

III. BIOFUELS

III.B. Technological Assessment

III.B.1. Ethanol

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Ethanol is produced by the fermentation of sugars by yeast. The following review describes the basic technologies used for the industrial production of ethanol for fuel from corn and sugar cane. In addition, some description of developing technologies for the production of ethanol and other liquid fuels from cellulosic plant material is presented. The processing steps for producing fuel ethanol falls into four major categories which are common to all feedstocks and processes: feedstock preparation, fermentation, distillation/recovery, and byproduct handling. Feedstock preparation involves any cleaning or conditioning steps as well as generating an aqueous solution high in simple sugars from the feedstock. The sugar solution is then fermented by yeast, *Saccharomyces cerevisiae*, which is also used for brewing beer and baking bread. After the fermentation is complete, the ethanol is separated and purified. Distillation is the standard industrial step for the major separation of ethanol from water. Additional steps are required to remove the final few percent of water from the ethanol to yield fuel-grade product. A denaturent also is added to avoid the Federal Alcohol Tax placed on potable ethanol. Byproduct handling includes technologies for producing secondary products from the residual feedstock not used for producing ethanol. Feedstock preparation and byproduct handling are largely what distinguishes one processing method from another.

This section will focus on technologies currently implemented in industry with some additional focus on second generation ethanol production technologies for cellulosic ethanol production. While ethanol is the dominant alcohol produced for fuel purposes, other alcohols are receiving interest for future development of effective production techniques for large scale fuel generation. Butanol production by anaerobic bacteria is receiving significant attention. A process is being developed by DuPont and British Petroleum is investigating possible use of butanol as a liquid fuel (Hessley, 2006). Production of mixed alcohols fuels, by mixed-acid fermentation with a mixed culture followed by hydrogenation of the mixed acids into mixed alcohols (e.g., ethanol, propanol, butanol, etc), a method known as the MixAlco process, can utilize an array of low or negative value feedstocks, such a sorted municipal solid waste, sewage sludge, industrial biosludge, manure, agricultural residues, energy crops (Chan & Holtzapple, 2003; Holtzapple et al., 1999).

III.B.1.a. Basic science and technology - This section outlines the basic chemistry, biochemistry, and microbiology that underlie the production of fuel ethanol. From this basis, the fundamental technologies for the processing steps are described in greater detail. Because the fermentation, distillation, and ethanol drying technologies are nearly identical for all fuel ethanol production technologies, the greatest detail for these technologies will be found here. Special considerations in fermentation, distillation, and ethanol drying for each processing method as well as the unique feedstock preparation and byproduct handling technologies will be described in the subsequent sections.

III.B.1.a.(i) Carbohydrates / sugars - Ethanol is produced from plant carbohydrates. Plant carbohydrates are grouped as soluble sugars, storage carbohydrates, and structural carbohydrates. An example of soluble sugars is sucrose, which for example is produced commercially by sugarcane. The major storage carbohydrate is starch such as produced commercially from grains (corn, wheat, rice and sorghum) and tubers (cassava and potatoes). Structural carbohydrates are what makes up most of the plant cell wall and quite literally holds up the plants. These include cellulose, hemicellulose, and pectin. Cellulose is the most abundant structural carbohydrate and plants generate more than 180 billion tons each year (Delmer, 1999).

III.B.1.a.(i)(a) Glucose polymers – Glucose in plants is found in two major polymers (polysaccharides), starch and cellulose. Starch is the major form of energy storage in plants. Plant seeds (wheat, rice, etc.) and/or tubers (potatoes, etc.) are rich in starch. Starch is found in two major forms, amylose and amylopectin (BeMiller & Whistler, 1996). Amylose is a straight chain polymer of glucose molecules joined by α 1-4 glycosidic bonds. This primary structure results in the long polymers coiling into a helical conformation (BeMiller & Whistler, 1996). Amylopectin is also primarily a straight chain of glucose. The characteristic distinguishing amylopectin from amylose is the presence of branches to the strain chain through α (1 \rightarrow 6) bonds occurring every 24 to 30 glucose units. Starch is semi-crystalline and transitions to an amorphous state at 60-70°C through gelatinization. Starch, especially gelatinized starch, is rather easily hydrolyzed to yield the individual glucose molecules. Thus starch, from corn, is the

feedstock used to produce more than 90% of the fuel ethanol in the United States as of 2006 (*Ethanol Industry Outlook*, 2006).

Cellulose is the major form of glucose polymer found in nature. Cellulose is a linear polymer of glucose joined by β 1-4 glycosidic bonds. The β 1-4 glycosidic bonds, in contrast to α 1-4 glycosidic bonds, does not result in coiling of the chain. Instead, cellulose assumes a straight rod-like structure (Gardner & Blackwel.J, 1974). The multiple hydroxyls along the cellulose chain hydrogen bond with the hydroxyls of other cellulose chains to form tight crystalline structures which make up microfibrils. Microfibrils have high tensile strength and are the major structural components of all plant cell walls (McCann et al., 2001). While cellulose is far more abundant than starch in the natural world, the high tensile strength and chemical stability of cellulose make it much more resistant to hydrolysis to yield glucose than starch.

III.B.1.a.(i)(b) Other sugars – Two other major plant sugars include fructose and sucrose. Fructose is a monosaccharide, like glucose, and is found in many fruits and other plants. Fructose is easily fermented to ethanol by *Saccharomyces* yeasts (e.g. distillers yeast). Sucrose is a dimer of fructose and glucose. Sucrose, commonly called table sugar, is the predominant sugar extracted from sugar cane and sugar beets. Sucrose, like fructose and glucose, is highly soluble in water. Sucrose is easily hydrolyzed to fructose and glucose by acid or enzymes. Most industrial strains of yeast naturally produce the necessary enzyme to hydrolyze sucrose into fructose and glucose. Ethanol production from sucrose is predominant in tropical regions with sugar cane production, such as Brazil.

Hemicellulose is another polysaccharide found in the cell walls of plants. Hemicellulose is a heterogeneous polymer with a xylose (grasses and hardwoods) or mannose (softwoods) polymer backbone. The polysaccharide backbone is highly substituted with sugars (glucose, galactose, arabinose, or rhamnose), organic acids (acetic, glucuronic, ferulic and p-coumaric acids). Xylose is a five carbon sugar (pentose) and represents approximately 40% of the simple sugars present in the polysaccharides that make up plant cell walls with xylose-rich hemicellulose (Somerville et al., 2004). *S. cerevisiae* is unable to metabolize xylose to produce ethanol; however efforts toward making the conversion of cellulosic biomass to ethanol feasible include the metabolic engineering of this yeast and other microorganisms to accomplish this goal.

III.B.1.a.(ii) Fermentation – Fermentation involves living microorganisms which consume sugars as a food source. Ethanol fermentation results in four major products: additional yeast cells (cell division), ethanol, carbon dioxide, and heat. One molecule of glucose will yield, stoichiometrically, 2 molecules of ethanol plus 2 molecules of carbon dioxide (Figure 1). On a mass basis, one pound of kilogram consumed for energy will theoretically produce 0.51 kilogram of ethanol and 0.49 kilogram of carbon dioxide. However, glucose consumed to generate additional yeast cells (cell mass) does not result in the production of ethanol. Most industrial fermentation processes operate at 90 – 95% of the theoretical yield of ethanol from glucose fed to the yeast.

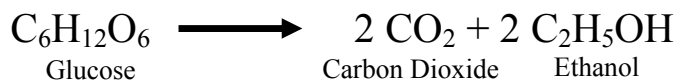


Figure 1. Fermentation of glucose to carbon dioxide and ethanol.

Fermentation for ethanol production at the industrial scale utilizes one of two major processing technologies, batch or continuous (or semi-continuous) fermentation. In batch fermentation, the fermentation process occurs in a single large fermentor which is filled with the sugar-rich liquid and living cells (inoculum), allowed to ferment, drained, and then cleaned in preparation for the next batch. There are usually multiple fermentors in batch operations, each in a different stage of the process (filling, fermenting, emptying, etc.). While the exact size of each fermentor varies between plant designs, common fermentor sizes range between 300,000 – 500,000 gallons (1-2 million liters) each (Kwiatkowski, Mcaloon, Taylor, & Johnston, 2006). The major advantage for batch fermentations is that they afford less opportunity for contamination, provided they properly sanitized between runs. Bacteria, especially species of *Lactobacillus*, can infect yeast fermentations and produce organic acids that lower ethanol yields and interfere with the *Saccharomyces* (Graves, Narendranath, Dawson, & Power, 2006).

In continuous fermentation, the process occurs through a series of cascading tanks where the liquid continuously flows through the process. New fermentation media is continuously added at the front end and fermented beer is continuously removed from the back end. While continuous fermentation has greater reactor productivity because it is continuously operating with high yeast loadings, much more care needs to be exercised to prevent contamination (Bayrock & Ingledew, 2001).

III.B.1.a.(iii) Distillation – Distillation is a common chemical separation method that is based upon differences in volatility (Wankat, 1988). If a mixture of ethanol and water are placed in a container at a given temperature and pressure, after time the mixture will reach equilibrium. At equilibrium, some of the ethanol will be a vapor in the gas above the liquid and some will be in the liquid phase. Similarly, some of the water will be in the vapor phase and some will be in the liquid phase. Because ethanol is more volatile than water (boils at a lower temperature), the ratio of ethanol to water in the vapor phase is greater than the ratio of ethanol to water in the liquid phase. This characteristic allows for the separation of the ethanol from the water.

Through subsequent vaporization of the mixture, condensation, re-vaporization, and re-condensation the mixture becomes higher and higher in ethanol content because the vapor at each vaporization step has higher ethanol concentration than the liquid from which it was vaporized. Thus, multiple fractionation steps can be used to purify ethanol from water. However, the basic principle by which this occurs – difference in boiling points between water and ethanol – ceases to exist when the mixture is 95.6 wt% ethanol (4.4% water). At this point, called the azeotrope, the ethanol and water both vaporize to the same degree and cannot be further fractionated by distillation. Either a third solvent can be introduced to break the azeotrope (e.g. benzene) or an alternate separation principle can

be applied such as absorption. The most popular method is absorb water away from ethanol using molecular sieves (section III.B.1.b.(iii)).

Industrial fractional distillation to produce fuel ethanol is one of the major energy inputs for the production of fuel ethanol. Process improvements that capture and recycle energy from the process has greatly reduced the cost of this step (Gulati, Westgate, Brewer, Hendrickson, & Ladisch, 1996; M. R. Ladisch & Dyck, 1979). All industrial fuel ethanol production uses continuous-feed distillation column systems.

III.B.1.a.(iii) Ethanol Drying - molecular sieves –Molecular sieves for drying ethanol are crystalline metal aluminosilicates (zeolites) with 3-dimensional porous structure of silica and alumina tetrahedra. Zeolites will strongly and preferentially adsorb water from vapor/gas mixture. The adsorbed water can be removed by increasing the temperature of the zeolite and passing dry gas over the particles, thus allowing this rather expensive desiccant (~\$10/lb) to be reused (Al-Asheh, Banat, & Al-Lagtah, 2004).

