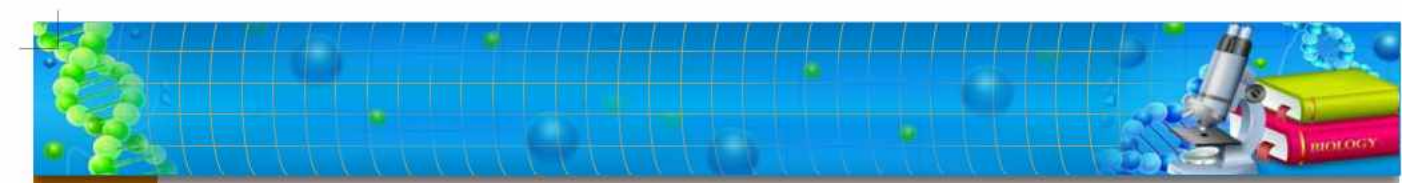


Major Concept





8.1 INTRODUCTION

The earliest biotechnologists were farmers who developed, improved species of plants and animals using cross-pollination or cross-breeding techniques. The use of these techniques to manufacture products intended to improve the quality of human life. To improve the situation and achieve global stability, it will be necessary to use the best available knowledge and understanding of the biotechnology will be an essential requirement for those who want cost-effective products for their successful future.

The term **“Biotechnology”** was used before the twentieth century for traditional activities such as making dairy products like cheese and curd, as well as bread, wine, beer, etc. In 1919, a Hungarian agriculture engineer called Karl Ereky coined the word Biotechnology. There are several definitions for biotechnology. One simple definition is that it is the commercialization of cell and molecular biology. According to the National Science Academy of United States, biotechnology is the “controlled use of biological agents like cells or cellular components for beneficial use”. It covers both classical as well as modern biotechnology. Generally, biotechnology can be defined as “the use of living organisms, cells or cellular components for the production of compounds or precise genetic improvement of living things for the benefit of man”.

Even though biotechnology has been in practice for thousands of years, but credit goes to Indus and Indo-Aryan civilizations, which first practiced biotechnology in 5000 BC to produce fermented foods, medicines and to keep the environment clean.

Genetic engineering has been defined as the artificial manipulation, modification, and recombination of DNA or other nucleic acid molecules in order to modify an organism or population of organisms. It refers to any process in which an organism’s genome is intentionally altered. Genetic engineering does not encompass traditional breeding techniques because it requires manipulation of an organism’s genes through cloning or transformation via the addition of foreign DNA. For example, a DNA fragment may be isolated from one



organism, spliced to other DNA fragments, and put into a bacterium or another organism. This process is called cloning because many identical copies can be made of the original DNA fragment. In 1970s, the scientists were able to alter the DNA of the organisms and in 1978 the first genetically engineered drug, human insulin was produced by bacteria.

In another example of genetic engineering, a stretch of DNA, often an entire gene, may be isolated and its nucleotide sequence determined, or its nucleotide sequence may be altered by *in vitro* mutagenic methods. The related activities in genetic engineering have two basic objectives:

1. To learn more about the ways nature works, and
2. To make use of this knowledge for practical purposes.

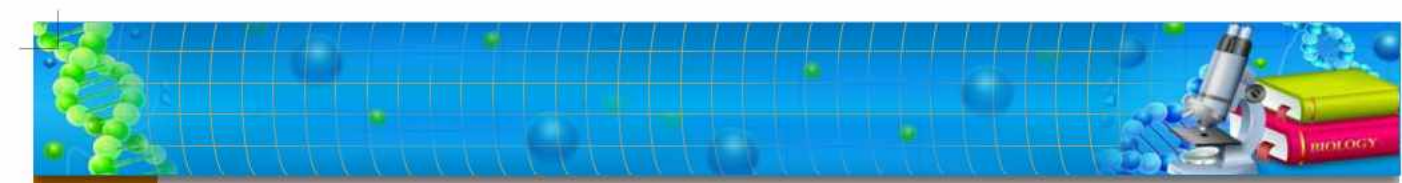
A useful genetic approach to increase the efficiency of production. The fermentation was then shown to be useful in its production; and, now genetic engineering promises to make the fermentation process economically competitive. There are several ways that DNA can be cut, spliced, or otherwise altered. But engineered DNA by itself is a static molecule. To be anything more than the end of a laboratory exercise, the molecule must be integrated into a system of production; to have an impact on society at large; it must become a component of an industrial or otherwise useful process.

