Microbial production of Ethanol

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Ethanol production

- Ethanol (Ethyl alcohol) CH_3CH_2OH may be produced by either synthetic chemical method or by fermentation.
- Ethanol (also called bioethanol) is produced by fermentation from a glucose or sucrose rich medium, the production of alcohol occurs best in the absence of oxygen.
- The most common ethanologenic microorganisms are yeasts, which include *Saccharomyces cerevisiae*, *Schizosaccharomyces* spp., *Candida* spp., *Kluyveromyces lactis*, *Pichia* spp., etc. Bacteria such as *Zymomonas mobilis*, *Clostridium sporogenes*, and *Leuconostoc mesenteroides* are also involved in alcoholic fermentation.
- The yeast involved in these alcoholic fermentation is mostly strains of *Saccharomyces cerevisiae*, which cannot directly ferment starch.
- Ethanol production from agricultural products has been in practice for more than 100 years.
- 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.

Uses of Ethanol

- **Chemical feed stock**: In the chemical industry, ethanol is an intermediate in many chemical processes.
- **Solvent use**: Ethanol is widely used in industry as a solvent for dyes, oils, waxes, cosmetics etc.
- **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.
- **Fuel**: Ethanol is mixed with petrol or gasoline up to 10% and known as gasohol.

Biochemistry of Ethanol Production

- The process starts with the breakdown of sugars into pyruvic acid via the Embden Meyerhof-Parnas (EMP) pathway, and then converted into acetaldehyde under anaerobic conditions through the enzyme pyruvate decarboxylase.
- Acetaldehyde further releases two molecules of carbon dioxide and forms ethanol by alcohol dehydrogenase.
- Yeast grows rapidly in a glucose- or sucrose-rich medium, with a doubling time of about 60 minutes.
- High growth rates result in O_2 depletion, and the neutral reaction products allow growth to be continued anaerobic mode.

Alcohol (Ethanol) Fermentation

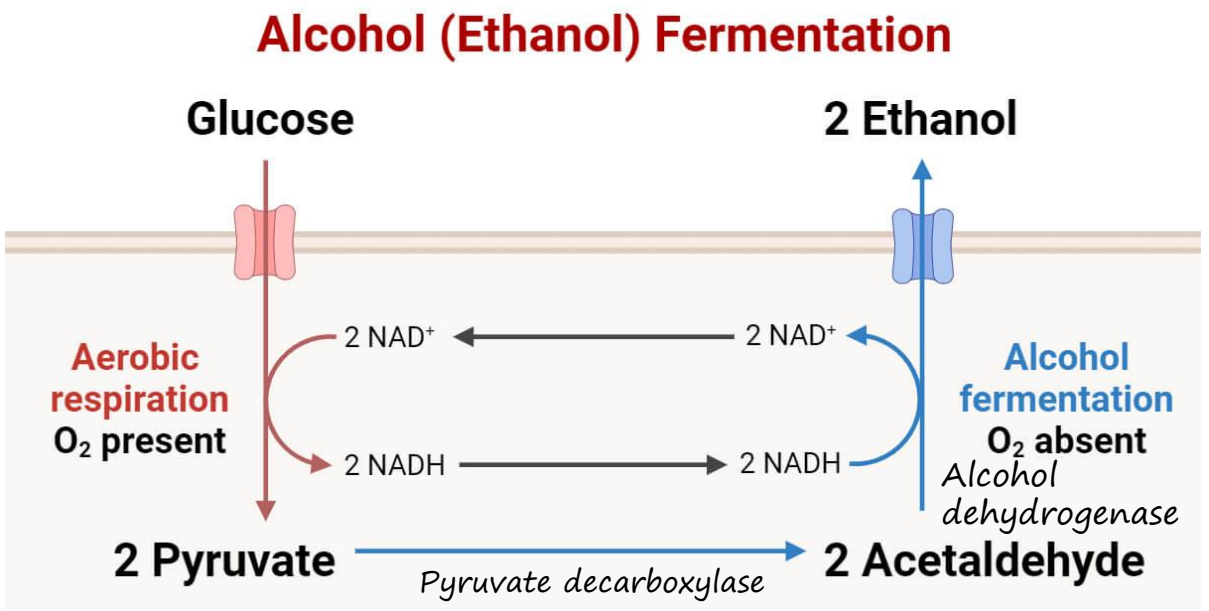

Figure 1.

Microorganisms Used for fermentation

- 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.

Ethanol Production process:

- Microbial production of ethanol from the organic feed stocks and from plant substances such as molasses is presently used for ethanol production.
- Ethanol is now-a-days produced by using sugar beet, potatoes, com, cassava, and sugar cane.
- 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.

...Ethanol Production

- If yeast strains are to be used, the starch must be hydrolysed as yeast does not contain amylases.
- About 1 ton of starch required 1 litre of amylases and 3.5 litre of glucoamylases.
- 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.

...Ethanol Production

- Regardless of whether the production is done in a mass quantity or a backyard, the basic steps for making ethanol are the same;
  - Procuring the grain or plant
  - Converting this to sugar
  - Fermentation
  - 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.
- 2) Addition of 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

...Ethanol Production

- 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.
- 5. Fractional distillation: Alcohol obtained by the fermentation is called "wash" which is about 15% to 18% pure.
- 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).

...Ethanol Production

- 6. 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.
- The remaining flow from the distillation column, known as vinasse, or stillage can be volatilized to produce co-products which may include process steam and electricity, products for feeding animals, stillage of moderate concentration used as fertilizer, and other valuable by-products.

Feed Preparation

- Cereal grains, such as corn, rye, rice, barley, soybeans, wheat, and plants like sugar cane are the major sources of feedstock's of fermentation.
- Sugars (e.g., from sugarcanes, molasses, sugar beet, and fruits) can be directly fermented using yeast to produce ethanol.
- It should be noted that if sugar materials such as molasses and sugarcane juice are used for fermentation, then processes like milling, pretreatment, hydrolysis, and detoxification are not necessary.
- For the production of fermentable sugar from starchy materials, processes like milling, liquefaction, and saccharification are used.
- Commercial enzyme cocktails used directly or a defined mixture of α-amylase, β-amylase, glucoamylase, cellulases, xylanases and pullunases of various origins used are more effective for FW saccharification.
- Sugar can be directly extracted from sugarcane, and the residual bagasse is used as a boiler fuel to provide much of the energy for the extraction and ethanol production and recovery operations.
- In a corn dry mill, corn is ground, and enzymes and heat are added to hydrolyze starch to sugars for conversion to ethanol, while the oil, protein, and fiber in corn are recovered after fermentation as an animal feed known as distillers dried grains with solubles (DDGS).
- Wet mills first fractionate corn to separate corn oil, corn gluten meal (CGM), and corn gluten feed (CGF) to capture value for food and animal feed, and the starch can then be hydrolyzed to sugars for fermentation to ethanol.
- For cellulosic biomass, heat and acids or enzymes hydrolyze the hemicellulose and cellulose portions to release sugars that can be fermented to ethanol, and the lignin and other remaining fractions can be burned to provide all the process heat and electricity for the conversion step with excess electricity left to export.
- 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 mins 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.

Schematic representation of production of ethanol from cane sugar, corn, and cellulosic biomass