APPLICATION OF FUNGI IN BREWERY INDUSTRY

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INDEX PAGE

SUMMARY

Since ancient time, fungi have been considered as important components in numerous biotechnological processes. These processes depend on the metabolic pathways and enzymes produced by fungi that are used by various industries including brewing, baking, and the production of antibiotics, enzymes, wine, alcohols, organic acids, and pharmaceuticals. However, the utilization of lignocellulosic wastes has proven to be a challenge in the brewing and wine industries. Lignocellulases occupy a key position among industrial enzymes. Filamentous fungi are among modern the top research topics. This chapter discusses the use of enzymes produced by different strains of Penicillium to improve the taste, yield of alcohol, flavor, and clarity of wine and beer.Although there is a distinction between beer, wine and liquor as well as other lesser known alcoholic beverages, they share one thing in common. They are the fermentation products of yeasts, mostly Saccharomyces cerevisiae or in the case of beers, usually S. carlsburgiensis. Yeasts, as you recall, are not mycelial. They are unicellular fungi that reproduce asexually by budding or fission. The reaction by which alcoholic beverages are produced is generally referred to as fermentation and may be summarized as:

Yeast + Glucose è Alcohol (Ethanol) + CO2

This reaction is also important in baking bread, but the desired product is then the carbon dioxide rather than alcohol. The production of alcohol occurs best in the absence of oxygen. However, from the yeast's point of view, alcohol and carbon dioxide are waste products, and as the yeast continues to grow and metabolize in the sugar solution, the accumulation of alcohol will become toxic when it reaches a concentration between 14-18%, thereby killing the yeast cells. This is the reason why the percentage of alcohol in wine and beer can only be approximately 16%. In order to produce beverages (liquor) with higher concentrations of alcohol, the fermented products must be distilled.

INTRODUCTION

Fungi are eukaryotic organisms which generally reproduce sexually and asexually and have characteristic rigid cell wall containing chitin or cellulose or both(Jůzlová et al.,1996). They are osmotrophic chemoheterotrophs which collectively utilize substrates ranging from simple sugars to complex carbohydrates. Although fungi are typically aerobic, some are facultative or obligate anaerobes. They produce extracellular enzymes (including cellulases, xylanases and pectinases) which release soluble components from insoluble materials. Fungi include hundreds of species which are of tremendous economic importance to man. In fact our lives are intimately linked with those of fungi. Hardly a day passes when we are not benefited or harmed directly or indirectly by these organisms (Alexopoulos et al., 1996). The English word fungusis directly adopted fromtheLatin fungus (mushroom). The word mycology, derived from the Greek mikes (mushroom) and logos (discourse), is used to denote the scientific study of fungi. Fungi have a worldwide distribution, and grow in a wide range of habitats, including extreme environments. A group of all the fungi present in a particular area or geographic region is known as microbiota. Around 120,000 species of fungi have been described by taxonomists. They play an important role in medicine yielding antibiotics, in agriculture by maintaining the fertility of the soil and causing crop and fruit diseases, forming basis of many industries and as important means of food. Some of the fungi are important research tools in the study of fundamental biological processes. Some of these fungi, particularlymould and yeasts, play a negative role by causing spoilage of stored goods such as foodstuffs, textiles, leather, rubber, plastic, timber and even glass (Bennet, 1996; Benka-Coker and Olumagin, 1996). They are used also as sources of antibiotics and industrially important chemicals (e.g., alcohols, acetone and enzymes) as well as for their role in fermentation processes (e.g., the production of alcoholic beverages, vinegar, cheese and bread dough). The fungi form the basis of many important industries. The shift from chemical processes to biological processing, achieved by using fungal enzymes instead of chemical processes in industries, such as textiles, leather, paper and pulp, has significantly reduced negative impacts on the environment Use of enzymes in the food and feed industry, such as animal feed, baking, brewing, and wine and juice, has significantly improved what we get out of biological raw materials. Microbial enzymes added to detergents, washing laundry clean even at low temperatures, has significantly reduced CO_2 emissions. There are a number of industrial processes in which the biochemical activities of certain fungi are harnessed to good account. Fungi play an important role in addressing major global challenges. Use of fungal processes and products can lead to increased sustainability through more efficient use of natural resources. Applications range from upgrading bio-waste for value added products to use of renewable plant biomass as a substitute for oil-based products such as biochemicals, plastics, fertilizer, and fuel. Fungal inoculum, introduced into soil together with seed, can promote more robust plant growth through increasing plant uptake of nutrients and water, a robustness of importance for maintaining crop yields under climate change condition. Fungal enzymes can lead to production of food ingredients with prebiotic effects for a healthier human gut biota and hence greater resilience towards life-style diseases. Similarly, use of fungi can be a short cut to healthier animal feed and less use of antibiotics in, for example, meat production, one of the current prime sources of multiple drug resistant bacteria. Fungi are one of nature´s most promising hotspots for finding new drug candidates and antimicrobials. Last but not least, fungi have interesting potential as the new way of manufacturing biological medicines and a wide spectrum of new value added bio-based products. The paper, therefore, examine the potential application of fungi in the petroleum, agro-allied and pharmaceutical industries.

