Unit 17

BIOTECHNOLOGY

Students Learning Outcomes

The students will be able to:

- Define biotechnology and explain its importance.
- Relate biotechnology with genetic engineering and fermentation.
- Define fermentation.
- Explain the method of fermentation by yeast and bacteria.
- Identify different fermentation products and their importance in daily life i.e. yogurt making, bread making, making of cheese and production of alcohol.
- Explain the use of fermenter in large-scale production of microorganisms and their products.
- Describe the procedure of using fermenters.
- Describe the advantages/profitability of using fermenters in preparing medical products.
- Define genetic engineering and describe its objectives.
- Describe how a gene is transplanted.
- Describe major achievements of genetic engineering with reference to improvement in agricultural crops (herbicide resistance, virus resistance and insect resistance).
- Describe major achievements of genetic engineering in curing animal diseases (foot-and mouth disease, Coccidiosis, Trypanosomiasis) and in animal propagation (animal cloning).
- Describe the application of genetic engineering in the production of human insulin and growth hormones.
- Describe single-cell protein and its importance.
- Describe the significance of single-cell protein in animal feed.
- State the significance of single-cell protein in human food.

Introduction

Biotechnology has been flourishing since prehistoric times. It all started when, the first human beings realised that they could plant their own crops and breed their own animals. These people were applying biotechnology. Biotechnology can be seen in action when milk is converted into cheese and yogurt. Or when vinegar is prepared by fermenting solutions of sugar. In the same way, long ago bakers learned and used biotechnology to make soft, spongy bread.

Tidbit

What do you think of when you hear the word "biotechnology"? May be things you have seen in the news, such as Dolly the cloned sheep or the controversy on genetically modified organisms, or gene therapy.
Research the topic and discuss your findings and opinions with your classmates.

More specifically, the term "biotechnology" refers to the use of living organisms or their products for the welfare of human beings. It has various applications in different fields such as Therapeutics, Diagnostics, Processed Food, Waste Management, Energy Production, Genetically Modified Crops etc. Many life saving drugs, higher yields of crops, stronger and better breeds of animals and livestock, and a variety of processed foods, are all a result of biotechnological advancements.

17.1 Importance of Biotechnology

In the 19th century after the discovery of Mendel's laws of heredity, scientists began to explore more about genes and characters. It resulted in new advancements in biotechnology. With the discovery of the structure and function of DNA in 1953, modern biotechnology emerged. The techniques being used in modern biotechnology include fermentation, genetic engineering, and tissue culture.
Biotechnology has its positive impacts almost in every field of life. It finds out the best possible technological measures that prove beneficial for the humankind without disturbing nature. Biotechnologists use microorganisms in various ways for obtaining benefits related to food production, health and the environment.

17.2 Fermentation

The term "fermentation" is used for two concepts i.e. (i) as a type of cellular respiration, and (ii) as a technique in modern biotechnology.

17.2.1 Fermentation as a type of cellular respiration

Anaerobic respiration is also called fermentation. It happens in the absence of oxygen. In this process, glucose is oxidized incompletely and a little amount of energy is released. It occurs in different organisms and is of two types.

a- Alcoholic fermentation by yeast cells

Do You Know?

The science of fermentation is known as zymology.

Alcoholic fermentation is a two-step process. In this fermentation, the glucose molecule is first broken down into two molecules of pyruvic acid. This step is called glycolysis. In the next step, pyruvic acid is further broken down into carbon dioxide and ethanol. This fermentation occurs in yeast *(Saccharomyces cerevisiae)*. Scientists use this process to produce alcohol during the making of alcoholic products, ethanol fuel and bread.

b- Lactic acid fermentation by bacteria

For Your Information

Lactic acid fermentation also occurs in our skeletal muscles, during exercise or other hard jobs.

The first step in this fermentation is similar to alcoholic fermentation. The glucose molecule is broken down into two molecules of pyruvic acid. In the second step, hydrogen is added to pyruvic acid (reduction), which changes it to lactic acid. This fermentation occurs in bacteria e.g. *Lactobacillus* and *Streptococcus*. Lactic acid fermentation done by bacteria is used to turn milk into yogurt.