In 1990, The Human Genome Project was launched to map all the genes of the human cell. The complete draft of the human genome sequence was published in 2002 with the following objectives:

- Determining the human DNA sequence
- Understanding the function of the human genetic code
- Identifying all of the genes
- Determining their functions
- Understanding how and when genes are turned on and off throughout the lifetime of an individual

Scope and importance of biotechnology

Biotechnology is controlled use of biological agents for beneficial use. It is integrated use of biochemistry, molecular biology, microbiology to achieve technological application of the capabilities of biological agents. Therefore, biotechnology has emerged as a science with immense potential



for human welfare ranging from food processing, human health to environmental protection.

Biotechnology in medicine

Production of a monoclonal antibody, DNA, RNA probes for diagnosis of various diseases; valuable drugs like insulin and interferon have been synthesized by bacteria for the treatment of human diseases. DNA fingerprinting is utilized for identification of parents and criminals. Development of recombinant vaccines like human hepatitis B etc. by genetically engineered microbes; includes the list of notable achievements in medicine

Biotechnology in Agriculture

In agriculture, plant cell, tissue, and organ culture are used for rapid and economic clonal multiplication of fruit and forest trees, for production of virus-free genetic stocks and planting material as well as in the creation of novel genetic variations through soma-clonal variation. Genetic engineering techniques are utilized to produce transgenic plants with desirable genes like disease resistance, herbicide resistance, an increased shelf life of fruits etc. Also, molecular breeding has hastened the process of crop improvement.

Biotechnology in Industry

Industrial Biotechnology is an area with which biotechnology was initiated for large scale production of alcohol and antibiotics by microorganisms. Even today, a variety of pharmaceutical drugs and chemicals like lactic acid, glycerine etc. are being produced by genetic engineering for better quality and quantity.

Biotechnology in the environment

Environmental problems like pollution control, depletion of natural resources for nonrenewable energy, conservation of biodiversity etc. are being dealt with using biotechnology. For example bacteria are being utilized for detoxification of industrial effluents, in combating oil spills for treatment of sewage and for biogas production. Bio-pesticides

give an environmentally safer alternative to chemical pesticides for control of insect pests and diseases.

8.2 FERMENTATION

The classical biotechnology that emerged during the early twentieth century was basically a microbial-based fermentation process in which the principles of biochemical engineering have been applied to change it into an industrial process. In short, it is a hybrid of fermentation and biochemical engineering. Today, most living things use oxygen to make ATP from glucose. However, many living things can also make ATP without oxygen. This is true of some plants and fungi and also of many bacteria. These organisms use aerobic respiration when oxygen is present, but when oxygen is in short supply, they use **anaerobic respiration** instead. Certain bacteria can only use anaerobic respiration. In fact, they may not be able to survive at all in the presence of oxygen.

Fermentation is the process by which living organisms such as yeast or bacteria are employed to produce useful compounds or products.

There are two major types of anaerobic fermentation: lactic acid fermentation and ethanol fermentation. Both restore NAD^+ to allow a cell to continue generating ATP through glycolysis.

Lactic Acid Fermentation

In lactic acid fermentation, pyruvic acid from glycolysis changes to lactic acid. This is shown in Figure 8.1. In the process, NAD^+ forms NADH. NAD^+ , in turn, lets glycolysis continue.



Lactic acid fermentation produces lactic acid and NAD^+ . The NAD^+ cycles back to allow glycolysis to continue so more ATP is made. Each circle represents a carbon atom.

Fig 8.1 Lactic acid fermentation

This type of fermentation is carried out by the bacteria *Streptococcus* and *Lactobacillus* species for souring milk into yogurt and production of various types of cheese. It is also used by your own muscle cells when you work them hard and fast.

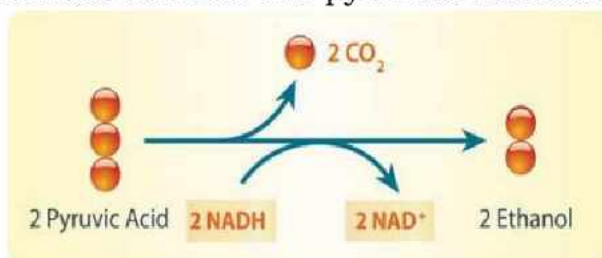


Did you ever run a race and notice that your muscles feel tired and sore afterward?

