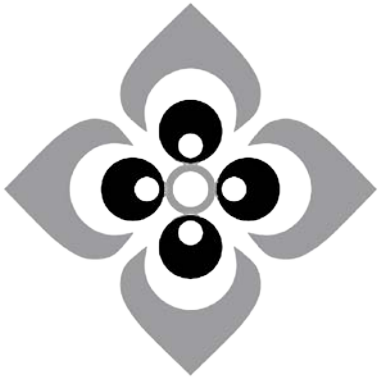

Consortium for Educational Communication

Module
on
Fermentation, Basic Concepts, Types And Industrial Importance

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Introduction

The term fermentation has come to have somewhat different meanings as its underlying causes have become better understood. The derivation of the word fermentation signifies a gentle bubbling condition. The term was first applied to the production of wine more than a thousand years ago. The bubbling action was due to the conversion of sugar to carbon dioxide gas. When the reaction was defined following the studies of Gay-Lussac,fermentation came to mean the breakdown of sugar into alcohol and, carbon dioxide. Pasteur later demonstrated

the relationship of yeast to this reaction, and the word fermentation became associated with microorganisms, and still later with enzymes. The early research on fermentation dealt mostly with carbohydrates and reactions that liberated carbon dioxide. It was soon recognized, however, that microorganisms or enzymes acting on sugars didnot always evolve gas. Further, many of the microorganisms and enzymes studied also had the ability to break down non carbohydrate materials such as proteins and fats, which yielded carbon dioxide other gases, and a wide range of additional materials.

Fermentation is a metabolic process that converts sugar to acids, gases, and/or alcohol. It occurs not only in yeast and bacteria, but even in oxygen-starved muscle cells, as in the case of lactic acid fermentation. Fermentation is also used more broadly to refer to the bulk growth of microorganisms on a growth medium, often with the goal of producing a specific chemical product. French microbiologist Louis Pasteur is often remembered for his insights into fermentation and its microbial causes. The science of fermentation is known as zymology.

Fermentation takes place in the lack of oxygen (when the electron transport chain is unusable) and becomes the cell's primary means of ATP (energy) production (Klein et al 2006). It turns NADH and pyruvate produced in the glycolysis step into NAD^+ and various small molecules depending on the type of fermentation. In the presence of O_2, NADH and pyruvate are used to generate ATP in respiration. This is called oxidative phosphorylation, and it generates much more ATP than glycolysis alone. For that reason, cells generally benefit from avoiding fermentation when oxygen is available. Exceptions include obligate anaerobes, which cannot tolerate oxygen.

The first step, glycolysis, is common to all fermentation pathways:

$$C_6H_{12}O_6 + 2\ NAD^+ + 2\ ADP + 2\ Pi \rightarrow 2\ CH_3COCOO^- + 2\ NADH + 2\ ATP + 2\ H_2O + 2H^+$$

Pyruvate is CH_3COCOO^-. Pi is phosphate. Two ADP molecules and two Pi are converted to two ATP and two water molecules via substrate-level phosphorylation. Two molecules of NAD+ are also reduced to NADH.

In oxidative phosphorylation the energy for ATP formation is derived from an electrochemical proton gradient generated across the inner mitochondrial membrane (or, in the case of bacteria, the plasma membrane) via the electron transport chain. Glycolysis has substrate-level phosphorylation (ATP generated directly at the point of reaction).

Fermentation has been used by humans for the production of food and beverages since the Neolithic age. For example, fermentation is employed for preservation in a process that

produces lactic acid as found in such sour foods as pickled cucumbers, kimchi and yogurt, as well as for producing alcoholic beverages such as wine and beer. Fermentation can even occur within the stomachs of animals, such as humans. Auto-brewery syndrome is a rare medical condition where the stomach contains brewer's yeast that break down starches into ethanol; which enters the blood stream.

Industrial fermentation is the intentional use of fermentation by microorganisms such as bacteria and fungi to make products useful to humans. Fermented products have applications as food as well as in general industry. Some commodity chemicals, such as acetic acid, citric acid, and ethanol are made by fermentation . The rate of fermentation depends on the concentration of microorganisms, cells, cellular components, and enzymes as well as temperature, pH and for aerobic fermentation oxygen.

Benefits of Fermentation

In addition to the roles of fermentation in preservation and providing variety to the diet, there are further important consequences of fermentation. Several of the end products of food fermentation, particularly acids and alcohols, are inhibitory to the common pathogenic microorganisms that may find their way into foods. The inability of *Clostridium, botulinum* to grow and produce toxin at pH values of 4.6 and below has already been cited. Increasing the acidity of foods by fermentation is very common. Foods as diverse as yogurt, hard sausages, and sauerkraut all contain acid as a result of fermentation. When microorganisms ferment food constituents, they derive energy in the process and increase in numbers. To the extent that food constituents are oxidized, their remaining energy potential for humans is decreased. Compounds that are completely oxidized by fermentation to such end products as carbon dioxide and water retain no further energy value. Most controlled food fermentations yield such major end products as alcohols, organic acids, aldehydes, and ketones, which are only slightly more oxidized than their parent substrates, and so still retain much of the energy potential of the starting materials. Fermentation processes are attended by temperature increases. The energy dissipated as heat represents a fraction of the total energy potential of the original food material no longer recoverable for nutritional purposes. Fermented foods can be *more* nutritious than their unfermented counterparts. This can come about in at least three different ways. Microorganisms not only are catabolic, breaking down more complex compounds, but they also are anabolic and synthesize several complex vitamins and other growth factors. Thus, the industrial production of such materials as riboflavin, vitamin B_{12} and the precursor of vitamin C is largely by special fermentation processes.