DISCUSSION

The production of alcohol beverages is a process that involves the active participation of microorganisms, most often yeasts.

➢ Learning Objectives :

Explain why microorganisms are used for beer, wine, and sake production.

- Yeasts are the main fermenter and alcohol producer in the production of wine, beer and other alcohol drinks.
- The main yeast species used is Saccharomyces cerevisiae. It ferments the sugars, coming from different sources, e.g., grapes for wine, barley for beer, to alcohol and carbon dioxide.

- Both wild and cultivated strains are used. The species or strains used in the fermentation play an important role in giving the final taste properties of the drink.

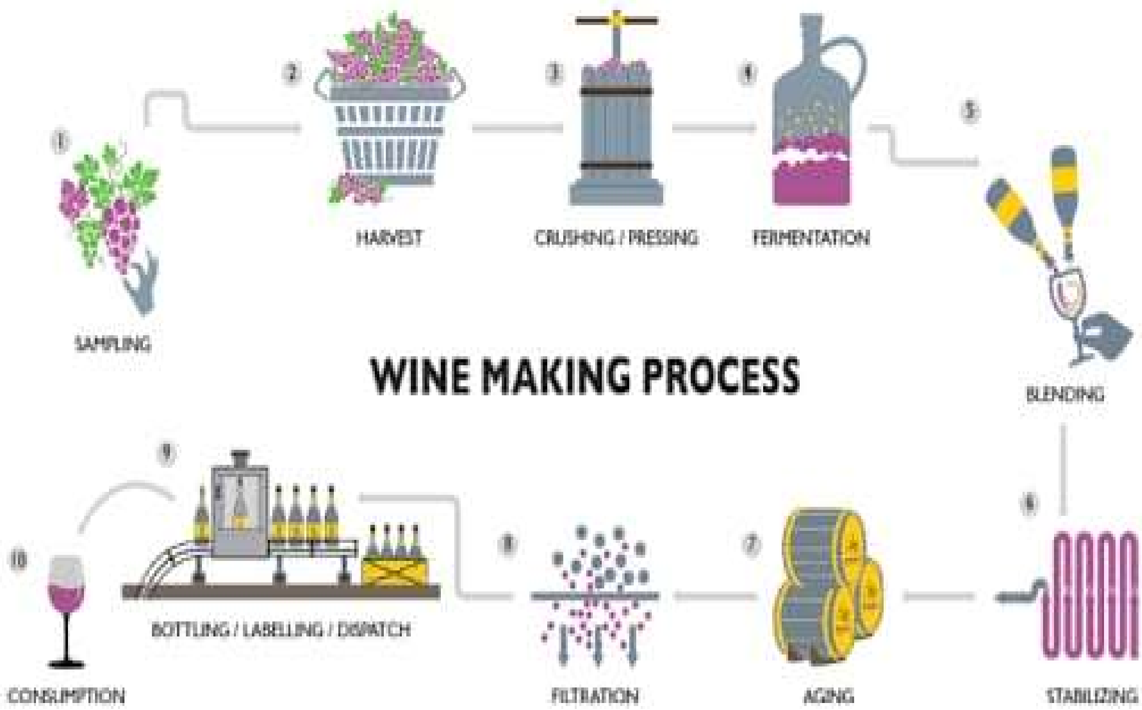
- The unfermented grape juice of crushed grapes that contains fruit, seeds and skins.