This is because your muscle cells used lactic acid fermentation for energy. This causes lactic acid to build up in the muscles. It is the buildup of lactic acid that makes the muscles feel tired and sore.

Alcoholic Fermentation

In alcoholic fermentation, pyruvic acid changes to alcohol and carbon dioxide. This is shown in figure below. NAD^+ also forms from NADH , allowing glycolysis to continue making ATP. This type of fermentation is carried out by yeast *Saccharomyces cerevisiae* and some bacteria. Ethanol fermentation converts two pyruvate molecules, the products of glycolysis into two molecules of ethanol and two molecules of carbon dioxide. It is used to make bread, wine, and biofuels.



Alcoholic fermentation produces ethanol and NAD^+ . The NAD^+ allows glycolysis to continue making ATP.

Fig 8.2 Alcoholic fermentation



This type of fermentation also explains why bread dough rises. Yeasts in bread dough use alcoholic fermentation and produce carbon dioxide gas. The gas forms bubbles in the dough, which cause the dough to expand. The bubbles also leave small holes in the bread after it bakes, making the bread light and fluffy.



The small holes in bread are formed by bubbles of carbon dioxide gas. The gas was produced by alcoholic fermentation carried out by yeast. Do you see the small holes in the slice of bread?

Fermentation or Industrial Biotechnology

In the world of biotechnology, the term fermentation is used rather loosely to refer to the growth of microorganisms forming on food, under either aerobic or anaerobic conditions. The industrial microorganisms are grown under controlled conditions with an aim of optimizing the growth of the organism for the production of targeted microbial products such as foods (cheese, yogurt, fermented pickles & sausages, soy sauce), beverages (beers, wines) and spirit.

Application of fermentation

Fermentation changes the chemical environment of a food. Before man knew much about fermentation, they simply used a small portion of food to add to new batches. This ensured that the microorganisms that are needed were included in the recipe. Fermentation is an important process in the preparation of foods for human consumption. Many plant products undergo a fermentation process in order to produce the final product and also Pre-sterilized (pasteurized) material assembled into packages and aseptically filled (Aseptic packaging) for the grocery shelf.

Fermentation as a food preservation technique

Fermented foods are foods that have been prepared in a way so that the bacteria naturally found within them starts to ferment. Fermentation, also known as lacto-fermentation, is a chemical process in which bacteria and other micro-organisms break down starch and sugars within the foods, possibly making them easier to digest, and resulting in a product that is filled with helpful organisms and enzymes. This process of fermentation is a natural preservative, which means that fermented foods can last a long time.

Products of fermentation

Foods that undergo fermentation include dairy products: yogurt, cheese, cereal products: bread, cakes fruit and vegetable products: flavorings, candy, fruit juice, silage, and beverages: beer, wine, cider. Pickling involves fermentation. Foods that are frequently pickled include beans, onions, cauliflower, cucumbers, tomatoes, and cabbage. Fermentation helps preserve the food and lowers the need of refrigerator.



Fig 8.3 Fermented foods

Traditionally, only food and beverages were produced using fermentation but now many non-food products are being produced. Non-food items that undergo fermentation include antibiotics, laundry detergent, insulin, growth hormones, cellulose, monoclonal antibodies, compost, chemicals and medicine to dissolve tumors and to clot blood.



Fig. 8.4

Chemicals produced through fermentation Fermentor

Fermentor, in general terms, as its name suggests, are containers used to grow bacteria and fungi on large scale. The general idea behind the fermentor is to provide a stable and optimal environment for microorganisms to reproduce and interact with substrate for required product.

Advantages of fermenter

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 organisms by controlling many factors like nutrients, oxygen, growth inhibitors, pH and temperature.

A fermenter may hold several thousand liters of growth medium. So, fermenters allow the production of material in bulk quantities. Massive amounts of medicines, insulin, human growth hormone and other proteins are being produced in fermenters and thus production proves much inexpensive.