The second important way in which fermented foods can be improved nutritionally has to do with the liberation of nutrients locked into plant structures and cells by indigestible materials. This is especially true in the case of certain grains and seeds. Milling processes do much to release nutrients from such items by physically rupturing cellulosic and hemicellulosic structures surrounding the endosperm, which is rich in digestible carbohydrates and proteins. Crude milling, however, practiced in many less developed regions, often is inadequate to release the full nutritional value of such plant products; even after cooking, some of the entrapped nutrients may remain unavailable to the digestive processes of humans. Fermentation, especially by certain molds, breaks down indigestible coatings and cell walls both chemically and physically.

Molds are rich in cellulose-splitting enzymes; in addition, mold growth penetrates food structures by way of its mycelia. This alters texture and makes the structures more Permeable to the cooking water as well as to human digestive juices. Similar phenomena result from the enzymatic actions of yeasts and bacteria. A third mechanism by which fermentation can enhance nutritional value, especially of plant materials, involves enzymatic splitting of cellulose, hemicellulose, and related Polymers that are not digestible by humans into simpler sugars and sugar derivatives. This goes on naturally in the rumen of the cow through the enzymatic action of protozoa and bacteria. It also occurs in the process of preparing silage for animal feeding. Cellulosic materials in fermented foods similarly can be nutritionally improved for humans by the action of microbial enzymes. Of course, such changes are accompanied by gross changes in texture and appearance of the starting food materials, just as all fermented foods are markedly altered from their unfermented counterparts. Such changes are not looked upon as quality defects. Quite the contrary; particularly in areas of the world where most of human nutrients are derived from plant sources, food materials markedly altered by fermentation commonly are more frequent and relished items of diet than are the natural plant components.

Types of Fermentation Processes:

Some of the most important types of fermentation are as follows:

1. Solid State Fermentation

2. Submerged Fermentation

3. Anaerobic Fermentation

4. Aerobic Fermentation

5. Immobilized Cell Bioreactors

6. Immobilized Enzyme Bioreactors.

1. Solid State Fermentation:

In such fermentations, microbial growth and product formation occur at the surface of solid substrates. Examples of such fermentations are mushroom cultivation, mold-ripened cheeses, starter cultures, etc. More recently, this approach has been used for the production of extracellular enzymes, certain valuable chemicals, fungal toxins, and fungal spores (used for biotransformation). Traditional substrates are several agricultural products, e.g., rice, wheat, maize, soybean, etc.

The substrate provides a rich and complex source of nutrients, which may or may not need to be supplemented. Such substrates selectively support mycelial organisms, which can grow at high nutrient concentrations and produce a variety of extracellular enzymes, e.g., a large number of filamentous fungi, and a few bacteria (actinomycetes and one strain of Bacillus).

According to the physical state, solid state fermentations are divided into the following two groups: (i) low moisture solids fermented without or with occasional/continuous agitation, and (ii) suspended solids fermented in packed columns through which liquid is circulated.

Solid state fermentations on large scale use stationary or rotary trays. Temperature and humidity controlled air is circulated through the stacked solids. Less frequently, rotory drum type fermenters have been used. Solid state fermentations offer certain unique advantages but suffer from some important disadvantages. However, commercial application of this process for biochemical production is chiefly confined to Japan.

2. Submerged Fermentation:

Batch Culture:

Batch culture is a closed culture system, which contains limited amount of nutrient medium. After inoculation, the culture enters lag phase, during which there is increase in the size of the cells and not in their number. The culture then enters lag phase or exponential growth phase during which cells divide at a maximal rate and their generation time reaches minimum.

The increasing population of bacterial cells, after sometime, enters into a stationary-phase due to depletion of the nutrients and the accumulation of inhibitory end products in the medium. Eventually, the stationary, phase of bacterial population culminates into death-phase when the viable bacterial cells begin to die.

Fed-Batch Culture:

When a butch culture is subsequently led with fresh nutrient medium without removing the growing microbial culture, it is called fed-batch culture. Fed-batch culture allows one to supplement the medium with such nutrients that are depleted or that may be needed for the terminal stages of the culture, e.g., production of secondary metabolites.

Therefore, the volume of a fed- batch culture increases with time. Fed-batch cultures achieve higher cell densities than batch cultures. It is used when high substrate concentration causes growth inhibition. It allows the substrate to be used at lower nontoxic levels, followed by

subsequent feeding. It allows the maximum production of cellular melabolities by the culture.

Continuous Culture:

Contrary to the batch culture where the exponential growth of microbial population is restricted only for a few generations, it is often desirable to maintain prolonged exponential growth of microbial population in industrial processes.

This condition is obtained by growing microbes in a continuous culture, a culture in which nutrients are supplied and end products are continuously removed. A continuous culture, therefore, is that in which the growth of bacterial population can be maintained in a steady state over a long period of time.

3. Anaerobic Fermentation:

In anaerobic fermentation, a provision for aeration is usually not needed. But in some cases, aeration may be needed initially for inoculum build-up. In most cases, a mixing device is also unnecessary, but in some cases initial mixing of the inoculum is necessary. Once the fermentation begins, the gas produced in the process generates sufficient mixing.

The air present in the headspace of the fermenter should be replaced by CO_2, H_2, N_2 or a suitable mixture of these; this is particularly important for obligate anaerobes like Clostridium. The fermentation usually liberates CO_2 and H_2, which are collected and used, e.g., CO_2 for making dry ice and methanol, and for bubbling into freshly inoculated fermenters. In case of acetogens and other gas utilizing bacteria, O_2-free sterile CO_2 or other gases are bubbled through the medium. Therefore, when recovery of such enzymes is the objective, cells must be harvested under strictly anaerobic conditions.