While this drying property was discovered with naturally occurring zeolites, commercial molecular sieves are synthetically produced to have highly uniform pores within a tight size distribution. Industrial molecular sieve drying systems consist of multiple columns each filled with a bed of uniform sized zeolite sieves. The ethanol/water vapor mixture leaving the fractional distillation system is super-heated and forced through the molecular sieve bed. The water vapor is selectively adsorbed to the particles while ethanol passes through the column, where it is recovered and condensed to liquid at high purity. After the capacity of the zeolites to adsorb water from the ethanol vapor is reached, feed vapor is stopped and the flow through the column is reversed. Dry gas (usually CO₂ produced by the fermentation process) passes over the zeolites while the system is placed under a slight vacuum to drive the desorption of the water from the solid particles. The water vapor and residual ethanol vapor exiting the column is condensed and returned to the stripper in the distillation system to re-vaporize the residual ethanol to improve the efficiency of ethanol recovery by the plant (M. R. Ladisch & Dyck, 1979).

To achieve continuous processing, molecular sieve dehydration systems consist of pairs of beds. As the first bed in the pair processes wet ethanol, a second molecular sieve bed undergoes regeneration to remove the adsorbed water. When the capacity of the first column to remove water is filled, the duties of the columns are switched so that the wet column begins regeneration and the fresh column continues to process wet ethanol vapor.

An alternative to zeolite molecular sieves is using ground corn (corn grits) in a packed bed, similar in design to conventional molecular sieve beds (Chang, Yuan, Tian, & Zeng, 2006; Neuman, Voloch, Bienkowski, & Ladisch, 1986; Westgate, Lee, & Ladisch, 1992). In this system, currently in commercial use by ADM, water is selectively adsorbed to corn starch from a water-ethanol vapor mixture (Beery, Gulati, Kvam, & Ladisch, 1998; Beery & Ladisch, 2001; M. R. Ladisch & Dyck, 1979). In similar design to the zeolite molecular sieve beds, the corn grit system operates in pairs of beds – one drying ethanol vapor while the other(s) are undergoing regeneration to remove the adsorbed water. The major advantages of the bio-based adsorbents that they are easily available, less

expensive than molecular sieves, mechanically stable, and easily disposable (M. R. Ladisch, 1997). Purified ethanol is then denatured by the addition of gasoline or other similar combustible organic solvent to render the product non-potable.

III.B.1.b. Conversion processes and technologies

III.B.1.b.(i) From grain (corn) – Corn is currently the primary feedstock for fuel ethanol production in the U.S., representing >90% of the raw material for ethanol production (*Ethanol Industry Outlook*, 2006). Approximately 1.1×10^{10} bushels (2.8×10^8 metric tons) of corn were produced in the U.S (*U.S. Grains Supply and Distribution: Wheat, Corn, Sorghum, Barley, Oats, Rye, and Rice*, 2006) of which ?% was directed for ethanol production. Corn is largely composed of starch (Table 1) which can be rather easily hydrolyzed to fermentable sugars and fermented to ethanol.

Table 1. Proximate chemical analysis of corn grain (Watson, 1987).

Compound	Average % Mass (dry basis)
Starch	71.7
Hemicellulose	6.2
Cellulose + Lignin	3.3
Protein	9.5
Fat	4.3
Ash	1.4

Two major processing technologies are used for converting corn into fuel ethanol: dry-grinding and wet milling. The major difference between these two processes are the process complexity and associated capital costs (dry-grind plants facilities are simpler and less expensive), number of co-products (more from wet-milling) and the flexibility in products that can be produced (wet milling is more flexible). Currently, U.S. fuel ethanol is mostly produced by dry-grinding and current expansion in the industry is through construction of new or expansion of existing dry-grind facilities. A description of the two processes follows.

III.B.1.b.(i)(a) Dry-grind – Dry-grind corn-ethanol production technology produces high ethanol yields (2.7-2.8 gal/bu) at minimal capital investment (Figure 2). However, the only major co-product –other than CO₂- is the fermentation residuals, which is sold as animal feed. This product is commonly called distillers dried grains with solubles, or DDGS.

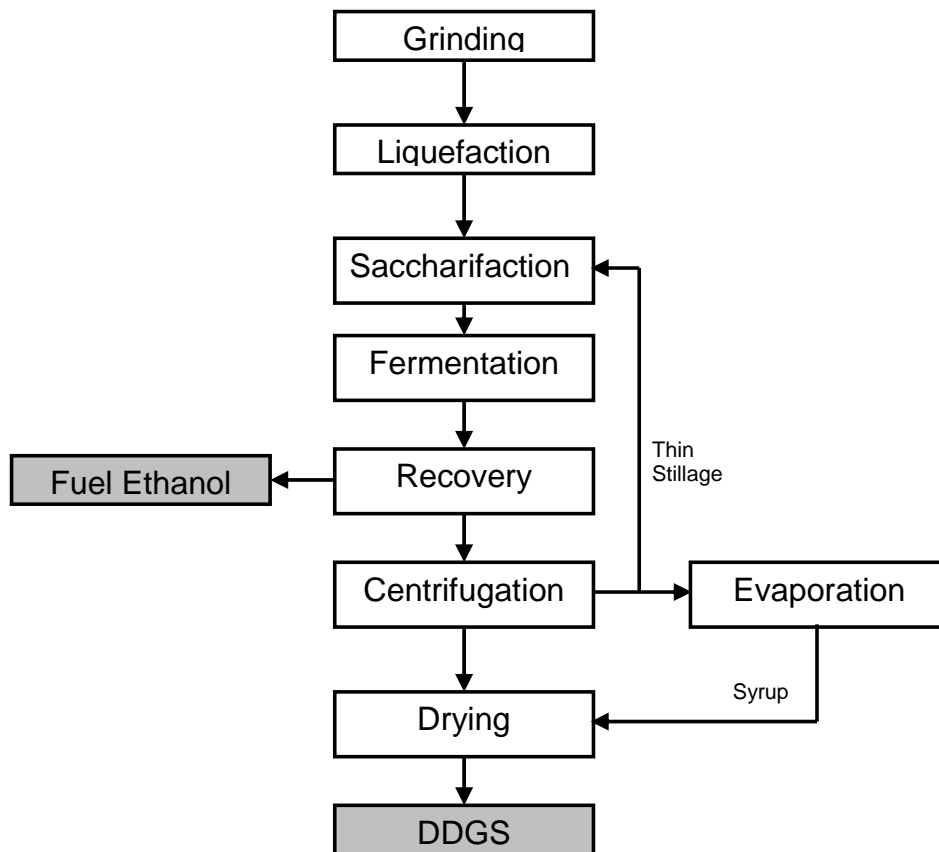


Figure 2. Overview of dry-grind ethanol process.

III.B.1.b.(i)(a)(1) Grinding – Enough grain is stored onsite in bins for 8-12 days of plant operation (Kwiatkowski et al., 2006). Broken kernels and foreign materials (metal, dirt, cobs, etc.) are removed by blowers and screens. The cleaned corn is then ground in hammer mills. The hammer mills are fitted with screens with openings ranging between 3.2 – 4.8 mm in diameter. The grain usually passes through after a single milling step, unlike corn dry milling which uses multiple milling steps to fractionate the components of the grain. The ground corn passing through the screens has a distribution of sizes with more than 90% of the ground corn (by weight) having a diameter between 0.5 to 2 mm (Rausch et al., 2005). Grinding serves to break the tough seed outer coating and increased the surface area of exposed corn starch.

III.B.1.b.(i)(a)(2) Liquefaction – The ground corn is slurried with process water to approximately 30% solids by weight (Kwiatkowski et al., 2006). Ammonia and lime are also added at this step to adjust the pH of the slurry to 6.5. The ammonia has the additional benefit of serving as a nutrient for the yeast in the subsequent fermentation step. Thermostable alpha-amylase is then added. Alpha-amylase (EC 3.2.1.1) is an enzyme that cleaves starch molecules at random points along the middle of the polymer chain.

The slurry is then heated by direct steam injection using a “jet-cooker” to 88°C. Thermostable alpha-amylase then break the starch into small, water soluble fragments

called dextrans. After approximately one hour, the output from the first step of liquefaction is combined with “backset”, which is recycled water from the end of the ethanol distillation process. The backset accounts for approximately 15% of the final volume of the corn mash (McAloon, Taylor, Yee, Ibsen, & Wooley, 2000). Critical nutrients for the yeast are also carried in the backset. As the liquefied slurry is cooled to 60°C, the removed heat is recovered to heat the incoming slurry going to the jet-cooker (Kwiatkowski et al., 2006). A new enzyme technology developed by Genencor allows for the rapid hydrolysis of granular starch. This eliminates the need for gelatination of starch slurry by jet-cooking, thus significantly lowering the energy requirements for ethanol production from corn (Shetty, Lantero, & Dunn-Coleman, 2005).

III.B.1.b.(i)(a)(3) Saccharification – Sulfuric acid is added to the liquefied slurry to lower the pH to 4.5. An additional enzyme, glucoamylase, also called beta-amylase (EC 3.2.1.3), is then added. Glucoamylase breaks starch and dextrans into glucose by the stepwise hydrolysis of glucose from the molecule ends. The slurry is held at 60°C for 5-6 hours as the glucoamylase hydrolyzes the dextrans to fermentable glucose (Schenck, 2002). Most of the dextrans are converted to glucose during this step, however the glucoamylase remains active throughout the fermentation step and will continue to hydrolyze any residual dextrans during the fermentation. After saccharification, the slurry (now called mash) is cooled to 32°C with heat being transferred to other process streams. The cooled mash then enters the fermentation tanks. A popular alternative to mash-presaccharification is to add the glucoamylase during the filling of the fermentor and saccharify the starch simultaneously with fermentation (SSF or Simultaneous Saccharification and Fermentation). An additional advantage to this approach is that reversion reactions where glucose re-polymerizes are much less likely to occur (Power, 2003).

III.B.1.b.(i)(a)(4) Fermentation - In the fermentation step, yeast grown in seed tanks are added to the corn mash to ferment the simple sugars (glucose) to ethanol. The other components of the corn kernel (protein, oil, etc.) remain largely unchanged during the fermentation process, though the corn oil helps to prevent foaming during the fermentation. In most dry-grind ethanol plants, the fermentation process occurs in batches. A fermentation tank is filled and ferments completely before being drained and refilled with a new batch. The up-stream processes (grinding, liquefaction, and saccharification) and downstream processes (distillation and recovery) occur continuously (grain is continuously processed through the equipment). Thus, dry-grind facilities of this design usually have three or more fermentors. At any given time, one fermentor is filling, one is fermenting (for approximately 46-68 hours), and one is emptying and resetting for the next batch. Carbon dioxide is also produced during fermentation. Usually, the carbon dioxide is not recovered as a sellable product. If recovered, this carbon dioxide can be cleaned, compressed and sold for carbonation of soft drinks or frozen into dry-ice for cold product storage and transportation. If the carbon dioxide is not recovered, it passes through a water scrubber to remove evaporated ethanol and other volatile organic compounds (VOCs) carried in the gas. The water from the scrubber, containing the recovered ethanol, is sent to the distillation system. The cleaned carbon dioxide is vented to the atmosphere.

