# Industrial Microbiology

Microbial production of one of the organic feed stocks from plant substances such as molasses is presently used for ethanol production. This alcohol was produced by fermentation in the early days but for many years by chemical means through the catalytic hydration of ethylene. In modem era, attention has been paid to the production of ethanol for chemical and fuel purposes by microbial fermentation. Ethanol is now-a-days produced by using sugar beet, potatoes, com, cassava, and sugar cane. The commercial production is carried out with Saccharomyces cerevisiae. On the other hand, uvarum has also largely been used. The Candida utilis is used for the fermentation of waste sulphite liquor since it also ferments pentoses. Recently, experimentation with Schizosaccharomyces has shown promising results. When whey from milk is used, strain of K. fragilis is recommended for the production of ethanol. It is also found that Fusarium, Bacillus and Pachysolen tannophilus (yeast) can transform pentose sugars to ethanol. Theoretically, it is interesting to note that fermentation process retains most of the energy of the sugar in the form of ethanol. The heat of combustion of solid sucrose is 5.647 MJ mol-1, the heat of combustion of glucose is 2.816 MJ $mol^{-1}$ but the heat release is 1.371 MJ mol-1.

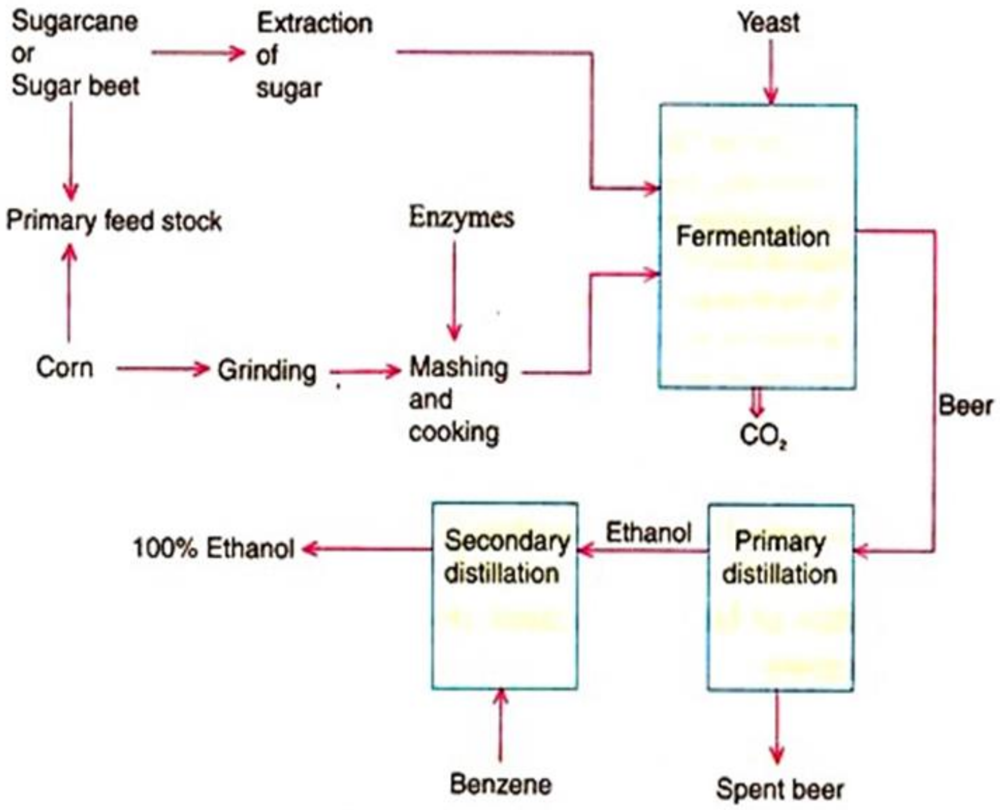



Fig. Ethanol Production From Molasses

Three types of substrates are used for ethanol production:

(a) Starch containing substrate

(b) Juice from sugarcane or molasses or sugar beet,

(c) Waste products from wood or processed wood.

(d) Production of ethanol from whey is not viable.

• If yeast strains are to be used, the starch must be hydrolysed as yeast does not contain amylases. After hydrolysis, it is supplemented with celluloses of microbial origin so as to obtain reducing sugars. About 1 ton of starch required 1 litre of amylases and 3.5 litre of glucoamylases. Following steps are involved in conversion of starch into ethanol.

• On the other hand, if molasses are used for ethanol production, the bagasse can also give ethanol after fermentation.

• Several other non-conventional sources of energy such as aquatic plant biomass, wood after hydrolysis with celluloses gives ethanol.

• Sulphite waste-liquor, a waste left after production of paper, also contains hexose as well as pentose sugar. The former can be microbially easily converted.