4. Aerobic Fermentation:

The main feature of aerobic fermentation is the provision for adequate aeration; in some cases, the amount of air needed per hour is about 60-times the medium volume. Therefore, bioreactors used for aerobic fermentation have a provision for adequate supply of sterile air, which is generally sparged into the medium. In addition, these fermenters may have a mechanism for stirring and mixing of the medium and cells.

Aerobic fermenters may be either of the

(i) stirred-tank type in which mechanical motor-driven stirrers are provided or

(ii) (ii) of air-lift type in which no mechanical stirrers are used and the agitation is achieved by the air bubbles generated by the air supply.

Generally, these bioreactors are of closed or batch types, but continuous flow reactors are also used; such reactors provide a continuous source of cells and are also suitable for product generation when the product is released into the medium.

5. Immobilized Cell Bioreactors:

Bioreactors of this type are based on immobilized cells. Cell immobilization is advantageous when (i) the enzymes of interest are intracellular, (ii) extracted enzymes are unstable, (iii) the cells do not have interfering enzymes or such enzymes are easily inactivated/removed and (iv) the products are low molecular weight compounds released into the medium.

Under these conditions, immobilized cells offer the following advantages over enzyme immobilization:

(i) enzyme purification is not needed,

(ii) (ii) high activity of even unstable enzymes,

(iii) (iii) high operational stability,

(iv) (iv) lower cost and

(v) possibility of application in multistep enzyme reactions.

In addition, immobilization permits continuous operation of bioreactor, which reduces the reactor volume and, consequently, pollution problems. Obviously, immobilized cells are used for such bio-transformations of compounds, which require action of a single enzyme.

Cell immobilization may be achieved in one of the following ways.

(1) Cells may be directly bound to water insoluble carriers, e.g., cellulose, dextran, ion-exchange resins, porous glass, brick, sand, etc., by adsorption, ionic bonds or covalent bonds. (2) They can be cross-linked to bi- or multifunctional reagents, e.g., glutaraldehyde, etc.

(3) Polymer matrices may be used for entrapping cells; such matrices are polyacylamide gell, K-Carrageenan (a polysaccharide isolated from a seaweed), calcium alginate (alginate is extracted from a seaweed), polyglycol oligomers, etc. Out of these approaches, calcium alginate immobilization is the most commonly used since it can be used for even very sensitive cells, e.g., plant cells; K-Carrageenan is also a useful entrapping agent.

Cell immobilization has been used for commercial production of amino acids, e.g., E. coli cells entrapped in polyacrylamide gel for the production of L-aspartic acid, L-alanine production using a mixture of E. coli and Pseudomonas dacunhae immobilized in K-Carrageenan, organic acids, e.g., L-malic acid from fumaric acid using Brevibacterium ammoniagenes cells immobilized in polyacrylamide gel/K-Carrageenan (subsequently, B. flavum was used in the place of B. ammoniagenes)-, NADP production by B. ammoniagenes and yeast (Saccharomyces cerevisiae) cells immobilized together in polyacrylamide gel.

6. Immobilized Enzyme Bioreactors:

Continuous flow reactors are based on immobilized enzymes. They offer the following advantages:

(i) greater productivity per unit amount of enzyme,

(ii) can be used for substrates having low solubility, and

(iii) uniform product quality, i.e., lack of batch to batch variation.

These reactors may be of the following three types:

(i) continuous flow stirred tank reactor,

(ii) (ii) packed bed reactor and

(iii) fluidized bed reactor.

Continuous Fermentation

In continuous fermentation, an open system is set up. Sterile nutrient solution is added to the bioreactor continuously and an equivalent amount of converted nutrient solution with microorganisms is simultaneously removed from the system. Two basic types of continuous fermentations can be distinguished:

Homogeneously Mixed Bioreactor –

This is run as either a chemostat or a turbidostat. In the chemostat in the steady state, cell growth is controlled by adjusting the concentration of one substrate. Any required substrate (carbohydrates, nitrogen compounds, salts, O2) can be used as a limiting factor. In the turbidostat, cell growth is kept constant by using turbidity to monitor the biomass concentration and the rate of feed of nutrient solution is appropriately adjusted.

Plug Flow Reactor - In this type of continuous fermentation, the culture solution flows through a tubular reactor without back mixing. The composition of the nutrient solution, the number of cells, mass transfer, and productivity vary at different locations within the system. At the entrance to the reactor, cells must be continuously added along with the nutrient solution.

In a continuous process under steady state conditions, cell loss as a result of outflow must be balanced by outgrowth of the organism.

In the conventional batch process, the reactor is filled with a sterile nutrient substrate and inoculated with the microorganism. In the course of the entire fermentation, nothing is added except oxygen (in the form of air), an antifoam agent, and acid or base to control the pH. The culture is allowed to grow until no more of the product is being made, at which point the reactor is "harvested" and cleaned out for another run. Four typical phases of growth are observed: lag phase (when the organisms adapt to their surroundings), exponential growth (when they grow in numbers), stationary phase (when they stop growing), and death phase.

An enhancement of the closed batch process is the fed-batch fermentation, which is used in the production of substances such as penicillin. In the fed-batch process, substrate is added in increments as the fermentation progresses. This is employed to avoid the inhibition of substrate consumption at high substrate concentrations due to catabolite regulation. In this process, fermentation is started batchwise with a small substrate concentration. When all the initial substrate is consumed, a new addition of fermentation medium is made in an amount such that the substrate concentration remains just below the point where it produces inhibitory effects. At the same time, some of the fermentation product is removed and taken off for processing.