Humans have been producing alcoholic beverages for thousands of years. The production of alcohol in these drinks is based primarily on yeast fermentation. Yeasts are eukaryotic microorganisms that ferment variety of sugars from different sources into the final products of carbon dioxide and alcohol.

➢ WINE PRODUCTION

Wine is made from grapes or other fruit. The grapes are first cleaned of leaves and stems and the fruit is crushed into must that is ready for fermentation. The yeasts used for the fermentation grow a film on the fruit or in the environment. These wild strains play an important role in the final properties of the drink. However, cultivated strains of *Saccharomyces cerevisiae* are often added to improve the consistency of the final product. There are hundreds of commercially available yeast strains for wine fermentation.

Wine grapes: The white film that covers the grapes contains wild yeasts.



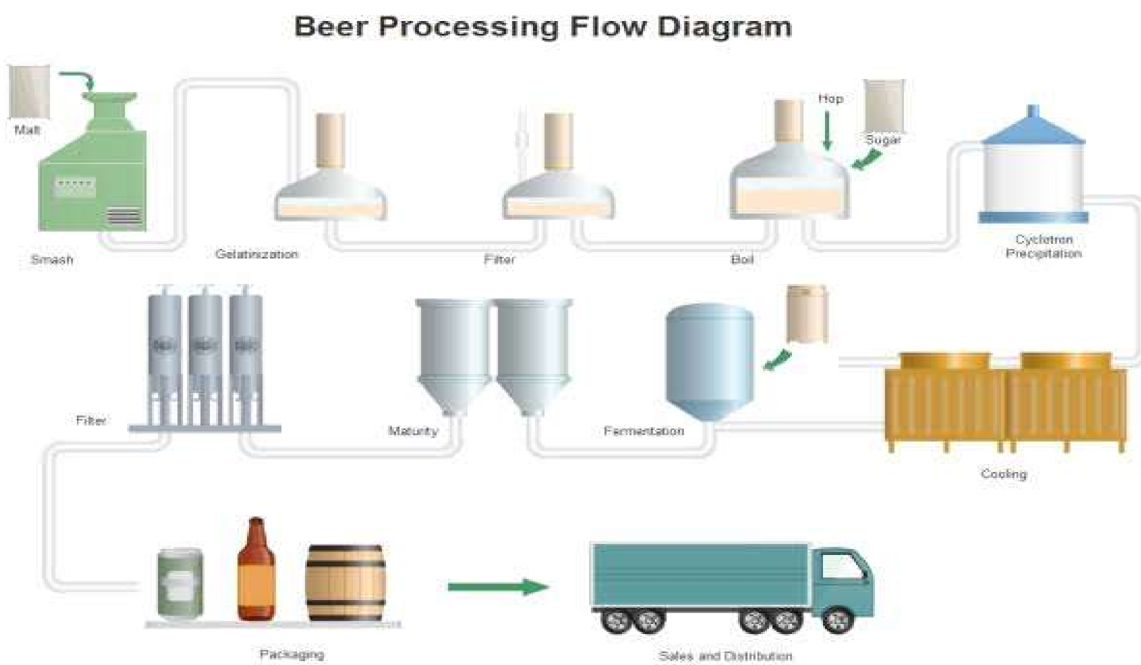
In the fermentation process, energy that is converted to heat is produced as well. It is important to keep the temperature in the fermentation vessel lower than 40ºC to keep the yeasts alive. To improve yeast growth, additional nutrients, like diammonium phosphate, are sometimes added in the fermentation step.

When making red wine, there is an additional fermentation step after alcoholic fermentation. Malic acid, naturally present in grape juice, can be converted to lactic acid by lactic acid bacteria naturally found in wineries or added artificially.

