenting substrate, like starch, molasses, whey and many cereals (Fig. 15.7). The choice of fermentation material plays an important role in production of enzymes as it is the source of carbon and nitrogen. At completion of the process, the enzyme is either present inside the microorganism or is secreted into the medium. The enzyme is then extracted and purified (Fig. 15.7). We will take up fermentation biotechnology is some detail in section 15.5.

15.4.2 Immobilised Enzymes

In principle, any catalyst may be used over and over again because it is not consumed in the catalysed reaction. The pre-requisite for this that the catalyst should be recovered in an active form the reaction mixture. It is particularly difficult to recover active enzymes from reaction mixtures, because of their solubility in water and their stability characteristics. It may be recalled that the enzymes, being proteins, are readily denatured and inactivated

unless the conditions of pH and temperature are rigorously controlled. The latter may not always be easy while isolating the reaction products. The lack of recovery increases the cost of using enzymes on a large scale. This difficulty may be overcome to a large extent by attaching the enzyme to a water-insoluble soild support, called matrix (immobilisation of enzymes). The immobilised enzyme may be easily recovered by filtration and washing and my be reused.

A number of materials and processes are used for enzyme immobilization, which constitutes a major part of the enzyme technology. Both physical and chemical methods are in practice for enzyme immobilization. Physically, enzymes are immobilised by mixing them with the immobilizing material, whereby they may be adsorbed on to an insoluble matrix. Many chemical methods have been developed whereby covalent bonds are established between the nonessential functional groups of the enzyme and the immobilizing material ranging form inorganic carriers like ceramics, glass, iron, zirconium, titanium and natural polymers such as sepharose, cellulose etc. to synthetic polymers such as nylon, and poly acrylamide.

15.5 FERMENTATION BIOTECHNOLOGY

Fermentation has been defined as breakdown or decomposition of complex organic molecules (other than proteins) to simpler compounds by the action of living cells, generally bacteria or yeasts. The latter are called Ferments. Such processes have been employed for a long time of industrial and domestic purposes as in brewing industry, bread making or preparation of yoghurt from milk. More recently, the term fermentation technology has been extended also to preparation of complex biomolecules on a large scale as in the manufacture of antibiotics.

The oldest process, given the name of fermentation, is the production of ethanol on the degradation of sugars by yeast.

$$C_6H_{12}O_6 \xrightarrow{\text{yeast}} 2C_2H_5OH + 2CO_2$$

Steps involved in this process and its physiological significance, e.g., capture of metabolic energy as ATP, were discussed in Unit 9. In the present unit, we are concerned with the application of this and other fermentation processes.

15.5.1 Alcoholic Fermentation

The alcohol industry is the largest sector of fermentation industries in terms of production, number of commercial units and number of the persons employed directly or indirectly. Alcohol being a very good solvent, it finds used in many synthetic reactions and for this reason the demand of alcohol in many industrial processes has increased sizably. Ethanol is also the basic/essential constituent of alcoholic beverages like beer, wine, whisky, etc. In this form, it is generally taxed in all countries. Thus fermentation industry is good source of state revenue. "Spirit" which is commonly sold in the market for paint/varnish purposes is made unfit for drinking by mixing the methanol and other materials and is given the name methylated or industrial spirit.

In the world as a whole most of ethanol is manufactured chemically from ethylene. However, alcoholic fermentation accounts for a large fraction of the total ethanol production. This is specially true in countries like India having a large sugar industry and limited petroleum resources. The syrupy by-product of sugar industry, called molasses, is rich in sugars and is a cheap starting material for alcoholic fermentation. The alcoholic beverages are entirely produced by fermentation.

Beer is primarily produced from barley, but recently potatoes, corn and other cereals have also been employed for its production. Yeast are not able to ferment starch directly. Therefore, an essential step in the production of alcohol from grain is the "saccharification" of the starch to maltose or glucese by means of enzymes. Almost all distilleries use malted barley (barley germinated for a short time). This process called malting. activates amylases which are helpful in breaking down starch to oligo-and mono-saccharides.

The malted barley is then crushed and simmered with hot water (around 340 K) to effect breakdown of starch to smaller carbohydrate molecules. The crushed grains are separated and the extract (malt wort) is boiled with hops to provide to flavour typical of the beer. Besides it also stops further action of enzymes and proteins are precipitated. The resulting malt wort is then inoculated with yeast. The commonly used yeast strains are of *Saccharomyces cerevisiae, Saccharomyces ellipsoideus* or "wine yeasts". The inoculation

which initiates the conversion of sugar with yeast is the crucial step when sugar is converted to alcohol into ethanol and corbon dioxide.

