UNIT-4 BENEFICIAL ROLE OF MICRO-ORGANISMS

4.1 Fermentation

In the biochemical sense, the term fermentation refers to **a metabolic process in which organic compounds (particularly carbohydrates) are broken down to release energy without the involvement of a terminal electron acceptor such as oxygen**. Partial oxidation of the substrate occurs so that only a relatively small amount of ATP energy is released compared with the energy generated if a terminal acceptor is involved. Partial oxidation of a carbohydrate can give rise to a variety of organic compounds. The compounds produced by micro-organisms vary from organism to organism and are produced via different metabolic pathways.

The term fermentation can also be applied to any industrial process that produces a material that is useful to humans and if the process depends on the activity of one or more micro-organisms. These processes, known as **industrial fermentations**, are usually carried out on a large scale and in vessels in which the organism are normally grown in liquid media. Some industrial fermentations are fermentation's in the biochemical sense but the majority of microorganisms are aerobic and use oxygen and metabolize carbohydrates completely.

A vast range of material are produced by industrial fermentations. These include:

- Organic chemicals used as fuels, food additives, antibiotics and enzymes for use in the food and other industries. Vinegar is an example of a food additive produced by an industrial fermentation.

- Organisms produced on a large scale for the extraction of protein (single cell protein) that can be used as part of the human diet. Quorn is an example of a single cell protein, produced from the fungus *Fusarium graminearum*. The mycoprotein, purified from the fungus, is currently available for use as a food and is incorporated into a range of dishes that appear on supermarket shelves. Meatless dishes with a high protein content made from Quorn are particularly appealing to vegetarians.

- Yeast cells produced for use in industries such as the baking industry, which relies on the mass production of large amounts of baker's yeast.

- Foods produced on a large scale as a result of the activities of micro-organism, e.g. cheese, yogurt and bread.

- Production of alcoholic beverages.

- Cells extracts used as food additives, e.g. yeast extracts from yeast cells produced as a byproduct of the brewing industry.

- Mushroom production is another process that can be considered an industrial fermenatation.

Industrial fermentation are now often considered under the heading of biotechnology, i.e. technology that uses living organisms and their products in the manufacturing and service industries

4.2 Fermented food and Their importance

Fermented foods are those foods produced by the modification of a raw material of either animal or vegetable origin by the activities of micro-organisms. Bacteria, yeast and moulds can be used to produce a diverse range of products that differ in flavour, texture and stability from the original raw material. The production of many fermented foods involves organisms that are biochemically fermentative. Lactic acid bacteria that ferment carbohydrates to

produce lactic acid are particularly important, but yeasts also play a major role in some food fermentations, fermenting carbohydrate to produce ethanol and other organic chemicals. Mould that do not ferment carbohydrate, also play an essential part in some food fermentations, for example, the production of blue cheeses and soy sauce.

Fermented foods are an extremely valuable addition to the human diet for a whole variety of reasons:

- **Increase in variety :** Fermented foods increase the variety of foods that are available, adding to our diet a group of highly nutritious products with unique characteristics. There are, for example, about 1000 different types of cheeses.

- **Use of ingredients:** Fermented foods form important ingredients for wide variety of dishes and are often used to impart special flavours, e.g. pepperoni in pizzas, yoghurt in curries, cheeses in a whole range of dishes, including soups, and soy sauce in stir-fry dishes.

- **Improvement in nutritional quality:** The fermentation process may improve the nutritional quality of a raw material. Here are some examples:

(a) Tempeh fermentation raises the vitamin B12 content of the original soyabean form 0.15□g / g to 5.0 □g/ g.

(b) Tape fermentation doubles the protein content of cassava and increases the level of essential amino acids.

(c) The presence of yeasts in a fermented food will increase the vitamin B content.

(d) Antinutritional factors such as phyates, glucosinolates and lectins may be removed by the fermentation process.

