Starch-Based Ethanol Production

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Key Points

- Pay a premium for high starch, low moisture grain
- Increase dissolved solids in the mash (in stages with comfort)
- Increase fermentation rates and ethanol contents Build plants with excess cooling capacity in fermentation and excess evaporative capacity to handle thin stillage
- Reduce backset – reduce organic acids ions and other stress agents
- Use heat exchangers on fermentors to cool mash – recycle energy
- Know your yeast and its properties and contaminants
- Don’t attempt continuous or continuous-intermittent propagation. Batch is the answer to propagation
- Select your yeast for quality, lab backup, service and integrity
- Understand the effects of yeast foods
- Reduce harmful recycled waters – know their contents of chemicals
- Buy the best enzymes you can and know how to use them. Store properly.
- Understand cleaning and sanitation
- Use antibiotics with care and investigate alternate antimicrobials
- Keep learning. Improve the knowledge of your personnel.

Saccharomyces cerevisiae is the most exploited microbe known to man and produces more than 51 billion L/year of beverage, industrial and fuel alcohol around the world. In the total US industry, 25.5 billion liters are made in 127 plants at this time (08/29/07). Canada is much smaller with 10 plants making 809 million liters - expanding to more than twice this volume in future. By 2008-9, fuel alcohol in North America alone will expand to more than 50 billion liters/year, doubling in volume as the number of plants increase from 127 to about 207.

How much alcohol is required? A 10% substitution of the 450 billion L of gasoline used yearly in NA requires 45 billion liters. At the current conversion at a modest 387 L/tonne corn, we will need 116 million metric tonnes. The yearly production of corn is ~ 250 million tonnes so we would need about 46% of the corn crop to supply a 10% v/v substitution of gasoline. From this data, it is clear ethanol will only be an octane enhancer and fuel extender – not a total replacement for gas, and using the complete corn crop we could eliminate ~ 20% of the volume of gasoline now consumed. At the same time, over 80 billion tonnes of distillers grain would be available for animal feed. Research will lead to supplementation of DDG to make it more suitable for ruminant animals, monogastrics and birds. This is all we can expect until cellulosic is usable.

Our industry is young - resurging for real in 1980 from beginnings with Henry Ford’s ethanol-powered automobiles in the 1900’s. Some 2800 alcohol stations existed across America until cheaper but more toxic gasoline forced ethanol out, even though lead and later ETBE - used as octane enhancers/oxygenates - were found toxic or carcinogenic. Just a short time ago, alcohol plants produced 5-10% v/v ethanol concentrations in fermentation – needing more energy to distill and dewater the "beer". Now, factories exist that make 20% v/v by end fermentation. Others are producing near or over 15% v/v. These beer concentrations are less expensive to distill.

Moreover, scientists have published that the energy gained in ethanol manufacture is now 1.34 times the energy used to make it. Although in dispute, most believe that this has been made possible by novel advances in engineering (distillation, energy recovery, molecular sieve) and microbiology (VHG fermentation) as well as upgrades to plant design. Research continues to discover ways to lower costs and increase productivity.

Today, the workplace, on average, has little official training. New plants hire workers from older plants – increasing manpower costs. Most training of new employees is on-the-job with vendor training. Two alcohol schools are run yearly – to older one with 27 years of continuous training in Europe and North America poised for training of experience operators, middle and upper managers, while the new one poises their school more at the operator level. Only one textbook is available – The Alcohol Textbook - soon going into its 5th Edition. Alcohol production is a multidisciplinary business encompassing the fields of engineering, biochemistry, microbiology, plant science, food science, chemistry, cleaning/sanitation, animal nutrition, feedlot operation, commerce, accounting, politics and marketing - to name only some.

Process flow is different in dry grind and wet milling operations, and further made complex by the choice of batch or continuous fermentations, cook or no cook systems and all other ancillary equipment (mills, stills, molecular sieves, evaporators, dryers, thermal oxidizers, methanators, etc).

In both dry grind and wet milling ethanol plants, mash is made continuously. In the former, corn is slurried with backset and other recycled waters in a mash mingler or slurry tank. In most plants, a cooking procedure follows – the amount of heat depending on whether or not the design calls for high temperatures for liquefaction/gelatinization or lowered heat to avoid the Maillard reaction known to lower yields of ethanol.