Yeast cells grow and multiply during fermentation. Amyl alcohol, isoamyl alcohol, phenyl ethyl alcohol, acetic acid and butyric acid are important by-products of years metabolism. These trace compounds affect the flavour of the beer. After completion of the fermentation, the yeast cells are removed by filteration, the beer is matured for a period of time followed by further filteration and pasteurisation. Normal fermentation requires approximately one week after which the "young" beer is transferred to bates for primary and secondary ageing, which may take additional six months. If the beer is to be canned or bottled, it is pasteurised at 333 K for fifteen minutes to kill all the yeast cells, and is then filtered to remove the yeast. The alcoholic content of the beer is usually 4 per cent.

Wine production is relatively simple. Wine is produced from fruits, fruit juice or extract of parts of plants, such as dandelions. The grapes juice is the common source for production of wine, because of its high nutrient content, natural acidity which inhibits growth of other microorganisms, high sugar content and very special flavour and aroma. The juice is extracted and is incubated with strains of *S. cerevisiae* at 280-287 K. Previously fermentation was done by the microbes present on the skin of the fruit, but now-a-days external addition of microorganism is the normal practice, because it enables rigorous controls and uniform results. Many techniques are applied to avoid participation of the other microbes. Addition of sulphur dioxide is one such process which inhibits growth of non-wine yeast. The grapes variety is responsible for particular flavour in the wine. The fermentation usually requires only a few days. However, ageing may continue for months or even years. During ageing, wine develops flavour, aroma and bouquet. There is a common belief that the quality of wine improves as the ageing period increases. The red wine comes from the skin of the fruit. In colourless wine, grape juice is used. The alcohol percentage varies from 12-16% in different wines. In certain fortified wines, it may be as high as 22 per cent.

The stronger beverages or spirits contain much more alcohol than beer or wine. These are obtained by distillation.

In addition to the production of alcoholic beverages, fermentation is also employed for the manufacture of pure ethanol which may be used as a solvent or as the raw material for the sysnthesis of many other organic compounds. The fermented liquor, containing 12-16% ethanol, is subjected to fractional distillation to obtain "rectified spirit". The latter is a constant boiling, i.e., azeotropic, mixture of 95.6% ethanol and 4.4% water by weight. It boils at 351.2 K which is slightly lower than the boiling point of pure (anhydrous) ethanol (351.3). Therefore, the latter cannot be obtained by further fractional distillations. Other methods are employed to remove the remaining water, which you must have read with alcohols and their manufacture in Unit 12 of the course "Organic Chemistry"

15.5.2 Vinegar Production

Vinegar, is a fermentation product that conertains more than 4% acetic acid (w/v). The production of vinegar essentially requires two steps.

i) Conversion of the sugar containing material to alcohol using *S. cerevisiae* by steps similar to those in wine production.

Practically any substrate capable of being converted to alcohol can be used.

ii) The second step involves the conversion of alcohol obtained from first step to acetic acid via acetaldehyde. The process requires collection of *Acetobacter schnetzenbanchii,*

A. curvim, A. oreleanense or other related microbes. The process is exothermic and requires excess of oxygen.

The above procedure was used for vinegar manufacture in earlier days. These days a cheaper variety called "sythetic vinegar" is also marketed which is obtained by appropriate dilution of pure ethanoic acid (acetic acid).

15.5.3 Antibiotic Production

With the discovery of penicillin by Alexander Flemming, the antibiotics have become one of the most potent clinical agents. Antibiotics are biologically active compounds synthesised by some micro-organisms which prevent the growth of other micro-organisms. Such antibiotics which are either non-toxic or exhibit only low toxicity towards human beings, are evidently useful as important drugs for various diseases. Some of these are listed in Table 15.3

Table 15.3: Some economically important antitiotics

Antibiotic compound	Producer microorganism	Activity spectrum
Actinomycin D	*Streptomyces* s_p	Antitumour
Asparaginase	*Erwinia* s_p	Antileckemia
Badcilracin	*Bacillus* s_p	Antibacterial
Bleomycin	*Streptomyces* s_p	Antibactial
Cephalosporin	*Acremonium* s_p	Anticancer
Chloramphenicol	*Cepalosporium* s_p	Antibacterial
Dounorubicin	*Streptomyces* s_p	Antibacterial
Fumogillin	*Aspergillus* s_p	Amoebicidal
Griseoflvin	*Pencillium* s_p	Antifungal
Mytomycin C	*Streptomyces* s_p	Antitumour
Natamycin	*Streptomyces* s_p	Food preservative
Nisin	*Streptococcus* s_p	Food preservative
Penicillin G	*Penicillium* s_p	Antibacterial
Rilamycin	*Nocardia* s_p	Antitubrculosis
Streptomycin	*Streptomyces* s_p	Antibacterial