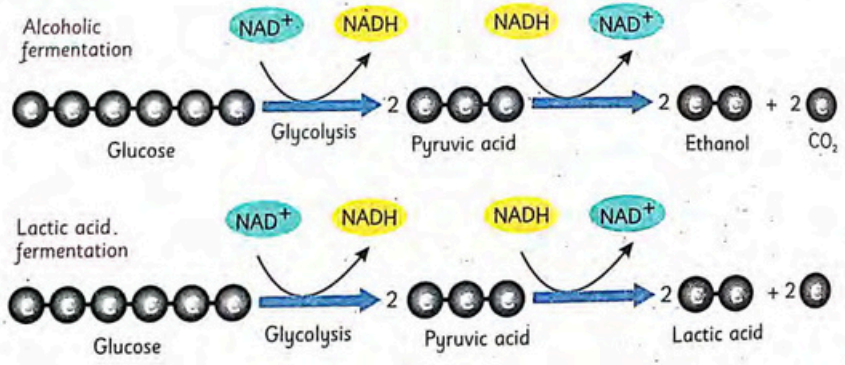

Fig.17.2 Types of fermentation

Fig: 17.3 a. *Lactobacillus bulgaricus*

b. *Streptococcus thermophiles*

Yogurt is produced from whole or skimmed milk. It is inoculated with a starter culture, which usually contains *lactobacillus bulgaricus* and *streptococcus thermophiles*. These bacteria ferment the lactose in the milk to lactic acid which acts on the milk protein to give yogurt its texture and flavour. In this way milk is converted to curd and forms yogurt.

Fermentation Products

The following are the most consumed fermentation products worldwide:

1. **Alcohol** is made as a result of the fermentation of a natural source of sugar with a catalyst, which is usually yeast. During fermentation, the carbohydrates (starch and sugars) are the main source which are converted into carbon dioxide and ethyl alcohol. Alcohol is widely used in medicines.

For Your Information

Cheese, vinegar, pickles, chocolate, coffee, linen methane, and liquor are just a few of the items produced through fermentation. Chemical companies are researching new ways to create vitamins, medicines, organic chemicals (acetic acid, ethylene glycol, amino acids) through biological processes.

2. **Yogurt** is lactic acid containing milk, fermented by bacteria. Lactose present in milk is converted to lactic acid during fermentation.
3. **Bread:** Wheat dough is fermented to make bread. Fermentation plays an important role in softening the bread and is also responsible for its aroma. This fermentation is mainly carried out by yeast and sometimes by bacteria.
4. **Cheese** is formed when bacteria converts milk lactose into lactic acid, due to which milk proteins are also coagulated. In this way, milk changes into cheese.
5. **Pickles** are made as a result of fermentation of fruits and vegetables. The by-products of fermentation turn fruits and vegetables into pickles.
6. **Soy sauce** is made by fermentation of soybeans by fungi.
7. **Chocolate is produced** when cacao beans undergo the process of fermentation.
8. **Many chemical products** are also produced through the fermentation process e.g. formic acid, glycerol and acrylic acid etc.

17.2.2 Fermentation in Modern Biotechnology

In modern biotechnology, the term **"fermentation"** means the large-scale production of any product by the massive culture (population) of microorganisms. Such microorganisms are grown in a large container called a **fermenter**.

Fermenter

This is a large container in which populations of microorganisms are grown to produce large quantities of products. Fermenters (also called bioreactors) provide suitable environment (temperature and pH etc.) for quick metabolism in microorganisms. It provides a specialized medium in which all essential nutrients of microorganisms are present. When raw material is

added to the medium, microorganisms carry out metabolic reactions to make products. Fermenters are used for the manufacture of many products e.g. medicines (antibiotics, vaccines, interferon, hormones etc.), enzymes (cellulose, protease, lipase etc.), and other products (ethanol, lactic acid etc.).