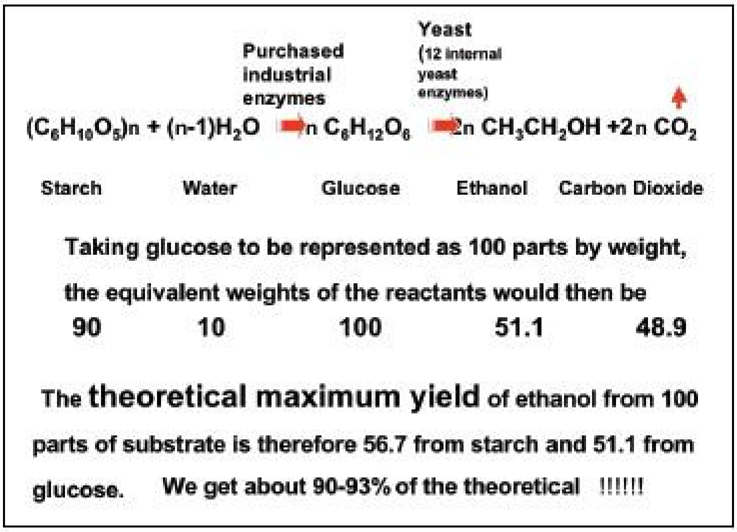
During temperature ramp-up, the addition of heat tolerant alpha-amylase is made such that the viscosity of the gelatinizing starch is broken down as the HT-amylase cuts α-1,4 glucosidic linkages, adding water across bonds. More enzyme is added when high heat processed mash is taken to the flash tank where temperatures decrease to 80-86°C. Low molecular weight dextrins are made in this process and taken to the fermentor - with additions of backset or acid, and yeast foods. Proper yeast nutrition is needed to ensure proper levels of usable nitrogenous "foods" (amino acids, ammonium salts, ammonia, or urea). Other elements essential to yeast growth may also be required.

The second segment of the process is the fermentation. Fermentation in most plants today takes place coincidently with the use of the industrial enzyme, glucoamylase. Yeasts (active dry direct pitch, active dry conditioned, compressed, or stabilized liquid yeast) are added, and oxygen (as air) is injected to permit synthesis of unsaturated fatty acids and sterols to create strong membranes in both mother and daughter yeasts. The recommended rate is ~ 20 ppm over 3-4 hours when the yeasts are actively growing. Carbon dioxide, ethanol and glycerol (and yeast cells) are the major products of fermentation, and the beer after fermentation is taken to the beer still.

This last and perhaps largest of the process segments includes a two stage distillation of beer. The stillage from the bottom of the first still is centrifuged to produce wet grain (taken for sale or for drying – with or without solubles added - and thin stillage, while the alcohol of ~ 100 Proof is taken to the rectifying still to create 190 Proof. This alcohol is taken to the mol sieve to remove residual water and the near 200 Proof alcohol is denatured and stored for use. Some of the thin stillage is returned as makeup water for the next corn mashing. The rest is either used as water for ruminants or dried to a syrup that will be added to the grains as they dry to form distillers' dried grains with solubles (DDGS). The dry grind process using a tonne of grain results in 374-402 L of 190 Proof ethanol, 323 kg of DDG/DDGS and ~287 kg of CO2.

The wet milling process used prior to the resurgence of alcohol production to make pure starches and syrups is a more complex process only carried out by a small number of plants in North America. The products of the wet mill from a tonne of dry corn are corn oil (27 kg via 43.3 kg of germ), starch (570 kg) or 622 kg of a wide variety of sweeteners (made by specialized enzymes), gluten meal (55 kg), gluten feed (157 kg), CO2 (287 kg) and ~374 L of alcohol if all starch was sent to the fermentor.

From this information, we can calculate the yield of alcohol from the amount of starch available (if we can measure it accurately). This is shown below in two charts and shows the theoretical maximum yield.



$$(C_6H_{10}O_5)n + (n-1)H_2O \xrightarrow{\text{Purchased industrial enzymes}} n\ C_6H_{12}O_6 \xrightarrow{\text{Yeast (12 internal yeast enzymes)}} 2n\ CH_3CH_2OH + 2n\ CO_2 \uparrow$$