(e) Fermentation may produce an increase in the bio-availability of minerals.

These improvements in the nutritional value of raw material will have little effect in the balanced diets of Western populations. However, of population that subsist on diets consisting largely of polished rice, maize or other starches, such as in Africa and Asia, the contribution that fermented foods make to the intake of B group vitamins and proteins is highly significant.

- **Preservation :** Fermentation often preserves a raw material , improving safety with regard to food- borne pathogens and increasing shelf-life; compare the shelf-life of raw milk (only a few days) with the shelf-life of yoghurt (several weeks).

- **Health benefits:** Some fermented foods are said to have definite health benefits, although the scientific evidence for this is limited. Reports suggest that fermented milk products such as yoghurt can reduce serum cholesterol levels and help avoid cancers, particularly those associated with the colon. 'Bio' yoghurts (AB and ABT yoghurts) are said to have a restorative effect on a normal microflora, assisting recovery of a normal balanced flora after oral antibiotic therapy.

- **Improve digestibility:** Some fermented foods are more easily digested than the original raw material. People who cannot digest lactose properly (show lactose intolerance) can often consume some types of fermented dairy products (particularly yoghurts) without harmful effects. Lactose intolerance is due to the absence of the enzyme galactosidase in digestive juices, which converts lactose to glucose and galactose. Ingestion of dairy products leaves unabsorbed lactose in the gut, which is fermented by the normal gut flora giving flatulence, abdominal pain and diarrhoea. The fermentation of milk converts the harmful lactose to the more easily digested lactate, and the galactosidase in live starter culture organisms appears to assist in the

digestion of any residual lactose. Legumes, e.g. soybean, contain oligosaccharides such as stachyose which are fermented in the gut to give gas and the associated socially embarrassing flatus. The oligosaccharides are broken down to readily digestible monosaccharides and disacccharides during mould fermentation of legumes, thus removing the problem.

- **Detoxification of raw materials:** The fermentation process may remove toxic chemicals present in the raw material. Cassava fermentation, for example, removes a cyanogenic (cyanide producing) glycoside; cassava is toxic if eaten raw.

4.3 TYPES OF FOOD FERMENTATION

A number of different types of food fermentation can be recognized.

4.3.1 Acid Food Fermentations

Acid food fermentations include:

- acid fermented dairy products, e.g. cheese, butter, yoghurt and kefir;
- acid fermented vegetable products, e.g. sauerkraut, olives and various pickles;
- acid fermented meat products, e.g. the semidry fermented meats such as cerevelat and the dry fermented meats such as salami and pepperoni;
- sourdough breads

The common feature of all these products is the use of lactic acid bacteria to carry out the basic fermentation process. Modern production usually involves the use of starter cultures. The one exception is the fermentation of sauerkraut for which the process depends on lactic acid bacteria that are natural inhabitants of the surface of cabbage leaves. Sometimes sugar is added to raw-material to allow the lactic acid to produce sufficient acid for a successful fermentation. This is the case with fermented meats in which the sugar content

of the raw material is very slow. Salt may be added to suppress the growth of the normal spoilage microflora and allow the lactic acid bacteria to dominate, e.g. sauerkraut, pickles and fermented meats. The raw materials may be pasteurized to eliminate pathogens and suppress natural contaminants that compete with the lactic acid bacteria used in the starter culture.

4.3.2 Yeast Fermentation

Yeasts are important in food fermentation because of their ability to produce carbon dioxide and ethanol. Carbon dioxide is the important metabolic product in the manufacture of leavened bread whereas ethanol is metabolized in the production of beers, wines and spirits. Carbon dioxide and yeast itself are important byproducts of beer manufactutre.