Procedure to use Fermenter

Discontinuous (Batch) fermentation

In this fermentation, the whole process is divided into batches. The tank of the fermenter is filled with the raw materials and nutrients. The population of microorganisms is added which performs fermentation and makes the products. During the reactions occurring in the fermenter, no microorganisms or nutrients are added or removed from the culture. The contents of the fermenter are taken out for further processing after the required time has elapsed/passed. The fermenter is cleaned and the process is repeated.

For Your Information

In a fermenter, temperature, pH and oxygen meters are linked to a computer which monitors the conditions inside the vessel. Paddle stirrers ensure that the microorganisms, and raw material are well mixed.

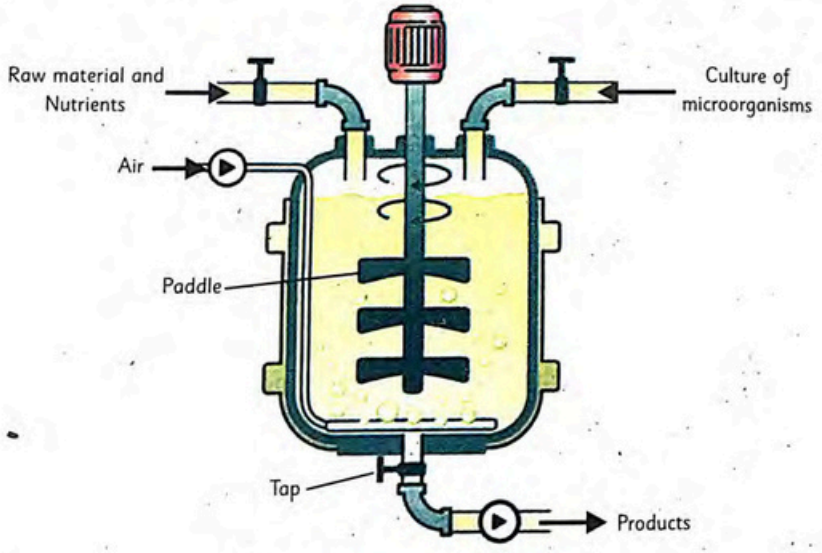

Fig.17.4 Discontinuous (Batch) fermentation

Continuous fermentation

In continuous fermentation, the exponential growth of microbes is maintained in the fermenter for prolonged periods of time. While the population of microorganisms is added to the fermenter once, raw material and nutrients are added continuously at regular intervals.

Thus unlike batch fermentation (above) the, continuous fermentation process never stops in between and it continues to run for a longer period. The fermentation products are also taken out continuously.

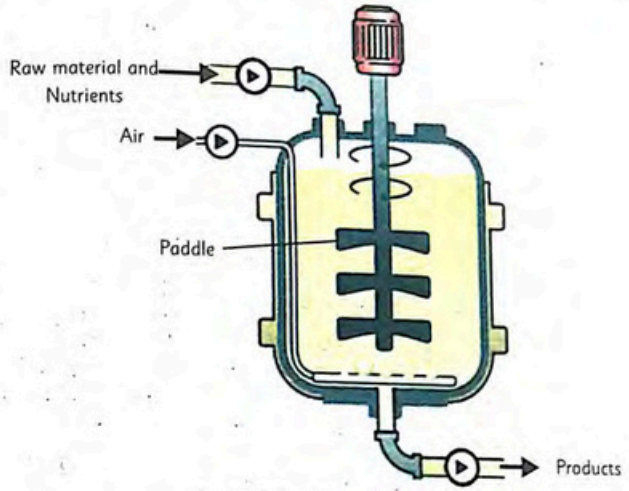

Fig.17.5 Continuous fermentation

Advantages of a Fermenter

1. Fermenters have and auto control system so environmental changes cannot harm microbial growth.
2. Separation of products is easy and safe.
3. Inoculation of microbes is easy.
4. Wastage of materials in handling is minimised by fermenters.
5. They can be installed with ease and take up very little space.
6. Single fermenters can be used for production of a wide range of products.

Fermenters enable the production of medical products, such as penicillin, Insulin Erythromycin, Streptomycin, Griseofulvin (Antifungal antibiotic) and hundreds of othe products from microbes.