Since it is usually not possible to measure the substrate concentration directly and continuously during the fermentation, indirect parameters which are correlated with the metabolism of the critical substrate must be measured in order to control the feeding process. For instance, in the production of organic acids, the pH value may be used to determine the rate of glucose feeding. In the production of ethanol, the rate of CO_2 production can be measured and used to continuously vary the glucose feed rate based on the following formula:

$C_6H_{12}O_6 \rightarrow 2\ C_2H_5OH + 2\ CO_2$

In fermentations with critical osmotic valves, feeding can be regulated by monitoring the pO_2-value or the CO_2 content in the exhaust air.

An extension of fed-batch fermentation is continuous fermentation.

There are also industrial considerations related to the fermentation process. For instance, to avoid

biological process contamination, the fermentation medium, air, and equipment are sterilized. Foam control can be achieved by either mechanical foam destruction or chemical anti-foaming agents. Several other factors must be measured and controlled such as pressure, temperature, agitator shaft power, and viscosity. An important element for industrial fermentations is scale up. This is the conversion of a laboratory procedure to an industrial process. It is well established in the field of industrial microbiology that what works well at the laboratory scale may work poorly or not at all when first attempted at large scale. It is generally not possible to take fermentation conditions that have worked in the laboratory and blindly apply them to industrial-scale equipment. Although many parameters have been tested for use as scale up criteria, there is no general formula because of the variation in fermentation processes. The most important methods are the maintenance of constant power consumption per unit of broth and the maintenance of constant volumetric transfer rate.

Alcoholic Fermentation

Industrial alcohol

Ethyl alcohol can be produced by fermentation of any carbohydrate containing a fermentable sugar, or a polysaccharide that can be hydrolysed to a fermentable sugar. The equation that describes the net result of alcoholic fermentation by yeast is:

$C_6H_{12}O_6 \rightarrow 2C_2H_5OH + 2\ CO_2$

It indicates that a sugar is the substrate and that the process is anaerobic. Selected strains of Saccharomyces cerevisiae are commonly employed for fermentation. It is imperative that the strain must have a high tolerance for alcohol, must grow vigorously and produce a large quantity of alcohol.

In recent years the production of industrial alcohol by the fermentation process has declined because of the-increased cost of raw materials and the rapid developments of synthetic ethanol production. Industrial alcohol will probably continue to be obtained on a diminished scale from certain processes. For example alcohol is obtained as the end product in the processes designed to reduce biological oxygen demand (BOD) of some industrial wastes, including whey and sulphite waste of paper mills. The large amount of carbon dioxide evolved from decarboxylation of pyruvate during the fermentation period is recovered and converted to solid carbon dioxide.

Alcoholic beverages

These products of alcoholic fermentations originated in spontaneous fermentation processes are of great antiquity. However, it is only in recent years that modern methods of industrial microbiology have been applied to their manufacture. In principle, the production of alcoholic beverages is similar to the production of industrial ethyl alcohol. These fermentation processes do not suffer competition from synthetics products. This is because the character of the beverage is dependent upon interactions between a variety of biological factors that have not yet been denned in chemical or physical terms. In beverage production refinements are introduced

with respect to flavour, aroma, colour, and sanitation that are not necessary in the making of industrial alcohol.

The type of beverage produced is determined by the nature of the plant material employed for fermentation. In all these processes the method of preparing the fermentation medium is a factor of prime importance.

Beer. *Beef* is made by the yeast fermentation of grains to ethanol and carbon dioxide. There are five major steps in the manufacture of *beer* or *ale* from grain. These are *malting, mashing, 'fermenting, maturing,* and *finishing. Malting* and *mashing* are concerned with the conversion of starch into fermentable form such as maltose or glucose. The chief raw material is *malt,* which is germinated barley that has been dried and ground. It contains stanch, proteins, and high concentration of amylases and proteinases. Amylases convert the starch into fermentable sugar. Mould amylase derived from *Aspergillus oryzae* is sometimes used for the same purpose. Ground malt is mashed in warm water to bring about the digestion of starch and proteins. The aqueous extract contains dextrins, maltose, and other sugars, protein breakdown products, minerals and various growth factors. This is a rich nutrient medium and is called *beer wort.* The beer wort is filtered and *hops* are added, *Hops* are the flowers of *Humulus lupulus.* They are added for flavour, colour, and for aroma and for mild antibacterial activity to prevent the growth of spoilage bacteria.

A large inoculum of selected strain of *Saccharomyces cerevisiae* is added to the wort to bring about a vigorous fermentation. Yeasts are classified as *'top yeasts'* or *'bottom yeasts. Top yeasts* float on the surface of a fermenting mixture and are employed in making *ale.* Bottom yeasts settle in the fermentation tank and are used in making *beer.* Beer fermentation takes place at 6 to 12°C., whereas ale fermentation is complete in five to seven days at 14 to 23°C. The alcoholic content of beer is between 3 to 6 percent, that of ale is somewhat higher.

The fermented wort is refrigerated at 0°C for two weeks to several months to remove the harsh flavour and other undesirable characteristics. Some of the harshness attributed to higher alcohols disappears as they are oxidized or esterified during *aging.* Finishing process consists of carbonation, cooling, filtering and dispensing into barrels, bottles, and cans. Bottled or canned beer is usually pasteurized at 60°C for 20 minutes to kill yeasts and other microorganisms. As an alternative, the beer may be passed through a filter to remove microorganisms, and then aseptically dispensed into sterile cans. The composition of American lager beer is as follows :

Alcohol	3.8 percent
Dextrins	4:3 percent
Proteins	0.3 percent
Ash	0.3 percent
$C0_2$	0.4 percent

It also contains appreciable amounts of vitamins, particularly riboflavin. In addition, there are

a number of minor constituents, some of which are important for flavour and aroma.