Heat is another by-product of fermentation. Approximately 12000 kJ are released for each kg of ethanol produced (516 BTU per pound) (Kwiatkowski et al., 2006). This heat must be continuously removed from the fermentors. This is usually accomplished in one of two ways. Either cooling water is passed through a cooling coil that is within the fermentors or the fermenting mash is continuously pumped from the fermentors through a large heat exchanger where the heat is transferred to cooling water before the mash is returned to the fermentor. If the heat is not removed, the temperature in these large fermentors will quickly rise to the point that will kill the yeast. The cooling system attempts to maintain the fermentation temperature between 27-32°C which is the optimum temperature for ethanol fermentation.

After the fermentation is nearly complete, the fermented corn mash (now called beer) is emptied from the fermentor into a beer well. The beer contains 8-10% ethanol by weight. The beer well stores the fermented beer from individual batches so a continuous stream can be supplied to the ethanol recovery system

III.B.1.b.(i)(a)(5) Separation – Separation of the ethanol from the water slurry is accomplished through a continuous process containing a number of steps. The complex recycling of liquid and vapor streams between the beer column, rectifier, stripper, and molecular sieves is illustrated in Figure 3. In the first step, the beer is processed through a beer column where steam is used to strip off almost all of the ethanol, along with some water, from the slurry. The ethanol and water vapor exit the top of the beer column and the whole stillage exits from the bottom. Whole stillage leaving the bottom of the beer column for the decanter contains less than 0.1% ethanol by weight. The overhead vapor flows to a rectifier column where the ethanol is concentrated from 45% to 91% through fractional distillation (section II.B.1.a.(iii)). The bottoms from the rectifier pass through a stripping column to remove the residual ethanol. Liquid exiting the bottom of the stripper has less than 0.1% ethanol by weight, and is recycled as process water for slurring the ground corn. The overhead vapor from the rectifier (91% ethanol by weight) is superheated and passes through molecular sieves, as described above in section III.B.1.a.(iii). The final product from the molecular sieve system is ethanol vapor that is at least 99.6% pure. This vapor is condensed and mixed with a denaturant (e.g. gasoline) to render it as non-potable fuel ethanol. Denatured fuel ethanol stored on site generally varies between 8-12 days of production (Kwiatkowski et al., 2006).

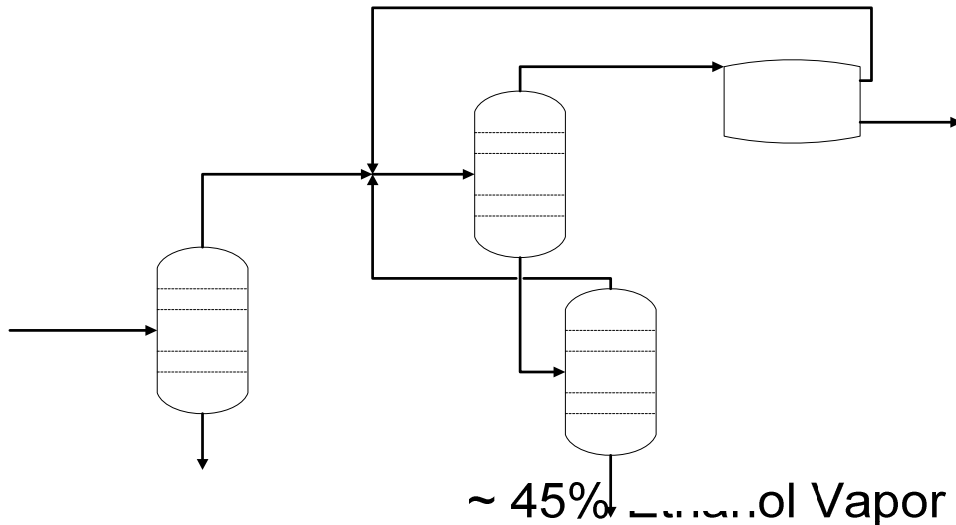


Figure 3. Simplified flow diagram of dry-grind ethanol distillation process.

III.B.1.b.(i)(a)(6) Byproduct (DDGS) – Whole stillage leaving the bottom of the beer column (Figure 3) contains approximately 15% solids. The whole stillage is centrifuged to remove approximately 83% of the water. The centrifuged solids, termed wet distillers grains or wet cake, is 35 – 40% solids. The liquid stream, called thin stillage, is partially recycled as backset to the second stage of the liquefaction as described above. The remaining thin stillage passes to a surge tank which supplies a steady feed to the evaporators, where they are concentrated.

Beer Column

Thin stillage passes through a multiple effect evaporator which removes a significant amount of the water as steam (Figure 2). This steam is used to vaporize the ethanol in the reflux for the rectifier (Figure 3). The steam, now condensed to liquid, is mixed with other condensates and recycled to the beginning of the process and added to the ground corn. The concentrated product of the evaporators is a syrup containing 55% solids by weight (McAloon et al., 2000). The syrup is mixed with wet distillers grains and sent to a large rotary drum dryer. The rotary drum dryer dries the mixture from 64% moisture to 9-10% moisture. The hot gas from the dryers needs to be processed prior to venting to the atmosphere to remove volatile organic compounds (VOCs) released during drying. Thermal oxidation is commonly used to convert VOCs to carbon dioxide and water (Vij, 2003).

Whole Stillage

III.B.1.b.(i)(a)(7) DDGS issues and research needs – As fuel ethanol production from corn through dry-grind technology continues to increase, efficient use and capturing the value of the residuals becomes increasingly important (Rausch & Belyea, 2006). The effects of large-scale feeding of DDGS in animal rations and the optimization of cattle, dairy, swine, and poultry diets for inclusion of DDGS will become critical (Belyea, Rausch, & Tumbleson, 2004). DDGS has long been used in cattle feed rations. Current recommendations are inclusion rates up to 40% (Ham et al., 1994; Peter et al., 2000). However, much less research has been conducted on including DDGS in non-ruminant animal feed rations such as swine and poultry. Recent published studies recommend inclusion rates (based on amino acid) for DDGS up to 20% for swine (Whitney, Shurson,

Johnston, Wulf, & Shanks, 2006). However, in laying hens the recommended inclusion rate is less than 10% (Lumpkins, Batal, & Dale, 2005).

Handling and flowability properties of DDGS also require additional attention. These properties are critical for efficient transportation of DDGS to markets by barge, rail, or truck (Ganesan, Muthukumarappan, & Rosentrater, 2006; MCGA, 2005). Improved methods for measuring nutritional quality are currently under development to help insure feed quality for this product in the marketplace (*Evaluation of Analytical Methods for Analysis of Dried Distillers Grains with Solubles*, 2007).

Table 2. Composition of DDGS (Belyea et al., 2004).

Compound	Average % Mass (dry basis)
Crude Fat	11.9
Crude Protein	31.3
Crude Fiber	10.2
Acid Detergent Fiber	17.2
Starch	5.1
Ash	4.6
Total	80.3

In addition to protein, DDGS also contains a lot of fiber (Table 2), which is largely cellulose and hemicellulose (M. Ladisch et al., 2006), Integrating cellulose conversion technologies in dry-grind facilities might further increase the value of DDGS by lowering its fiber content and increasing its relative protein content, not to mention increasing the plants over-all ethanol yield per bushel (N. S. Mosier et al., 2005).

III.B.1.b.(i)(b) Wet mill – Corn wet milling is designed to fully fractionate corn grain so that the major constituents (Table 1 - carbohydrates, lipids, and protein) can be efficiently recovered and purified for the production of value-added products. Corn wet mills are generally larger in size than dry-grind facilities due to the higher capital intensity of the process. While fuel-grade ethanol is produced by some wet milling facilities, most corn wet mills produce a number of food-grade products such as specialty starches, high-fructose corn syrup (HFCS), corn oil, acidulants (citric acid) and thickeners (xanthan gum). The upstream processing steps (steeping and fractionation) are common to all wet mills (Figure 4). Various technologies can be used to further process the starch into the variety of value-added products produced by corn wet mills. When the starch is converted to fuel ethanol, the processing steps (liquefaction, saccharification, fermentation, and recovery) are very similar to what is done in a dry-grind operation.

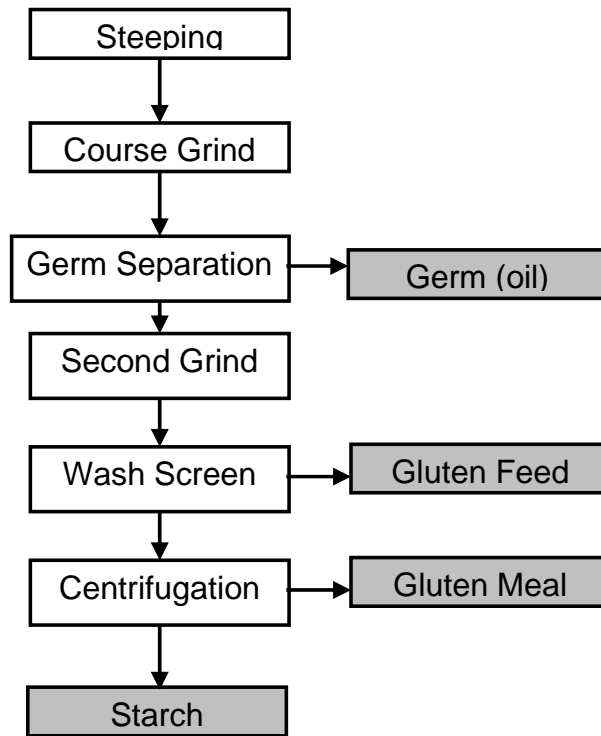


Figure 4. Overview of wet milling process.

III.B.1.b.(i)(b)(1) Steeping – The first step in the wet milling process, steeping, is what differentiates this process from dry milling (for cereal processing) and dry-grinding (for ethanol production) processes. In the steeping process, the corn kernels are soaked in water acidified with SO₂. at the corn is typically steeped at 52°C (125°F) for 22-50 hours. Steeping softens the kernels by thoroughly wetting the grain from 15% to 45% moisture content. Sulfur dioxide (typically at 0.12 – 0.20% in the water) and possibly lactic acid produced by lactic bacteria breaks down the protein-starch matrix in the endosperm to aid in the separation of the starch from the protein and other components of the grain (May, 1987).