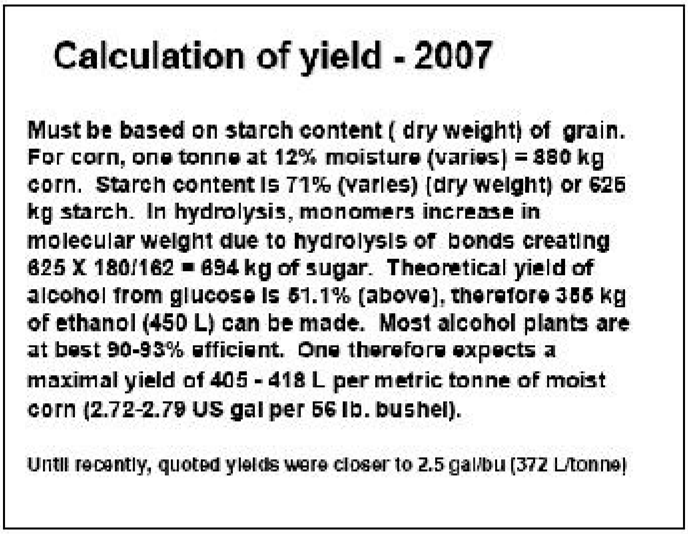
Starch	Water	Glucose	Ethanol	Carbon Dioxide

Taking glucose to be represented as 100 parts by weight, the equivalent weights of the reactants would then be

90	10	100	51.1	48.9

The **theoretical maximum yield** of ethanol from 100 parts of substrate is therefore 56.7 from starch and 51.1 from glucose. We get about 90-93% of the theoretical !!!!!!

The calculation below is given in metric and based on one tonne of corn at 12% moisture and 71% (DM). Both these amounts are very variable due to corn purchase, and the basis of comments made many times that relate to purchase of grain of any kind as trucks/trains unload. Many dollars are lost through purchase of moisture, dockage, mold infestation and low quality grain.If grain with 14% moisture and 67% (DM) starch was used due instead, the ethanol yield would now be 2.46 gal/bu (370 L/tonne) instead of the yields above of 2.7 gal/bu or 405 L/tonne – possible with 12% moisture and 71% starch. This may be more like the case with prairie wheats or Ontario corn that may have higher moisture, lower starch and higher dockage.



Calculation of yield - 2007

Must be based on starch content (dry weight) of grain. For corn, one tonne at 12% moisture (varies) = 880 kg corn. Starch content is 71% (varies) (dry weight) or 625 kg starch. In hydrolysis, monomers increase in molecular weight due to hydrolysis of bonds creating 625 X 180/162 = 694 kg of sugar. Theoretical yield of alcohol from glucose is 51.1% (above), therefore 356 kg of ethanol (450 L) can be made. Most alcohol plants are at best 90-93% efficient. One therefore expects a maximal yield of 405 - 418 L per metric tonne of moist corn (2.72-2.79 US gal per 56 lb. bushel).

Until recently, quoted yields were closer to 2.5 gal/bu (372 L/tonne)

The question of why yeasts do not operate at 100% of theoretical yield often comes up. The answer is that new cells are made (the yeast's objective), glycerol and other end products in smaller amounts are made, losses in factory operation occurr, some starch is retrograded by heat or reacts in the Maillard reaction, infection by bacteria or wild yeasts takes place, sluggish or stuck fermentations happen or the yeast is under stress.

The overall economics of a yeast plant are, in part, based on these biochemical and microbiological events, but also are impacted greatly by the design and cost of the plant and other fixed costs. The improvement in economics made in the last few years have changed due to: increases in alcohol concentration and faster rates of production, use of enzymes that eliminate process problems, eliminate need for calcium supplements, lower viscosity or generate additional glucose from otherwise recalcitrant substrates,lowered distillation costs, use of molecular sieves to remove the last 5% water rather than azeotropic stills, energy recycling, fractionated grains, high starch varieties or special grains, methanators and thermal oxidizers to reduce pollutions, plant diversification in use of byproducts or energy sources, microbiological advances and control including ethanol tolerant or temperature tolerant yeasts, understanding of yeast stresses and conditioning requirements, elimination of losses of yield, lowering energy costs using new technologies (eg no cook), and use of antimicrobials to control bacteria.

Most factories attempt to increase productivities by trying to increase the stated 90-93% efficiency to a value like 93-95%. This is done by eliminating known losses of substrate or ethanol, by reducing numbers of bacterial end products, eliminating stuck fermentations, adding yeast foods, or preprocessing N compounds and unusable carbohydrates (with enzymes). Other ways to increase production are to build more plants, expand old plants, or develop more usable substrates. Forgotten is the possibility of increasing production by increasing throughput. **Throughput** becomes the word - to increase rate of fermentation, and/or increase ethanol concentration, and/or remove insolubles that need cooling in fermentation and heating in the still where they occupy ~ 11 % of the volume of the vessels. They take no part in fermentation.