4.3.3 Solid State Fermentation

Solid state fermentation involve the use of a solid substrate into which the fermenting organism is inoculated. The organisms used are often moulds. Examples are the 'koji' process and the second stage of tempeh fermentation

4.4 ORIENTAL AND INDIGENOUS FERMENTED FOODS

A large number of fermented foods can be grouped under the heading of oriental and indigenous fermented foods. Fermented foods of this type are produced in Asia and Africa and are often associated with specific countries or areas. Most of the products are unknown in the West but frequently have major nutritional role in the diets of the local population. Lactic acid bacteria are involved in some of the fermentation but yeast and moulds are often the main organisms responsible. Many are sold state fermentations or involve fermentations of more than one type. Some of the products are manufactured on large scale but many are carried out on a cottage industry or household basis. The major groups of these products with example are given in Table 4.1.

Table 4.1 : Example of oriental and indigenous fermented foods.

Food Type	Example	Country of origin
Foods fermented by fungi followed by brine process	Soy sauce, miso	Southeast Asia
Meat- flavoured pastes produced by one-step bacterial fermentation	Natto	Japan, China, Thailand
Legume-based foods produced by bacterial fermentation or acid soak followed by fungal fermentation	Tempeh	Indonesia
Doughs fermented by lactic acid bacteria	Idli	India
Alcoholic foods produced by fermentation with yeasts or other fungi	Tape	Indonesia

4.5 ROLE OF LACTIC ACID BACTERIA

The manufacture of acid fermented foods is based on the ability of lactic acid bacteria (LAB) to ferment carbohydrates to produce lactic acid. Mesophilic lactic acid bacteria *Lactococcus, Leuconostoc, Lactobacillus* and *Pedicococcus* are used in fermentation that take place between 20and 30° C., e.g. most cheeses, fermented meats, sourdough breads and fermented vegetable production, whereas theremophilic *Lactobacillus spp* and *Streptococcus* spp are

used in fermentation carried out at higher temperature up to 45°C.e.g. in the manufacture of yoghurt.

4.6 METABOLISM OF LACTIC ACID BACTERIA

Two types of lactic acid bacteria are recognized in terms of the metabolic pathways used to break down carbohydrates to release energy, with the fermentation products formed as biproducts: the homolactic bacteria that produce mainly lactic acid and the heterolactic bacteria that produce a mixture of lactic acid, acetic acid, carbon dioxide and ethanol. The metabolic pathways involved are illustrated in Fig. 4.1.

Lactic acid bacteria are also important in the production of organic molecules that impart flavour to fermented foods in addition to the typical flavour of lactic acid. Although these substances are produced in very small quantities, they impart flavours to fermented diary products that are often responsible for their unique flavour characteristics, for example, *Lactococcus* var *diacetylactis* and *Leuconostoc* spp can covert citrate to diacetyl which is the main flavouring constituent of cottage cheese and butter. The characteristics flavour of yoghurt is associated with the production of acetaldehyde by *Lactobacillus delbreukii* subsp. *bulgraicus*. Bacteria used in starter cultures for the specific production of aromas and flavours are known as **aroma bacteria**. Some lactic acid bacteria produce extracellular polymers (slimes) that contribute to the texture of the final product also.

