SCRIPT

YEAST FERMENTATION AND ITS PRODUCTS

The topic Yeast fermentation and its products will be discussed under 5 sub units such as

1. INTRODUCTION

2. BAKER'S YEAST

3. FOOD AND FEED YEASTS

4. ALCOHOL YEASTS

5. OTHER YEAST PRODUCTS

1. INTRODUCTION

Yeast is a single-celled microorganism, that is classified along with molds and mushrooms, as members of the Kingdom Fungi. Although yeasts are single-celled organisms, they possess a cellular organization similar to that of higher organisms, including humans. Specifically, their genetic content is contained within a nucleus. This classifies them as eukaryotic organisms, unlike their single-celled counterparts, bacteria, which do not have a nucleus and are considered prokaryotes. Yeasts are widely dispersed in nature with a wide variety of habitats. They are commonly found on plant leaves, flowers, and fruits, as well as in soil.

Yeast has been used as an industrial microorganism for 1000's of years. The ancient Egyptians used yeast fermentation to leaven bread. There is evidence of grinding stones, baking chambers and drawings of 4000-year-old bakeries. Archaeological digs have uncovered evidence in the form of jars containing the remains of wine that is 7,000 years old. Yeasts were first visualized in 1680 by Antoni van Leeuwenhoek using high quality lenses. However, he thought that these globules were starchy particles of the grain used to make wort, the liquid extract used in brewing, rather than fermenting yeast cells. In the 1850s Louis Pasteur discovered that fermented beverages resulted from the conversion of glucose to ethanol by yeast and defined fermentation as "respiration without air".

Yeast has long been considered to be the organism of choice for the production of alcoholic beverages, bread, and a large variety of industrial products. Yeast strains are commonly used in many industries because they have been developed with the following traditional and new physiological properties.

(a) Ability to grow rapidly at room temperature of about 20-25°C
(b) Easy dispensability in water
(c) Ability to produce large amounts of CO_2 in flour dough, rather than alcohol
(d) Good keeping quality, i.e., ability to resist autolysis when stored
(e) High potential glycolytic activity
(f) Ability to adapt rapidly to changing substrates
(g) High invertase and other enzyme activity to hydrolyze the higher glucofructans rapidly
(h) Ability to grow and synthesize enzymes under the anaerobic conditions

(i) Ability to resist the osmotic effect of salts and sugars
(j) High competitiveness i.e. high yielding in terms of dry weight per unit of substrate used.

2. BAKER'S YEAST

The use of yeasts in bread making is an ancient art, although man did not always recognize the mechanism of the rise of dough. It is of interest to give a brief historical account of the development of the yeast industry. The dough of leavened bread whose antiquity is testified by biblical records, was probably raised by a mixture of yeasts and lactic acid bacteria. A small piece of successful dough was used as the inoculum for the next batch, providing a type of early continuous culture.

From about the Middle Ages, bakery yeasts were obtained from winemaking and brewing. But the quality of the yeast was variable and in the case of yeast obtained from beer the product was bitter because of the hops in the beer. This period lasted until the latter part of the 19th century when the work of Pasteur from 1855 to 1857 elucidated the nature of yeasts.

The first major step in the development of baker's yeast technology can be said to be the so-called Vienna process introduced about 1860 in which grain mash meant for anaerobic alcohol production was gently aerated so that a good quantity of yeast was obtained. The work of Pasteur later led to more vigorous aeration, thus yielding more cells and less alcohol. As a result of grain shortages resulting from World War I a shift was made from the use of grain to the use of beet molasses, supplemented by ammonia and phosphate.

The next major step in the development of baker's yeast technology was the introduction of *fed-batch* or incremental addition of nutrients rather than the introduction of all nutrients at the beginning, as is the case in the classical batch method. The essence of this system known as the Z*ulaut* method is still used today in baker's yeast manufacture and ensures that an excess of molasses sugar which might lead to alcohol production is avoided.

Another important development, the production of dried active yeast, was necessitated by the need to provide troops, fighting in far off lands a means of producing bread, instead of the compressed yeast normally used in temperate countries.

Today's production methods for baker's yeast do not allow alcohol production because of the vigorous aeration used. Furthermore the yield has increased from 3% in the mid-19th century through 13% early in this century to the present-day yield of over 50% dry weight of yeast.

Yeast strains used: Nowadays specially selected strains of *Saccharomyces cerevisiae* are used however non-sporulating 'torula' yeasts have occasionally been used for baking. For some time two strains of baker's yeasts were available: one was highly active but had poor stability during storage; the other had poor activity but was highly stable in storage. The specially selected baking strains of *Saccharomyces cerevisiae* are apt to mutate and therefore proper storage is most important. Of the various methods used, storage in liquid nitrogen and the oil culture method in which sterile oil is placed over a slant of yeast and refrigerated at 4°C are most widely used.

Production of baker's yeast

Substrate: The substrate usually used for baker' yeast production is molasses. Where these are not available or are too expensive any suitable sugar-containing substrate e.g. corn steep liquor may of course be used. In the Soviet Union for example sulphite liquor is used for both alcohol and baker's yeast production.

Phosphorous, ammonium and smaller amounts of magnesium, potassium, zinc, and thiamin are added for maximum productivity to the mixed molasses. Antifoam is sometimes added.

Beet and cane molasses, when they are simultaneously available, are treated separately: clarified, pH adjusted and sterilized. They are then mixed in equal amounts so that the nutritional deficiency of one type is made up by the other. Cane sugar is particularly richer in biotin, thiamin and magnesium and calcium; while beet molasses is much richer in nitrogen.

The molasses is clarified to remove inert colored material arising from colloidal particles and which can impart undesirable color to the yeast. Clarification may be achieved by precipitation with alum or calcium phosphate or by poly-electrolyte flocculating agents such as alginates and polyacrylamides. Clarification also helps reduce foaming. 'Sterilization' is achieved by heating at 100°–110°C for about an hour, after the pH has been adjusted to 6-8 to prevent caramelization of the sugar.

Fermentation process: The fermentor for baker's yeast propagation is nowadays made of stainless steel. Generally, baker's yeast fermentors are aerated only by spargers which are so arranged that large volumes of air pass through per unit time: about one volume of air per volume of broth per minute. It is most important that aeration be high and constant. When the oxygen falls below 0.2 ppm anaerobic conditions set in and alcohol is formed.Water, mineral nutrients, yeasts and the blended molasses containing 1% glucose are mixed. Molasses is added incrementally during the course of the fermentation as it is used up by the yeast, beyond the 0.1% ceiling. The pH is maintained at 4-6 by the addition of alkali and the temperature at 30°C by cooling. Large amounts of heat are evolved and the cooling of the fermentor is very important.

Harvesting the yeast: The period of fermentation in the trade or production fermentor varies from 10 to 20 hours depending on how much yeast is pitched into it; cells form from 3.5% to 5% dry wt. of the broth. In some processes aeration is allowed to continue for 30-60 minutes at the end of the feeding to allow unused nutrients to be used up, budding cells to divide so that most cells are 'resting' at the beginning of the budding cycle. This ensures that the cells divide somewhat 'synchronously' when growth resumes.

The fermentation broth is cooled and cells concentrated in centrifugal separators; they are washed by resuspension in water and centrifugation until they are lighter in color. The yeast cream resulting from this treatment contains 15-20% yeast cells. It is further concentrated by passing over a rotary vacuum filter or through a filter press. Sometimes the Mautner process is used to ensure a friable dry cream during vacuum filtration. This latter process consists of adding before filtration 0.2-0.6% (w/v) sodium chloride, which causes cell shrinking by osmosis. Excess salt is removed during filtration by spraying water over the filtered yeast, so that the cells swell again. The resulting product has a dry matter content of 28-30%.

The yeast may then be packaged as compressed yeast or active dry yeast. It may also be converted into *dried yeast* for human or animal feeding.

Baker's yeasts may be produced as moist (compressed) yeasts or as dried active yeast.

