ETHANOL

Microbial production of industrial products (micro-organisms involved, media, fermentation conditions, downstream processing and uses).

ETHANOL-PRODUCING MICROORGANISMS

In the production of fermentation ethanol, the microorganism being employed should possess a number of important characteristics:

(1) Rapid and relevant carbohydrate fermentation ability.

(2) Appropriate flocculation and sedimentation characteristics.

(3) Genetic stability.

(4) Osmotolerance (i.e., The ability to ferment concentrated carbohydrate solutions).

(5) Ethanol tolerance and the ability to produce elevated concentrations of ethanol.

(6) High cell viability for repeated recycling.

(7) Temperature tolerance.

Microorganisms Involved

Microorganisms

Certain yeasts and bacteria are employed for alcohol fermentation. The type of the organism chosen mostly depends on the nature of the substrate used. Among the yeasts, ***Saccharomyces cerevisiae*** is the most commonly used, while among the bacteria, ***Zymomonas mobilis*** is the most frequently employed for alcohol production.

Microorganism	*Source of carbohydrate*
Yeasts	
Saccharomyces cerevisiae	Starch, sugar
S. ellipsoideus	Starch, sugar
Kluyveromyces fragilis	Starch, sugar
Bacteria	
Zymomonas mobilis	Starch
Candida pseudotropicalis	Lactose, whey
C. utilis	Sulfite waste liquor

Media

Media (Raw materials)

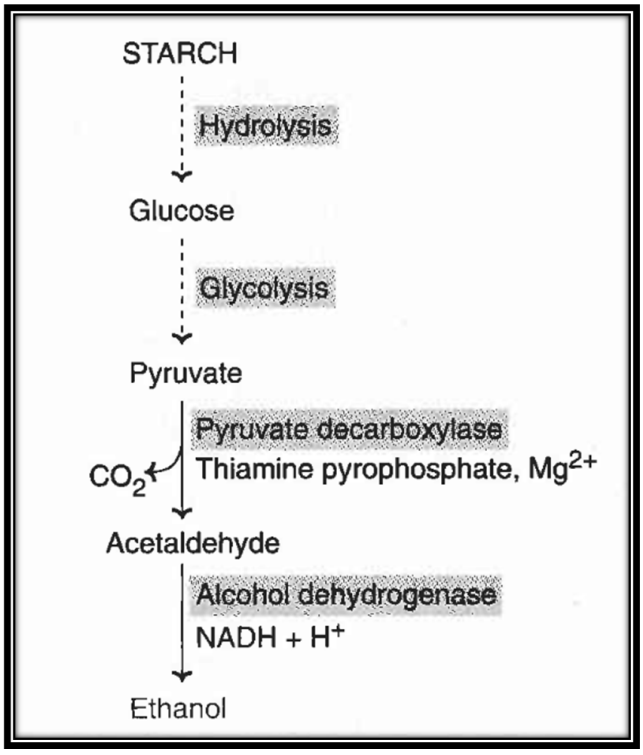
There are a large number of raw materials that can serve as substrate for alcohol fermentation. They may be broadly categorized as sugary materials (e.g., molasses, whey, glucose, sucrose) starchy materials (wheat, rice, maize, potato) and cellulosic materials (wood, agricultural wastes).

Sugary materials	*Starchy materials*	*Cellulosic materials*
Molasses	**Cereals**	Wood
Sugar cane	Wheat	Saw dust
Sugar beet	Maize	Agricultural wastes
Sweet potato	Barley	Paper wastes
Sweet sorghum	Sorghum	Municipal solid wastes
Whey	Corn	
Sulfite waste	Rice	
Sucrose	**Starchy roots**	
Lactose	Potato	
Glucose	Tapioca	
	Milled products	
	Wheat flour	
	Corn feed	

Pretreatment of Raw Materials

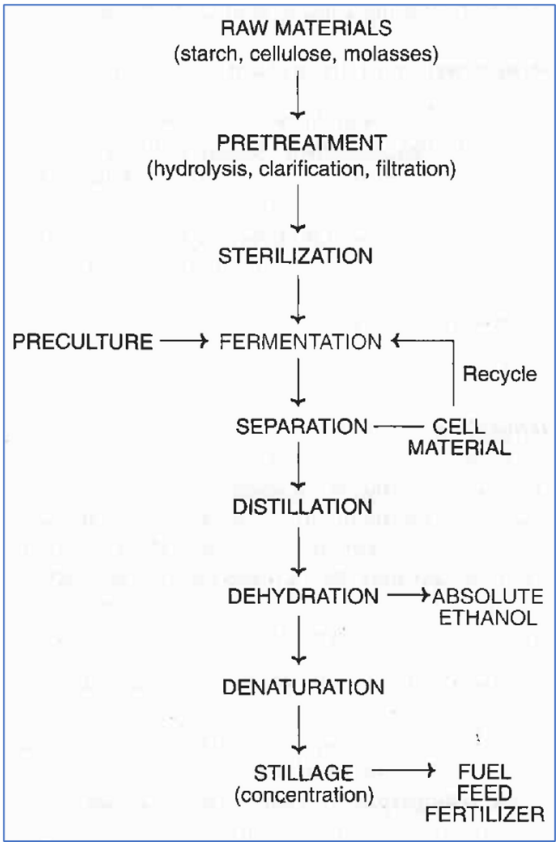
Pretreatment of raw materials:

Most of the raw materials of alcohol fermentation require some degree of pretreatment. The actual process depends on the chemical composition of the raw material. In general, the sugary raw materials require mild or no pretreatment while the cellulosic materials need extensive pretreatment. This is because the cellulosic substances have to be subjected to acidic or enzyme hydrolysis to release monosaccharide units that are needed for alcohol production.



Biosynthesis of Ethanol

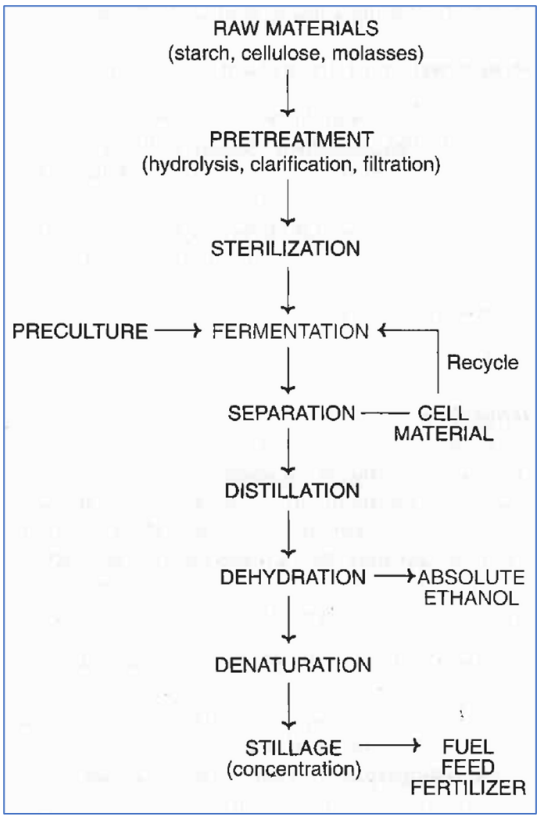
- Glucose gets broken down to pyruvate by glycolysis Under anaerobic conditions (i.e. in the absence of O_2), pyruvate is converted to acetaldehyde by the enzyme pyruvate decarboxylase Acetaldehyde is then reduced by alcohol dehydrogenase to form ethanol.
- Under aerobic conditions (adequate O_2 supply) with excess glucose content in the medium, the microorganisms (e.g., *S. cerevisiae*) grow well without producing alcohol.
- Under anaerobic conditions, growth slows down and alcohol production occurs.



Regulation of synthesis:

- Ethanol at high concentration in the medium inhibits its own biosynthesis.
- This is particularly observed when yeasts are the fermentation organisms.
- It is generally seen that growth of yeasts ceases at 5% ethanol concentration (volume/volume in water).
- Yeasts are sensitive to inhibition by endogenously synthesized ethanol and not to the ethanol added to the medium.
- Some biotechnologists prefer to use the bacterium ***Zymononas mobilis***, since it can tolerate a high concentration of alcohol (up to 13% by some strains against 5% by yeasts.

Production Process of Ethanol:



Ethanol production can be carried out in three stages-preparation of nutrient solution and inoculum, fermentation proper and recovery.

Preparation of nutrient solution medial.

- The most commonly used raw materials are molasses, whey, grains, potatoes and wood wastes.
- Where molasses is used for fermentation, it is diluted with water so that the sugar concentration is in the, range of 10-18%. A concentration higher than this is detrimental to the yeast.
- Where starchy materials (corn, barely) are used, they have to be first hydrolysed by pretreatment for use as nutrients.
- This may be done by barley malt, dilute acids or fungal amylases (e.g. ***Aspergillus* sp, *Rhizopus* sp**).
- The pH of the medium is adjusted to 4-5.
- The initial temperature is kept between 21-26°C.

Production Process of Ethanol:

Preparation of inoculum

- After selection of the desired organism (yeast or bacteria) and its isolation in pure form, the inoculum is prepared under aseptic conditions.
- For this purpose, the organisms are first cultured in flasks under aerobic conditions to increase the size of the inoculum which can be used for inoculation.

Fermentation proper

- Batch fermentation process was originally adopted in Brazil.
- There are several advantages of continuous fermentation. These include the retention of the fermenting organisms in the bioreactor" by separation and recycling, and the continuous evaporation of fermentation broth.
- It has been possible to increase alcohol production by 10-12 fold by continuous fermentation compared to conventional batch fermentation.

Production Process of Ethanol:

Recovery of ethanol

- The cell mass is separated by centrifugation or sedimentation.
- Ethanol from the fermentation broth can be recovered by successive distillations. By these processes, it is easy to obtain ethanol of around 95%.
- For a concentration above 95%, special techniques of distillation have to be adopted. For preparation of absolute (100%) alcohol, an azeotropic mixture of benzene, water and alcohol is first prepared.
- This mixture is then distilled by gradually increasing the temperature.
- By this technique, it is possible to first remove benzene ethanol- water mixture, and then ethanol-benzene mixture. Thus, absolute alcohol is left out.

Production Process of Ethanol:

Stillages in alcohol production

Large volumes of wastes which are technically referred to stillages are formed during the course of alcohol fermentation. Attempts are made to fruitfully utilize stillages for various purposes.

- To use as feed or fertilizers.
- For converting to single-cell protein.
- To use a fuel.
- For production of methanol.

Glucose to alcohol-conversion profile:

Theoretically, one gram of glucose can be converted to 0.511grams of ethanol. ln fact, a conversion yield of 95% was observed when pure substrates (glucose, lactose, sucrose) are used. For industrial grade raw materials (corn starch) the yield is around 90%. It is estimated that for 100 g pure glucose, 48.5 g of ethanol is produced, along with 46.5 g of CO_2, 3.3 g glycerol and 1.3 g of biomass (yeast cells).

The uses of ethanol

The uses of ethanol can be divided into a number of categories:

(1) Potable ethanol in beer, wine, sake, cider, and Perry, a variety of fermented fruit juices, and in distilled beverages such as whiskey, gin, vodka, brandy, rum, and liquors.

(2) Solvent ethanol in the laboratory, in pharmaceutical preparations such as tonics and cough syrups, as a solvent for hop constituents, and in cosmetics.

(3) As a cosurfactant in oil-water microemulsions.

(4) As an antiseptic and sterilant .

(5) As a fuel in automobiles either on its own or more usually admixed with gasoline.