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Paper: Industrial and Environmental Microbiology

Unit 3: Microbial production of industrial products

Topics: **Ethanol production**

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Alcohol (Ethanol) Production

Ethanol:

- Ethanol (ethyl alcohol, EtOH) is a clear, colourless liquid with a characteristic, pleasant odour. Ethyl alcohol is the intoxicating component in beer, wine and other alcoholic beverages.
- In dilute aqueous solution, it has a somewhat sweet flavor, but in more concentrated solutions it has a burning taste.
- It is also being used as a biofuel in several countries across the world.
- Large industrial plants are the primary sources of ethanol production, though some people have chosen to produce their own ethanol.
- Ethanol production from agricultural products has been in practice for more than 100 years. Ethanol **can** be produced from many kinds of raw materials that contain starch, sugar or cellulose etc.
- In general there are three groups of raw materials from which ethanol can be produced:

  1) beet, sugar cane, sweet sorghum and fruits

  2) starchy material such as corn, milo, wheat, rice, potatoes, cassava, sweet potatoes etc.

  3) cellulose materials like wood, used paper, crop residues etc.
- The third group of materials mostly include biomass. Recently, biomass is being considered as an important biological resource for the production of ethanol.

Alcohol (Ethanol) Production

Uses of Ethanol:

(i) ***Use as a chemical feed stock*** : In the chemical industry, ethanol is an intermediate in many chemical processes because of its great reactivity. It is thus a very important chemical feed stock.

(ii) ***Solvent use*** : Ethanol is widely used in industry as a solvent for dyes, oils, waxes, explosives, cosmetics etc.

(iii) ***General utility*** : Alcohol is used as a disinfectant in hospitals, for cleaning and lighting in the home, and in the laboratory second only to water as a solvent.

(iv) ***Fuel*** : Ethanol is mixed with petrol or gasoline upto 10% and known as gasohol and used in automobiles.

Manufacture of Ethanol

- Ethanol may be produced by either synthetic chemical method or by fermentation.
- Fermentation was until about 1930 the main means of alcohol production.
- In 1939, for example 75% of the ethanol produced in the US was by fermentation, in 1968 over 90% was made by synthesis from catalytic hydration of ethylene.
- Due to the increase in price of crude petroleum, the source of ethylene used for alcohol production, attention has turned worldwide to the production of alcohol by fermentation.

Alcohol (Ethanol) Production

Microbial production of Ethanol:

- Microbial production of ethanol from the organic feed stocks and from plant substances such as molasses is presently used for ethanol production.
- 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 (Fig. 20.6).

Alcohol (Ethanol) Production

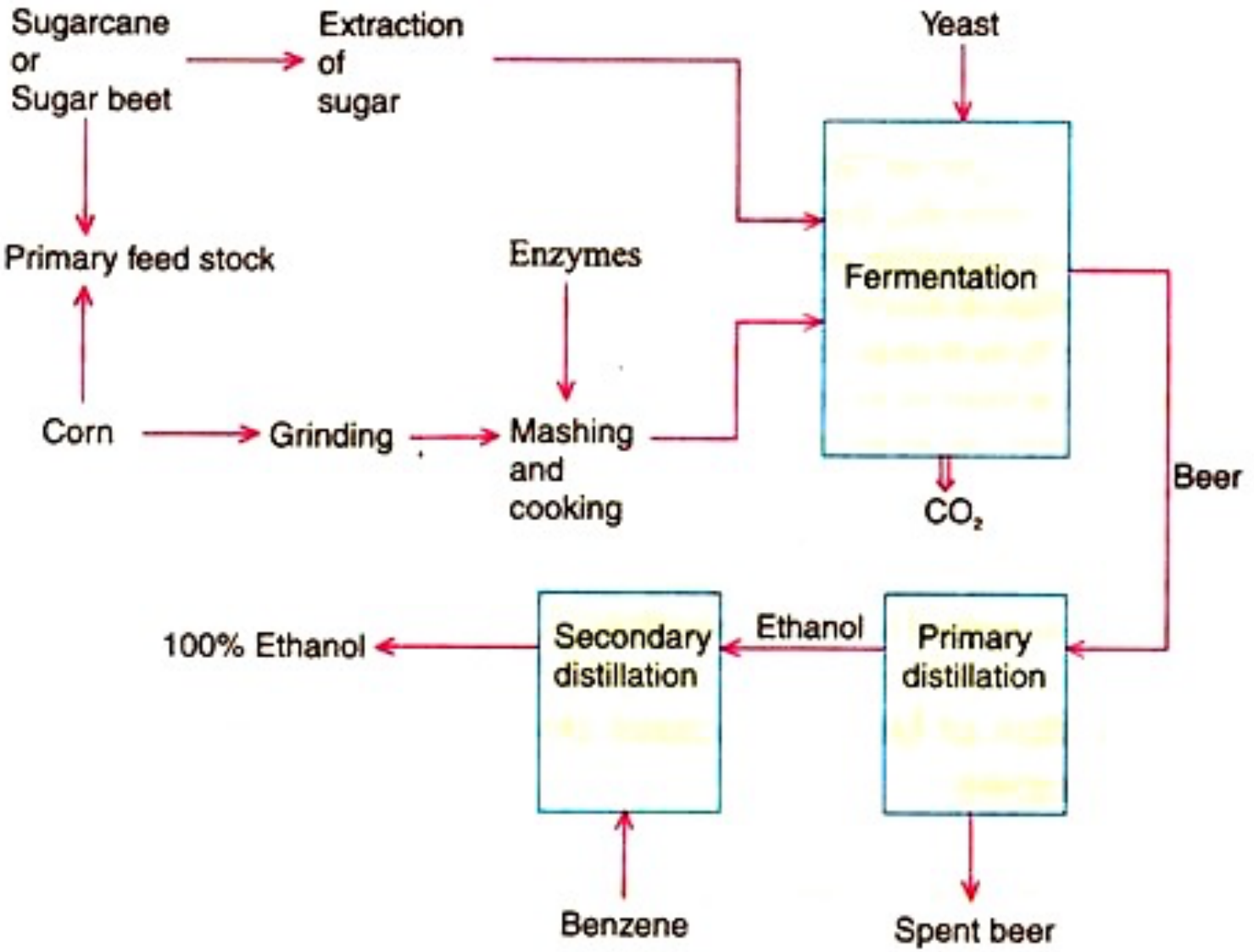

Fig. 20.6: Ethanol production from molasses.

Alcohol (Ethanol) Production

- Both yeasts (Saccharomyces cerevisiae, S. uvarum S. carlsbergensis, Candida brassicae, C. utilis, Kluyveromyces fragilis, K. lactis) and bacteria (Zymomonas mobilis) have been employed for ethanol production in industries.
- The commercial production is carried out with Saccharomyces cerevisiae. On the other hand, S. 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.
- It is noteworthy that the ethanol at high concentration inhibits the yeast. Hence, the concentration of ethanol reduces the yeast growth rate which affect the biosynthesis of ethanol.
- The bacteria Zymomonas mobilis has a merit over yeast that it has osmotic tolerance to higher sugar concentration. It is relatively having high tolerance to ethanol and have more specific growth rate.

Alcohol (Ethanol) Production

1. Preparation of Medium:

- **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 (Fig. 20.7).
- 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.

Alcohol (Ethanol) Production

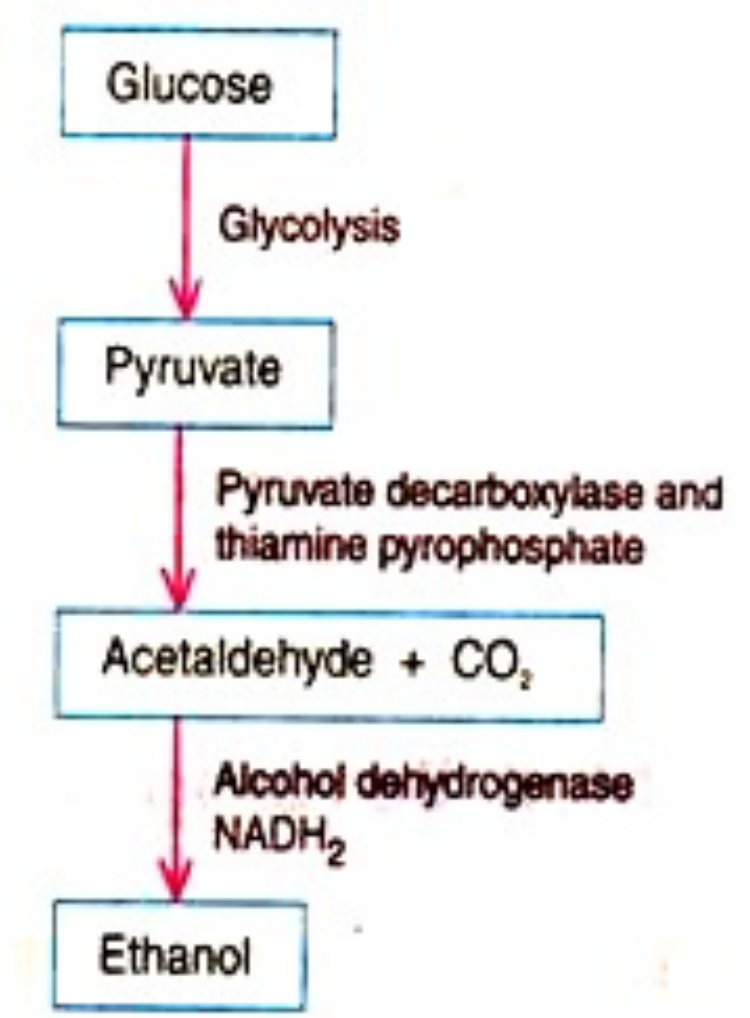

Fig. 20.7 : Biosynthesis of ethanol.

Alcohol (Ethanol) Production

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.

Alcohol (Ethanol) Production

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.

Alcohol (Ethanol) Production

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.

Alcohol (Ethanol) Production

- **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

$$C_{12}H_{22}O_{11} + H_2O \rightarrow C_6H_{12}O_6 + C_6H_{12}O_6$$
$$C_6H_{12}O_6 \rightarrow C_2H_5OH + 2CO_2$$

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, $\mathbf{CO_2}$ 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.

Source:

- Modern Industrial Microbiology and Biotechnology, Nduka Okafor, Science Publishers, p: 373-377.
- Ethanol Production (Shodhganga) p: 1-28
- http://www.biologydiscussion.com/industrial-microbiology-2/fermentation-industrial-microbiology-2/production-of-ethanol-microbiology/66072.