Wine. *Wine* is the product made by the normal alcoholic fermentation of the juice of sound, ripe grapes and the usual cellar treatment. Beverages produced by the alcoholic fermentation of other fruits and certain vegetable products-are also called wines for example, peach wine, orange wine, cherry wine. Wine making is a much simpler process. It can be made by a direct fermentation of sugars, i.e. glucose and fructose, instead of starch which requires hydrolysis to yield sugars. Many fruits have the wine yeast *Soccharomyces cerevisiae var. ellipsoideus* on them. All that is necessary is to crush the fruits. An alcoholic fermentation starts spontaneously. The characteristic qualities of famous wines are attributed in part to strains of yeast found in certain localities. However, undesirable moulds, wild yeasts, and bacteria are also likely to be present and the fermentation may not give a predictably good product. Many wine makers now destroy natural yeasts by adding sulphur dioxide to the raw juice.

The grapes are crushed carefully and the juice is collected. To the raw juice or *must;* sulphur dioxide is added as sodium meta-bisulphite. The *must* is then inoculated with a starter culture-of a selected strain *of S. cerevisiae var. ellipsoideus.* At the start the *must* is aerated slightly to promote vigorous yeast growth. Once the fermentation sets in, the rapid production of carbon dioxide maintains anaerobic condition. The temperature of fermentation is usually 25 to 30°C and the process may extend from few days to 2 weeks. The yield of ethanol varies from 7 to 15 percent (by volume). The wine is placed in large casks to settle, clarify and age for two to five years to develop a good flavour and aroma.

Wines are endless in their varieties and differ in so many attributes that it is difficult to classify them. According to colour, the two most basic types are *red* and *white wine.* In making *red wines* the grapes are crushed and stemmed but the skins and seeds are left in the must. *White wines* are made from white grapes or from the juice of grapes from which the skins have been removed. *Dry wines* are those which contain too little sugar to be detected by taste. In *sweet wines* the sugar content is high enough to be detected by taste. *Sparkling wines* contain carbon dioxide. They are made effervescent by secondary fermentation in closed containers, generally in the bottle itself. *Still wines* are those which do not contain carbon dioxide. *Fortified wines* contain added alcohol in the form of *brandy.*

Distilled liquors. Yeast action is limited by the amount of alcohol present, and at about the level of 18 percent by volume its action ceases. To produce the so called *hard liquor,* for higher levels of alcohol, distillation is required. Distilled alcoholic beverages may be divided into three major classes depending on the nature of the solution distilled:—

1. The products starting from a starchy substance and needing enzymes.

2. The products starting directly from a sugar substrate.

3. The type of liquor produced by adding flavour substances to quite pure ethanol, which has been obtained by distillation and rectification.

Malt whisky is prepared by fermentation and subsequent distillation of malted barley. *Grain whisky* is prepared in a similar manner from a mixture of malted and unmalted barley with

unmalted maize. Malt and grain whisky are matured and finally blended to form *Scotch whisky. Bourbon* is whisky prepared from a mash in which maize is the predominant grain. *Irish Whisky* is manufactured from a mash in which rye grain predominates. *Arrak* (Far East) and *sake* (Japan) are fermented beverages prepared from rice. Rice starch is hydrolysed by amylases derived from moulds, principally *Aspergills oryzae.*

Brandy is obtained from distillation of fermented fruit juice, that is wine. Rum is produced by distillation of fermented molasses or other sugarcane by products. Gin is prepared by extracting juniper berries with alcohol and distillation of alcohol. Cordials and liqueurs are sweetened alcoholic distillates from fruits flowers, leaves, etc.

Cheese

The origin of cheese making is lost in unrecorded history. There is evidence to suggest that cheese was made as far back as 7000 BC. There are numerous references to cheese making in the Bible while the writings of Homer and Aristotle indicate that cheese was made from the milk of cows, goats, sheep, mares and asses. Around 300 AD trade in cheese between countries especially on sea routes became so great that the Roman emperor Diocletian had to fix maximum prices for the cheese. Cheese is made in almost every country of the world and there exist more than 2000 varieties. Despite the large number of varieties cheese may be classified into different groups, i.e. ripened and unripened cheese, cheese with low or high fat content and cheese with soft or hard consistency. Unprocessed milk held at high ambient temperatures has a shelf-life from 2–3 hours up to 24 hours. Cheese, however, has a shelf-life from 4–5 days up to five years depending on the variety. Cheese therefore provides an ideal vehicle for preserving the valuable nutrients in milk and making them available throughout the year. Cheese is an excellent source of protein, fat and minerals such as calcium, iron and phosphorus, vitamins and essential amino acids and therefore is an important food in the diet of both young and old people.

Single Cell Proteins:

SCP is the dried cells of selected micro-organisms such as algae, yeast, bacteria molds, and higher fungi,that can be used as rich protein sources to humans and animals. There are various angles at which the sources of the SCP can be looked at. Some use simpler methods such as suggesting whether the source is a bacteria or fungi or yeast etc. While others select on the basis of the photosynthetic ability of producing organisms. Photosynthetic microorganisms which could be exploited commercially as source of SCP include algae such as *Scenedesmus, Spirulina, Chlorella* etc, and bacteria such as those belonging to the such as *Rhodopseudomonas, Bacillus* and *Azatobacteria*. Non-photosynthetic microorganisms useful for SCP production include bacteria, such as *Methylococcus* and *Axinetobacteria* species etc. Yeasts belonging to the genera *Saccharomyces*, *Candida* and *Kluyveromyces*. However, it is important to note that these organisms must not be pathogenic to plants, animals and humans besides not containing any toxins.