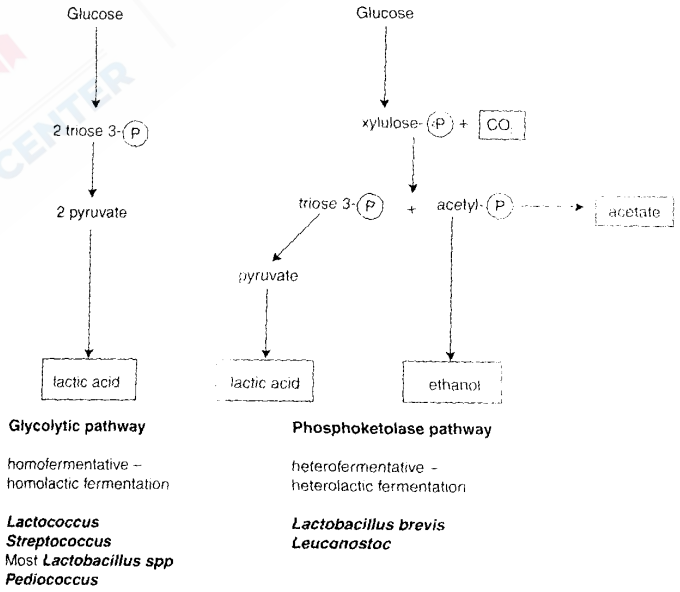

Fig. 4.1 : Metabolic pathways associated with lactic acid fermentations

4.7 EXAMPLES OF FOOD FERMENTATIONS

4.7.1 Fermented baked preparations

In baked products such as bread and bun, the yeast *Saccharomyces cerevisiae* which is popularly known as "baker's yeast", helps by raising the dough, giving it the texture and also adding flavours. The different ingredients further added contribute distinct different tastes to each of these products. The naan which is popular in India is made from maida (refined wheat flour) to which salt, yeast or curd is added. It is kneaded vigorously for 15 minutes adding vegetable oil for softening. It is allowed to ferment for 30 minutes to 1 hour. It is then baked rapidly on metal trays for 5 to 10 minutes. Intense heat causes centre of the dough to expand rapidly and create a central pouch. *Saccharomyces cerevisiae* is mainly responsible for leavening by carbondioxide production.

4.7.2 Fermented vegetable foods

Sauerkraut

Sauerkraut is fermented fresh cabbage product. It is popular in USA and Europe. The main organism involved in the fermentation of this pickle is lactic acid bacteria. *Leuconostoc mesenteroides* followed by *Lactobacillus plantarum*.

Cucumber pickle

Cucumber pickle is a fermentation product of fresh cucumbers. Several lactic acid bacteria are involved in preparation of this pickle. *Lactobacillus plantarum* is the most important organism required for fermentation of cucumber pickle.

4.7.3 Fermented soyabean products

Tempeh

Tempeh is a highly popular soyabean preparation in Indonesia. The chief organism in this preparation is the mould species of *Rhizopus oligosporus*. The

soybean mash is wrapped in a banana leaves or kept in boxes and the mash is inoculated with tempeh fungus by addition of a portion of previous batch and allowed to ferment for about 24-48 hours at a temperature 30-40^{o}C until there is a good mycelium growth which is then sliced and prepared as per the taste such as roasting or frying. The taste of the tempeh is considered to be bland but it is highly nutritious.

Soya sauce

Soya sauce is a very popular preparation of Japan which has received wide acceptance world over. This is prepared by inoculating an aspergillus species mostly *Aspergillus oryzae* in a mixture of soaked and steamed soya bean with roasted wheat in the ratio of 2:1. The mixture is allowed to be incubated for 3 to 5 days. Subsequently, it is subjected to various processing steps using *Lactobacillus bacteria* and the yeast *Saccharomyces rouxii*. After 3 months, the final product is filtered, pasteurized and bottled for use.

4.7.4 Fermented Dairy Products

The fermented dairy products assume greater importance in the human diets as invariably most of the diets consist of milk byproducts especially the cheese, butter, yoghurt, curd etc.

Cheese

There are several varieties of cheese which manufactured all over the world. All types of cheese are the byproducts of lactic acid fermentation of milk. There are several varities of cheese which are classified as hard, semihard and soft cheese. These are prepared with culturing of the milk either with bacteria or mould species. Among the several varieties, the popular ones are Cheddar cheese and Swiss cheese which are known as hard cheese whereas Roquefort cheese (blue cheese) is a semi-soft cheese and the soft variety is the Camembert cheese. The Cheddar cheese originated from England and the colour of which ranges from white to orange-yellow depending upon the colour added. The curing is done with the help of *Streptococcus* and *Lactobacillus*.