One of the methods which permits the production of 20% or more ethanol is a process developed in my lab called **Very High Gravity** (VHG) fermentation. In this published process, all the carbohydrate in the fermentor is there at zero time, commercial active dry yeast is used, and yeast foods are added to promote yeast growth to the maximum cell number possible so that alcohol product proceeds – first at the fastest rate/cell as conducted by growing cells, and then by the larger numbers of cells grown but at a slower rate/cell but almost equal rate as previously due to the much larger cell numbers. Twenty % v/v is almost the optimum concentration with regards to use of steam in distillation. Eliminated is an amount of water in all vessels in the process. This leads to increased plant capacity or reduction in capital costs in new construction, 20% v/v alcohol, increased fermentor space (if grain is removed), reduction in labor and energy per L ethanol, reduced survival and proliferation of bacteria (loss of yield), and provides opportunities for food quality byproducts and for harvesting high protein yeasts. It is not possible to recycle yeasts as done in Brazil as they are dead. The higher alcohol levels are made with understanding of yeast handling and yeast nutrition.

Here we will cover nitrogenous nutrition for yeasts – practically the use of "yeast foods" containing amino acids, urea, ammonia or inorganic ammonium salts (phosphate or sulfate). Usable nitrogen for yeasts is only one of many possible nutrients that may be deficient in corn or wheat mashes or mashes made from other substrates not the least of which are the isolated starch or sugar fractions from grains or sugar cane. These latter substrates have virtually NO nutrients that support growth other than carbohydrate. Some treatment of this subject is found in the article below (Ingledew, 1999).

In our example of nitrogen deficiency, we now know that **all grains** used to make alcohol are low in usable nitrogen.

We also know (Ingledew, 2003) that the amount of free amino nitrogen (supplied by amino acids in wort or replaced/supplemented by urea, ammonium ion or ammonia) as recommended by brewers for worts of only 12% sugar is 150 mg/L. More stimulates fermentation rate. Our fuel alcohol mashes range from about 22% carbohydrate to more than 35% - when VHG fermentation is used. Studies in our lab show that in a corn dry grind plant, FAN arises from backset use (FAN is already yeast-scavenged in one or more fermentations) or from mash, the total in a 24% carbohydrate mash is near 85 mg/L but much is in peptide form. Similar deficiencies are shown in wet milling mashes where nutrients added back to starch slurries prior to fermentation are also low in usable nitrogen, and so backset is not a good source of FAN due to its use in one or more other fermentations where the FAN that is usable was scavenged. Note also that FAN is measured on peptides and proteins even though yeast are not able to use these N sources.

So, in conclusion, mashes are all usable nitrogen-deficient and require supplementation with usable N for adequate rates of ethanol production – especially if high levels of carbohydrate are present as in VHG mashes. We now assume that the levels are inadequate and we have “titrated” such mashes determining along the way that, with urea, some 500 – 1000 mg of urea supplying 224-448 mg of usable nitrogen are necessary for normal and VHG mashes, respectively. This is a very large amount of urea (500 to 1000 kg of urea in a million liters of mash, but this is what is needed to grow the almost 10,000 kg of new yeast that will be anaerobically propagated in this size vessel during fermentation using energy obtained from fermenting 90% of the sugar supplied in the mash.

The proof of the need for increased usable nitrogen is best shown in the very interesting experiments conducted by Jones and Ingledew (1994) that shows not only the effect of the excess usable nitrogen on fermentation rate over 5 temperatures, but also shows the importance of the nitrogen added in achieving high alcohol levels from the mashes used at highest dissolved solids (30 and 36.5 % w/v. This is the evidence that prompted industry to invoke “temperature staging” – a procedure where fermentation temperature was dropped in later stages of fermentation when alcohol became a stress agent in combination with the higher temperatures.

Nutrition is the answer to faster fermentations, increased yeast growth and catalytic conversion of glucose into ethanol, as well as increased tolerance of yeast to stress agents. In this work it is easy to see that as long as temperatures near the end of fermentation do not exceed 27°C, over 20 % ethanol can be achieved in less than 50 hours.

This lab (Bellissimi and Ingledew, unpublished data) has been able to create a new yeast food that we hope will be available industrially soon. It allows faster fermentations to occur over and above the rates normally seen, and the overall fermentation times were reduced significantly. The yeast food works under both normal gravity and very high gravity conditions. Although the composition of this yeast food is not published, concentrations of the ingredients were determined by experiments that optimized each ingredient in relation to the others.

The concentrations of each ingredient seem on first glance to be high, but were based on the fact that in a 950,000 L fermentor, over 10,000 lbs of new yeast are formed. The nutrition is added to increase the rate of growth of these yeasts and the number of them such that the fermentation will carry on long enough to convert very large amounts of produced glucose to ethanol and carbon dioxide.

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