III.B.1.b.(i)(b)(2) Fractionation – Two milling processes are employed in wet milling to fully fractionate the kernel components. The first milling process is a course grinding mill. This mill consists of a stationary disk and a rotating disk. The disks have knobs that break up the kernels. The course grind mill is adjusted so that few kernels exit without being broken while few germs inside the kernels are damaged during the grinding (May, 1987). After the course grind, the oil-rich germ is separated from the slurry based upon differences in densities. Hydrocyclones are commonly used to float the germ out of the top while the other components leave the bottom of this processing step. The separated germ is then dewatered and dried before removal of the corn oil by solvent extraction.

The underflow material passes through a wedge-wire screen to separate the pericarp fiber from the starch and protein, called gluten. Approximately 30-40% of the starch is recovered in this step (May, 1987). The recovered fiber, containing attached starch, is next milled using either a entoleter or a disk mill. An entoleter mill forces the fiber

against pins at high speed to shear the starch from the fiber. Disk mills, similar to the course mill described above, have knobs or grooves that remove the starch from the fiber. Both milling processes are tuned to maximize starch separation from the fiber, while gentle enough to keep the fiber intact. The newly separated starch is once again separated from the fiber by passing it through a screen sized to small for the fiber to pass through. The processed fiber is then dewatered by pressing to 40-60% moisture. This material is mixed with evaporated stillage and steep (check this statement?), and dried to a product marketed as corn gluten feed – despite the absence of gluten in the product! Corn gluten feed is an inferior feed to DDGS because it has a lower protein content and energy value.

The starch/protein slurry that is fractionated from the fiber is further processed to separate the starch from the protein. Gluten is lower in density than starch (1.06 SG compared with 1.6 SG for starch) (May, 1987). Thus centrifugation or hydrocyclones can be used to efficiently separate the protein from the starch. The fractionated protein is dewatered, dried, and marketed as animal feed under the label of corn gluten meal. The remaining starch after the primary centrifugation also contains 3-5% protein. This may be further purified depending on the end use of the starch. In fuel ethanol production, this primary starch slurry is usually processed as described above for liquefaction (III.B.1.b.(i)(a)(3)) and saccharification (III.B.1.b.(i)(a)(3)) in the dry-grind process.

III.B.1.b.(i)(b)(3) Fermentation – The fermentation of the saccharified starch in a wet mill is similar to the process described above for dry-grind facilities. The only major differences are the lower amount of insoluble solids in the fermentation liquid, since the fiber and germ have been removed, and the use of steep water. The liquid used in the steeping process contains a significant amount of protein and micronutrients from the corn (Rausch, Thompson, Belyea, & Tumbleson, 2003). Sometimes, the steep is sold in a condensed form as a complex nitrogen/nutrient source for industrial fermentations (Kapen, 1996). In ethanol production at wet milling facilities, the saccharified starch is diluted to the desired concentration (16 – 22 wt% sugar) using steep water before the yeast is added to the fermentor. The fermented beer is then processed to separate and purify the ethanol using fractional distillation and molecular sieves as described above (May, 1987).

III.B.1.b.(i)(b)(4) Byproducts – There are three main byproducts from the wet milling of corn to produce ethanol: germ, corn gluten meal, and corn gluten feed. The germ, which is separated from the other kernel constituents after the course grind, contains the majority of the oil found in maize. The germ may be processed to extract the oil, which can be further refined into food-grade corn oil. This may be done in the same facility as the ethanol plant or sold to a secondary processor.

Corn gluten meal is the protein rich product separated from the gluten-starch slurry by centrifugation or hydrocyclones as described above. Corn gluten meal is approximately 60% protein and is sold as high-protein animal feed (Ham et al., 1994). The residual fiber separated from the starch-gluten slurry after the second milling step is called corn fiber. Corn fiber is mixed with evaporated light stillage and dried to produce a middle-

grade protein product (20-26% of dry matter) called corn gluten feed. This product is also sold as an animal feed (Loe, Bauer, & Lardy, 2006). Corn gluten feed is similar in fiber content but lower in protein content when compared to DDGS. Corn gluten feed is much lower in oil content than DDGS because the germ has been fractionated and separated from the fibrous solids (Ham et al., 1994).

III.B.1.b.(ii) From sugar cane

III.B.1.b.(ii)(a) Process overview – Sugar cane is the principal feedstock for the fuel ethanol industry in Brazil. In 2004, 4.2 billion gallons (15 billion liters) of fuel ethanol was produced in Brazil from sugar cane (Barros, 2005). The upstream processing of sugar cane is nearly identical to standard sugar cane processing for sugar production. Once the sugar is recovered, the fermentation and ethanol recovery process is very similar to corn ethanol production, although the fermentation and distillation technology is based upon large-scale beverage alcohol production (Rosillo, 1986). Fermentation can be done directly with the sugar cane juice (without any evaporation) or from the molasses, after some granulated sugar has been produced. Most ethanol production plants in Brazil produce ethanol from the molasses. Most plants in Brazil use molasses A, which has had sugar removed by a single crystallization step. The ethanol concentration in the fermented molasses is about 9% v/v (7% w/w), which is lower than the concentration for corn fermentation. This translates into shorter fermentation times. Free amino nitrogen is low or quite variable in sugar cane molasses, so additional nutrients are often added to insure proper yeast performance. For fuel grade ethanol production, urea is the typical nitrogen source that is added. Depending upon the quality of the molasses, phosphorous, biotin, pantothenic acid, and inositol may be added to improve yeast performance (Piggot, 2003).

III.B.1.b.(ii)(b) Byproducts – bagasse for heat and power – One major difference between sugar cane and corn-based ethanol production processes is the byproducts associated with each feedstock. The residuals left after the starch in corn is converted to ethanol are rich in oil (found in the germ) and protein, both of which are valuable as human or animal food products. The residual left after sugar cane processing is called bagasse. Bagasse is rich in cellulose, hemicellulose, and lignin (Okano et al., 2006; Pandey, Soccol, Nigam, & Soccol, 2000; Silva, Matos, & Carvalho, 2005), but has no food value. However, bagasse can be burned to produce heat and steam. Traditional sugar cane processing plants use this heat to evaporate the water from the sugar in the crystallization process that is used to produce granulated sugar. In fuel ethanol plants, this energy supplies heat to the distillation steps. In fact, excess heat beyond the needs of the processing plant are produced which can also supply steam-powered generators for electrical generation for the plant with excess sold to the power grid (Bhatt & Rajkumar, 2001; Goldemberg, Coelho, Nastari, & Lucon, 2004). The stillage left after the distillation of ethanol is used as a fertilizer for sugar cane fields.

III.B.1.b.(iii) From cellulosic biomass – Plant matter, such as grasses and trees, represent a large reservoir of carbohydrates that could potentially be converted to fuel ethanol. These materials are largely inedible by humans, though forage grasses are fed to cattle. A joint study by the U.S. Department of Agriculture and U.S. Department of

Energy concluded that approximately 1.3 billion dry tons of cellulosic biomass not currently utilized could potentially be collected each year as a feedstock for producing ethanol. This represents approximately 100 billion gallons of ethanol which could replace 30% of current U.S. demand for gasoline (Perlack et al., 2005). However, a number of technological hurdles must be overcome before cellulosic ethanol is economically competitive with grain alcohol or gasoline.

III.B.1.b.(iii)(a) Plant Cell Walls –The two major carbohydrate polymers found in all plant cell walls are cellulose and hemicellulose. These two carbohydrate polymers, along with pectins (another carbohydrate polymer), protein, and lignin, form the complex matrix of plant cell walls that give them structural stability and protection from the environment (Somerville et al., 2004).

III.B.1.b.(iii)(b) Pretreatment – Pretreatment is the general term for processing steps that precede hydrolyzing the polysaccharides in cellulosic biomass into fermentable sugars. The primary purpose of pretreatment is to convert the cellulose and hemicellulose in the plant cell walls into a more reactive form that breaks down into fermentable sugars more rapidly and more completely (Grohmann et al., 1984; Lynd, Wyman, & Gerngross, 1999; McMillan, 1994). Pretreatment methods usually require rather harsh conditions and non-selectively hydrolyze the polysaccharides in the plant material. Sugars that are produced during pretreatment can be further degraded to compounds that act as inhibitors to the subsequent fermentation (Klinke, Thomsen, & Ahring, 2004; Olsson & Hahn-Hagerdal, 1996; Palmqvist & Hahn-Hagerdal, 2000; Taherzadeh, Eklund, Gustafsson, Niklasson, & Liden, 1997).

The goal of any pretreatment technology is to alter or remove structural and compositional factors present in plant biomass that hinder hydrolysis of cell wall polysaccharides to fermentable sugars (N. Mosier et al., 2005). Effective pretreatments improve the rate of enzyme hydrolysis and increase yields of fermentable sugars from cellulose or hemicellulose. These methods cause physical and/or chemical changes in the plant biomass in order to achieve this result. Pretreatment methods are either physical or chemical. Some methods incorporate both effects (Hsu, 1996; McMillan, 1994). For the purposes of classification, steam and water are excluded from being considered chemical agents for pretreatment since extraneous chemicals are not added to the biomass. Acids or bases are chemical additives that promote hydrolysis and improve the yield of glucose recovery from cellulose by removing hemicellulose or lignin during pretreatment. The most commonly used acids and bases are H_2SO_4 and NaOH or ammonia. Cellulose solvents are another type of chemical additive. Alkaline H_2O_2 , ozone, organosolv (uses Lewis acids, $FeCl_3$, $(Al)_2SO_4$ in aqueous alcohols), glycerol, dioxane, phenol, or ethylene glycol are among solvents known to disrupt cellulose structure and promote hydrolysis (Wood & Saddler, 1988). Further objectives and successes of promising pretreatment processes are summarized and evaluated by (Lynd et al., 1999), (N. Mosier et al., 2005), and (Kim, Hendrickson, Mosier, & Ladisch, 2005).

A commonly used pretreatment method is dilute sulfuric acid (Hinman, Schell, Riley, Bergeron, & Walter, 1992; Lynd et al., 1999; Thompson & Grethlein, 1979). However,

major drawbacks are the need for expensive reactors constructed from exotic steels, formation of unwanted salts, and the need for neutralizing agents (Hinman et al., 1992; Lynd, 1996). Liquid hot water pretreatment with pH control effectively dissolves hemicellulose and lignin while minimizing degradation of monosaccharides without the need for costly and potentially dangerous pretreatments and neutralizing agents (Kim et al., 2005; Weil, Brewer, Hendrickson, Sarikaya, & Ladisch, 1998). Other pretreatment methods include: steam explosion (Abatzoglou, Chornet, Belkacemi, & Overend, 1992; Heitz et al., 1991; Ramos, Breuil, & Saddler, 1992), ammonia fiber expansion (AFEX) (Gollapalli, Dale, & Rivers, 2002; Teymouri, Laureano-Perez, Alizadeh, & Dale, 2005; Wang, Dale, Yurttas, & Goldwasser, 1998), and other chemical solvents (Hsu, 1996; McMillan, 1994). Few of these pretreatment processes have been fully commercialized or tested at industrial scales (N. S. Mosier et al., 2005). Scaling pretreatments and the associated reactor design issues remains a major barrier to commercial production of fuel ethanol from lignocellulosic biomass (Lynd et al., 1999; N. Mosier et al., 2005).