Activities

1. Investigate the role of yeast in the fermentation of flour. Use flour, water, salt and yeast to perform your experiment.
2. Investigate the role of bacteria in the fermentation of milk.

17.3 Genetic Engineering and its Uses

Genetic engineering is considered as the area of biotechnology that induces DNA alterations, artificial manipulation and transfer of genetic material from one organism to another. Genetic engineers have the ability to find specific genes, to cut them away from chromosomes and insert them into the chromosomes of other organisms.

For Your Information

The bacterium that is found in the bowels of humans namely *Escherichia coli* is drawing the attention of genetic engineers. This bacterium has become one of the most powerful tools known in genetic manipulation.

Recombinant DNA technology is a method used in genetic engineering. This technology involves the selection of DNA of one organism (donor) and its introduction to combine with the DNA of another organism (recipient). As a result, the recipient organism acquires the genetic abilities of the donor, and is called Genetically Modified Organism (GMO). The DNA that is a combination of genes from two different sources is called recombinant DNA.

17.3.1 Objectives of Genetic Engineering

The main objectives of genetic engineering are;

1. Identify and isolate genes that cause disease, with a view to repair or eradicate them so that their harmful effects are negated.
2. Find remedies and therapies to treat non-genetic diseases.
3. Develop more sophisticated and effective medicine through genetic engineering for example bio-engineered insulin and human growth hormone (somatotropin).
4. Genetically Modified Organisms (GMOs). GMOs are produced to enhance the food production. Genetically modified crops and cattle produce more food in lesser time. They
5. are made disease resistant so that losses due to pathogens can be reduced.
6. Plants are genetically modified to get substances which they do not produce naturally such as antibiotics, certain proteins, hormones, etc.

Fig.17.6 Genetically modified orange

17.3.2 Basic Techniques in Genetic Engineering

Bacterial cells have different kinds of enzymes. Some of these can cut DNA into fragments and others can join such fragments. For example, **restriction endonucleases** are involved in cutting DNA at specific sites. Hence they are called molecular scissors. The enzyme **DNA ligase** acts like a paste molecule to join DNA fragments. Thus the restriction endonuclease and the DNA ligase are the basic tools required for genetic engineering.

Basic techniques of genetic engineering are as follows.

1. Isolation of gene of interest: The gene (DNA) of donor organism or **gene of interest** is identified. It is then isolated from the chromosome of the donor by using restriction endonucleases.

2. Making Recombinant DNA: The gene of interest is attached to a suitable **vector** (cloning vehicle), to carry the gene to the host organism. The most common vector used in genetic engineering is plasmid. It is the extra chromosomal circular DNA of *Escherichia coli*. Bacteriophages. (viruses that can enter bacteria) are also used as vectors. The DNA of the vector is cut into fragments by the restriction endonucleases. Using the enzyme DNA ligase, the DNA fragments of the donor and vector are joined together. As a result recombinant DNA is obtained.

3. Gene Cloning: The recombinant DNA is introduced into the host bacterial cell. The host cell is treated with enzymes so that their cell wall becomes permeable for the recombinant DNA. The host bacterial cell continues to multiply with the foreign DNA or gene of interest. After a short time, this results in a colony of bacteria having the recombinant DNA. Each colony is grown separately to get a number of colonies having identical copies of recombinant DNA. This is called **gene cloning**.

As a result of the transfer of the donor's gene into the bacteria, the bacteria are genetically modified. They start preparing the product by using the donor's gene.

17.3.3 Major Achievements of Genetic Engineering

Benefits of genetic engineering are experienced in many fields especially in agriculture, in the production of valuable proteins and vaccine production.

1. Achievements in the field of Agriculture

With respect to agriculture, modern biotechnology is seen as the second phase of the green revolution. Many genetically modified organisms (GMOs) have been produced. Some useful benefits of genetically modified plants are: improved nutritional quality; better nitrogen fixation; virus resistance; herbicidal resistance; insect resistance; disease resistance; and enhanced efficiency to use minerals. Details on some of these are provided below.