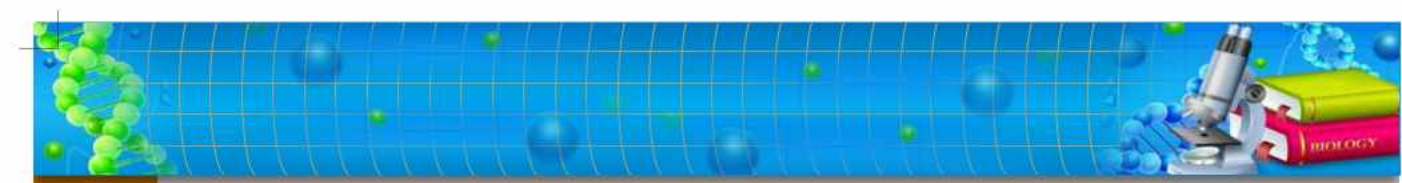


Fig. 8.5

Aseptic packaging



Fig. 8.6 Fermenter



8.3 GENETIC ENGINEERING AND ITS USES

Genetic engineering is the cornerstone of modern biotechnology. It is based on scientific tools, developed in recent decades that enable researchers to:

1. Identify the gene that produces the protein of interest.
2. Cut the DNA sequence that contains the gene from a sample of DNA.
3. Place the gene into a vector, such as a plasmid or a bacteriophage.
4. Use the vector to carry the gene into the DNA of the host cells, such as *Escherichia coli* (*E coli*) or mammalian cells grown in culture.
5. Induce the cells to activate the gene and produce the desired protein.
6. Extract and purify the protein for therapeutic use.

Genetic Engineering Tools

To manipulate cells and DNA, scientists use tools that are borrowed from nature, including:

- **Restriction enzymes:**

These naturally occurring enzymes are used as a defense by bacteria to cut up DNA from viruses. There are hundreds of specific restriction enzymes that researchers use like scissors to snip specific genes from DNA.

- **DNA Ligase:**

This enzyme is used in nature to repair broken DNA. It can also be used to paste new genes into DNA.

- **DNA vector:**

- a. **Plasmids:**

These are mostly circular units of DNA. They can be engineered to carry genes of interest.

- b. **Bacteriophages (also known as phages):**

These are viruses that infect bacteria. Bacteriophages can be engineered to carry recombinant DNA.

- When segments of DNA are cut and pasted together to form new sequences, the result is known as recombinant DNA. When



recombinant DNA is inserted into cells, the cells use this modified blueprint and their own cellular machinery to make the protein encoded by the recombinant DNA. Cells that have recombinant DNA are known as **genetically modified organism (GMO) or transgenic cells**.

- The GMO contains the gene of interest and manufactures the desired product.

Major achievements of genetic engineering

Genetic engineering means making changes to DNA in order to change the way living things work. So many things have been made through genetic engineering such as; Vaccines, Monoclonal antibodies, Gene therapy, Interferons, Interleukins, Recombinant human proteins, Human growth hormones, Clotting factors and Erythropoietins.

1. The creation of a new **synthetic vaccine** for **foot-and-mouth disease** is a strange and more impressive tale. The trick was to carve up the virus genome to make a DNA copy that codes only for the three capsid proteins.
2. **Coccidiosis** is a disease of both invertebrates and vertebrates caused by parasitic protozoa which invade the epithelial cells lining the alimentary tract and the cells of associated glands. A vaccine made against coccidiosis by using avian protein to immunize chicken against avian coccidia.
3. Sleeping sickness (**trypanosomiasis**) is caused by a parasite called *Trypanosoma brucei*. The availability of a genetic transformation made possible the treatment of this disease.
4. Molecular biology has introduced in modern medicine a new way to cure diseases, namely **genetic therapy**, direct intervention in the genetic makeup of an individual. Gene therapy can be somatic or germ line.

5. **Cloning Humans** In the second half of the 20th century, as dramatic advances were taking place in genetic knowledge, as well as in the genetic technology. Some proposals suggested that persons of great intellectual or artistic achievement or of great virtue be cloned.
6. Genetically modified (GM) foods possess specific traits such as tolerance to **herbicides or resistance to insects or viruses**.
7. Adding a gene from **insect-killing bacteria** to cotton so that insects, eat cotton will be poisoned!
8. Genetic engineering also includes insertion of human genes into sheep so that they secrete alpha-1 antitrypsin in their milk - a useful substance in treating some cases of lung disease.
9. By inserting a gene for **human insulin** into an *E. coli* bacterium, the *E. coli* will make lots of insulin, which scientists and doctors can collect and use.
10. Scientists have found a gene called **p-53** which normally keeps cells under control and works best to suppress cancer cells.

