CHAPTER



Biotechnology

Animation 17.1: Biotechnology Source & Credit: dailykos

Humans have been making use of biotechnology since they discovered farming. This use extended from the planting of seeds to the control of plant growth and crop production. Animal breeding is also a form of biotechnology. Cross-pollination of plants and cross-breeding of animals were major techniques in biotechnology. These techniques were used to enhance product quality and to meet specific requirements. In this chapter we will get basic knowledge about the techniques being used in biotechnology.

17.1 Introduction Of Biotechnology

Biotechnology is defined as the use of living organisms in processes for the manufacture of useful products or for services. Although the term biotechnology is new, the discipline itself is very old. Fermentation and other such processes, which are based on the natural capabilities of organisms, are commonly considered as old biotechnology.



In Scotland, in 1997, an embryologist lan Wilmut produced a sheep (Dolly) from the body cell of an adult sheep.

Genetic engineering i.e. the artificial synthesis, modification, removal, addition and repair of the genetic material (DNA) is considered as modern biotechnology. It is done to alter the characteristics of organisms. The work on genetic engineering started in 1944 when it was proved that DNA carries the genetic information. Scientists isolated the enzymes of DNA synthesis and then prepared DNA outside cells. In 1970s, they were able to cut and paste the DNA of organisms. In 1978, scientists prepared human insulin by inserting the insulin gene in bacteria. Human growth

hormone was also synthesized in bacteria. In 1990, the **Human Genome Project** was launched to map all the genes in human cell. The complete map of human genome was published in 2002. Human began using microorganisms as early as 4000 BC for making wine, vinegar, cheese, yogurt etc. Some of these processes have become a part of every home that we may even hesitate to refer them as biotechnology.

17.1.1 Scope And Importance Of Biotechnology

In recent years, biotechnology is growing as a separate science. It has attracted the attention of many intellectuals from diverse fields like agriculture, medicine, microbiology and organic chemistry. The scope for biotechnology is so wide that it is difficult to recognize the limits. The following are some areas of the application of biotechnology.

Biotechnology in the Field of Medicine

In the field of medicine, biotechnologists synthesized insulin and interferon (antiviral proteins) from bacteria and released for sale. A large number of vaccines and antibodies; human growth hormone and other medicines have also been produced. Various enzymes are being synthesized for medicinal as well as industrial use. Gene therapy (treatment through genes) has become important in recent years. Biotechnology also proved much beneficial in forensic medicine. The study of DNA helps in the identification of criminals.

Biotechnology in the Field of Food and Agriculture

Fermented foods (e.g. pickles, yogurt), malted foods (e.g. powdered milk: a mixture of barley, wheat flour and whole milk), various vitamins and dairy products are produced by using microorganisms. Wine and beer are produced in beverage industry. Biotechnology has also revolutionized research activities in the area of agriculture. Transgenic (organisms with modified genetic set-up) plants are being developed, in which desirable characteristics are present e.g. more yields and resistance against diseases, insects and herbicides. Transgenic goats, chickens, cows give more food and milk etc. Many animals like mice, goats, cows etc. have been made transgenic to get medicines through their milk, blood or urine.

Biotechnology and Environment

Biotechnology is also being used for dealing with environmental issues, like pollution control, development of renewable sources for energy, restoration of degraded lands and biodiversity conservation. Bacterial enzymes are used to treat sewage water to purify. Microbes are being developed to be used as biopesticides, biofertilizers, biosensors etc. Such transgenic microorganisms are also used for the recovery of metals, cleaning of spilled oils and for many other purposes.

Fears are also being expressed about the advances in biotechnology in terms of release of harmful organisms developed through recombinant DNA technology.

17.2 Fermentation

We know that in cellular respiration, glucose molecule goes through oxidation-reduction reactions to release energy in the form of ATP. Fermentation is the process in which there is incomplete oxidation-reduction of glucose. Fermentation has been in the knowledge of man since centuries, but it was believed that it is purely a chemical process.

In 1857, Pasteur convinced the scientific community that all fermentations are the results of microbial activity. He showed that fermentation is always accompanied by the development of microorganisms. There are many kinds of fermentation and each kind is a characteristic of particular microbial group.

Fermentations are classified in terms of the products formed. The initial steps of **carbohydrate fermentation** are identical to those of respiration. The process begins with glycolysis, in which the glucose, molecule is broken into two molecules of pyruvic acid. Different microorganisms proceed the further reactions in different ways. It results in the formation of various products from pyruvic acid.