III.B.1.b.(iii)(c) Saccharification - After pretreatment, plant cell wall polysaccharides are now more susceptible to chemical or enzymatic hydrolysis. In the saccharification step the polysaccharides are further broken down into their substituent monomeric sugars that the yeast can use for fermentation. The goal of the hydrolysis step is produce high-concentrations of monosaccharides that are available for the yeast to ferment quickly (Lynd et al., 1999). Depending on the pretreatment method and how well the pretreatment softened the biomass, this step will take more than 24-48 hours before the material is ready to be inoculated for fermentation (Lin & Tanaka, 2006; Olsson & HahnHagerdal, 1996). In an effort to cut down on this processing time, the enzymes can be added with the yeast inoculum in a method called simultaneous saccharification and fermentation (SSF) (Lynd et al., 1999). The enzymes hydrolyze oligomers while the yeast is present to ferment the monosaccharides directly. This provides the yeast with a just-in-time supply of fermentable sugars. SSF is meant to reduce or eliminate valuable processing time during the hydrolysis step and lower the amount of enzyme required in separate hydrolysis due to end product inhibition. Since fermentable sugars are being consumed as they are produced, this also could avoid sugar degradation into inhibitory compounds as well as inhibition of hydrolysis enzymes by these sugars (Lin & Tanaka, 2006).

III.B.1.b.(iii)(d) Fermentation – The fermentation process for producing fuel ethanol from lignocellulosic biomass is very similar to the process described above for dry-grind ethanol production from corn. The fermentation media will contain insoluble solids from the plant biomass in addition to the yeast and soluble products from the pretreatment and saccharification steps. Hexoses (glucose and galactose) liberated from the plant biomass can be fermented to ethanol by industrially strains of *S. cerevisiae*, as these sugars are identical to sugars utilized in other ethanol fermentations. The relatively large amounts of pentoses (xylose and arabinose) found in plant biomass necessitate the development of microbes able to convert these sugars to ethanol in order to make the overall process economically feasible (Eggeman & Elander, 2005; Nagle, Ibsen, & Jennings, 1999).

III.B.1.b.(iii)(d)(1) Fermentation of pentoses by yeast - Yeast are highly effective in fermenting glucose to ethanol at high rates and high yields. However, *Saccharomyces* yeast are unable to ferment xylose and other pentoses to ethanol. The only feasible option to make *S. cerevisiae* ferment xylose is by recombinant DNA techniques.

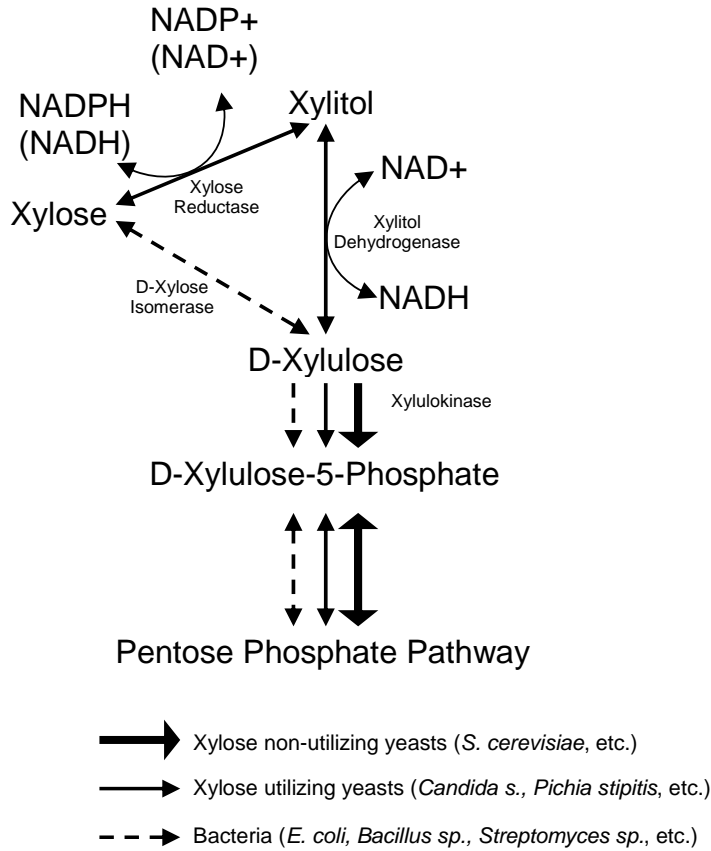


Figure 5. Xylose utilization pathways compared in yeast and bacteria. From Figure 1, pg. 166,(N. Ho, Chen, Brainard, & Sedlak, 1999).

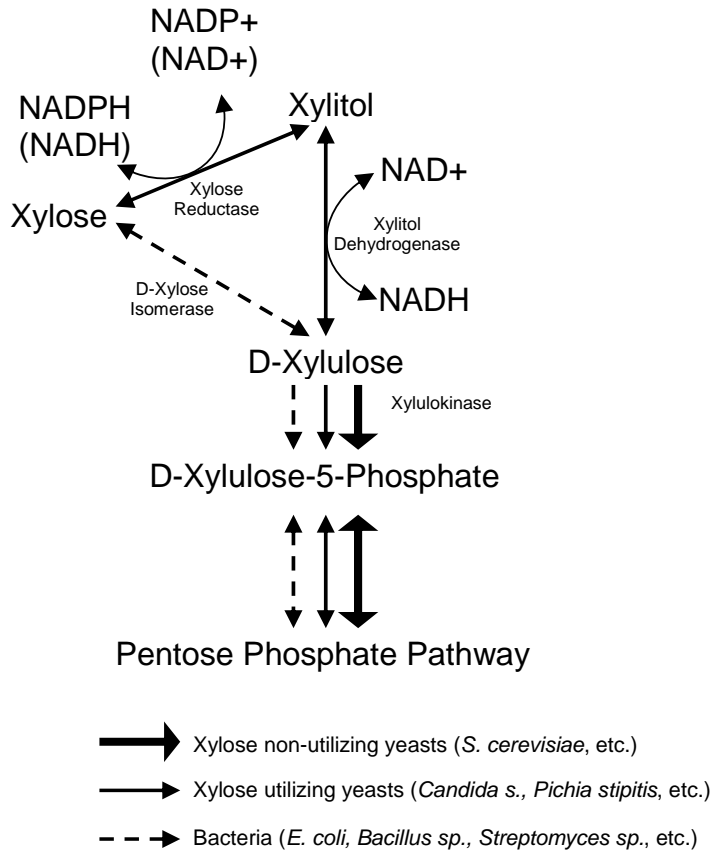


Figure 5 shows the different xylose utilization paths employed by yeast and bacteria.

Industrial ethanol producing *S. cerevisiae* strains can ferment xylulose to ethanol via the pentose phosphate pathway, but lack the enzymes necessary to convert xylose to xylulose. Xylose utilizing yeasts convert xylose to xylulose in two steps through an intermediate compound, xylitol. Bacteria, however, convert xylose directly to xylulose. Early efforts to metabolically engineer *S. cerevisiae* to utilize xylose transferred a bacterial xylose isomerase (XI) gene (Figure 5) into yeast (N. W. Y. Ho, Rosenfeld, & Stevis, 1983; N. W. Y. Ho, Stevis, Rosenfeld, Huang, & Tsao, 1983; Morgan & Nicolaidis, 1983). Unfortunately these efforts did not result in genetically modified yeast strains that were efficient in fermenting xylose to ethanol.

Later attempts at genetic modification focused on cloning genes from more closely related yeasts to *S. cerevisiae*. Successful cloning and over-expression of the yeast xylulokinase (XK) gene in *S. cerevisiae* was completed in 1987 (N. W. Y. Ho & Chang, 1989; Xue & Ho, 1990). This strain of *S. cerevisiae* could not only ferment xylose to ethanol, but also effectively co-ferment both glucose and xylose to ethanol. This was

accomplished by first cloning the properly modified (fused to glycolytic promoters) xylose reductase (XR), xylitol dehydrogenase (XD) (both from *Pichia stipitis*), and XK (from *S. cerevisiae*) genes on a high copy-number plasmid or integrative plasmid and using the resulting plasmids to transform *S. cerevisiae*. In addition, four other groups (one each in Sweden, Finland, Germany, and Japan) have also genetically engineered *S. cerevisiae* to ferment xylose. These groups cloned only the XR and XD genes from *P. stipitis* (Kotter, Amore, Hollenberg, & Ciriacy, 1990; Tantirungkij, Nakashima, Seki, & Yoshida, 1993; Walfridsson, Anderlund, Bao, & HahnHagerdal, 1997).

Using the new gene integration method developed at the Laboratory of Renewable Resources Engineering (LORRE) at Purdue University, scientists there successfully integrated sufficient copies of the XR-XK-XD (or KDR) cassette (at least ten copies per cell) into the original polyploid yeast 1400 strain used to develop a more effective glucose/xylose-cofermenting recombinant yeast. The resulting “stable” 1400(LNH-ST) could coferment glucose and xylose to ethanol even more effectively than 1400(pLNH32) (N. Ho et al., 1999; N. Ho & Tsao, 1998; N. W. Y. Ho, Chen, & Sedlak, 2000; Toon et al., 1997). More than ten different yeast strains, which are all effective glucose-fermenting yeast, to glucose/xylose co-fermenting yeast, have been successfully transformed. Several companies have used one of these strains, 424A (LNH-ST), to successfully ferment their particular cellulosic biomass feedstock hydrolysates to ethanol (Corrington & Abbas, 2003; Dennison & Abbas, 2003; Helle, Murray, Lam, Cameron, & Duff, 2004). Additional efforts are underway to improve the tolerance of *S. cerevisiae* to inhibitory compounds found in lignocellulose or generated during processing (Liu et al., 2004; Olsson & HahnHagerdal, 1996; Palmqvist & Hahn-Hagerdal, 2000; Zaldivar, Nielsen, & Olsson, 2001).

III.B.1.b.(iii)(d)(3) Genetically modified bacteria – Many bacteria, such as *E. coli* and *Klebsiella oxytoca* are natively able to utilize a wide range of sugars, including xylose. However, these bacteria are not efficient producers of ethanol. Another bacteria, *Zymomonas mobilis*, is an efficient ethanol producer but can only utilize glucose and fructose. These three bacteria, *E. coli*, *K. oxytoca*, and *Z. mobilis*, have all been successfully modified to produce ethanol from xylose (Dien, Cotta, & Jeffries, 2003; Zaldivar et al., 2001).