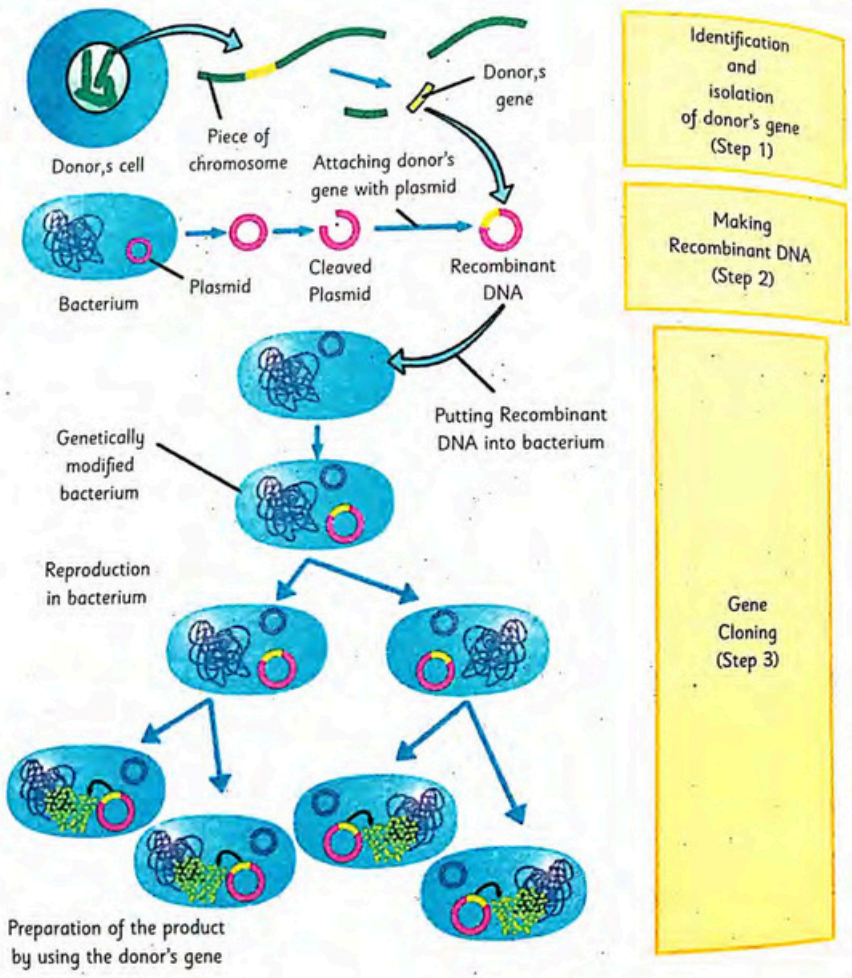

Fig.17.6 Transfer of a gene to an organism and its cloning

Resistance against Herbicides

Herbicide resistant genes have been incorporated into many crops of soybean, corn, and cotton. Therefore, herbicides can be used around these plants to kill weeds without harming the crops.

NOT FOR SALE

Resistance against Viruses

Virus resistant traits have been introduced into many crops, including squashes, tomatoes, potatoes, tobacco etc. These plants are not affected by viruses and survive viral attacks.

Resistance against Insects

Widespread use of insecticides, fungicides and pesticides for crop protection has damaging effects on the environment. It is important to improve the control of pests by genetic means. Genetic engineers have introduced pest resistant genes into several crops, including tomato and cotton. Such modified plants show resistance against pests.

2. Achievements in Curing Animal Diseases

Human Insulin was the first genetic engineering product. In 1982, the human gene for insulin was inserted into a bacterium. Since then, the modified bacteria are providing large amounts of human insulin.

Vaccine against hepatitis B virus has been produced from yeast through genetic modification.

Human growth hormone was produced in genetically modified bacteria. It is used to treat dwarfism.

Interferon (anti-virus protein) is made in genetically modified bacteria.

Vaccine against Foot and mouth Diseases are being developed for foot-and-mouth disease, a highly contagious viral disease that infects cattle, sheep, and other animals.

Vaccine against Coccidiosis (a parasitic disease of the intestinal tract of animals). This vaccine kills protozoan that causes coccidiosis.

