Ethanol production:

Ethanol (Ethyl alcohol) CH3 CH2 OH may be produced by either synthetic chemical method or by fermentation. The yeasts involved in these alcoholic fermentations are mostly strains of *Saccharomyces cerevisiae*, which cannot directly ferment starch. They require prior hydrolysis of the polysaccharide to simple sugars and small dextrins (not greater than three glucose units). Traditionally, this is achieved by using fungal or plant amylases. These enzymes may be inherent elements of the carbohydrate source or added during processing.

Uses of Ethanol

(1) *Use as a chemical feed stock*: In the chemical industry, ethanol is an intermediate in many chemical processes .

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

(3) *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.

(4) *Fuel*: Ethanol is mixed with petrol or gasoline up to 10% and known as gasohol.

Principles in the Ethanol Production:

- *Preparation of the medium*

The grain starch is hydrolyzed to sugars with microbial enzymes or with the enzymes of barley malt. In all the others no hydrolysis is necessary as sugars are present in the fermenting substrate as in brandy (grape sugar) and rum (cane sugar). First, starch should be contact with water, this is typically accomplished with two hot water processes. First, grain is treated with hot water, typically 85°C for between 20 to 60 minutes. Then, super heated water, typically 110°C, is introduced with high pressure. With the first mixing with hot water, the starch absorbs water. During the heating step starch is hydrolyzed into fermentable sugars. In an ethanol industry, two enzymes are usually employed, endoenzyme *alpha-amylase* and exoenzyme *glucoamylase*. *Alpha-amylase* attacks the alpha-1, 4 linkages of starch. Then, starch is converted into dextrin . After the first hydrolysis with *alpha-amylase, glucoamylase* works. *Glucoamylase* removes one glucose from dextrin. Thus, *glucoamylase* cuts linkage of dextrin from its end.

- ***Propagation of yeast inoculum***

In general the inocula are made of selected alcohol-tolerant yeast strains usually *Saccharomyces cerevisiae* grown aerobically with agitation and in a molasses base. Yeast inculum with up to 5% (v/v). Progressively larger volumes of culture may be developed before the desired volume is attained.

-Fermentation:

Yeast is a facultative anaerobe. In an aerobic environment, it converts sugars into carbon dioxide and water. In an anaerobic environment, it converts sugars into carbon dioxide and ethanol. Thus, for an ethanol industry, it is important to exclude significant oxygen from its system.
Alcohol-resistant yeasts, strains of *Saccharomyces cerevisiae* are used, and nutrients such as nitrogen and phosphate lacking in the broth are added.When the nitrogen content of the medium is insufficient nitrogen is added usually in the form of an ammonium salt. As in all alcohol fermentations the heat released must be reduced by cooling and temperatures are generally not permitted to exceed 35-37°C. The pH is usually in the range 4.5-4.7, when the buffering capacity of the medium is high. Higher pH values tend to lead to higher glycerol formation. When the buffering capacity is lower, the initial pH is 5.5 but this usually falls to about 3.5. During the fermentation contaminations can have serious effects on the process: sugars are used up leading to reduced yields.

-Distillation
Distillation is one of the steps of the purifications. Distillation is the method to separate two liquid utilizing their different boiling points.After fermentation the fermented liquor contains alcohol as well as low boiling point volatile compounds such as acetaldeydes, esters and the higher boiling, fusel oils. The alcohol is obtained by several operations. **First**, steam is passed through the liquor which is said to be steam-stripped. The result is a dilute alcohol solution which still contains part of the undesirable volatile compounds. **Secondly**, the dilute alcohol solution is passed into the center of a multi-plate aldehyde column in which the following fractions are separated: esters and aldehydes, fusel oil, water, and an ethanol solution containing about 25% ethanol. **Thirdly**, the dilute alcohol solution is passed into a rectifying column where a constant boiling mixture, distils off at 95.6% alcohol concentration.
In practice, the condensate is not allowed to separate out, but the arrangement of plates within the columns enable separation of the alcohol. Four columns are

usually used. The first and second columns remove aldehydes and fusel oils, respectively, while the last two towers are for the concentration of the alcohol.

Some Developments in Alcohol Production

(1) Developments of new strains of yeast of *Saccharomyces uvarum* able to ferment sugar rapidly, to tolerate high alcohol concentrations, flocculate rapidly.
(2) The use of continuous fermentation with recycle using the rapidly flocculating yeasts.
(3) Continuous vacuum fermentation in which alcohol is continuously evaporated under low pressure from the fermentation broth.

(4) The use of *Zymomonas mobilis,* a Gram-negative bacterium which is found in some tropical alcoholic beverages. The advantages claimed for the use of *Zymomonas* are the following:
(a) Higher specific rates of glucose uptake and ethanol production than reported for yeasts.
(b) Higher ethanol yields and lower biomass than with yeasts.
(c) Ethanol tolerance is at least as high or even higher [up to 16% (v/v)] in some strains of the bacterium than with yeast.
(d) *Zymomonas* also tolerates high glucose concentration and many cultures grow in sugar solutions of up to 40% (w/v) glucose which should lead to high ethanol production.
(e) *Zymomonas* grows anaerobically and, unlike yeasts, does not require the controlled addition of oxygen for viability at the high cell concentrations used in cell recycle.
(f) The many techniques for genetic engineering already worked out in bacteria can be easily applied to *Zymomonas* for greater productivity.