Production: It is significant enough to consider the production of SCP from various microbial sources independently owing to the variation in the process used. Algae grown photosynthetically in a medium containing organic compounds and inorganic nutrients such as Iron, Magnesium, Sodium, Potassium etc, sources of Nitrogen such as ammonia and nitrates and sources of Phosphorous and Sulfur. A pH value of about 11 is considered optimum for *Spirulina* while a value of 8 is considered best for *Scenedesmus*.

Artificial illumination for a light source has not been cost-effective and hence natural light is preferred. Culture system could be batch, pond or semi-continuous types, all types using an agitator to ensure uniform supply of nutrients. Mixed cultures and contaminated by bacteria are looked upon as major problems in the production. Bacteria are also grown photosynthetically as pure cultures with an organic carbon source or in mixed cultures with other bacteria. The most suitable growth parameters are temperature range of 25 to 300°C and a pH range of 6 to 8.5. Non-photosynthetic bacteria are cultured aerobically using many designs of bioreactors and fermenters. The cultures are operated as batch, continuous or fed-batch modes. Yields from non-photosynthetic bacteria such as *Methylococcus* are in the orders of one gram per gram of substrate used (menthol). However, bacterial SCP is extremely difficult to recover.

Yeasts are cultivated using variety of carbon sources such as xylose, glucose, sucrose and lactose. Important factors for selection of a yeast strain (some as those for any other source of SCP) are its growth rate, yield, pH and temperature tolerance, aeration requirements, genetic stability and non-toxigenicity. Typical yeast cultures yield 10-15g (dry weight) per litre of medium used. Some higher fungi such as *Coprinus* have also been propagated as a source of SCP. The optimal parameters include a temperature of 25 to 300°C and a pH of 3 to 7 units.

Applications:

1) SCP forms one of the richest sources of proteins or amino acids. They had been used as a part of feeds to live stocks, chickens, eggs-laying hens and pigs. The SCP provide the essential amino acids at various concentrations depending on the source, which is expressed as Biological value (BV) and Protein Efficiency Ratio (PER).

2) Human feeding studies have been conducted with algae, yeasts and molds. However, the palatability has remained a question. Also digestibility and amino acids content have remained points of controversy. Since SCP's are significant sources of proteins for man, it is advised to avoid an intake exceeding 2g per day, which would otherwise lead to stone formation in kidneys and gut.

Modifications of SCP's such as succinylation (addition of succinic acid) and Phosphorylation are being attempted to improve functional properties. However, licensed approval is awaited.

Production of antibiotics

The **production of antibiotics** has been widespread since the pioneering efforts of Florey and Chain in 1938. The importance of antibiotics to medicine has led to much research into their discovery and production.

Identifying useful antibiotics

Despite the wide variety of known antibiotics, less than 1% of antimicrobial agents have medical or commercial value. For example, whereas penicillin has a high therapeutic index as it does not generally affect human cells, this is not so for many antibiotics. Other antibiotics simply lack advantage over those already in use, or have no other practical applications.

Useful antibiotics are often discovered using a screening process. To conduct such a screen, isolates of many different microorganisms are cultured and then tested for production of diffusible products that inhibit the growth of test organisms. Most antibiotics identified in such a screen are already known and must therefore be disregarded. The remainder must be tested for their selective toxicities and therapeutic activities, and the best candidates can be examined and possibly modified.

A more modern version of this approach is a rational design program. This involves screening directed towards finding new natural products that inhibit a specific target, such as an enzyme only found in the target pathogen, rather than tests to show general inhibition of a culture.

Industrial production techniques

Antibiotics are produced industrially by a process of fermentation, where the source microorganism is grown in large containers (100,000–150,000 liters or more) containing a liquid growth medium. Oxygen concentration, temperature, pH and nutrient levels must be optimal, and are closely monitored and adjusted if necessary. As antibiotics are secondary metabolites, the population size must be controlled very carefully to ensure that maximum yield is obtained before the cells die. Once the process is complete, the antibiotic must be extracted and purified to a crystalline product. This is easier to achieve if the antibiotic is soluble in organic solvent. Otherwise it must first be removed by ion exchange, adsorption or chemical precipitation.

Strains used for production

Microorganisms used in fermentation are rarely identical to the wild type. This is because species are often genetically modified to yield the maximum amounts of antibiotics. Mutation is often used, and is encouraged by introducing mutagens such as ultraviolet radiation, x-rays or certain chemicals. Selection and further reproduction of the higher yielding strains over many generations can raise yields by ٢٠-fold or more. Another technique used to increase yields is gene amplification, where copies of genes coding for enzymes involved in the antibiotic production can be inserted back into a cell, via vectors such as plasmids. This process must be

closely linked with retesting of antibiotic production.