Treatment of Trypanosomiasis (sleeping sickness) may be possible through use of biotechnological techniques. Trypanosomiasis is a parasitic infection transmitted by a fly in humans and other animals. Genetic engineers are doing research to develop such proteins which can kill the parasite of this disease.

Gene Therapy enables the treatment of genetic disorders. Through this technique genetic engineers treat genetic disorders by introducing a gene into the patient's cells. It is being used to treat genetic disorders of the blood (e.g. thalassaemia).

Animal Cloning has become possible due to biotechnology. Genetic engineers have successfully cloned mice, sheep, cows and other mammals. The basic idea behind cloning of animals is to transfer the readymade DNA into the egg and create an identical organism. In this method, the genetic information from a cell of the animal to be cloned, is transferred to an egg whose nucleus has been removed. Then the egg with the new genetic material is stimulated to divide and make a multicellular embryo. When this embryo is sufficiently developed, it is implanted into the uterus of a female host, who acts as its surrogate mother. The rest of the development of the embryo happens just like a normal organism. When the development is complete, the new animal is the exact clone of the parent whose nucleus was used. Dolly the sheep was cloned using this process by the Roslin Institute in Scotland in 1977.

Science, Technology and Society

Human beings rely on genetic engineering for many benefits such as medicines, improved varieties of organisms with novel products, treatment, solutions to environmental problems etc. However, these benefits are tagged with peril. In humans, when the foreign gene is inserted, it may interfere with the normal genes and therefore, affect the normal functioning. It may develop antibiotic resistance in human beings as all GM plants carry antibiotic resistance genes. The genetically modified organism may affect the normal balance in the ecosystem. Critics of rDNA fear that disease-producing organisms used in some rDNA experiments might develop extremely infectious forms that could cause worldwide epidemics.

17 .4 Single Cell Protein

For Your Information

The term 'single cell protein' was coined in 1966. Previously, the dried cells of microorganisms used as food or feed for animals and they were collectively known as Microbial proteins. This term was replaced by a new term 'single cell protein'

For centuries microorganisms have been widely used for preparation of a variety of fermented foods, for example cheese, butter etc. Some microorganisms have long been used as human food, e.g. the blue green alga spirulina, and the fungi like yeasts. More recently, efforts have been made to produce microbial biomass using low-cost substrates. These are used as a supplementary food for humans or as feed for animals. The isolated protein or the total cell material from microorganisms like bacteria, yeasts, filamentous fungi and algae used as food or feed is called single cell protein (SCP).

In view of the insufficient world food supply and the high protein content of microbial cells, the use of biomass produced in the fermenter or bio-reactor would be an ideal supplement for conventional food. Some of the major uses of SCP are as follows:

1. It is a rich source of protein (60 to 72 %), vitamins, amino acids, minerals and crude fibres.
2. It is a popular healthy food.
3. It provides valuable protein-rich supplement in human diet.
4. It lowers blood sugar level of diabetics and prevents the accumulation of cholesterol in human body.

Tidbit

Nowadays, Spirulina tablets are prescribed as enriched vitamin for most people.

In many countries, however people hesitate to use SCP as a major food source because of the following.

1. The high nucleic acid content (4 to 6 % in algae, 6 to 10 % in yeast of SCP) can cause health problems like uric acid formation and kidney stones.
2. Toxic or carcinogenic (cancer causing) substances absorbed from microbial growth substrate may be present.
3. The slow digestion of microbial cell in the digestive tract may cause vomiting, indigestion and allergic reaction.

High cost of production will also be a deciding factor in determining the ultimate place of SCP in the human or animal diet.