- BEER PRODUCTION

Beer is the most consumed alcoholic beverage in the world. It is made most often of malted barley and malted wheat. Sometimes a mixture of starch sources can be used, such as rice. Unmelted maize can be added to the barley or wheat to lower cost. Potatoes, millet and other foods high in starch are used in different places in the world as the primary carbohydrate source.

The process of making beer is called brewing. It includes breaking the starch in the grains into sugary liquid, called wort, and fermenting the sugars in the wort into alcohol and carbon dioxide by yeasts. Two main species are used in the fermentation process: *Saccharomyces cerevisiae* (top-fermenting, since it forms foam on top of the wort) and *Saccharomyces uvarum* (bottom-fermenting). Top-fermenting yeasts are used to produce ale, while bottom-fermenting produce lagers. The temperature used for top-fermenting (15-24ºC) leads to the production of a lot of esters and flavour products that give beer a fruity taste. Hops are added to introduce a bitter taste and to serve as a preservative.

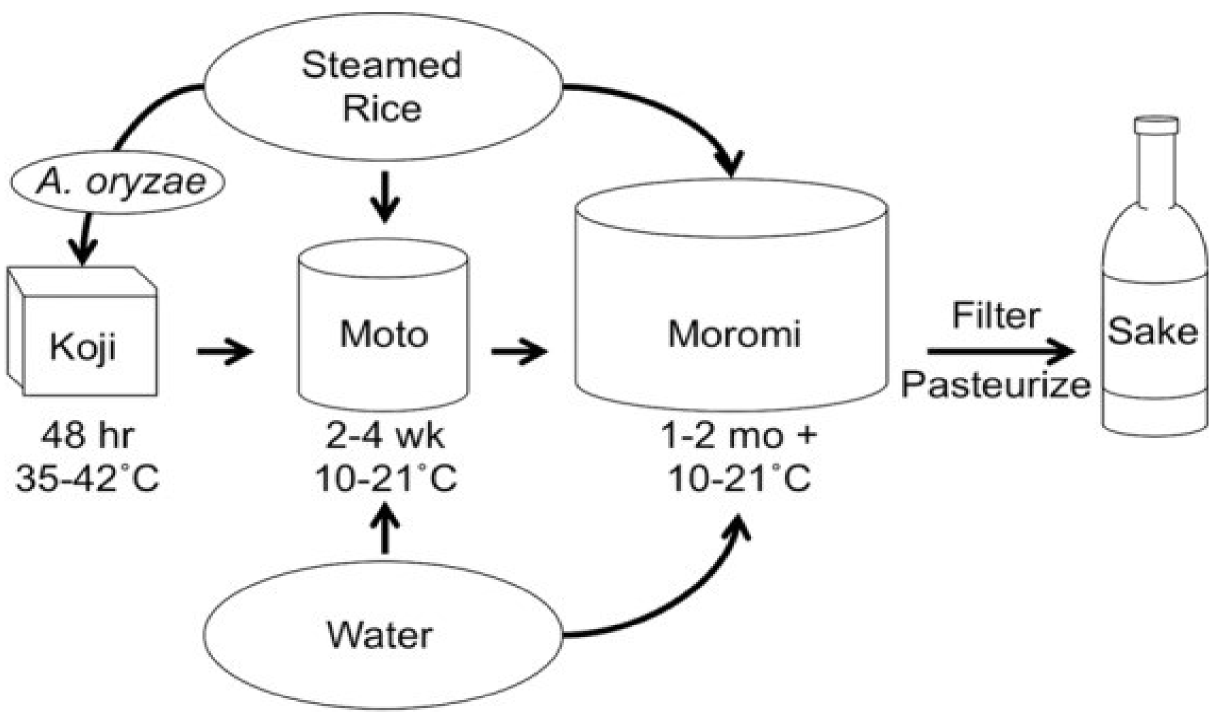


Brewer's yeasts are very rich in essential minerals and B vitamins, with the exception of vitamin B12. Beer brewing in modern days is performed by added pure cultures of the desired yeast species to the wort. Additional yeasts species that are used in making beer are *Dekkera/Brettanomyces.* After the fermentation is finished, the beer is cleared of the yeasts by precipitation or with the use of clearing additives.