At the same time initial successes were reported on the modification of *S. cerevisiae* to ferment xylose to ethanol, *E. coli* was successfully metabolically engineered to selectively produce ethanol (Ingram, Conway, Clark, Sewell, & Preston, 1987). The bacteria was a natural choice because it has been extensively studied, is easily cultured, has been used industrially for the production of recombinant proteins, and can ferment a wide array of sugars without the need for complex growth factors or nutrients. However, *E. coli* has the significant disadvantages of narrow pH growth range (pH 6.0 – 8.0) and being less tolerant than yeast to harsh conditions (heat, salt, shear, acid, etc.) (Dien et al., 2003). Continued efforts have improved the performance of *E. coli* for the fermentation of sugars from lignocellulose to ethanol. Strain K011, resulting from genetic modifications to improve ethanol yields, has been shown in the lab to successfully ferment hydrolysates from pine, sugarcane bagasse, and corn stover (Asghari, Bothast,

Doran, & Ingram, 1996; Barbosa, Beck, Fein, Potts, & Ingram, 1992; Dien, Hespell, Ingram, & Bothast, 1997). Strain FBR5 has been shown at the lab scale to successfully ferment sugars derived from corn hulls and germ meal (Dien, Nichols, O'Bryan, & Bothast, 2000). Further effort are needed to improve ethanol tolerance to increase the concentrations of ethanol that can be produced by genetically modified *E. coli* (Gonzalez et al., 2003).

Another bacteria that has received considerable attention as a candidate for metabolic engineering for fuel ethanol production is *Z. mobilis* (Zaldivar et al., 2001). This organism has several properties that make it appealing: native homo-ethanol fermentation pathway, high tolerance to ethanol (up to 12 wt%), ethanol fermentation is high yield and high productivity, and is considered generally regarded as safe (GRAS) (Dien et al., 2003). However, unlike *E. coli*, *Z. mobilis* is only able to consume glucose and fructose. Researchers at the National Renewable Energy Laboratory (U. S. Department of Energy) have successfully engineered strains of *Z. mobilis* to ferment xylose and arabinose to ethanol, in addition to glucose and fructose. The first recombinant strain to ferment xylose required the insertion and expression of four *E. coli* genes (xylose isomerase, xylulose kinase, transketolase, and transaldolase). This resulted in a strain that was capable of co-fermenting glucose and xylose (Zhang et al., 1995). A similar strategy was employed to engineer a strain capable of fermenting arabinose to ethanol (Deanda, Zhang, Eddy, & Picataggio, 1996). These initial efforts have led to the development of *Z. mobilis* AX101. This strain carries all seven genes necessary to ferment both xylose and arabinose, in addition to glucose, as part of its chromosomal DNA (Mohagheghi, Evans, Chou, & Zhang, 2002; Mohagheghi, Evans, Finkelstein, & Zhang, 1998). Integration of these genes into the chromosome eliminates the need for antibiotics to maintain exogenous DNA carried on plasmids (Dien et al., 2003). Additional efforts are underway to improve the low tolerance of *Z. mobilis* to acetic acid, an organic acid naturally found in lignocellulose (Mohagheghi et al., 2002; Tao, Miao, Shi, & Zhang, 2005).

While great strides have been made in genetically modifying yeast and bacteria to ferment xylose to ethanol, additional efforts are still required in order to meet all of the following traits that are important for economical production of ethanol from lignocellulosic biomass (Zaldivar et al., 2001). Ethanol yields from consumed sugars must be high (> 90% of theoretical) and productivity (rate at which ethanol is produced) must be high (> 1 g L⁻¹ hr⁻¹) in order for this biotechnology to be practical (Dien et al., 2003). In addition, the organisms must possess a high tolerance for the ethanol produced (> 4 wt%) in order to make the energy requirements of distillation and recovery of the ethanol practical (M. R. Ladisch & Dyck, 1979). A number of fermentation inhibiting compounds, such as acetic acid, furfural, and lignin degradation compounds, are found in sugar streams derived from lignocellulosic biomass (Palmqvist, Grage, Meinander, & Hahn-Hagerdal, 1999). Substantial resistance to these inhibitors and tolerance of acidic pH (pH 4 – 5) is also required of industrially practical strains (Palmqvist & Hahn-Hagerdal, 2000).

III.B.1.b.(iii)(e) Distillation – Recovery of the ethanol produced by fermentation will likely occur using similar technology found in the corn dry-grind facility or in the sugar

cane to ethanol facility. Most proposed designs for cellulose-to-ethanol facilities include burning of the residual material which is high in lignin in order to supply heat and electricity to the rest of the processing steps (McAloon et al., 2000). This process design shares a number of commonalities with sugar cane processing to ethanol.

III.B.1.b.(iii)(f) Byproducts – Unlike processes that produce ethanol from corn, the residual solids from ethanol production from lignocellulose has little or no value as an animal feed (McAloon et al., 2000). The residual material is very high in lignin while low in fiber and protein. This residual material shares many characteristics with the byproduct of sugar or ethanol production from sugar cane (bagasse). Like sugar processing and paper pulping, the residual from lignocellulosic ethanol production can be burned for heat to produce steam. This steam can then be used to drive the distillation and pretreatment steps in the process while also providing energy for electrical generation. Current estimates for models of lignocellulosic ethanol plants predict that sufficient steam and electricity could be produced to fully supply the needs of the facility while also generating excess electricity which could be sold to the electrical grid (Nagle et al., 1999).

III.B.2. Biodiesel

Biodiesel is a bio-based fuel produced from lipids. It is comprised of mono- alkyl esters of long chain fatty acids derived from vegetable oils or animal fats, and is produced throughout the United States as an alternative and/or additive to petroleum diesel fuel. The percent of biodiesel in a fuel is classified using the nomenclature BXX, with XX being the percent of biodiesel in the blend. Pure biodiesel, or biodiesel with no petroleum mixed in, is designated B100 (Biodiesel 101, n.d.). At the present time, most fleets that run on biodiesel use blends of B2 through B20. Through a process called transesterification, biodiesel is made by reacting vegetable oils or fats with alcohol in the presence of a catalyst to produce fatty acid methyl esters (FAME) or ethyl esters (FAEE) and glycerol (Meher, Sagar, & Naik, 2006). There are many different types of vegetable oils that can be used in the production of biodiesel, and some of the most common oils include canola, castor, peanut, palm, rapeseed, soybean, and sunflower. The choice of which oil to use is usually determined by the local and national economies of where the biodiesel is being produced. Much of the biodiesel efforts in Europe and Asia focus on rapeseed oil because of its abundance in those locations, while a studies from Brazil focus on castor and soybean oil because both of these plants are grown in Brazil in large quantities (de Oliveira et al., 2005). In the Unite State, soybean oil is the predominant feedstock.

III.B.2.a. Conversion processes and technologies - The process of making biodiesel utilizes triglycerides and alcohol to produce esters and glycerol. Common sources of triglycerides include plant oils, animal fats, and used cooking grease. As transesterification (Figure 6) proceeds, the reaction can be altered by temperature, reaction length, molar ratio of alcohol to triglyceride, type of catalyst used, and amount of water and free fatty acids present. The mechanism in which biodiesel is formed

consists of three consecutive reversible reactions, with the kinetics varying based on experimental factors.

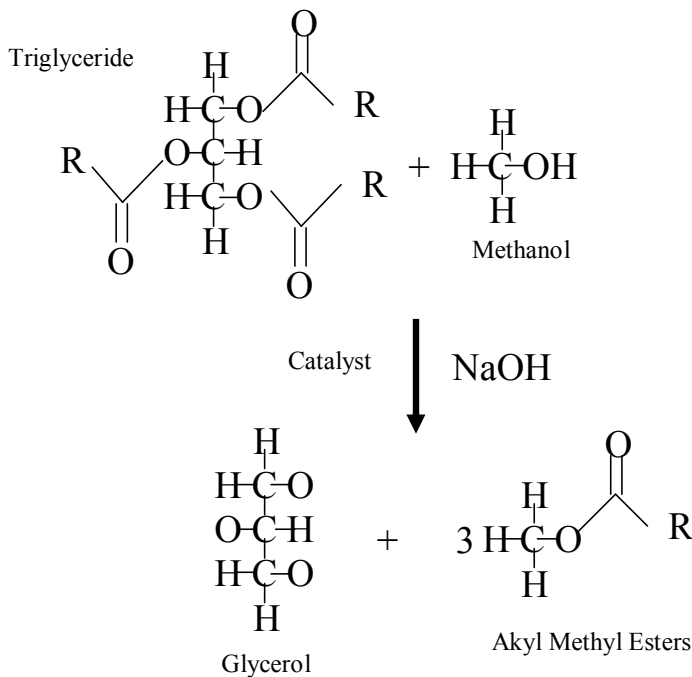


Figure 6. Transesterification of triglyceride with methanol. ‘R’ is alkyl chain of fatty acid.

III.B.2.a.(i) Production process - Producing biodiesel is a fairly straightforward process. Alcohol and catalyst (usually sodium hydroxide) are mixed together first, and then added to the vegetable oil. Often, 80% of the alcohol/catalyst mixture is added and allowed to react initially, and then the last 20% is added in order to obtain a maximal yields (Ma & Hanna, 1999). Transesterification can take place in a batch reactor, however larger plants sometimes employ a continuous stirred-tank reactor or plug flow reactor. Two products are formed: glycerol and biodiesel. These are separated by drawing the glycerol off the bottom of a settling vessel with the help of a centrifuge as needed. Once phase separation has occurred, the two by-products must be cleaned. Alcohol is removed from the biodiesel by flash evaporation or distillation, distilled to remove water, and re-used. The biodiesel is usually washed several times to remove excess catalyst and glycerol. Glycerol is neutralized with acid in order to split any residual soap into fatty acids and salts, which can then be separated, leaving about 85% pure glycerol that can be sold to a glycerol refiner (Van Gerpen, 2005).

III.B.2.a.(iii) Biodiesel potential – In 2005/2006 the world production of vegetable oils topped 123 million metric tons, of which 10.3 million metric tons were produced in the U.S. (*Oilseeds: World Supply and Distribution*, 2006). If the entire U.S. production of vegetable oils were converted to biodiesel, it would yield approximately 2.9 billion gallons (11 billion liters) of fuel. In 2005, on-road transportation consumed

approximately 38 billion gallons of diesel fuel while off-road use consumed an additional 3 billion gallons of diesel fuel (*U.S. Sales of Distillate Fuel Oil by End Use*, 2005). Thus, biodiesel could displace a maximum of 7% of the current diesel consumption in the U.S. While the potential impact of biodiesel on petroleum use is far less than the potential impact of ethanol and other fuels derived from plant polysaccharides, a niche market for biodiesel is expected to continue growing world-wide.