2. Fermentation:

• Ethanol is produced by continuous fermentation. Hence, large fermenters are used for continuous manufacturing of ethanol. The process varies from one country to another. India, Brazil, Germany, Denmark have their own technology for ethanol production.

• The fermentation conditions are almost similar (pH 5, temperature 35°C) but the cultures and culture conditions are different. The fermentation is normally carried out for several days but within 12h starts production.

• After the fermentation is over, the cells are separated to get biomass of yeast cells which are used as single cell protein (SCP) for animal's feed. The culture medium or supernatant is processed for recovery of ethanol (Fig. 20.6).

• Ethanol is also produced by batch fermentation as no significant difference is found both in batch and continuous fermentation.

• Although as stated earlier within 12h Saccharomyces cerevisiae starts producing ethanol at the rate of 10% (v/v) with 10-20g cells dry weight/lit. The reduction in fermentation time is accomplished use of cell recycling continuously in fermentation.

**3. Recovery**:

Ethanol can be recovered upto 95% by successive distillations. To obtain 100%, it requires to form an azeotropic mixture containing 5% water. Thus 5% water is removed from azeotropic mixture of ethanol, water and benzene after distillation. In this procedure, benzene water ethanol and then ethanol-benzene azeotropic mixture are removed so that absolute alcohol is obtained.

**General procedure for production of ethanol from Sugarcane**

Regardless of whether the production is done in a mass quantity or a backyard, the basic steps for making ethanol are the same;

1. Procuring the grain or plant

2. Converting this to sugar

3. Fermentation

4. Distillation

• On industrial scale, ethanol is produced by the fermentation of molasses. Molasses is the mother liquor left after the crystallization of sugarcane juice. It is a dark colored viscous liquid. Molasses contains about 60% fermentable sugar.

1) Dilution of molasses

Molasses is first diluted with water in 1:5 (molasses: water) ratio by volume Addition of

• 2) Ammonium sulphate. If nitrogen content of molasses is less, it is fortified with ammonium sulphate to provide adequate supply of nitrogen to yeast.

3) Addition of sulphuric acid Fortified solution of molasses is then acidifies with small quantity of sulphuric acid. Addition of acid favours the growth of yeast but unfavours the growth of useless bacteria

• 4) Fermentation The resulting solution is received in a large tank and yeast is added to it at 35°C and kept for 2 to 3 days. During this period, enzymes sucrose and zymase which are present in yeast, convert sugar into ethyl alcohol

$C_{12}H_{22}O_{11}$ + H2O      C6H12O6 + C6H12O6

C6H12O6      C2H5OH + 2CO2

5. Fractional distillation Alcohol obtained by the fermentation is called "wash" which is about 15% to 18% pure. By using fractional distillation technique, it is converted into 92% pure alcohol which is known as rectified spirit or commercial alcohol. Production of ethanol from

various feed stocks involves the following steps. I) Feed preparation 2) fermentation 3) distillation 4) dehydration and 5) denaturing.

i) Feed preparation The first step in making ethanol is to prepare the feedstock to enter the fermentation process. Cereal grains, such as corn, rye, rice, barley, soybeans, wheat, and plants like sugar cane are the major sources of feedstock's of fermentation. Some producers use high starch plants such as potatoes. Many different methods are used to prepare the feedstock to enter the fermentation process. All of the different processes ultimately produce a liquid solution that contains fermentable sugars. These solutions are clarified and heated to high temperature for 20 to 30 minutes to reduce the bacterial levels which can harm the performance of the process. After this treatment the liquid mixture is removed and subjected to fermentation process. If sugar cane is used as a feedstock, the liquid mixture is said to be sugarcane juice or molasses.

ii) Fermentation of sugars The liquid mixture obtained in the above process is subjected to fermentation process by adding yeast cells. Zymase, an enzyme from yeast, changes the simple sugars into ethanol and carbon dioxide. The enzymatic reaction carried over by the yeast in fermentation produces mainly ethanol, CO2 and heat. The fermentation reaction is actually very complex and the resulting product is similar to beer or wine. The impure culture of yeast Produces varying amounts of other substances, including glycerin, methanol and various organic acids. After fermentation, the liquid is subjected to distillation to separate alcohol from water.

iii) Distillation

Ethanol produced by fermentation ranges in concentration from a few percent up to about 14 percent balance is being water and other components. The boiling point of ethanol (78.4'C) is slightly lower than the boiling point of water (100°C). Since the difference in the boiling point of these materials is low they cannot be completely separated by distillation. Instead, an azeotropic mixture (i.e. a mixture of 96%ethanol and 4% water) is obtained. Azeotropic mixture of alcohol cannot be further concentrated by distillation. Distillation is used to produce Rectified Spirit (RS, 94%v/v ethanol).

iv) Dehydration of Alcohol Pure alcohol can be obtained from distillation since it forms azeotrope with water at 96% (vlv). Ethanol or absolute alcohol is produced by dehydration of rectified spirit. Commercially available technologies for dehydration of rectified spirit are a) Azeotropic distillation and b) Molecular Sieve Technology.