Enzyme Production

The production of enzymes is central to the modern biotechnology industry. The traditional industrial enzymes continue to have expanding markets, and the recognition of potential to use biocatalysis in various industrial sectors for new applications generates demand for enzymes with novel activities and/or improved stability (see also Microbial enzymes for food processing. The history of modern enzyme production really began in 1874 when the Danish chemist Christian Hansen first produced rennet by extracting it from dried calves´ stomachs with saline solution. Apparently, this was the first enzyme preparation of relatively high purity used for industrial purposes. During the early part of the last century, in the Far East, an age-old tradition involving the use of mould fungi called koji (see also Food fermentation and processing) in the production of certain foodstuffs and flavour additives based on soya protein and fermented beverages, formed the basis on which the Japanese scientist Takamine developed a fermentation process for the industrial production of fungal amylase. The process included the culture of Aspergillus oryzae on moist rice or wheat bran, and the product was called 'Takadiastase' which is still used as a digestive aid. The value of the industrial enzymes market was estimated to $ 2 billion, and has increased at an average annual rate of 3-5 percent during the past decade. A number of companies are competing in the industrial enzymes business, Novozymes dominating with 45 percent of sales, followed by Danisco that holds a 20 percent share of the market. The industrial or bulk enzymes include proteases, amylases, lipases, etc. which are required in large volumes, but have an inherently low unit value so that they demand significantly lower manufacturing costs. On the other end of the scale is the therapeutics sector with products such as urokinase, which are produced in lower volumes and at inherently greater manufacturing cost. In between these two lie the diagnostic enzymes.

Enzyme Source

The primary consideration in the production of any enzyme relates to the choice of source. In most cases, the desired activity can be obtained from several sources. Traditionally, however, the choice of source has been more restricted for some enzymes. For example, the enzyme rennet was until recently obtained from the stomach of suckling calves; the corresponding microbial enzyme led to an off-flavor in the cheese produced. Today, recombinant DNA technology is used to produce the calf enzyme in microorganisms. Microorganisms represent an attractive source of enzymes as they can be cultured in large quantities in a relatively short period by established methods of fermentation (see also Microbial Cell Cultivation). However, the level of production of a particular enzyme varies in different microorganisms, and moreover the enzymes often differ in. composition and properties. One usually finds that the closely related organisms have enzymes with nearly similar properties, while unrelated organisms have enzyme systems that differ widely. The most critical feature of the organisms for producing industrially significant enzymes is their GRAS (generally regarded as safe) status, which implies that they must be non-toxic, non-pathogenic and generally should not produce antibiotics. The GRAS listed microorganisms include fewer than 50 bacteria and fungi. Examples are the bacteria including Bacillus subtilis, B. licheniformis, and various

other bacilli, lactobacilli, Streptomyces species, the yeast Saccharomyces cerevisiae, and the filamentous fungi belonging to the genera Aspergillus, Mucor, Rhizopus, etc. In case of Bacillus, mutants are selected that can no longer form spores. Since Aspergillus cultures are frequently inoculated with conidia, enzyme production using these fungi relies on good spore formation. Most of the bulk enzymes (hydrolases) are secreted by the microorganisms directly into the culture medium, while some enzymes e.g. penicillin acylase and glucose isomerase are intracellular. For some applications, it may not be necessary to isolate the enzymes but the microbial cells themselves are used as enzyme source. The organism is preferred which gives high yields of enzyme in shortest possible fermentation time.

Amino Acids

Amino acids such as lysine and glutamic acid are used in the food industry as nutritional supplements in bread products and as flavor enhancing compounds such as monosodium glutamate (MSG). Amino acid production is typically carried out by means of **regulatory mutants,** which have a reduced ability to limit synthesis of an end product. The normal microorganism avoids overproduction of biochemical intermediates by the careful regulation of cellular metabolism. Production of glutamic acid and several other amino acids in large quantities is now carried out using mutants of *Corynebacterium glutamicum* that lack, or have only a limited ability to process, the TCA cycle intermediate _-ketoglutarate. A controlled low biotin level and the addition of fatty acid derivatives results in increased membrane permeability and excretion of high concentrations of glutamic acid. The impaired bacteria use the glyoxylate pathway to meet their needs for essential biochemical intermediates, especially during the growth phase. After growth becomes limited because of changed nutrient availability, an almost complete molar conversion (or 81.7% weight conversion) of isocitrate to glutamate occurs. Lysine, an essential amino acid used to supplement cereals and breads, was originally produced in a two-step microbial process. This has been replaced by a single-step fermentation in which the bacterium *Corynebacterium glutamicum,* blocked in the synthesis of homoserine, accumulates lysine. Over 44 g/liter can be produced in a 3 day fermentation. Although not used extensively in the United States, microorganisms with related regulatory mutations have been employed to produce a series of 5′purine nucleotides that serve as flavor enhancers for soups and meat products.

Organic Acids

Organic acid production by microorganisms is important in industrial microbiology and illustrates the effects of trace metal levels and balances on organic acid synthesis and excretion. Citric, acetic, lactic, fumaric, and gluconic acids are major products.Until microbial processes were developed, the major source of citric acid was citrus fruit from Italy. Today most citric acid is produced by microorganisms; 70% is used in the food and beverage industry, 20% in pharmaceuticals, and the balance in other industrial applications.

The essence of citric acid fermentation involves limiting the amounts of trace metals such as manganese and iron to stop *Aspergillus niger* growth at a specific point in the fermentation. The medium often is treated with ion exchange resins to ensure low and controlled concentrations of available metals. Citric acid fermentation, which earlier was carried out by means of

static surface growth, now takes place in aerobic stirred fermenters. Generally, high sugar concentrations (15 to 18%) are used, and copper has been found to counteract the inhibition of citric acid production by iron above 0.2 ppm. The success of this fermentation depends on the regulation and functioning of the glycolytic pathway and the tricarboxylic acid cycle .After the active growth phase, when the substrate level is high, citrate synthase activity increases and the activities of aconitase and isocitrate dehydrogenase decrease. This results in citric acid accumulation and excretion by the stressed microorganism.

In comparison, the production of gluconic acid involves a single microbial enzyme, glucose oxidase, found in *Aspergillus niger. A. niger* is grown under optimum conditions in a corn-steep liquor medium. Growth becomes limited by nitrogen, and the resting cells transform the remaining glucose to gluconic acid in a single-step reaction. Gluconic acid is used as a carrier for calcium and iron and as a component of detergents.