This cheese is without the gas holes (the eyes) which characterizes the swiss cheese. The swiss cheese is cultured with the help of mixed culture. *L.bulgaricus* and *Streptococus-thermophilus* and *Propionbacterium shermanii* which imparts the characteristics eye formation. The Roquefort cheese is prepared by the use of Penicillium *roqueforti* where as the camembert cheese is produced by *Penicillum camemberti*.

Dahi / curd

Dahi is an Indian fermented product which resembles yoghurt and is widely used in the daily menu of an average Indian. The dahi / curd is obtained from the milk by use of mixed culture of heterolactic bacteria

Yoghurt

Yoghurt is the preparation which uses the action of two lactic acid bacteria *Streptococcus thermophilus* and *Lactobacillus bulgaricus*. The ideal ratio of these two organisms is 1:1 for the best result.

Butter

The microorganisms which are involved in the preparation of butter are *Streptococus lactis* and *Streptococcus cremoris* which convert the lactose in the milk to lactic acid. Then the organisms like *Streptococcus* diacetylactis, *Leuconostoc dextranicum*, *Laeuconostoc citrovorum* are involved in imparting the aromatic flavours to the butter. The preparation of commercial butter involves the ageing of cream overnight at 5-10°C and subculturing for 15-16 hours with lactic acid bacteria followed by churning.

4.7.5 Other Fermented Foods

Idli

Idli is prepared from rice and urad lentil (dhal). They are cleaned, washed and grounded in equal proportions and left overnight for fermentation. The microorganism involved in this preparation are *Leuconostoc mesenteroides* which grows first in the batter which is followed by *Streptococcus faecalis* and

Pediococus cerevisiae. Once the batter raises sufficiently, it is steam cooked and served.

Vada

Vada is prepared by soaking urad lentil (dhal) in water for sometime, then ground to paste. It is then left to ferment at 23-32°C for 12-48 hours, usually overnight. It is made into balls and deep fried in vegetable oil. The dal paste is fermented by lactic acid bacteria. *Leuconostoc mesenteroides* being the major organism. The organism produces carbondioxide which aerates the product and contributing to its texture..

Dosa

Dosa is a light, shallowed-fried thin pan-cake. It is prepared from fermenting rice and urad dhal batter (ratio 2:1) followed by overnight fermentation. The aeration of batter is caused by lactic acid bacteria fermentation by *Streptococcus faecalis* with carbondioxide production by *Leuconostoc mesenteroides***.**

Bhatura

Bhatura is prepared from wheat flour, salt and sometimes with pepper or cumin and made into dough with water and curd. Curd is used as a starter and the dough is then fermented at 20-30°C overnight. It is rolled and flattened into discs and deep fried in vegetable oil. The major organism involved in fermentation are *Streptococcus* and *Lactobacillus species* introduced by curd.

Dhokla

Dhokla is similar to idli except that rice and bengal gram are used in this preparation. It is steamed into large sheets, then cut into pieces and seasoned. It is yellow in colour unlike idli.

4.7.6. Economically important fermentation products.

Apart from helping man in preparing his foods, the microrganisms have contributed to a great extent in perpetuating man's desire for alcoholic beverages which are usually made up of plant products.

Beer

Beer is an alcoholic product produced by brewing. It is a principal malt beverage where the fermentation of carbohydrate to alcohol takes place. Barley is used in the preparation of beer. Yeasts play a major role in the preparation of beer. Lagerbeer is produced by *Saccharomyces uvarum* which settles at the bottom of the fermenting vat and is known as bottom yeast.

Ale

The ale is produced by strains of *S.cerevisiae* which is collected at the top and is called top yeast.

Rice Beer

Rice beer is a low-alcohol beverage made from rice which is more popular in North Eastern India. The rice is milled, water is added and cooked. It is cooled and then starter is added and fermented for 18 hours at 20-28^{o}C. Rice beer is then decanted from solid residue, which often is used as a breakfast cereal.