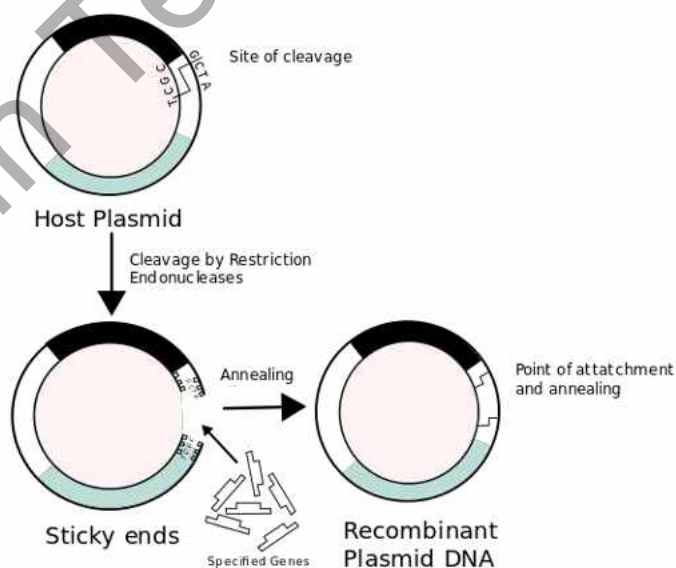


Fig. 8.7 Plasmid with insulin gene

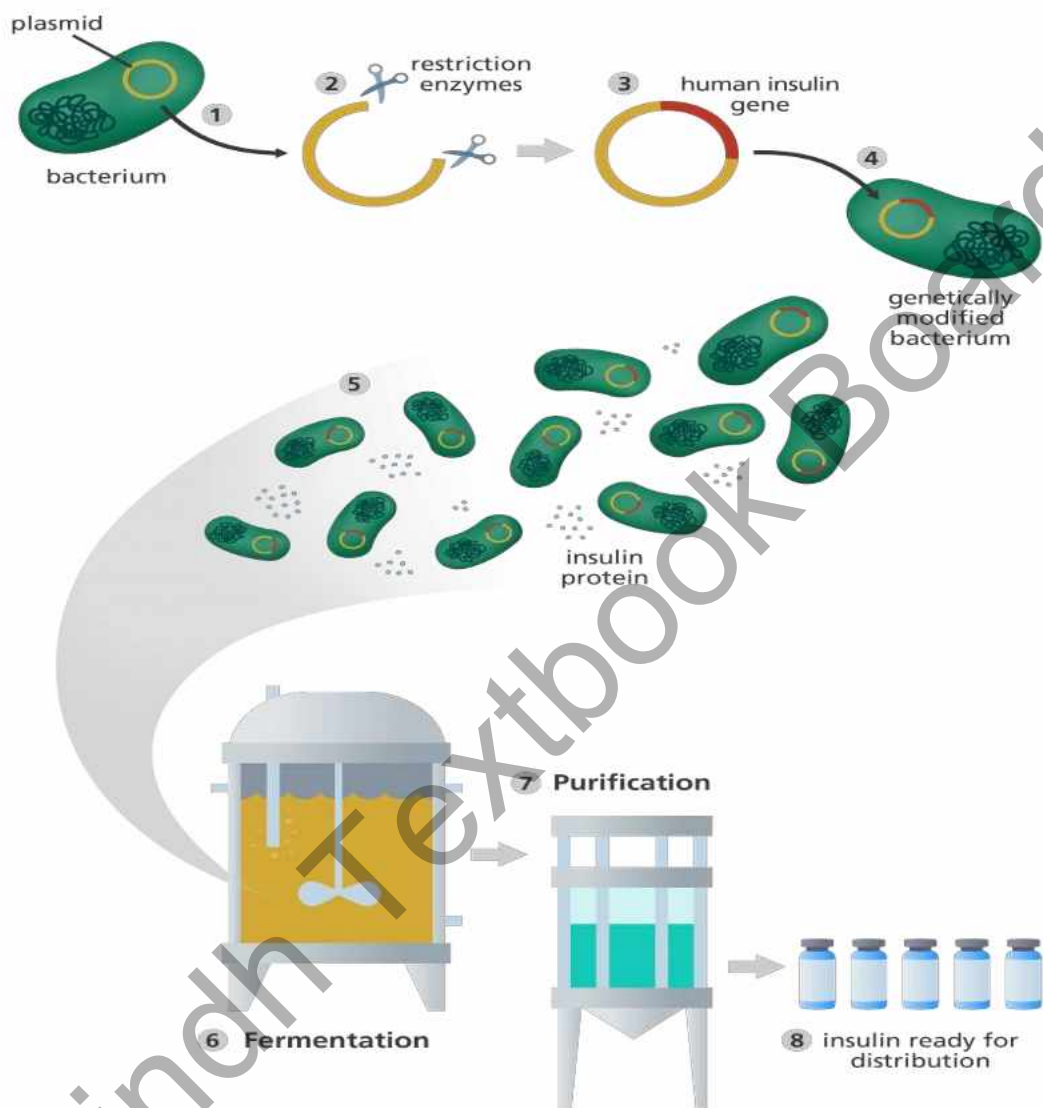
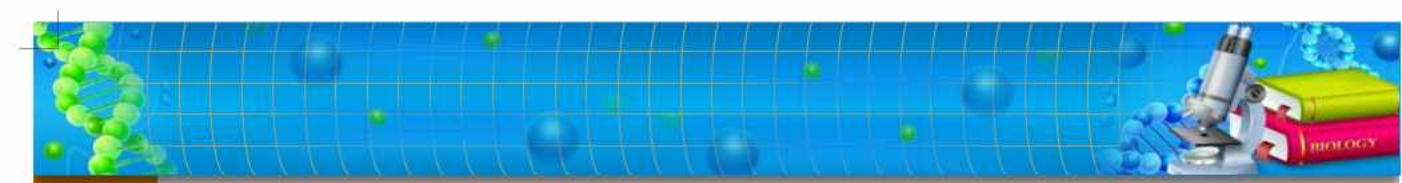


Fig. 8.8 Production of insulin through *Escherichia coli* (*E. coli*)

8.4 SINGLE CELL PROTEIN AND ITS USES

During World War II, when there were shortages in proteins and vitamins in the diet, the Germans produced yeasts and a mould (*Geotrichum candidum*) in some quantity for food; this led to the idea to



produce edible proteins on a large scale by means of microorganisms during the 1970s. Several industrial giants investigated the possibility of converting cheap organic materials into protein using microorganism.

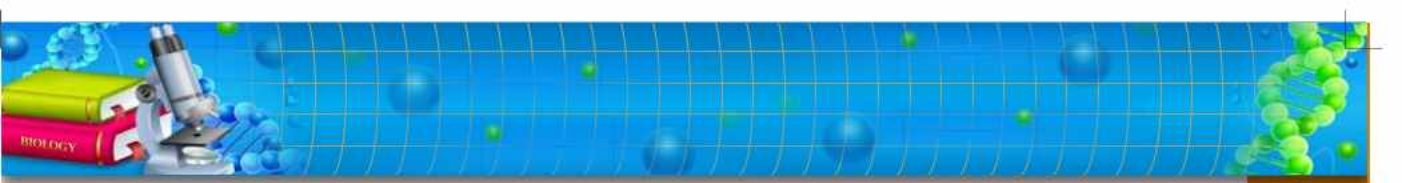
Single-Cell Protein (SCP) is a term coined at Massachusetts Institute of Technology by Prof. C.L. Wilson (1966) and represents microbial cells (primary) grown in mass culture and harvested for use as protein sources in foods or animal feeds.

Single Cell Protein (SCP) is not a pure protein but refers to whole cells of bacteria, yeasts, filamentous fungi or algae contains carbohydrates, lipids, nucleic acids, mineral salts, and vitamins. Carbon substrates: major substrates used in commercial SCP production are alcohols, *n*-alkanes, molasses, sulphite, liquor and whey.

Single cell protein is a protein extracted from cultured algae, yeasts, or bacteria and used as a substitute for protein-rich foods, especially in animal feeds or as dietary supplements.

Many types of animal feeds contain single cell proteins. 60-80% dry cell weight; contains nucleic acids, fats, CHO, vitamins, and minerals rich in essential amino acids (Lys-Met).

Microbes can be used to ferment some of the vast amounts of waste materials, such as straws; wood and wood processing wastes; food, cannery and food processing wastes; and residues from alcohol production or from human and animal excreta.