References

- Abatzoglou, N., Chornet, E., Belkacemi, K., & Overend, R. P. (1992). Phenomenological Kinetics of Complex-Systems - the Development of a Generalized Severity Parameter and Its Application to Lignocellulosics Fractionation. *Chemical Engineering Science*, 47(5), 1109-1122.
- Al-Asheh, S., Banat, F., & Al-Lagtah, N. (2004). Separation of ethanol-water mixtures using molecular sieves and biobased adsorbents. *Chemical Engineering Research & Design*, 82(A7), 855-864.
- Asghari, A., Bothast, R. J., Doran, J. B., & Ingram, L. O. (1996). Ethanol production from hemicellulose hydrolysates of agricultural residues using genetically engineered *Escherichia coli* strain KO11. *Journal of Industrial Microbiology*, 16(1), 42-47.
- Barbosa, M. D. S., Beck, M. J., Fein, J. E., Potts, D., & Ingram, L. O. (1992). Efficient Fermentation of Pinus Sp Acid Hydrolysates by an Ethanologenic Strain of *Escherichia-Coli*. *Applied and Environmental Microbiology*, 58(4), 1382-1384.
- Barros, S. (2005). *Brazil Sugar Semi Annual* (No. GAIN Report BR5020): USDA Foreign Agricultural Service.
- Bayrock, D., & Ingledew, W. M. (2001). Changes in steady state on introduction of a *Lactobacillus* contaminant to a continuous culture ethanol fermentation. *Journal of Industrial Microbiology & Biotechnology*, 27(1), 39-45.
- Beery, K. E., Gulati, M., Kvam, E. P., & Ladisch, M. R. (1998). Effect of enzyme modification of corn grits on their properties as an adsorbent in a skarstrom pressure swing cycle dryer. *Adsorption-Journal of the International Adsorption Society*, 4(3-4), 321-335.
- Beery, K. E., & Ladisch, M. A. (2001). Chemistry and properties of starch based desiccants. *Enzyme and Microbial Technology*, 28(7-8), 573-581.
- Belyea, R. L., Rausch, K. D., & Tumbleson, M. E. (2004). Composition of corn and distillers dried grains with solubles from dry grind ethanol processing. *Bioresource Technology*, 94(3), 293-298.
- BeMiller, J. N., & Whistler, R. L. (1996). Carbohydrates. In *Food Chemistry* (3rd ed., pp. 157-223.). New York, New York: Marcel Deker.
- Bhatt, M. S., & Rajkumar, N. (2001). Mapping of combined heat and power systems in cane sugar industry. *Applied Thermal Engineering*, 21(17), 1707-1719.
- Chan, W. N., & Holtzapple, M. T. (2003). Conversion of municipal solid wastes to carboxylic acids by thermophilic fermentation. *Applied Biochemistry and Biotechnology*, 111(2), 93-112.
- Chang, H., Yuan, X. G., Tian, H., & Zeng, A. W. (2006). Experimental investigation and modeling of adsorption of water and ethanol on cornmeal in an ethanol-water binary vapor system. *Chemical Engineering & Technology*, 29(4), 454-461.
- Corrington, P., & Abbas, C. (2003). *Screening ethanoglogens on corn fiber hydrolysate*. Paper presented at the 25th Symposium on Biotechnology for Fuels and Chemicals, Breckenridge, Colorado, USA.
- de Oliveira, D., Di Luccio, M., Faccio, C., Rosa, C. D., Bender, J. P., Lipke, N., et al. (2005). Optimization of alkaline transesterification of soybean oil and castor oil for biodiesel production. *Applied Biochemistry and Biotechnology*, 121, 553-560.

- Deanda, K., Zhang, M., Eddy, C., & Picataggio, S. (1996). Development of an arabinose-fermenting *Zymomonas mobilis* strain by metabolic pathway engineering. *Applied and Environmental Microbiology*, 62(12), 4465-4470.
- Delmer, D. P. (1999). Cellulose biosynthesis: Exciting times for a difficult field of study. *Annual Review of Plant Physiology and Plant Molecular Biology*, 50, 245-276.
- Dennison, E., & Abbas, C. (2003). *Evaluation of recombinant microorganism ethanol fermentation of corn fiber hydrolysate*. Paper presented at the 25th Symposium on Biotechnology for Fuels and Chemicals, Breckenridge, Colorado, USA.
- Dien, B. S., Cotta, M. A., & Jeffries, T. W. (2003). Bacteria engineered for fuel ethanol production: current status. *Applied Microbiology and Biotechnology*, 63(3), 258-266.
- Dien, B. S., Hespell, R. B., Ingram, L. O., & Bothast, R. J. (1997). Conversion of corn milling fibrous co-products into ethanol by recombinant *Escherichia coli* strains K011 and SL40. *World Journal of Microbiology & Biotechnology*, 13(6), 619-625.
- Dien, B. S., Nichols, N. N., O'Bryan, P. J., & Bothast, R. J. (2000). Development of new ethanologenic *Escherichia coli* strains for fermentation of lignocellulosic biomass. *Applied Biochemistry and Biotechnology*, 84-6, 181-196.
- Eggeman, T., & Elander, R. T. (2005). Process and economic analysis of pretreatment technologies. *Bioresource Technology*, 96(18), 2019-2025.
- Ethanol Industry Outlook*. (2006). Renewable Fuels Association.
- Evaluation of Analytical Methods for Analysis of Dried Distillers Grains with Solubles*. (2007). American Feed Industry Association.
- Ganesan, V., Muthukumarappan, K., & Rosentrater, K. A. (2006). *Effect of Flow Agent Addition on the Physical Properties of DDG with Varying Moisture Content Soluble Percentages*. Paper presented at the 2006 ASABE Annual International Meeting, Portland, OR.
- Gardner, K. H., & Blackwell, J. (1974). Structure of Native Cellulose. *Biopolymers*, 13(10), 1975-2001.
- Goldemberg, J., Coelho, S. T., Nastari, P. M., & Lucon, O. (2004). Ethanol learning curve - the Brazilian experience. *Biomass & Bioenergy*, 26(3), 301-304.
- Gollapalli, L. E., Dale, B. E., & Rivers, D. M. (2002). Predicting digestibility of ammonia fiber explosion (AFEX)-treated rice straw. *Applied Biochemistry and Biotechnology*, 98, 23-35.
- Gonzalez, R., Tao, H., Purvis, J. E., York, S. W., Shanmugam, K. T., & Ingram, L. O. (2003). Gene array-based identification of changes that contribute to ethanol tolerance in ethanologenic *Escherichia coli*: Comparison of KO11 (Parent) to LY01 (resistant mutant). *Biotechnology Progress*, 19(2), 612-623.
- Graves, T., Narendranath, N. V., Dawson, K., & Power, R. (2006). Effect of pH and lactic or acetic acid on ethanol productivity by *Saccharomyces cerevisiae* in corn mash. *Journal of Industrial Microbiology & Biotechnology*, 33(6), 469-474.
- Grohmann, K., Himmel, M., Rivard, C., Tucker, M., Baker, J., Torget, R., et al. (1984). Chemical-Mechanical Methods for the Enhanced Utilization of Straw. *Biotechnology and Bioengineering*, 137-157.
- Gulati, M., Westgate, P. J., Brewer, M., Hendrickson, R., & Ladisch, M. R. (1996). Sorptive recovery of dilute ethanol from distillation column bottoms stream. *Applied Biochemistry and Biotechnology*, 57-8, 103-119.

- Ham, G. A., Stock, R. A., Klopfenstein, T. J., Larson, E. M., Shain, D. H., & Huffman, R. P. (1994). Wet Corn Distillers by-Products Compared with Dried Corn Distillers Grains with Solubles as a Source of Protein and Energy for Ruminants. *Journal of Animal Science*, 72(12), 3246-3257.
- Heitz, M., Capekmenard, E., Koeberle, P. G., Gagne, J., Chornet, E., Overend, R. P., et al. (1991). Fractionation of Populus-Tremuloides at the Pilot-Plant Scale - Optimization of Steam Pretreatment Conditions Using the Stake-Ii Technology. *Bioresource Technology*, 35(1), 23-32.
- Helle, S. S., Murray, A., Lam, J., Cameron, D. R., & Duff, S. J. B. (2004). Xylose fermentation by genetically modified *Saccharomyces cerevisiae* 259ST in spent sulfite liquor. *Bioresource Technology*, 92(2), 163-171.
- Hessley, R. K. (2006). Butanol as fuel. *Chemical & Engineering News*, 84(46), 7-7.
- Hinman, N. D., Schell, D. J., Riley, C. J., Bergeron, P. W., & Walter, P. J. (1992). Preliminary Estimate of the Cost of Ethanol-Production for Ssf Technology. *Applied Biochemistry and Biotechnology*, 34-5, 639-649.
- Ho, N., Chen, Z. D., Brainard, A. P., & Sedlak, M. (1999). Successful Design and Development of Genetically Engineered *Saccharomyces* Yeasts for Effective cofermentation of Glucose and Xylose from cellulosic Biomass to Fuel ethanol. In G. T. Tsao (Ed.), *Advances in Biochemical Engineering/Biotechnology*. Berlin, Heidelberg: Springer-Verlag.
- Ho, N., & Tsao, G. T. (1998). Recombinant Yeasts for Effective Fermentation of Glucose and Xylose. United States.
- Ho, N. W. Y., & Chang, S. F. (1989). Cloning of Yeast Xylulokinase Gene by Complementation of *Escherichia-Coli* and Yeast Mutations. *Enzyme and Microbial Technology*, 11(7), 417-421.
- Ho, N. W. Y., Chen, Z. D., & Sedlak, M. (2000). Strategies for successful metabolic engineering of *Saccharomyces* yeasts to effectively co-utilize glucose and xylose from renewable cellulosic biomass for the production of ethanol and other industrial products. *Abstracts of Papers of the American Chemical Society*, 219, U178-U178.
- Ho, N. W. Y., Rosenfeld, S., & Stevis, P. (1983). Cloning of the *Escherichia-Coli* Xylose Isomerase Gene in Yeast. *Federation Proceedings*, 42(7), 2167-2167.
- Ho, N. W. Y., Stevis, P., Rosenfeld, S., Huang, J. J., & Tsao, G. T. (1983). Expression of the *Escherichia-Coli* Xylose Isomerase Gene by a Yeast Promoter. *Biotechnology and Bioengineering*, 245-250.
- Holtzapple, M. T., Davison, R. R., Ross, M. K., Aldrett-Lee, S., Nagwani, M., Lee, C. M., et al. (1999). Biomass conversion to mixed alcohol fuels using the MixAlco process. *Applied Biochemistry and Biotechnology*, 77-9, 609-631.
- Hsu, T.-A. (1996). Pretreatment of biomass. In C. Wyman (Ed.), *Handbook on Bioethanol: Production and Utilization* (pp. 179-195). Washington: Taylor & Francis.
- Ingram, L. O., Conway, T., Clark, D. P., Sewell, G. W., & Preston, J. F. (1987). Genetic-Engineering of Ethanol-Production in *Escherichia-Coli*. *Applied and Environmental Microbiology*, 53(10), 2420-2425.
- Kapen, W. H. (1996). Chapter 2: Nutritional requirements in fermentative processes. In H. C. Vogel & C. C. Todaro (Eds.), *Fermentation and Biochemical Engineering*