Biopolymers

are microbially produced polymers used to modify the flow characteristics of liquids and to serve as gelling agents. These are employed in many areas of the pharmaceutical and food industries. The advantage of using microbial biopolymers is that production is independent of climate, political events that can limit raw material supplies, and the depletion of natural resources. Production facilities also can be located near sources of inexpensive substrates(e.g., near agricultural areas). Bacterial exo-polysaccharides. At least 75% of all polysaccharides are used as stabilizers, for the dispersion of particulates, as film-forming agents, or to promote water retention in various products. Polysaccharides help maintain the texture of many frozen foods, such as ice cream, that are subject to drastic temperature changes. These polysaccharides must maintain their properties under the pH conditions in the particular food and be compatible with other polysaccharides. They should not lose their physical characteristics if heated.

Biopolymers include (1) dextrans, which are used as blood expanders and absorbents;

(2) *Erwinia* polysaccharides that are inpaints; and

(3) polyesters, derived from *Pseudomonas oleovorans,*

which are a feedstock for specialty plastics. Cellulose microfibrils, produced by an *Acetobacter* strain, are used as a food thickener. Polysaccharides such as scleroglucan are used by the oil industry as drilling mud additives. Xanthan polymers enhance oil recovery

by improving water flooding and the displacement of oil. This use of xanthan gum, produced by *Xanthomonas campestris,* represents a large potential market for this microbial product.

The cyclodextrins have a unique structure**.** They are cyclic oligosaccharides whose sugars are

joined by _-1,4 linkages. Cyclodextrins can be used for a wide variety of purposes because these

cyclical molecules bind with substances and modify their physical properties. For example, cyclodextrins will increase the solubility of pharmaceuticals, reduce their bitterness, and mask chemical odors. Cyclodextrins also can be used as selective adsorbents to remove cholesterol from eggs and butter or protect spices from oxidation.

Enzymes commercially available now are at economically comparable to the chemical process. Hence, any substantial reduction in the cost of production of enzymes will be a positive stimulus for the commercialization of enzymatic depilation. Proteases are one of the most important groups of industrial enzymes and account for nearly 60% of the total enzyme sale. The major uses of free proteases occur in dry cleaning, detergents, meat processing, cheese making, and silver recovery from photographic film, production of digestive and certain medical treatments of inflammation and the virulent wounds . A wide range of microorganisms including them are available commercially; and, they have almost completely replaced chemical hydrolysis of starch in starch processing industry. Although many microorganisms produce this enzyme, the most commonly used for their industrial application are Bacillus licheniformis, Bacillus amyloliquifaciens and Aspergillus niger. Amylases stand out as a class of enzymes, which are of useful applications in the food, brewing, textile, detergent and pharmaceutical industries . Rhizopus oligosporous 1HS13, Aspergillus niger, Rhizopus oryzae, Saccromyce cerevisiae and Conidiobolus spp have ability to produce proteases. Their biomass can be easily determined after simple drying in oven as well as in dissector and weighing by digital balance. Fungal proteases are of particular importance in the food industry Aspergillus and Mucor have been studied intensively as protease producers although Rhizopus oligosporus also produces proteases, has a high proteolytic activity in the tempe fermentation and furthermore, does not produce toxins.Amylases are enzymes that break down starch or glycogen. The amylases can be derived from several sources such as plants, animals and microbes. The major advantage of using microorganisms for production of amylases is in economical bulk production capacity and microbes are also easy to manipulate to obtain enzymes of desired characteristics. The microbial amylases meet industrial demands; a large number of them are available commercially and, they have almost completely replaced chemical hydrolysis of starch in starch processing industry. Enzyme is protein which is synthesized as intra and extra cellular compounds. Enzymes energize and catalyze biochemical reaction with high specificity and enhance the reaction rate. Lipases are one of the highly commercialized enzymes; have an important role found and ranked after proteases and amylases. Lipases are widely used in industrial application such as, detergent industry, pharmaceutical industry, pulp and paper industry, production of biodiesel, dairy and bakery foods, fats and oils. Lipases are fond in animal, plant and microorganisms. Besides the stability, selectivity and broad substrate specificity microbial lipases are more promising in terms of availabilities and productivities. Among the various sources of lipases, fungi is recognized as the best enzyme producer and also used for industrial application.

Fermentation Enzymes have been used for thousands of years to produce food and beverages, such as cheese, yoghurt, beer and wine. Yeast is a fungus whose enzymes aid the breakdown of glucose into ethanol and carbon dioxide anaerobically. The enzymes in yeast break down sugar (glucose) into alcohol (ethanol) and carbon dioxide gas:

Glucose –Yeast – →– Ethanol + Carbon dioxide

$$C_6H_{12}O_6\ (aq) \xrightarrow{Yeast} 2\ C_2H_5OH\ (aq) + 2\ CO_2\ (g)$$

This reaction, which takes place in the absence of oxygen, is called fermentation. Fermentation works best when the yeast and glucose solution is kept warm. Enzymes will also become ineffective if the temperature becomes too high. Fermentation is used in all production of alcoholic drinks. For stronger alcohol, such as whiskey and vodka, these need to be distilled after fermentation to increase the concentration of ethanol in the fermented mixture. This is due to the fact that ethanol poisons the yeast and stops it working when the concentration builds up about 18% by volume. Fermentation is also used in the baking industry to make bread rise. After the dough has been prepared, it is left to rest in a warm place before going into the oven. This gives the enzymes in the yeast a chance to break down the sugar and make carbon dioxide.