(i) *Compressed yeast*: The yeast product obtained after harvesting, is mixed with fine particles of ice, starch, fungal inhibitors and processed vegetable oils which all help to stabilize it. It is then compressed into blocks of small blocks for household use or large for factory bakery operations, stored at – 7 to 0°C and transported in refrigerated vans.

(ii) *Active dry yeast*: Dry yeast is more stable than compressed yeast. In many developing countries baker's yeast is imported from abroad in the form of active dry yeast. For active dry yeast production special strains better suited for use and dry conditions may be used. It has been found that when regular strains are used they perform better as dry yeasts when they are subjected to a number of treatments.

3. FOOD AND FEED YEASTS

Yeasts are used for food by man for the following reasons: to provide protein; to impart flavor and to supply vitamins especially B-vitamins. Food yeasts are sometimes prescribed medically when a deficiency of B-vitamins exists in a patient. Food yeasts have several synonyms: dried yeast, inactive dried yeast, dry yeast, dry inactive yeast, dried torula yeast, sulfite yeast, wood sugar yeast, and xylose yeast.

Too high a consumption of yeasts is detrimental to health because of the high RNA content of yeasts, which the kidneys are unable to dispose of.

Production of Food Yeast: While baker's yeasts are usually produced from molasses using special strains of *S. cerevisiae* food yeasts are produced from a wide variety of yeasts and substrates.

Yeasts used as food yeasts: Yeasts used as food yeasts are *Saccharomyces cerevisiae, Saccharomyces carlbergiensis, Saccaromyces fragilis*, *Candida utilis*, and *Candida tropicalis.* Only *Saccharomyces fragilis* can utilize lactose hence it is used for the fermentation of whey. *Candida utilis* is the most versatile of all the yeasts and will utilize a wider range of carbon and nitrogen sources than any other, hence it is most widely used in food yeast preparations.

Substrates used for food yeast production: The most commonly used substrates are molasses, sulphite liquor, wood hydrolysate, and whey. Since interest developed in single cell protein other unconventional sources have been developed. These include hydrocarbons, alcohol and wastes of various types. However, commonly used substrates such as molasses, sulphite liquor, wood hydrolysate and whey will be discussed.

(i) *Molasses*: Bakers yeast grown on molasses as described above may, after separation from the spent liquor by centrifugation, be dried to yield food yeast. Drum-drying, spray-drying or fluidized bed drying may be used to reduce the moisture content to only about 5%. Sometimes food yeast is grown on molasses for that purpose. Thus *Candida utilis* is grown fed-batch in Taiwan in Waldhof fermentors. The fedbatch method using molasses is also used in South Africa. Recently food yeasts using *Candida utilis* in continuous culture in molasses has been grown in Cuba and Eastern Europe.

(ii) *Sulfite liquor*: The impetus to produce food and fodder yeast from sulfite liquor derived from an attempt to reduce the pollution which would arise if the wastes containing fermentable substrates were discharged directly into a stream. The use of continuous fermentation was attractive because the sulfite is produced almost continuously in the operation of the pulp factory. In general a Waldhof-type fermentor is used for the continuous production of yeasts from sulfite waste. Liquors from various sources are usually blended. Thereafter, the sulfite containing compounds are removed either by precipation with lime, by aeration or by passing steam through it (steam stripping). The pH is adjusted from about 2 to 5.5 using ammonia. The lowest pH consistent with high yield is usually preferred in order to lessen the chances of contamination.

Ammonium, phosphate and potassium are monitored and supplied continuously. The versatile and hardy yeast *Candida utilis* is usually used so that biotin is not added. The yeast is harvested continuously and recovered by removing liquor at the same rate as it is introduced.

(iii) *Production of food yeast from whey*: The effluent which drains from the coagulum from milk during cheese manufacture is known as whey. It contains approximately 4% sugar (lactose), 1% mineral and some of the lactic acid which enabled the coagulation of the milk protein. In countries where a lot of cheese is produced, whey is a waste product but it is sometimes turned into good use in the production of alcohol or yeasts. Very few yeasts metabolize lactose. Those which do include *Saccharomyces lactis, Kluyveromyces fragilis* and its imperfect or asporogeneous stage *Candida pseudotropicalis.* The whey is diluted, fortified with ammonia, phosphate, minerals, yeast extract and then pasteurized at 80°C for about 45 minutes. It is then inoculated with yeasts at pH 4.5 at an incubation temperature of 30° C. Any of the above yeasts could be used but in the United States the preference is for *Kluyveromyces fragilis.* In many establishments the fermentation is continuous and sugar, pH and minerals are monitored automatically. The yeast is recovered by centrifugation and may be drum or spray dried.

FEED YEASTS

Feed yeasts are the same as food yeasts described above. The only difference is that less rigid standards are imposed on the production of feed yeasts. Thus, feed yeasts intended for animal feeding are usually obtained by drying out the whole fermentation broth, often without washing.

Several thousands tons of yeasts are recovered from breweries around the world annually. To be used as food yeasts, such yeast is 'debittered' of hop resins by repeated washing with dilute alkali until the bitterness no longer exists. It is then slightly acidified to about pH 5.5. Cells are recovered by centrifugation and spray – or drum-dried.

4. ALCOHOL YEASTS

Alcohol yeasts are those to be used in beer brewing, wineries and distilleries for spirits of industrial alcohol. In the production of alcohol yeasts, the aim is cell production. The methods are generally similar to those already described for baker's yeasts. Beginning from a lypholized vial or tube, contamination is checked in a plate. A single colony (or preferably a single spore by micromanipulation) is picked and multiplied in sequentially increasing amounts.

The yeasts used are specially selected strains of the following:
Brewing: *Saccaromyces cerevisiae, Saccharomyces uvarum carlbergensis, S. uvarum.*

Wine: *Saccharomyces cerevisiae, Sacch. bayanus, Sacch. beticus, Sacch. elipsoides.*
Distillery Yeasts: *Saccharomyces cerevisiae.*

The medium used in the multiplication of the yeast is made of materials to be found in the final fermentation. Thus for growing brewery yeasts wort is used, for distiller's yeast a rye-malt medium is used, and for wine grape juice is used.

Alcohol yeasts are usually recovered and reused for several rounds of fermentation before being discarded.

5. OTHER YEAST PRODUCTS

Various products used in the food, pharmaceutical and related industries may be produced from yeasts.

Yeast extracts are used in the preparation of soups, sausages, gravies, to which they impart a meaty flavor. The extracts may be obtained by autolysing the yeasts and thereafter spraydrying or drum-drying with or without extracting soluble materials from the autolysate.

The extract may also be obtained by hydrolyzing the yeast cells in acid solution. It is neutralysed with sodium hydroxide, filtered, decolorized through charcoal and concentrated to a syrup or spray-dried. Yeast products are usually fortified with the flavoring compound, mono-sodium glutamate, extracts of animal or vegetable protein or with yeast cells.

In addition to these traditional uses yeast has also been used for many other commercial applications. Vegans often use yeast as a cheese substitute and it is often used as a topping for products such as popcorn. It is being used in the petrochemical industry where it has been engineered to produce biofuels such as ethanol, and farnesene, a diesel and jet fuel precursor. It is also used in the production of lubricants and detergents. Yeast is used in the food industry for the production of food additives including colorants, antioxidants, and flavor enhancers. It is the often used in the production of pharmaceuticals including antiparasitics, anticancer compounds, biopharmaceuticals such as insulin, vaccines, and nutraceuticals. Yeast is commonly used in the production of industrial enzymes and chemicals. In the field of environmental bioremediation strains have even been exploited for the removal of metal from mining waste.

Conclusion: Today yeasts are produced and used in all the six continents of the world and it is known that the single most produced micro-organisms in terms of weight. Yeast has been considered to be the organism of choice for the production of alcoholic beverages, bread, and a large variety of industrial products. This is mainly based up on that the metabolism of yeast can be manipulated using genetic techniques, easy and quick it can be grown to high cell yields (biomass), and the same biomass can be separated from products. Above all yeast is generally recognized as safe (GRAS).