- Handbook: Principles, Process Design, and Equipment* (2nd ed., pp. 122-160). Westwood, NJ: Noyes Publications.
- Kim, Y., Hendrickson, R., Mosier, N., & Ladisch, M. R. (2005). Plug-flow reactor for continuous hydrolysis of glucans and xylans from pretreated corn fiber. *Energy & Fuels*, 19(5), 2189-2200.
- Klinke, H. B., Thomsen, A. B., & Ahring, B. K. (2004). Inhibition of ethanol-producing yeast and bacteria by degradation products produced during pre-treatment of biomass. *Applied Microbiology and Biotechnology*, 66(1), 10-26.
- Kotter, P., Amore, R., Hollenberg, C. P., & Ciriacy, M. (1990). Isolation and Characterization of the *Pichia-Stipitis* Xylitol Dehydrogenase Gene, Xyl2, and Construction of a Xylose-Utilizing *Saccharomyces-Cerevisiae* Transformant. *Current Genetics*, 18(6), 493-500.
- Kwiatkowski, J. R., Mcaloon, A. J., Taylor, F., & Johnston, D. B. (2006). Modeling the Process and Costs of Fuel Ethanol Production by the Corn Dry-Grind Process. *Industrial Crops and Products*, 23, 288-296.
- Ladisch, M., Tyner, W., Mosier, N., Cotta, M., Dien, B., Blaschek, H., et al. (2006). *Research challenges and opportunities for cellulose conversion technology in a dry mill pathway*. Paper presented at the 28th Symposium on Biotechnology for Fuels and Chemicals, Nashville, TN.
- Ladisch, M. R. (1997). Biobased adsorbents for drying of gases. *Enzyme and Microbial Technology*, 20(3), 162-164.
- Ladisch, M. R., & Dyck, K. (1979). Dehydration of Ethanol: New Approach Gives Positive Energy Balance. *Science*, 205, 898-900.
- Lin, Y., & Tanaka, S. (2006). Ethanol fermentation from biomass resources: current state and prospects. *Applied Microbiology and Biotechnology*, 69(6), 627-642.
- Liu, Z. L., Slininger, P. J., Dien, B. S., Berhow, M. A., Kurtzman, C. P., & Gorsich, S. W. (2004). Adaptive response of yeasts to furfural and 5-hydroxymethylfurfural and new chemical evidence for HMF conversion to 2,5-bis-hydroxymethylfuran. *Journal of Industrial Microbiology & Biotechnology*, 31(8), 345-352.
- Loe, E. R., Bauer, M. L., & Lardy, G. P. (2006). Grain source and processing in diets containing varying concentrations of wet corn gluten feed for finishing cattle. *Journal of Animal Science*, 84(4), 986-996.
- Lumpkins, B., Batal, A., & Dale, N. (2005). Use of distillers dried grains plus solubles in laying hen diets. *Journal of Applied Poultry Research*, 14(1), 25-31.
- Lynd, L. R. (1996). Overview and evaluation of fuel ethanol from cellulosic biomass: Technology, economics, the environment, and policy. *Annual Review of Energy and the Environment*, 21, 403-465.
- Lynd, L. R., Wyman, C. E., & Gerngross, T. U. (1999). Biocommodity engineering. *Biotechnology Progress*, 15(5), 777-793.
- Ma, F. R., & Hanna, M. A. (1999). Biodiesel production: a review. *Bioresource Technology*, 70(1), 1-15.
- May, J. B. (1987). Wet Milling: Process and Products. In S. A. Watson, and Ramstad, Paul E. (Ed.), *Corn: Chemistry and Technology* (pp. 377-397). St. Paul, MN: American Association of Cereal Chemists.

- McAloon, A., Taylor, F., Yee, W., Ibsen, K., & Wooley, R. (2000). Determining the Cost of Producing Ethanol from Corn Starch and Lignocellulosic Feedstocks. *NREL/TP-580-28893*.
- McCann, M. C., Bush, M., Milioni, D., Sado, P., Stacey, N. J., Catchpole, G., et al. (2001). Approaches to understanding the functional architecture of the plant cell wall. *Phytochemistry*, *57*(6), 811-821.
- MCGA, A. a. (2005). *Methods to improve the flowability and pelleting of distillers dried grains with solubles (DDGS) In AURI and Minnesota Corn Growers Distillers Dried Grain Flowability Study Summary*.
- McMillan, J. D. (1994). Pretreatment of Lignocellulosic Biomass. In *Enzymatic Conversion of Biomass for Fuels Production* (Vol. 566, pp. 292-324).
- Meher, L. C., Sagar, D. V., & Naik, S. N. (2006). Technical aspects of biodiesel production by transesterification - a review. *Renewable & Sustainable Energy Reviews*, *10*(3), 248-268.
- Mohagheghi, A., Evans, K., Chou, Y. C., & Zhang, M. (2002). Cofermentation of glucose, xylose, and arabinose by genomic DNA-integrated xylose/arabinose fermenting strain of *Zymomonas mobilis* AX101. *Applied Biochemistry and Biotechnology*, *98*, 885-898.
- Mohagheghi, A., Evans, K., Finkelstein, M., & Zhang, M. (1998). Cofermentation of glucose, xylose, and arabinose by mixed cultures of two genetically engineered *Zymomonas mobilis* strains. *Applied Biochemistry and Biotechnology*, *70-2*, 285-299.
- Morgan, A. J., & Nicolaidis, A. (1983). Cloning of Bacterial D-Xylose Isomerase in Yeast. *Heredity*, *51*(OCT), 524-524.
- Mosier, N., Wyman, C., Dale, B., Elander, R., Lee, Y. Y., Holtzapple, M., et al. (2005). Features of promising technologies for pretreatment of lignocellulosic biomass. *Bioresource Technology*, *96*(6), 673-686.
- Mosier, N. S., Hendrickson, R., Brewer, M., Ho, N., Sedlak, M., Dreshel, R., et al. (2005). Industrial scale-up of pH-controlled liquid hot water pretreatment of corn fiber for fuel ethanol production. *Applied Biochemistry and Biotechnology*, *125*(2), 77-97.
- Nagle, N., Ibsen, K., & Jennings, E. (1999). A process economic approach to develop a dilute-acid cellulose hydrolysis process to produce ethanol from biomass. *Applied Biochemistry and Biotechnology*, *77-9*, 595-607.
- Neuman, R., Voloch, M., Bienkowski, P., & Ladisch, M. R. (1986). Water Sorption Properties of a Polysaccharide Adsorbent. *Industrial & Engineering Chemistry Fundamentals*, *25*, 422-425.
- Oilseeds: World Supply and Distribution*. (2006). U.S. Department of Agriculture.
- Okano, K., Iida, Y., Samsuri, M., Prasetya, B., Usagawa, T., & Watanabe, T. (2006). Comparison of in vitro digestibility and chemical composition among sugarcane bagasses treated by four white-rot fungi. *Animal Science Journal*, *77*(3), 308-313.
- Olsson, L., & HahnHagerdal, B. (1996). Fermentation of lignocellulosic hydrolysates for ethanol production. *Enzyme and Microbial Technology*, *18*(5), 312-331.
- Palmqvist, E., Grage, H., Meinander, N. Q., & Hahn-Hagerdal, B. (1999). Main and interaction effects of acetic acid, furfural, and p-hydroxybenzoic acid on growth and ethanol productivity of yeasts. *Biotechnology and Bioengineering*, *63*(1), 46-55.

- Palmqvist, E., & Hahn-Hagerdal, B. (2000). Fermentation of lignocellulosic hydrolysates. II: inhibitors and mechanisms of inhibition. *Bioresource Technology*, 74(1), 25-33.
- Pandey, A., Soccol, C. R., Nigam, P., & Soccol, V. T. (2000). Biotechnological potential of agro-industrial residues. I: sugarcane bagasse. *Bioresource Technology*, 74(1), 69-80.
- Perlack, R. D., Wright, L. L., Turhollow, A. F., Graham, R. L., Stokes, B. J., & Erbach, D. C. (2005). Biomass as Feedstock for a Bioenergy and Bioproducts Industry: the Technical Feasibility of a Billion-Ton Annual Supply.
- Peter, C. M., Faulkner, D. B., Merchen, N. R., Parrett, D. F., Nash, T. G., & Dahlquist, J. M. (2000). The effects of corn milling coproducts on growth performance and diet digestibility by beef cattle. *Journal of Animal Science*, 78(1), 1-6.
- Piggot, R. (2003). Treatment and fermentation of molasses when making rum-type spirits. In K. A. Jacques, T. P. Lyons & D. R. Kelsall (Eds.), *The Alcohol Textbook, 4th Edition*. Bath, England: Nottingham University Press.
- Power, R. F. (2003). Enzymatic conversion of starch to fermentable sugars. In K. A. Jacques, T. P. Lyons & D. R. Kelsall (Eds.), *The Alcohol Textbook, 4th Edition* (pp. 23-32). Bath, England: Nottingham University Press.
- Ramos, L. P., Breuil, C., & Saddler, J. N. (1992). Comparison of Steam Pretreatment of Eucalyptus, Aspen, and Spruce Wood Chips and Their Enzymatic-Hydrolysis. *Applied Biochemistry and Biotechnology*, 34-5, 37-48.
- Rausch, K. D., & Belyea, R. L. (2006). The future of coproducts from corn processing. *Applied Biochemistry and Biotechnology*, 128(1), 47-86.
- Rausch, K. D., Belyea, R. L., Ellersieck, M. R., Singh, V., Johnston, D. B., & Tumbleson, M. E. (2005). Particle size distributions of ground corn and DDGS from dry grind processing. *Transactions of the ASAE*, 48(1), 273-277.
- Rausch, K. D., Thompson, C. I., Belyea, R. L., & Tumbleson, M. E. (2003). Characterization of light gluten and light steep water from a corn wet milling plant. *Bioresource Technology*, 90(1), 49-54.
- Rosillo, F. (1986). The Brazilian Ethanol-Chemistry Industry (a Review). *Biomass*, 11(1), 19-38.
- Schenck, F. W. (2002). Starch hydrolysates - An overview. *International Sugar Journal*, 104(1238), 82-+.
- Shetty, J. K., Lantero, O. J., & Dunn-Coleman, N. (2005). Technological advances in ethanol production. *International Sugar Journal*, 107(1283), 605-+.
- Silva, S. S., Matos, Z. R., & Carvalho, W. (2005). Effects of sulfuric acid loading and residence time on the composition of sugarcane bagasse hydrolysate and its use as a source of xylose for xylitol bioproduction. *Biotechnology Progress*, 21(5), 1449-1452.
- Somerville, C., Bauer, S., Brininstool, G., Facette, M., Hamann, T., Milne, J., et al. (2004). Toward a systems approach to understanding plant-cell walls. *Science*, 306(5705), 2206-2211.
- