F03FP14 Fermentation in Industrial level

Introduction

Fermentation was originated from ancient times.Home fermentations were practiced in the form of baking, pickling, brewing and cheese making. Later on the production of large scale fermented foods rely on commercially available microbial cultures. The usage of defined cultures became the industrial standard in breweries by the 19th century. The well-defined cultures along with the technique of sterilization or pasteurization allowed the fermentation process to reach an industrial scale.

Objectives

On completion of this module, students will be able to

1. Explain the industrial fermentation process used in food processing industries
2. Identify the steps involved in the production of wine and soy sauce

14.1 Industrial Fermentations

Large scale fermentation in industries is the deliberate use of microorganisms such as bacteria, yeast and mold to produce fermented products. Industrial microbiology has made worthy contributions to the food industries for the economical production and thorough utilization of all byproducts. The products such as vinegar, citric acid, ethanol, enzymes (lipase, rennet, Invertase) are products of fermentation. The biomass production like starter cultures of baker's yeast and lactic acid bacteria are also manufactured in industries.

14.2 Types of industrial fermentations

The industrial fermentation processes are classified

1. Based on the type of substrate
2. Based on the feeding of substrate
3. Based on the need of aeration

14.2.1 Based on the type of substrate

The fermentation process based on the type of substrate is classified into two types. They are

1. Solid State fermentation
2. Submerged fermentation

14.2.1.1 Solid State Fermentation (SSF)

Solid state fermentation also referred as surface fermentation. It the process in which the microbial growth and product formation occurs on the surface of solid substrate. The microorganisms grow on the moist substrate with little or no 'free water'. This method is suitable for microorganisms the need lesser water for its growth. This method is economically viable, as the fermentation is carried out using agricultural byproducts. Several agro crops such as rice, wheat, maize, barley and industrial residues such as bran, straw, sugarcane bagasse, oil cakes, corn cobs, saw dust, fruit pulps etc. can be used as substrate.This method also uses an inert material as substrate, which requires nutrient supplement as well as a carbon source. These natural raw materials serve as a source of carbon and energy. Few examples of solid state fermentations are mushroom cultivation, bread making, production of miso, tempeh and soy sauce, cocoa processing.This method is considered to be cost effective due to the use ofagro-industrial residues as media for the growth and production. Little amount of water is utilized for the process which accordingly releases negligible or considerably less quantity of effluent, thus reducing pollution concerns. SSF processes use low volume equipment of lower cost but effective in producing concentrated products.There is an increased oxygen diffusion rate into the substrate hence aeration is easier in SSF. The process can be efficiently utilized at smaller scale, which makes them suitable for rural areas.

14.2.1.2 Submerged Fermentation (SmF)

Submerged fermentation occurs in the presence of liquid nutrient media seeded with microorganisms which trigger the fermentation process. The commonly used liquid medias are molasses, broth, corn steep liquor etc. This method is suitable for microorganisms that require high moisture. The substrates are utilized rapidly by the microorganisms and need to be constantly supplemented with nutrients. Also a steady flow of oxygen must be circulated throughout the process. As the microbe's breakdowns the nutrients in the media, they produce enzymes. The significance of submerged fermentation is that purification of products is easier and primarily used in the extraction of secondary metabolites that need to be used in liquid form. This process is industrially used for the production of enzymes, citric acid etc.

14.2.2 Based on the feeding of substrate to fermenter

The fermentation process based on the feeding of substrate to fermenter is classified into two types. They are

1. Batch fermentation
2. Continuous fermentation
3. Fed-batch fermentation

14.2.2.1 Batch fermentation

Batch fermentation is a closed process system. In this method, the fermenter tank is filled with the materials such as substrate and inoculum. The process parameters such as temperature and pH are set and occasionally nutritive supplements are added to the substrate. Until the process comes to an end, neither substrate is added northe product is removed from the fermenter. Fermentation proceeds and after desired period, the products are taken out from the fermenter. Once the process is over, the fermenter is cleaned and the process is repeated.

***14.2.2.2* Continuous fermentation**

In continuous fermentation, the sterilized liquid nutrients are added continuously to the fermenter at a fixed rate as the end products are continuously removed. In this type of process, the growth of bacterial population can be maintained in a steady state over a long period of time.

14.2.2.3 Fed-batch fermentation

Fed-batch fermentation employs both modes of operations such as batch and continuous process, where substrate is added at fixed time intervals during the fermentation process.

14.2.3 Based on the need of aeration

The fermentation process based on the need of aeration is classified into two types. They are

1. Aerobic fermentation
2. Anaerobic fermentation

14.2.3.1 Aerobic fermentation

Most of large scale fermentation processes are carried out in presence of aerobic conditions. In aerobic fermentation, the materials in fermenter are agitated with the help of impeller or sterile air is forced into the fermenter. In this process, it is necessary to maintain the dissolved oxygen concentration above the specified minimal level.

14.2.3.2 Anaerobic fermentation

The provision for aeration and mixing device is not needed in anaerobic fermentation. But in few cases, aeration and mixing may be needed in an initial period. Once the fermentation begins, the gas produced in the process generates sufficient mixing. The air present in head space should be replaced by CO_2 or N_2 or suitable combination. During this type of fermentation, the released CO_2 and H_2 are collected and reused.

14.3 Industrial Fermenter

A fermenter or a bioreactor is a vessel in which the living cells or biochemically active substances act on the substrate to produce product of higher quality. An industrial fermenter can hold up to 200,000 liters of cultures. They are usually made of stainless steel to withstand the acid produced during fermentation. The components in most of the fermenters are impellers (to mix the living cells and nutrients), sparger (aeration), Jacket or coils (cooling or heating), probes (to monitor pH, temperature, pressure and dissolved oxygen), valves and steam traps.

14.4 Range of Fermentation Process

Commercially important fermentation process can be divided into four groups. They are

1. Production of biomass or microbial cells
2. Production of extracellular metabolites
3. Production of intracellular components
4. Transformation of substrate

14.4.1 Production of biomass

Sometimes the microbial cells itself are the products of fermentation. The major biomass or microbial cell production is the production of yeast, lactobacillus to be used in the baking and dairy industry respectively and production of algaeto be used as human or animal food.

14.4.2 Production of extracellular metabolites

The extracellular metabolites are grouped as primary and secondary metabolites. The microbial metabolites produced during the growth phase are called primary metabolites. Many primary metabolites are economically importance and are being produced by fermentation. Examples of primary metabolites are ethanol, lactic acid, citric acid, nucleotides, vitamins and amino acids like lysine, tryptophan. Secondary metabolites are compounds produced in the stationary phase of the microbial growth. They are derived from the intermediates and products of primary metabolism. Secondary metabolites used as medicines, flavorings, and drugs. For example, antibiotics, antiseptics, fungicides are produced as secondary metabolites.

14.4.3 Production of intracellular components

In this method, cells are ruptured at the end of fermentation and the environment is engineered to maximize the production. The intercellular components such as microbial enzymes such as lipase, cellulose, lactase, recombinant proteins and microbial oils are produced through fermentation process.

14.4.4 Transformation of substrate

It is the process of conversion of a raw material into valuable finished product through fermentation. Productions of vinegar, steroids, antibiotics, prostaglandins are examples of microbial transformation process.

14.5Production of Wine

The conversion of grape juice to wine was practiced dating back to the dawn of civilization. Wine can also be made from other fruits. Red wine and white wine are the two major types of wine based on the color. All grape skins contain tannins whereas the skin of red grapes contains more tannin. The presence of tannins imparts the color, texture to red wine. The red color of wine is that the fermentation process occurs together with the grape skins. The prominent microorganisms involved in the wine making are *Saccharomyces cerevisiae* and Lactic acid bacteria which dominate the alcoholic fermentation and the malolactic conversion respectively. The following processes are involved in the production of red wine.

14.5.1 Harvesting

The grapes are harvested after 1-3 weeks of color change and when the pH is about 3.25. Harvesting can be performed either manually or mechanically.

14.5.2 Destemming and Crushing

Destemming is the process of separating grapes from the stems. Before the fermentation process, the stems are removed as the stem contains high level of tannins which also imparts a vegetal aroma to the wine. Crushing is the process of breaking the skins of berries by squeezing it to extract the contents of grapes.

14.5.3 Primary Fermentation

The crushed grapes and its skins are left to contact each other to extract the desired color. The squeezed juice along with skins and seeds are called must. Preparation of must is the first step in wine making. The most commonly used cultured yeasts in wine belong to Saccharomyces cerevisiae species.They are added to the must for the alcoholic fermentation to occur. The cultured yeasts are usually added in dried or inactive state. They are activated by mixing with warm water or diluted grape juice. For active yeast fermentation, they are continuously supplied with source of carbon, nitrogen, sulfur, phosphorus and also nutrient source such as vitamins and minerals.

During the primary fermentation process, the yeast cells feed on the sugar in the must and involve in the conversion of sugar into alcohol and carbon dioxide. The temperature is maintained at 22 -25°C. One gram of sugar will yield half a gram of alcohol in the product. Hence to obtain alcohol of 12 % concentration, the must should contain 24 % sugar. The duration of primary fermentation takes around 10-30 days depending on the final alcohol content in wine. Longer fermentation makes the wines dry because the majority of sugar is converted into alcohol, whereas shorter fermentation produces sweeter wine.

14.5.4 Secondary fermentation

Secondary malolactic fermentation takes place at the end of primary alcoholic fermentation. Malolactic fermentation is decarboxylation process which releases carbon dioxide. During this fermentation, specific strains of lactic acid bacteria such as *Lactobacillus*, *Oenococcus oeni* are added in addition to the one that is already available. This process turns malic acids into lactic acids which produces a more supple silky wine with buttery taste.The whole process is kept under anaerobic conditionto avoid oxidation of wine. The secondary fermentation is a slow process which takes around 3-6 months.

14.5.5 Pressing

Pressing is an optional process in which pressure is applied to the pomace to separate wine from the grapes and skins. In production of red wines, pressing is performed after primary fermentation. The juice obtained through crushing called free-run juice which accounts for about 60-70% of the total juice whereas 30-40% of juice comes from pressing called press juice. In practice, the free-run juice and press juice are blended in the ratio of 85-90% and 10-15% respectively to get a complete balanced wine.

14.5.6 Purifying or Clarifying

The sediments are removed after fermentation. The presence of solid portion could potentially harm the quality of wine in future. To remove the solids, the wine is filtered through a filter or siphoned off from the top of the vat after the solids sink to the bottom. Sometimes, gelatin, micronized potassium caseniate, skim milk powder, bentonite are used as fining or clarifying agent. The addition of these agents removes microscopic particles that could create cloudiness of wine, removes tannins and also reduces astringency of wine.

After fermentation,sedimentof tartrate crystals may appear in the wine formed due to the union of tartaric acid and potassium. These crystals are also known as ‘wine crystals’ or ‘wine diamonds’. This process is called cold stabilization to reduce the tartrate crystals in wine. Hence the temperature of wine is reduced to freezing cold for 1-2 weeks to separate the crystals from the wine.

14.5.7 Aging or Maturation

Immediately after fermentation, a period of maturation is required to get pleasant taste of wine. During the maturation process, the acidity of wine falls and softens the tannins. This process takes place in stainless steel vessel or oak barrels based on the desired taste of final product. Early drinking wines do not need maturation. For high quality wines, they must undergo maturation of about 9-22 months. During this time, the wine will undergo controlled oxygenation and produces oak aroma.

14.5.8 Bottling

The traditional wine bottles are sealed with cork. The closure types such as synthetic corks, screw caps are currently used. Before bottling of wine, sulfite is added to prevent the unwanted fermentation and to preserve the wine.

14.6Production of Soy Sauce

Soy sauce is produced from fermented soybeans, roasted grains like wheat, barley or rice, salt and special yeast molds. Soy sauce is considered as a traditional ingredient throughout Asia.

The consistency varies from thin to very thick and color ranges from lighter to dark brown. The modern method of soy sauce production involves the following steps.

14.6.1 Soaking and Steaming

The basic ingredients for soy sauce making are soybeans, wheat, salt and water. The carbohydrate in wheat imparts soy sauce a pleasant aroma. The starch in wheat is converted into glucose by the action of amylase from the mold which adds sweetness to the soy sauce. The addition of brine solution prevents the entry of destructive microorganisms and also acts as a preservative. In the modern methods the soybeans are soaked in water for 12 to 24 hours. The beans are drained and steam cooked at high temperature.Wheat is roasted and crushed to get coarse product. The coarsely grounded wheat is mixed with cooked soybeans in the ratio of eight parts of soy with two parts of wheat.

14.6.2 Inoculation and Koji Preparation

The steamed product is cooled and then mixture is inoculated with seed spores of Aspergillus Oryzae or/and Aspergillus sojae and kept for about 22-30 hours at 35°C. Sometimes the culture is grown on steamed polished rice. The inoculated mash is spread in a layer in wooden trays and the trays are stacked to allow air circulation over the mash. During fermentation, the mold grows over the mash and release off heat. The temperature of mash may reach up to 40°C or higher. After 3 days, the white mold surface turns yellowish as spore formation begins. The mixture is now called as '*koji'.* It is a concentrated source of amylolytic and proteolytic enzymes necessary for the decomposition of the carbohydrates and proteins.

14.6.3 Fermentation in brine

The molded mash is covered with 22% brine solution in a deep fermentation tanks. Lactic acid bacteria and yeast cultures are added to the *koji* to form slurry called '*Moromi*'. *Moromi*mash which is in a semi liquid state is allowed to ferment at controlled temperature and occasional aeration for every 2 to 3 days in the beginning. The high salt concentration effectively inhibits growth of undesirable microorganisms. The starch is broken down to sugars and fermented to produce lactic acid and alcohol. The pH drops from near-neutral to 4.7- 4.8. The *moromi*mash is held in the fermentation tanks for 6 to 8 months.

14.6.4 Pressing

The fermented mash is pressed to remove the solids from the liquid soy sauce. During pressing the moromi is strained through layers of fabric. After allowing the soy sauce to flow out under gravity, the mash is mechanically pressed for about 10 hours to obtain 'raw soy sauce'.

14.6.5 Clarification and Pasteurization

The raw soy sauce obtained from pressing is left in clarifier tank for 3-4 days. The clarified sauce is then pasteurized to about 70-80°C. Heat treatment of sauce halts the activity of enzymes, eliminates the growth of active yeasts and molds, and also favors the development the characteristic color and aroma. After final clarification the sauce is bottled.

Summary

- Fermentation on an industrial scale is used for the production of commercial products such as cheese, wine, vinegar, single cell protein, enzymes etc.

- The industrial fermentation processes are classified based on the type of substrate, feeding of substrate and based on the need of aeration
- Solid State fermentation is type of fermentation that occurs on the surface of substrate having little or near little moisture content
- Submerged fermentation occurs in the presence of liquid nutrient media seeded with microorganisms
- The industrial fermentation processes are preceded for the production of biomass or microbial cells, extracellular metabolites, intracellular components and for the transformation of substrate into new products