**BEER BREWING PROCESS**

The term **beer** is given to non-distilled alcoholic beverages produce from partially germinated cereal grains, known as malt. They include ales, lagers and stouts, which normally contain 3–8% (v/v) **ethanol**. Their other main ingredients are hops (giving beer a characteristic flavour and aroma), water and yeast.

| Substances | Concentration | Number of compounds |
| --- | --- | --- |
| Water | 90–94% | 1 |
| Ethanol | 3–5% v/v | 1 |
| Carbohydrates | 1–6% w/v | ∼100 |
| Carbon dioxide | 3.5–4.5 g/l | |

**The 5 essential beer ingredients**

- Water. It makes up 90% of the volume of a beer and it has a huge influence on the flavor. ...
- Malts. Malts are the sugar needed in the brewing process. ...
- Hops. It's called the "balancing agent" because hops add bitterness, counterbalancing the sweetness of the malt. ...
- Yeast. ...
- Fruit puree.

**2. Malts**

Malts are the sugar needed in the brewing process. Of all **beer ingredients**, this one is of the utmost importance. **(See also: Microbrewery )** Why? Because malts define the color, smell, taste, and head of the beer, and they come in different forms:

- Barley: It's the most common one around the world. The starch-protein ratio makes it perfect for the mashing process and the durable husks function as a natural filter during cleaning.
- Wheat: It's a soft malt with a slightly sour taste. The high content of protein results in a hazier liquid and thicker head on the beer.
- Oats: They have a smooth yet full mouthfeel. They are traditionally used in stouts, but brewers also experiment with other styles.
- Corn and rice: These are the cheapest source of starch, frequently used by big companies.

**3. Hops**

It's called the "balancing agent" because hops add bitterness, counterbalancing the sweetness of the malt. When you decide to incorporate it into the mix, it is decisive to the flavor. If you throw it in early, your beer will be more bitter than if you add it later in the process. For homebrewers, this is one of the beer ingredients that works as a natural preservative, so no other preservative is needed.

There was a time when bitterness was considered a bad trait until IPAs flooded the market. So, for the last 15 years, brewers have been using more hops to get the desired hoppiness flavor in their beer. The bitterness is measured in International Bitterness Units (IBUs).

**4. Yeast**

It's a microorganism that converts sugar to alcohol. Typically, isolated or cultivated yeasts are used, which guarantee producers the same cells in the batches and more control over fermentation. There are two types of yeast in **beer ingredients**.

- Bottom fermented: Works at lower temperatures (40-50ºF) and stays at the bottom of the tank during fermentation. The result is a clear drink with strong notes of malt and hops such as lager beers.
- Top fermented: Works at higher temperatures (60-75ºF), producing more fruity aromas that can be found in beers such as ales.

