EB 0800

FUEL ALCOHOL

FUEL ALCOHOL: CHEMISTRY AND MICROBIOLOGY

The chemistry and microbiology of alcohol production is a simple case of undoing what nature and the sun have done. Solar energy, water, and carbon dioxide are combined by green plant materials through a process called photosynthesis into sugar (glucose) and oxygen. The plant then converts this sugar into starch for storage or into cellulose for structural growth. Alcohol production is an attempt to reverse this process and regain some of the original energy converted by the plant into starch and cellulose. The production process takes advantage of the ability of yeast to convert the sugar into ethanol and carbon dioxide. The chemistry in alcohol production is the conversion of either starch or cellulose back into the original sugar (glucose). The microbiology is providing the optimum conditions for the highest yielding yeast strains to transform these sugars into ethanol.

Alcohol Production: Chemistry

Of the two sources of glucose (starch and cellulose), starch is by far the easiest to hydrolyze (break down into glucose). Cellulose can be hydrolyzed by either acid or enzymes, but either method has several problems. The major difficulty is that the cellulose is protected by the lignin, which requires expensive and difficult pretreatments to separate it. In addition, breaking down its crystalline structure requires strong acids at high temperatures which also can result in decomposition of the sought-after glucose. In short, cellulose is an excellent source of fermentable glucose, but its utilization is beyond the capital resources of the small-scale producer. Much innovative and significant research is being carried on that may alter its potential use.

Starches, on the other hand, are easily hydrolyzed into fermentable products. A starch molecule is made up of a chain consisting of individual sugar molecules. This chain can be short or quite long; a simple straight chain (amylase), or with numerous branches linked on (amylopectin). Most yeasts can ferment only single unit sugar molecules (glucose, fructose) or two-unit molecules (sucrose, lactose, maltose).

The starch molecule can be broken down (hydrolyzed) using several techniques. In some industrial applications, low concentrations of acids are employed. Traditionally in the brewery industry, malted barley was used. Later, the responsible hydrolyzing enzymes were isolated and commercially produced by a variety of manufacturers.

Enzymes are protein molecules that act as catalysts to accelerate the rate of normally slow chemical reactions. Two different enzymes, requiring two different environments (temperature, pH, etc.), are employed in starch hydrolysis. The first, amylase, converts starch (both amylose and amylopectin) into soluble dextrins (two-unit sugars) and small quantities of glucose and maltose by randomly hydrolyzing (breaking) the α-D-1,4 glucosidic linkage of the starch chain. This enzyme is often referred to as a liquefying enzyme because it converts the water-insoluble starch granules into soluble dextrins. Optimum pH and temperatures for alpha-amylase vary by strain and manufacturer, but normally range between a pH of 6.0 to 6.5, and a temperature around 200°F. In addition, some alpha-amylase

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enzymes require addition of calcium in the mash to assure stability.

The second, gluco-amylase, converts the dextrin into glucose by hydrolyzing both the α-D-1,6 glucosidic branch points and the predominating α-D-1,4 glucosidic linkages. These enzymes are often referred to as saccharifying enzymes. Optimum pH and temperatures are very different from those of the alpha-amylase enzymes. Optimum pH is significantly lower, around 4.0-4.5, and the maximum temperature is about 140^{o}F. Thus, both saccharification and fermentation can take place at the same time. With both enzymes, it is important to follow the supplier's recommendations regarding optimum temperatures, pH, and concentration.

Alcohol Production: Microbiology

In the microbiology of fermentation, of utmost importance is the active, healthy growth of the ethanol-producing yeast. But we must also be concerned about those organisms which could cause spoiling during the fermentation process. There are many different yeasts that can ferment sugars into a host of chemical products. The species called *Saccharomyces cereviseae* has the greatest capacity to ferment sugar to ethanol. Both bakers yeast (*S. cereviseae* var. *diactaticus*) and brewers yeast (*S. cereviseae* var. *carlsbergensis*) can be employed in ethanol production, although bakers yeast normally stops fermenting at about 12% alcohol and brewers yeast at about 16% alcohol by volume.

Yeasts are one-celled, plant-like organisms capable of growing under both aerobic and anaerobic conditions. The usual growth requirements include a carbon source, a nitrogen source, various minerals, and a mixture of vitamins. Most, if not all of these, are supplied by the feedstock used in the process. If not, they must be supplied by the producer.

During the initial growth period, sufficient oxygen is usually present to ensure aerobic conditions prevail, with rapid growth of the yeast cells. However, the major products of this metabolic stage are water and carbon dioxide. Once the oxygen is depleted, anaerobic conditions exist, and ethanol and carbon dioxide become the dominant byproducts. Since ethanol is a waste product of yeast metabolism, its concentration will effectively limit fermentation. Thus, the original concentration of glucose in the fermentation solution should be approximately twice the final ethanol concentration the yeasts can tolerate, i.e., approximately 20% glucose by volume.

Yeast growth is dependent upon a number of external factors as well, including pH, temperature, alcohol concentration, time, and the strain of yeast used. Of these, temperature is of prime importance because at low temperatures yeast activity is slowed and at high temperatures the yeast can be killed. Temperatures between 85^{o} and 100^{o}F seem to give optimum yields, although one should always follow the suppliers' suggestions.

Contaminants are organisms which compete for nutrients or which produce metabolic products which are not compatible. Most spoilage organisms are killed in the cooking process, except for thermophilic spore-forming bacteria called thermobacteria. These are normally killed by the dominating influence of the yeast in its early growth stage. Acetic acid bacteria ferment ethanol into acetic acid. Since they require oxygen to survive (aerobic), they do not normally survive during the fermentation process, but can cause spoilage after fermentation is complete. Lactic acid bacteria are a serious threat to the fermentation of beer since they propagate rapidly under very similar conditions to yeast growth. Yeast can overpower them, but careful cleaning and monitoring are required for control of these organisms. Most contaminants can be controlled by careful monitoring of the yeasts, early and vigorous yeast fermentation, and good sanitation practices.

References

Shelton, D. P., and R. L. Ogden, "Yeasts and Enzymes in Ethanol Production." Ethanol Production and Utilization for Fuel, Cooperative Extension Service, University of Nebraska, Lincoln, NB 1979.

"Fuel from Farms–A Guide to Small Scale Ethanol Production," SERI/SP-451-519, UC-61, Golden, CO 80401, February 1980.

"A Learning Guide for Alcohol Fuel Production," 1979. National Alcohol Fuel Producers Association, 1700 South 24th Street, Lincoln, NB 68502.

Sources of Yeasts and Enzymes

Anheuser-Busch, Inc.
Industrial Products Division
10877 Watson Road, Suite 200
St. Louis, MO 63127
(313) 996-7100

Universal Foods Corporation
Fermentation Products Division
433 E. Michigan Street
Milwaukee, WI 53201
(414) 271-6755

Biocon, Inc.
261 Midland Ave.
Lexington, KY 40507
(606) 254-0517

Miles Laboratories, Inc.
Enzyme Products Division
P.O. Box 932
Elkhart, IN 56515
(219) 564-8111

Nova Laboratory, Inc.
59 Danburg Road
Wilton, CT 06897
(203) 762-2401

Enzyme Development Corporation
2 Pennsylvania Plaza
New York, NY 10001
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