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Key Points

- The term "biotechnology" refers to the use of living organisms or their products for the welfare of human being.
- Genetic engineering is considered as an area of biotechnology that induces DNA alterations, artificial manipulation and transfer of genetic material from one organism to another.
- Alcoholic fermentation is the conversion of sugar into carbon dioxide gas (CO_2) and ethyl alcohol.
- Alcohol is the fermentation product of yeast or bacteria.
- Yogurt is lactic acid containing fermented dairy product obtained from bacterial fermentation of milk.
- An apparatus that maintains optimal conditions for the growth of microorganisms, used in large-scale fermentation and in the commercial production of antibiotics and hormones, is called a fermenter.
- Recombinant DNA technology is a technique where the selected DNA of one organism (donor) is introduced into the another organisms (recipient).
- The main objective in genetic engineering is to identify and isolate genes which cause disease, with a view to repair or eradicate them so that their harmful effects are negated.
- Extra chromosomal circular DNA found in the cytoplasm of *Escherichia coli* is called plasmid which is regarded as the most suitable vector.
- Organisms whose genes have been altered by manipulation are called genetically modified organism (GMO).

Exercise

A. Select the correct answer.

1. The enzymes which are used to cut the DNA in specified sequences is:
   a. Ligase b. Protease c. DNAase d. Endonuclease
2. The DNA molecule in which the gene of interest is inserted for carrying to the target cell is:
   a. Transforming DNA b. Carrier DNA c. Phage DNA d. Vector DNA
3. Endonucleases used in genetic engineering are naturally present in:
   a. Bacteriophages b. Bacterial cells c. Plasmids d. Blue green algae
4. Fermenter is a machine which is basically used for:
   a. Increasing fermentation b. Production of enzymes used in fermentation
   c. Production of microorganisms d. Making the conditions optimum
5. *Lactobacillus bulgaricus* is a type of bacteria in the production of:
   a. Alcohol b. Cheese c. Yogurt d. All of the above
6. In a technique for genetic engineering, rDNA stands for:
   a. Genetic makeup b. Replicative DNA c. Recombinant DNA d. Retro DNA
7. Interferons are chemical which are used to:
   a. Kill viruses b. Improve immunity
   c. Work as vaccine d. All of the above
8. Which is a molecule containing DNA from two different organisms?
   a. Vector DNA b. DNA clone c. Plasmid DNA d. Recombinant DNA
9. What term is used for inserting a healthy gene into a person who has a defective gene?
   a. Cloning b. Gene therapy
   c. Vaccination d. Fermentation
10. Which of these can be used as a vector for inserting a gene into bacteria?
    a. Plasmid b. Algae c. Yeast d. E. coli

B. Write short answers to the following questions.

1. Write about any four products developed through fermentation.
2. Name any four microorganisms used in biotechnology. Also name the process in which they are used.
3. Why are vectors used in genetic engineering?

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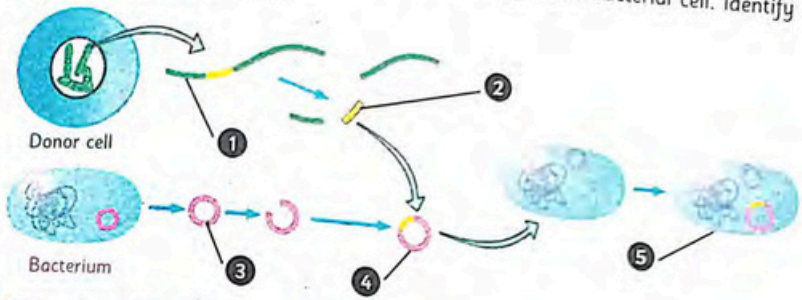
4. Write any five advantages of biotechnology in the field of agriculture.
5. How is yogurt produced through the use of biotechnology?
6. The following diagram shows how a gene is transferred to bacterial cell. Identify the structures labelled as 1 to 5.



C. Write detailed answers to the following questions:

1. Write a comprehensive note on the procedure of recombinant DNA technology.
2. How are single cell proteins produced and what is their significance?
3. What is a fermenter and how does it work?
4. Describe the advantages of using fermenters for getting products from genetically modified organisms.
5. Describe how biotechnology is helping humankind in the fields of food and health.

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 of some social and ethical issues related to genetic engineering.
3. Describe the ways in which society benefits from the knowledge of genetic engineering.
4. Interpret the data collected from the internet on viral resistant, insect resistant and high yielding varieties of agricultural crops in Pakistan.