Other types of alcohol beverages are made by the fermentation activity of microorganisms as well. A few examples are sake (uses the fungus *Aspergillus oryzae* to facilitate starch fermentation from rice), brandy, whiskey (both are distilled alcohol), and other alcohol beverages with higher percentage of alcohol compared to wine and beer.

- SAKE PRODUCTION

Sake is the traditional, national alcoholic beverage of Japan. Sake is produced from rice through the saccharification of starch by *Aspergillus flavus* var. oryzae and subsequent alcoholic fermentation by *Saccharomyces cerevisiae*. Sake brewing involves a serial propagation process, beginning with koji, a solid culture consisting of rice and *A. flavus* var. oryzae. Polished, steamed rice is mixed with the dried spores of *A. flavus* var. oryzae and incubated for approximately 2 days. Koji is then pitched with more steamed rice, water, and yeast into the moto (seed mash) tank, an open mashing vessel, wherein fermentation occurs for 10 to 25 days. Next, the moto is moved to a larger vessel and mixed with increasing amounts of water, rice, and koji in three additions to form moromi, the main fermentation. Moromi fermentation occurs for 20 to 30 days, after which it is pressed, filtered, and typically pasteurized to become finished sake. Originally, sake brewing was performed entirely by autochthonous microorganisms. However, as sake fermentations are conducted in open fermenters, such methods are prone to microbial contamination. Thus, most modern sake production is inoculated with pure yeast and acidified with lactic acid in the moto to inhibit the growth of undesirable organisms. In contrast, in traditional moto fermentations, the growth of undesirable bacteria and wild yeast is inhibited by several factors (low pH, high concentration of sugar and nitrite, and low temperature). In particular, lactic acid and nitrite produced by specific bacteria play an important role for inhibition of undesirable bacteria. After a decrease of undesirable microorganisms, the indigenous yeast that is suitable for sake fermentation grows spontaneously or pure culture yeast is added. This traditional method of moto process is called "kimoto."



Many studies have been conducted to reveal the microbial transitions that occur during kimoto-style sake production using culture-based techniques, but few have employed culture-independent techniques. In the early stages, bacteria (*Micrococcus*, *Escherichia*, *Pseudomonas*, *Enterobacter*, *Aerobacter*, and *Achromobacter*) and non-*Saccharomyces* yeasts (*Pichia* spp., *Candida* spp., *Zygosaccharomyces* spp.) have been detected. Among these, Gram-negative bacteria, including *Escherichia* and *Pseudomonas*, initially increase. Then, lactic acid bacteria such as *Leuconostoc mesenteroides* subsp. *sake* and *Lactobacillus sakei* grow and produce lactic acid, leading to decreased pH. In parallel with these microbial community changes, rice starches are saccharified by *A. flavus* var. oryzae amylase activity in the moto, and wild yeasts and bacteria are inhibited by the low pH, high sugar concentration, and high concentration of nitrite. Subsequently, sake yeast (*Saccharomyces cerevisiae*) increases in the moto and conducts the main alcoholic fermentation.

To improve our understanding of kimoto fermentations, we analysed the bacterial and fungal communities of koji and kimoto production in parallel with the processing environment of a North American sake brewery, using high-throughput marker gene sequencing, quantitative PCR (qPCR), and terminal restriction fragment length polymorphism (TRFLP). Results suggest that microbial transfer from the processing environment is responsible for driving microbial successions throughout sake fermentations.

➢ ROLE OF FUNGI INDUSTRY

The industrial uses of fungi are many and varied. In fact the fungi form the basis of many important industries. There are a number of industrial processes in which the biochemical activities of certain fungi are harnessed to good account.