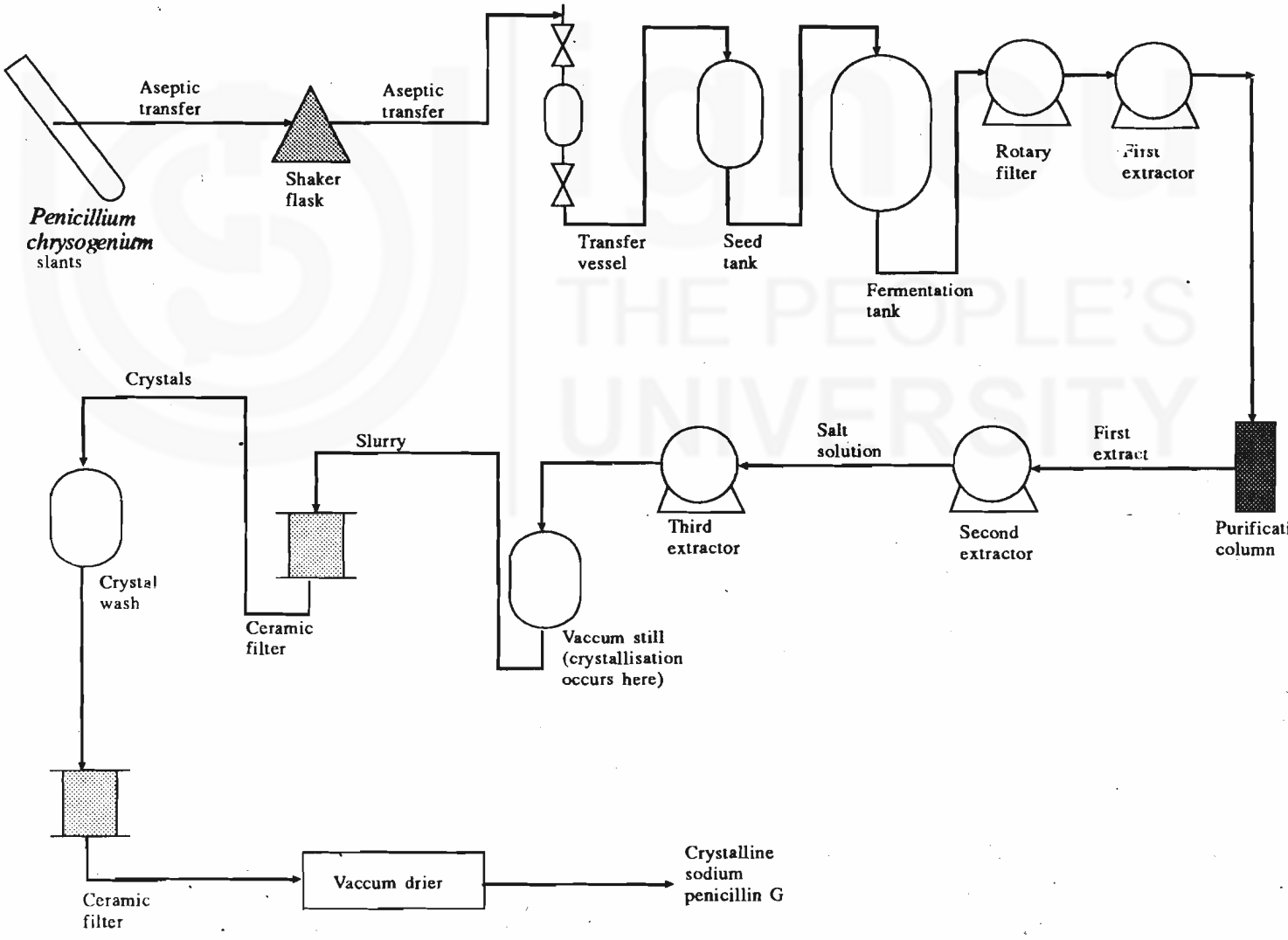

Fig. 16.8 : Flow diagram for production of penicillin.

We shall now briefly discuss the penicillin G production process as a typical example for the production of antibiotics. penicillin is produced by fermentation method. The steps in Fig. 15.8 may be separated into the following stages:

Fermentation : Various fermentation methods are known which are used to produce penicillin on a large scale. Out of them submerged fermentation method is commonly used for the commercial production. In this process, the *Penicillium chrysogenium* is used as seed for the fermentation. It is prepared by growing a master stock culture of the mold from lyophylized spores on a nutrient agar substratums with incubation. Several gallons of culture medium, generally constituting 5 to 10 per cent of the charge, are prepared in a series of seed tanks to seed a large tank.