SUMMARY

- ◆ Biotechnology can be defined as “the use of living organisms, cells or cellular components for the production of compounds or precise genetic improvement of living things for the benefit of man”.
- ◆ The related activities in genetic engineering have two basic objectives:
 - ◆ To learn more about the ways nature works and
 - ◆ To make use of this knowledge for practical purposes.
- ◆ Biotechnology is integrated use of biochemistry, molecular biology, microbiology to achieve technological application of the capabilities of biological agents.
- ◆ An important way of making ATP without oxygen is called **fermentation**.
- ◆ There are two major types of anaerobic fermentation: lactic acid fermentation and ethanol fermentation.
- ◆ Fermentation is carried out by the bacteria *Streptococcus* and *Lactobacillus* species for souring milk into yogurt and production of various types of cheese.
- ◆ Yeasts in bread dough use alcoholic fermentation and produce carbon dioxide gas.
- ◆ The gas forms bubbles in the dough, which cause the dough to expand.
- ◆ This process of fermentation is a natural preservative, which means that fermented foods can last a long time.
- ◆ The fermentor is to provide a stable and optimal environment for microorganisms to reproduce and interact with substrate for required product.
- ◆ To manipulate cells and DNA, scientists use tools
 - ◆ Restriction enzymes: These naturally occurring enzymes are used to cut up DNA use like scissors to snip specific genes from DNA.
 - ◆ DNA ligase: This enzyme is used in nature to repair broken DNA.
 - ◆ Plasmids: These are circular units of DNA. They can be engineered to carry genes of interest.

- ◆ Single-Cell Protein (SCP) is a term represents microbial cells (primary) grown in mass culture and harvested for use as protein sources in foods or animal feeds.
- ◆ Single Cell Protein (SCP) is not a pure protein but refers to whole cells of bacteria, yeasts, filamentous fungi or algae.
- ◆ Many types of animal feeds contain single cell proteins. 60-80% dry cell weight; contains nucleic acids, fats, CHO, vitamins, and minerals rich in essential amino acids (Lys-Met).



A. MULTIPLE CHOICE QUESTIONS

Choose the correct answer:

- The artificial manipulation, medication and recombination of DNA
 - Genetic engineering
 - Biotechnology
 - Molecular biology
 - Genetic
- The earlier biotechnologist were
 - Biologist
 - Agriculturist
 - Genetist
 - Farmers
- The complete graph of human genome was studied by
 - PCR
 - HGP
 - Medicine
 - soma-clonal
- Alcohol and antibiotics on large scale production by organism is an area of
 - Environmental biotechnology
 - Fermentation
 - Biotechnology in industry
 - Medicinal biotechnology
- Most of the living things use O_2 to produce
 - ATP
 - Alcohol
 - Organic acid
 - Ecological pyramid
- In acidic formulation lactic acid produced from
 - Pyruvic acid
 - Acetic acid
 - Citric acid
 - Glyceric acid



- vii)** The bread dough rises during alcoholic fermentation is due to
(a) Methyl alcohol (b) CO_2
(c) Ethyl alcohol (d) H_2O
- viii)** The container use to grow bacteria on large scale are called
(a) Chillers (b) Sterilizers
(c) Fermenters (d) Ferments
- ix)** Naturally occurring enzyme used as a defense chemical by bacteria
(a) Defense protein (b) Restriction enzyme
(c) Hydrolytic enzyme (d) ligase enzyme
- x)** Extra circular DNA which use as vector of gene is
(a) Genome (b) Plasmid
(c) Pilli (d) Ligase

B. ANSWER THE FOLLOWING QUESTIONS:

- i)** What kind of enzymes allows scientists to cut and paste pieces of DNA together to form recombinant DNA?
- ii)** Explain how making human tissue plasminogen activator (t-PA) in Chinese hamster ovary (CHO) cells is an example of genetic engineering.
- iii)** How do organisms obtain energy? How does fermentation work?
- iv)** What types of microorganisms cause fermentation to occur?
- v)** What food and non-food products are created by fermentation?
- vi)** What are some advantages and disadvantages of fermentation in food processing?
- vii)** What factors can affect the fermentation process?
- viii)** Define biotechnology?
- ix)** What is the classical biotechnology?
- x)** What is your justification in considering biotechnology an old technology?
- xi)** Describe the contribution of Louis Pasteur toward development of classical biotechnology.
- xii)** What are the different types of fermentation?
- xiii)** What is lactic acid bacillus? Explain its role in the formation of curd.
- xiv)** Name some of the fermented food products of Pakistan.
- xv)** Name four recombinant DNA products available on the market.