(i) Alcoholic fermentation:

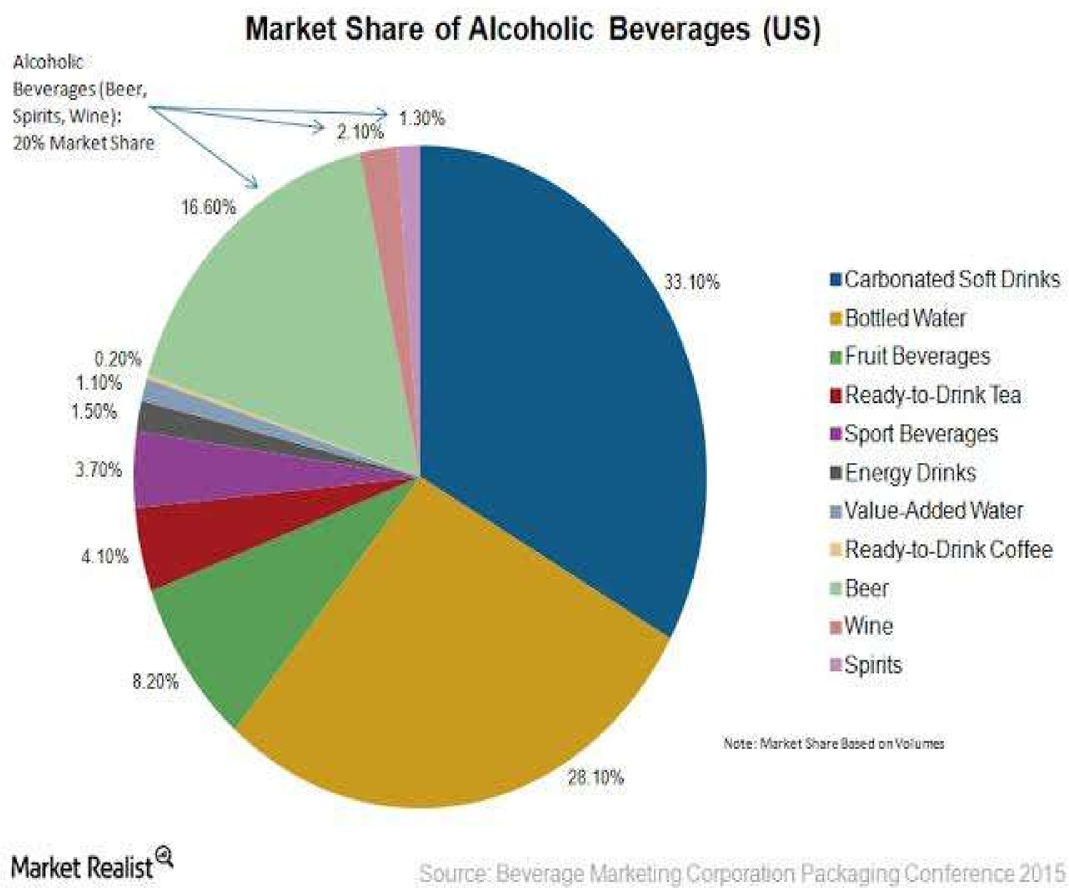
It is the basis of two important industries in India or rather all over the world. These are brewing and baking. Both are dependent on the fact that the fermentation of sugar solutions by yeasts produces ethyl alcohol and carbon dioxide. In brewing or wine making industry alcohol is the important product. The other by-product which is carbon dioxide was formerly allowed to escape as a useless thing.

The yeasts lack diastase. So they cannot break starch into sugar. There are a number of fungi popularly known as the moulds. They secrete a whole range of enzymes and thus bring about fermentation of complex carbohydrates.

In producing industrial alcohol moulds are employed as starters to bring about scarification of the starch. At the second stage yeast is employed to act on the sugar.

Although mould can complete the conversion to sugar but the yield is better if yeast is employed for the second stage. The moulds commonly used for purpose of scarification are Mucor racemosus.

M, rouxii and some species of Rhizopus. Aspergillus flavus is used in the production of African native beer.



CONCLUSION

As an organism, fungi influence our life knowingly or unknowingly.

It proves to be beneficial as it helps in maintain balance of the ecosystem by serving as an integral component in the ecological recycling.

It contributes to the economy also. However, the negative face of these organisms should also be well understood, its ability to spoil thing and cause disease to other organisms.

Through proper understanding and management few follies of the Fungi could be prevented and made to good use.

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