The starter introduces a mixture of moulds and yeasts. The rice starch is broken down to sugars by amylase enzymes of moulds of *Rhizopus, Mucor* and *Aspergillus* species. The yeasts like *Endomycopsis* and *Hansenula* then converts the sugars to ethanol and carbondioxide.

Wine

Wine is the oldest and well known fermented alcoholic beverage produced by the fermentation of good and sound grapes which is further processed, known as ageing, before consumption. Although there are other fruits wines, they are not as popular as grape wine. The grape fruits are crushed to give 'must'. The fermentation of 'must' is initiated by yeasts *Kloeckera apiculatus* and *Metschnikowia pulcherrima* together with yeasts of *Torulopis, Candida* etc. The

main fermentation is by the yeasts, *Saccharomyces cerevisiae* and *S. uvarum* or *S. bayanus* which converts sugars to ethanol and carbondioxide.

Champagne

Champagne, is a product of secondary fermentation. Fresh must, yeast and sugar are added to the wine selected for champagne preparation.

4.7.7 Distilled liquor products

The distilled alcoholic products of interest are rum, whisky and brandy. These products are manufactured from the distillation of yeast fermentation of sugar cane juice, molasses, grains and grain products.

Rum

The rum is an alcoholic distillate of fermented sugarcane juice or molasses.

Brandy

The brandy is produced by distilling grapes or other fruit wines.

Whisky

Whisky is produced by distilling the fermented mash of wheat, barley malt and other grains.

4.7.8 Other uses of microbes in industry

Vinegar

If the alcohol produced by the fermentation process is further oxidised to acetic acid producing bacteria, the product is vinegar. Vinegar is made by different processes. It can be made from fruit juices, starchy vegetables, malted cereals, sugars and alcohol. Vinegar is widely used as preservative in food preparation, wherein, the minimum percentage of acetic acid should be 3.75% as per PFA Act.

Enzymes

Enzymes, which are known as biocatalysts are very useful in the manufacturing of several products of commercial value. The enzymes are used

widely for manufacture of alcoholic beverage etc. The enzyme alpha-amylase used in bread making is commercially prepared from *Aspergillus oryzae*. The amyloglucosidase used as a substitute for malt in the production of beer and spirits is commercially prepared from *Aspergillus niger*. Pectolytic enzymes are produced from a number of fungi for use in fruit processing. Cellulase used for removing cellulose cloud and clarify the citrus juices is produced from the mould *Trichoderma viride* and proteases used in cheese making form *Aspergillus niger*.

Amino acids and Vitamins

The importance of amino acid and vitamins in human health is welll recognized. Several microbes have been used for their production which are biologically suitable. Yeast is one of the best sources of the vitamin B-complex. A number of preparations of high potency vitamin B-complex made from dried yeast, yeast extracts are available in the market. Ribolfavin one of the B group vitamins is produced from the yeast *Eremothecium ashbyii*. Ergosterol, the prescursor of vitamin D is synthesized by a number of moulds and yeasts. Betacarotene is produced commercially by fermentation using the yeasts *Rhodotorula.*

Citric acid

Citric acid is one of the widely used chemical which finds applications in several divergent industries such as pharmaceuticals, flavouring extractions in food preparations, dyeing etc. Citric acid is produced by mould, *Aspergillus niger* which converts sugars to citric acid where molasses is generally the raw material.

Antibiotics

Apart from giving man several food products, certain microroganisms especially moulds have given products which are life saving. The antibiotics are the products of living organisms which in small proportions could be acting as inhibitory agent for the growth of other microbes. The discovery of Penicillin produced by *Penicillium notatum* by Sir Alexander Fleming in 1929 has

triggered off the manufacturing of modern antibiotics. There are over 600 antibiotics derived from bacteria and over 150 from fungi. The genus *Streptomyces* has yielded a wide range of useful antibiotics such as strept, streptomycin, aureomycin , chloromycetin and terramycin.