

Module-33: Ethanol Fermentation

- In 1906 when the Industrial Alcohol Act was passed, the production of industrial alcohol, ethanol, became commercially feasible on a large scale.
- This act allowed the sale of tax-free alcohol, if alcohol has first denatured to avoid its use in various Alcohol beverages.
- There are various uses of alcohol such as,
 1. Ethanol is used as an organic solvent in many different products as Emulsified Paints, Perfumes, Markers Inks, varnishes, Soaps, and other Products like explosives.
 2. The most popular use of ethanol is as motor fuel and fuel additive (gasohol).
 3. Its renewable nature; makes it useful for fueling clean environmentally friendly Compartments.
 4. Ethanol is used as a potent food preservative in homes.
 5. Ethanol is used in making Antiseptic Soap and Cosmetics as it is effective against bacteria, fungi, and many viruses, but is not much effective against bacterial spores.
 6. Ethanol is routinely consumed in different forms around the World like Beer, Wine, Gin, Whisky, etc. Beer is the most popular Ethanol Beverage in the World.
 7. Ethanol plays an important role in making Drugs and Pharmaceuticals.
 8. Ethanol is used in the preparation of essences and flavorings
 9. Ethanol is used as the fluid in thermometers.
 10. Ethanol is used in preserving biological specimens.

The Chemical Process of Ethanol Fermentation:

- Ethanol fermentation is a biological process in which sugars are utilized to produce ethanol and carbon dioxide as metabolic waste products.
- This process is a complicate sequence of transformations carried out by yeast cells that converts glucose to CO₂ and alcohol.



- Before fermentation, one glucose molecule is converted into two pyruvate molecules by glycolysis.

- Under aerobic condition, some species of yeast (e.g., *Kluyveromyces spp*) will oxidize pyruvate completely to carbon dioxide and water which is termed as cellular respiration.
- These species of yeast will produce ethanol only under anaerobic condition.
- So ethanol fermentation is classified as anaerobic process.
- However, many yeasts such as *Saccharomyces cerevisiae* (baker's yeast), *Schizosaccharomyces pombe* (fission yeast) will produce ethanol even under aerobic conditions.

Microorganisms

- All yeasts are not suitable for brewing.
- Choice of yeast for the alcohol production depends upon the composition of the medium, particularly the type of carbohydrate used in the medium.
- Following table describes various raw materials used in medium with specific strain of yeast.

Raw materials in the medium	Fermentation Strain
Starch and sugar	<i>Saccharomyces cerevisiae</i>
Lactose of whey	<i>Candida pseudotropicalis</i>
Sulfur waste liquor	<i>Candida utilis</i>

- Fermentation strain should contain several properties like,
 1. They must grow rapidly.
 2. They should tolerate high concentrations of sugar.
 3. They must be able to produce abundant amounts of alcohol
 4. They must be resistant to the produced high concentrations of alcohol.
- This process is used in the production of alcoholic beverages, ethanol fuel, as well as in the rising of bread dough.

Media

- The media for the commercial production includes:
Blackstrap Molasses / Corn (Blackstrap molasses has greater use)

Potatoes

Grains

Wood Wastes

Sulfite waste liquor

Whey

- For the molasses fermentation, to keep final sugar concentration between 10 -18% molasses must be diluted with water.
- Concentrations greater than 20% are not employed as they could be detrimental to yeast.
- The pH of the medium is set between 4.8 to 5 with the help of sulfuric acids or lactic acids.
- Some starchy media like corn, rye and barley can be utilized but initial starch hydrolysis is necessary.
- This can be done by squashing with barley malt, by mixing with dilute acids or by utilizing amylolytic enzymes of fungi like *Aspergillus* and *Rhizopus*.

Fermentation:

- When ethanol fermentation is going on in large reactors, temperature should be between 21 - 27°C.
- But as heat gets evolved it raises the temperature up to 30°C, which bring down by cooling coils.
- Fermentation continues for 2-3 days, but exact time period is based on the substrate utilized and temperature.
- At the end, fermentation broth contains 6 -9 percent alcohol by volume.
- This alcohol yield reflects 90 -98% theoretical conversion of substrate sugar to alcohol.
- So yields should not be confused with "proof" as proofing means alcohol concentration designation and it will be twice the percentage in volume of ethanol as dissolved in water e.g. 70% ethanol is 140 proof.

Product Recovery:

- Ethanol is separated from fermentation broth in continuous stills.
- Ethanol of 95% concentration is obtained by successive distillations.
- To obtain 100% or absolute alcohol, special distillation technique is required.
- For that 5% water is removed by forming an azeotropic mixture of benzene, water & ethanol, which then is distilled with increasing temperature.
- This procedure removes first the azeotropic benzene- ethanol- water mixture, and then benzene-ethanol mixture, so that absolute ethanol remains.

References

- **Principles of Fermentation Technology:** (2nd edition, by Peter F. Stanbury, Allan Whitaker and Stephen J. Hall, Butterworth-Heinemann, An imprint of Elsevier Science.)
- **Industrial Microbiology:** (By Casida L. E. New Age international (P) Ltd publications)
- **A Text Book of Industrial Microbiology:** (2nd edition By Wulf Crueger & Anneliese Crueger)
- **Biotechnology:** Food Fermentation Microbiology, Biochemistry & Technology Vol. 1 & 2: (By V.K. Joshi & Ashok Pandey)
- **Manual of Industrial Microbiology and Biotechnology:** (2nd Edition by Arnold L. Demain and Julian E. Davies, Ronald M. Atlas, Gerald Cohen, Charles L. Hershberger, Wei-Shou Hu, David H. Sherman, Richard C. Willson and J. H. David Wu)
- **Industrial Microbiology-An introduction:** By Michael J. Waites, Neil L. Morgan, John S. Rockey and Gary Higton)
- **Comprehensive Biotechnology-The Principles, Applications and Regulations of Biotechnology in Industry, Agriculture and Medicine:** (By Mrray Moo Young)
- **Fermentation Technology : Up Stream Fermentation Technology- Vol-I:** (By H. A. Modi-Pointer Publications)
- **Fermentation Technology : Down Stream Fermentation Technology- Vol-II:** (By H. A. Modi-Pointer Publications)
- **Industrial Microbiology by Prescott and Dunn's:** (4th edition, edited by Gerald Reed, CBR publications)
- **Fermentation Technology:** (By M.L. Srivastava, NAROSA publications)
- **Industrial Microbiology:** (By A.H. Patel)
- **International student edition: Microbiology- A laboratory Manual:** (4th edition. By James G. Chappuccino & Natalie Sherman)
- **Bacteriological Techniques:** (By F.J. Baker)
- **Introduction to Microbial Techniques:** (By Gunasekaran)
- **Manual of Industrial Microbiology and Biotechnology:** (2nd Edition by Arnold L. Demain and Julian E. Davies, Ronald M. Atlas, Gerald Cohen, Charles L. Hershberger, Wei-Shou Hu, David H. Sherman, Richard C. Willson and J. H. David Wu)

Web references

- <http://www.homebrew.net/ferment/>
- <http://www.soyinfocenter.com/HSS/fermentation.php>
- http://www.ensymm.com/pdf/ensymm_fermentation_abstract.pdf
- <http://scialert.net/fulltext/?doi=jm.2007.201.208>
- <http://aem.asm.org/content/7/1/57.full.pdf>
- <http://www.slideshare.net/yongkangbirdnest/lecture-4-sterilization>
- http://www.ars.usda.gov/research/publications/publications.htm?seq_no_115=140721
- <http://www.scribd.com/doc/30706834/Fermentation-Design>
- http://www.wiley-vch.de/books/sample/3527318194_c01.pdf
- <http://www.engineersirelandcork.ie/downloads/Biopharmaceuticals%20Jan09%20-%20%20-%20Ian%20Marison%20DCU.pdf>
- www.yobrew.co.uk/fermentation.php
- <http://bioscipub.com/journals/bbb/pdf/19-24.pdf>
- <http://gertrude-old.case.edu/276/materials/web/immobilizedenzymereview.pdf>
- http://download.bioon.com.cn/upload/month_0902/20090223_b809d1c59ba2a6e2abfdJtWiJOiFDm02.attach.pdf
- <http://bioprocess-maulik.blogspot.in/2007/07/design-of-industrial-fermentation.html>
- <http://hsc.csu.edu.au/biology/options/biotechnology/3051/biotechnologyPart3.html>
- <http://www.rsc.org/ebooks/archive/free/BK9780854046065/BK9780854046065-00001.pdf>
- <http://www.biotech.upm.edu.my/academics/On%20Line%20Note/Bioprocess/BTK%205301/Lect6%28Inoculum%20Preparation%20and%20Development%29.pdf>
- <http://www.biotechresources.com/services-strain.shtml>
- <http://www.idosi.org/wjc/4%281%2909/14.pdf>
- <http://cheserver.ent.ohiou.edu/Paper-gu/DualFeed.pdf>