The fermentation medium is prepared from corn steep liquor, to which 2 to 4 per cent lactose has been added, in addition to inorganic materials such as calcium carbonate, potassium phosphate, magnesium sulphate and traces of iron, copper and zinc salts. After adjusting the pH to 4.5 to 5.0 the fermentation medium is fed with the fermenter which is equipped with a vertical agitator, a means of introducing air which has been sterilised by filtration and coils for controlling temperature. Following sterilisation of the fermenter, the 'seed' (mold) is introduced through sterile pipelines by air pressure. During fermentation, the temperature is maintained at 296 to 300 K. Sterile air permits growth of the aerobic mold, agitation distributes it uniformly in the batch. Fermentation is completed in 50 to 90 hours.

Separation of Penicillin : After fermentation, the batch is cooled to 278 K because of the instability of penicillin at room temperature and the mycelin (cells and insoluble metabolic products) are removed by filtration on a rotatory drum filter. The filtrates contain the antibiotic which initially is separated by solvent-extraction process. In this process, filtered liquor (beer) is adjusted to a pH 2.5 with phosphoric acid, resulting in an acid form of penicillin. Continuous counter current extraction is carried out with amyl acetate in specially designed extractors, the final liquor is treated with buffered phosphate and sodium bicarbonate to form the sodium salt. This material is made sterile by filtration and is freed of water and other solvents by crystallisation, crystalline penicillin is thereby formed which, when dried, may be packed in bulk in polyethylene bags, or stainless steel containers.

SAQ 3

Fill in the blanks:

a) Enzymes find applications in production of ..... pure physiologically active compounds.

b) Enzyme immobilisation can be achieved by ..... and ..... methods.

c) The essential preparatory step in production of alcohol from starchy material is ..... of the starch to maltose or glucose.

d) Barley germinated for short time are called ..... barley.

15.6 SUMMARY

Biotechnology, the package term of 'Biological Technology', is defined as the application of microbial, animal or plant cells or enzymes to transform or breakdown or synthesise useful materials. Biotechnical processes have been known to humans for centuries, e.g., brewing, bread making, etc. Biotechnology is an interdisciplinary science and involves integration of biology, biochemistry, microbiology and chemical engineering. In ths unit, only recombinant DNA (or gene cloning), enzyme technology and fermentation technology have been discussed here.

Recombinant DNA technique, or gene cloning, permits insertion of ''foeign'' genes into living cells such as years or bacteria. This requires identification, slicing and isolation the desired DNA fragment, i.e., the gene, from the donor species. This is combined with a vector or carrier DNA. Bacterial plasmids are the preferred vectors. This gives rise to ''recombinant'' DNA which is inserted into the host cell. When the recipient or the host cell grows and multiplies. The newly introduced ''foreign'' DNA is also replicated. At the same time this gene is also ''expressed'', i.e., the protein codded for by it is synthesised. This technique is being used for industrial preparation of many compounds that are important clinically and industrially, e.g., insulin, interferon, hormones, etc.

Isolation and production of enzymes on a large scale and their industrial application are referred to as enzyme technology. Enzymes may be applied in the preparation or isolation of specific stereo-isomer from prochiral starting material or racemic mixtures. Enzymes are also used as additives in detergents where they improve the washing qualities. Application of enzymes as industrial catalysts is facilitated by attaching them insoluble matrices. This process is referred to as enzyme immobilisation. The enzymes may be simply adsorbed on a matrix or may be covalently attached to it. The immobilised enzymes are easily separated from the reaction mixture by simple filtration and washing and may be reused.

Decomposition of complex organic compounds into simpler molecules by the action of micro-organisms, like yeasts or bacteria, has been referred to as fermentation. Now a days the term "fermentation technology" also includes the synthetic applications of micro-organisms. Most common examples of this technology are the manufacture of alcoholic beverages, pure ethanol and antibiotics. The last named are useful clinical agents.

15.7 TERMINAL QUESTION

1) Discuss briefly, what is meant by biotechnology.

2) Genetic engineering essentially involves alteration in gene. How the alterations are brought about ? Illustrate.

3) What does the term 'enzyme immobilisation mean' ?

4) Fermentation is one of the oldest processes known to mankind. Illustrate how fermentation has become industrial process of today.

5) Give the production of penicillin G.

15.8 ANSWERS

Self Assessment Questions

1) a) Plasmids b) DNA
   c) Diabetiecs d) Glycoproteins

2) a) F b) T c) T d) F e) F

3) a) Enantiomerically
   b) Physical, chemical.
   c) Saccharification
   d) Malted

Terminal Questions

1) See Section 15.2

2) See Section 15.3

3) Enzyme immobilisation is the means by which enzymes are attached or immobilised, without losing their specificity and activity to solid supports such as insoluble polymers, membranes and other particles, i.e. heterogeneous catalyst. It is a beneficial process as the catalytic enzymes can be removed from the system simply by physical methods and reused again.

4) See Section 15.5.

5) See Section 15.5