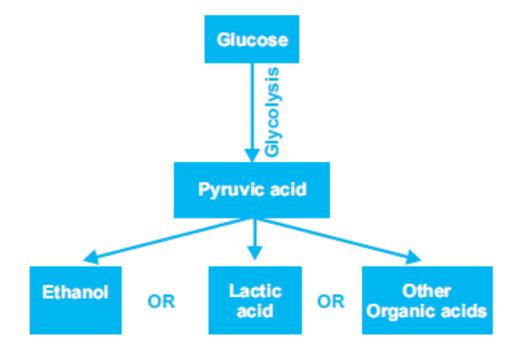


Figure 17.1: Carbohydrate fermentation and its products

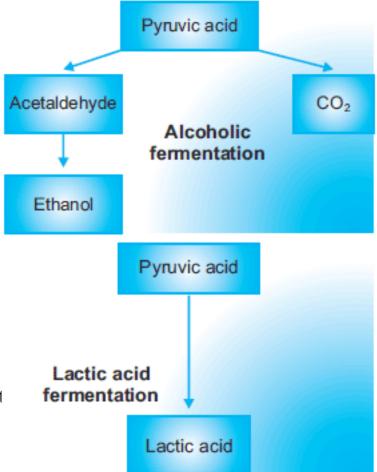
The two basic types of carbohydrate fermentation are described next.

1. Alcoholic Fermentation (by yeast)

This fermentation is carried out by many types of yeast such as Saccharomyces cerevisiae. This process is quite important and is used to produce bread, beer, wine and distilled spirits. In this process, carbon dioxide is removed from pyruvic acid. The product i.e. acetaldehyde is then reduced to ethanol. The carbon dioxide produced during this fermentation causes the rise of the bread.

2. Lactic Acid Fermentation (by bacteria)

In this process, pyruvic acid is reduced to lactic acid. It is carried out by many bacteria e.g. *Streptococcus* and many Lactobacillus species. It is quite important in dairy industry where it is used for souring milk and also for production of various types of cheese.



17.2.1 Fermentation In Biotechnology

In beginning, the meaning of fermentation process was the use of microorganisms for the production of foods (cheese, yogurt, fermented pickles and sausages, soy sauce), beverages (beers, wines) and spirits. However, in biotechnology the term "fermentation" means the production of any product by the mass culture of microorganisms.

Applications of Fermentation

In fermentation, maximum growth of an organism is obtained for the production of desired products of commercial value. Traditionally, only food and beverage products were produced by using fermentation. Now many other products e.g. industrial chemicals are also being produced.

a- Fermented Foods

Fermentation often makes the food more nutritious, more digestible and tastier. It also tends to preserve the food, lowering the need for refrigeration. The following groups are included in the fermented foods.

Cereal products: Bread is the commonest type of fermented cereal product. Wheat dough is fermented by *S. cerevisiae* along with some lactic acid bacteria.

Dairy products: Cheese and yogurt are important fermentation products. Cheese is formed when a milk protein is coagulated. This happens when the acid produced by lactic acid bacteria reacts with milk protein. Yogurt is made from milk by different lactic acid bacteria.

Fruit and vegetable products: Fermentation is usually used, along with salt and acid, to preserve pickle, fruits and vegetables.



Figure 17.2: Fermented foods

Beverage Products:

Beer is produced from cereal grains which have been malted, dried and ground into fine powder. Fermentation of the powder is done by yeast. This process breaks the glucose present in powder into pyruvic acid and then into ethanol. Grapes can be directly fermented by yeasts to wine.

b- Industrial Products

The following are the important industrial products produced through the process of fermentation.

Products	Microorganisms used	Some uses	
Formic acid	Aspergillus	Used in textile dyeing, leather treatment, electroplating, rubber manufacture	
Ethanol	Saccharomyces	Used as solvent; used in the production of vinegar and beverages	
Glycerol	Saccharomyces	Used as solvent; used in the production of plastics, cosmetics and soaps; used in printing; used as sweetener	
Acrylic acid	Bacillus	Used in the production of plastics	

17.2.2 Fermenter

Fermenter is a device that provides optimum environment to microorganisms to grow into a biomass, so that they can interact with a substrate, forming the product. Fermentation is carried out in fermenters, in the following two ways.

Batch Fermentation

In this process, the tank of fermenter is filled with the raw materials to be fermented. The temperature and pH for microbial fermentation is properly adjusted, and nutritive supplements are added.

In fact, the fermenter constitutes the heart of any industrial fermentation process.

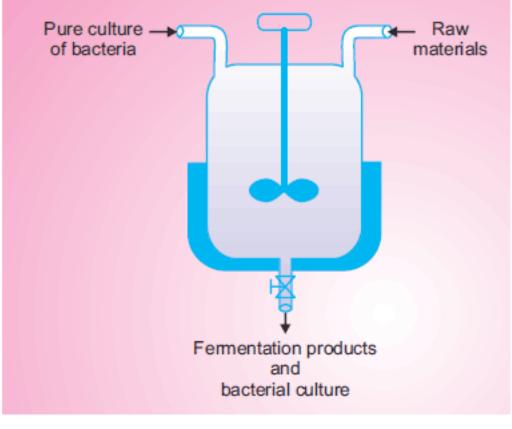
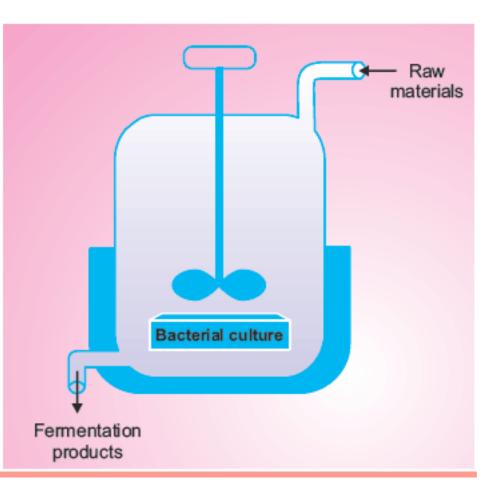


Figure 17.3: A batch fermenter

All the material is steam sterilized. The pure culture of microorganisms is added to fermenter from a separate vessel (Fig. 17.3). Fermentation proceeds and after the proper time the contents of fermenter are taken out. Fermenter is cleaned and the process is repeated. Thus, fermentation is a discontinuous process divided into batches.

Continuous Fermentation

In this process, the substrate is added to fermenter continuously at a fixed rate. This maintains the microorganisms in growth phase. Fermentation products are taken out continuously (Fig. 17.4).





Advantages of using Fermenters

For each biotechnological process, the environment provided to the organisms must be monitored

and controlled. Such a controlled environment is provided by fermenters. A fermenter optimizes the growth of the organisms by controlling many factors like nutrients, oxygen, growth inhibitors, pH and temperature. A fermenter may hold several thousand litres of the growth medium. So, fermenters allow the production of materials in bulk quantities. Massive amounts of medicines, insulin, human growth hormone and other proteins are being produced in fermenters and this production proves much inexpensive.



Figure 17.5: Fermenters used in food and pharmaceutical industry

Practicals:

- Investigate the role of yeast in the fermentation of flour.
- Investigate the role of bacteria in the fermentation of milk.

17.3 Genetic Engineering

Genetic engineering or recombinant DNA technology involves the artificial synthesis, modification, removal, addition and repair of the genetic material (DNA). Genetic engineering developed in the mid-1970s when it became possible to cut DNA and to transfer particular pieces of DNA from one type of organism into another. As a result, the characteristics of the host organism could be changed. If host organism is a microorganism, such as a bacterium, the transferred DNA is multiplied many times as the microorganism multiplies. Consequently, it is possible to obtain millions of copies of a specific DNA inside a bacterial cell.

17.3.1 Objectives of Genetic Engineering

The important objectives of genetic engineering are as follows.

- Isolation of a particular gene or part of a gene for various purposes such as gene therapy
- Production of particular RNA and protein molecules
- Improvement in the production of enzymes, drugs and commercially important organic chemicals
- Production of varieties of plants having particular desirable characteristics
- Treatment of genetic defects in higher organisms

17.3.2 Basic Steps in Genetic Engineering

All the above mentioned objectives can be obtained by some basic methodologies, such as:

1. Isolation of the gene of interest

In the first step, the genetic engineer identifies the gene of interest in a donor organism. Special enzymes, called restriction endonucleases, are used to cut the identified gene from the total DNA of donor organism.

2. Insertion of the gene into a vector

A vector is selected for the transfer of the isolated gene of interest to the host cell. The vector may be a plasmid (the extra-chromosomal DNA present in many bacteria) or a bacteriophage. The gene of interest is attached with the vector DNA by using endonuclease (breaking enzymes) and ligase (joining enzymes). The vector DNA and the attached gene of interest are collectively called **recombinant DNA**.

3. Transfer of recombinant DNA into host organism

Recombinant DNA is transferred to the target host. In this way, host organism is transformed into a genetically modified organism (GMO).

4. Growth of the GMO

The GMO are provided suitable culture medium for growth to give as much copies of the gene of interest as needed.

5. Expression of the gene

The GMO contains the gene of interest and manufactures the desired product, which is isolated from culture medium.

17.3.3 Achievements of Genetic Engineering

Various achievements of genetic engineering are as follows.

- Human **insulin** gene was transferred into bacteria. The genetically modified bacteria became able to synthesize insulin. Diabetics are now receiving this insulin. The steps of genetic engineering for the production of insulin are shown in figure 17.7.
- In 1977 an E. coli bacterium was created that was capable of synthesizing the human growth hormone.
- The hormone **thymosin** which may prove effective against brain and lung cancer has been produced by genetically modified microorganisms.
- **Beta-endorphin**, a pain killer produced by the brain, has also been produced by genetic engineering techniques.
- Genetic engineers produced a safe **vaccine** against the foot and mouth disease (a viral disease in cattle, goats and deer). Similarly many vaccines have been produced against human diseases such as hepatitis B.

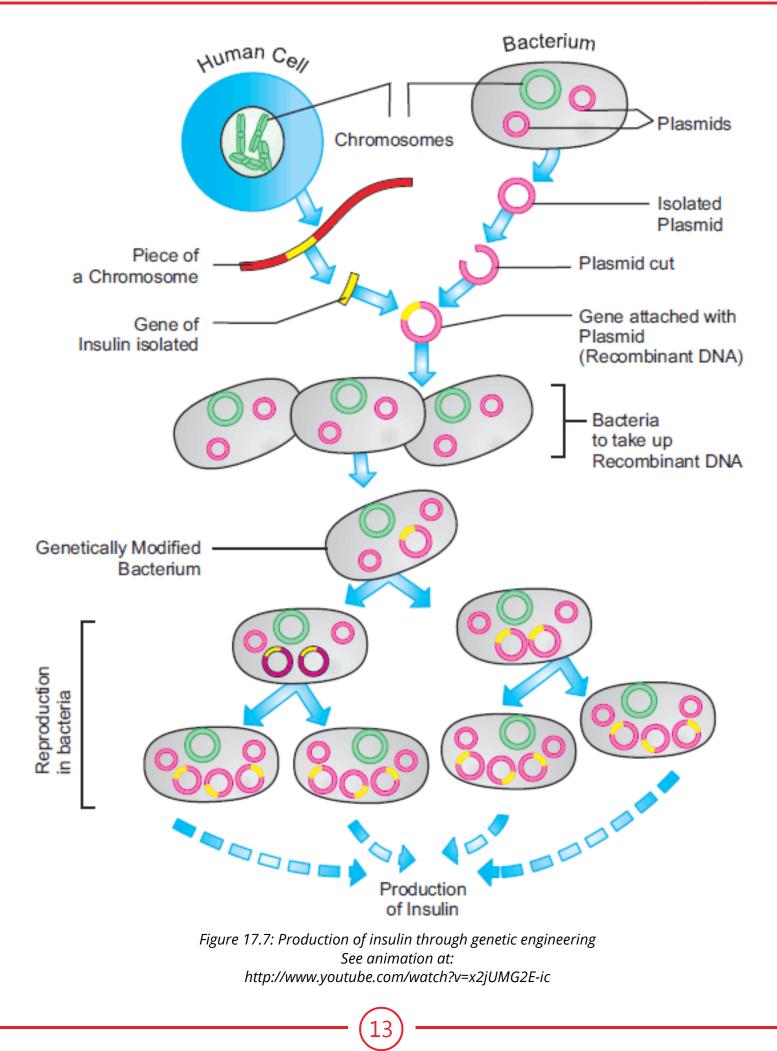
Before genetic engineering, 500,000 sheep brains were required to produce 5 mg human growth hormone.



Figure 17.6: Some medicines produced by genetic engineers

- **Interferons** are anti-viral proteins produced by cells infected with viruses. In 1980, interferon was produced in the genetically modified microorganisms, for the first time.
- The enzyme **urokinase**, which is used to dissolve blood clots, has been produced by genetically modified microorganisms.
- Now it has become possible to modify the genes in the human egg cell. This can lead to the elimination of inherited diseases like **haemophilia**.
- Genetic engineering techniques can also be used to cure **blood diseases** like thalassemia and sickle-cell anaemia, which result from defects in single genes.
- Normal genes could be transferred into the bone marrow.
- Genetic engineers have developed plants that can **fix nitrogen** directly from the atmosphere. Such plants need less fertilizers.

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17.4 Single-Cell Protein

In genetic engineering, we have studied about the transformation of microorganisms by the introduction of genes of beneficial proteins e.g. insulin. Single-Cell Protein (SCP) refers to the protein content extracted from pure or mixed cultures of algae, yeasts, fungi or bacteria. For the production of single-cell proteins, the microorganisms are grown in fermenters. These microorganisms utilize a variety of substrate like agricultural wastes, industrial wastes, natural gas like methane etc. Microorganisms grow very vigorously and produce a high yield of protein. The protein content produced by microorganisms is also known as novel protein or minifood.

We know that due to over-population, the world is facing the problem of food shortage. In future, the conventional agricultural methods might not be able to provide a sufficient supply of food (especially proteins). For a better management of food shortage problems (in humans and domestic animals), the use of microbes as the producers of single-cell proteins has been successful on experimental basis. This technique was introduced by Prof. Scrimshow of Massachusetts Institute of Technology. Scientist and food technologists believe that single-cell proteins will substitute the other protein-rich foods in human and animal feeds.

All scientists recognize the significance of the production of single-cell proteins. The microorganisms grow very vigorously and produce a high yield. It has been calculated that 50 kilogram of yeast produces about 250 tons of protein within 24 hours. Algae grown in ponds produce 20 tons (dry weight) of protein per acre/year. This yield of protein is 10-15 times higher than soybeans and 20-50 times higher than corn. When single-cell proteins are produced by using yeasts, the products also contain high vitamin content. In the production of single cell proteins, industrial wastes are used as raw materials for microorganisms. It helps in controlling pollution. The use of single-cell proteins has good prospects in future because they contain all essential amino acids. Moreover, the production of single-cell proteins is independent of seasonal variations.

It is known as single cell protein because the microorganisms used as producers are unicellular or filamentous individuals.

SCP is gaining popularity day by day because it requires limited land area for production.

UNDERSTANDING THE CONCEPT

- 1. Define biotechnology and describe its importance.
- 2. What is a fermenter? What are the two types of fermentation carried out in fermenters?
- 3. Describe the achievements of genetic engineering in medicine, agriculture and environment.
- 4. What basic steps a genetic engineer adopts during the manipulation of genes?
- 5. What are single cell proteins? Describe their importance.

SHORT QUESTIONS

- 1. How would you define fermentation with reference to biotechnology?
- 2. Name any two industrial products made by fermentation. Also describe their uses in the industry.
- 3. What are the products of the two types of carbohydrate fermentation?
- 4. Give an example how biotechnology is helping for better environment.
- 5. In biotechnology, what is meant by Genetically Modified Organism (GMO)? How is it made?

THE TERMS TO KNOW				
Batch fermentation Biotechnology Continuous fermentation Fermentation Fermenter	Genetically modified organism Recombinant DNA Restriction endonucleases Single-cell protein	<u>Transgenic</u> <u>Vector</u>		

ACTIVITIES

- 1. Investigate the role of yeast in the fermentation of flour.
- 2. Investigate the role of bacteria in the fermentation of milk.

SCIENCE, TECHNOLOGY AND SOCIETY

- 1. Apply knowledge to identify different products of animal and human food having single cell proteins.
- 2. Develop awareness among the students of other classes about important social and ethical issues of genetic engineering.
- 3. Describe the ways in which our society can benefit from the knowledge of genetic engineering.
- 4. Interpret the data collected from internet on virus resistant, insect resistant and high yielding varieties of agricultural crops in Pakistan.

ON-LINE LEARNING

- 1. www.sciencedaily.com/news/plants_animals/biotechnology/
- 2. http://www.youtube.com/watch?v=x2jUMG2E-ic
- 3. www.pakissan.com/biotech/institutes.biotech.engineering.shtm