**5. Fruit puree**

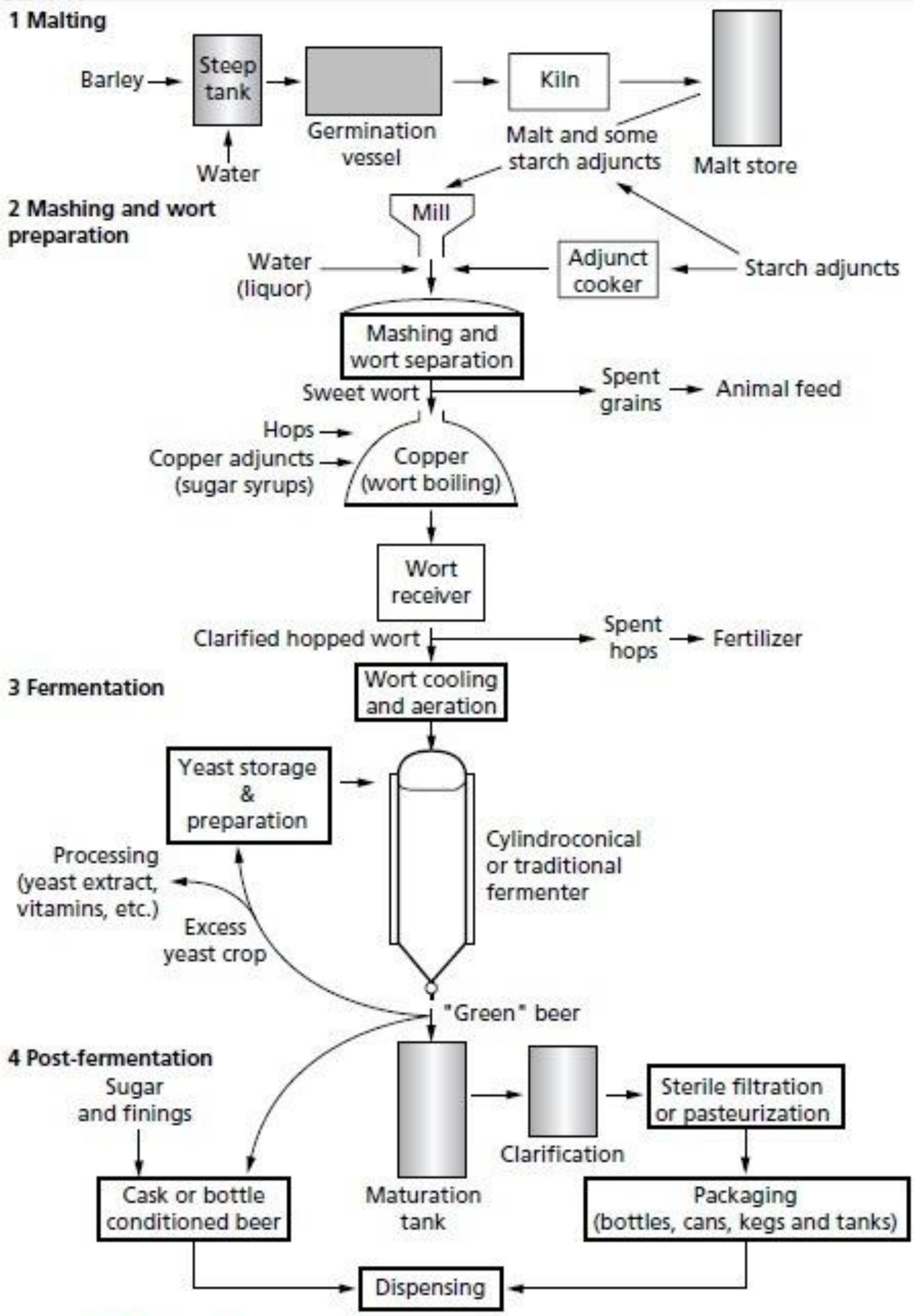
Among flavored beers, the fruity kind has conquered the market. The options are endless, but you have to be aware of the acidity and sugar content of the fruit. The base brew should be light in hops (under 20 IBUs), so it doesn't overpower the sweetness. The moment to add it will depend on the taste you are looking for. When using fruit puree as one of the beer ingredients, there are two options:

1. After the boil but before the wort gets cooled down. Since you are not using fresh products, there is no risk of contamination.
2. During the secondary fermentation while the beer is racked. The mix needs to sit for a week and then be strained to remove any residual solids.

Spices, herbs, coffee, chocolate, and so many other beer ingredients can be added to the mix. But the ones listed above are the core elements to making a standard beer. If you want to try them all, a fun session of bar hopping will do the trick.

THE BREWING PROCESS IS ESSENTIALLY DIVIDED INTO FOUR MAIN STAGES:-

1. **Malting**
2. **Mashing and wort preparation**
3. **Yeast fermentation**
4. **Post-fermentation**




## The beer brewing process

**Malting** is the partial germination of cereal grain for 6–9 days to form malt. This is the primary beer ingredient and contains mostly starch, some protein and hydrolytic enzymes. Malted barley is predominantly used, but beers are also made with malted wheat, occasionally malted oats and even malted sorghum.

**Mashing and wort preparation** involves the production of the aqueous **fermentation** medium, otherwise known as wort. It contains fermentable sugars, amino acids and other nutrients, and is prepared by solubilizing malt components through the action of endogenous hydrolytic enzymes.

A proportion of adjuncts are now also added, which are unmalted cereal and non-cereal starch sources, and sugar syrups. The resulting liquid wort is then ‘sterilized’ by boiling.

At the same time hops are added to impart their bitter flavour and characteristic aroma. Overall, wort preparation takes approximately 5–8 h.

**Yeast fermentation** is a non-aseptic batch process that uses a starter culture of a selected brewing strain of *S.cerevisiae*. The inoculated wort undergoes an alcoholic fermentation to produce ethanol, $CO_2$ and minor metabolites that contribute to flavour and aroma. Fermentations usually last for 2–7 days depending upon the type of beer being produced.

**Post-fermentation** treatments are conducted to mature or condition the new beer to make it ready for consumption, which may take from one to several weeks.

In beer brewing, ethanol production is rather less crucial, as development of flavour profile and other quality factors is equally important.

Traditionally, all necessary enzyme activities for the process were provided by the malt, which generates the wort, along with yeast enzymes, that convert wort components to ethanol, $CO_2$ and flavour compounds.

However, commercial enzymes are now employed when raw materials are enzyme deficient, or to produce novel products, or act as aids in processing and in product stabilization.

## Source: Online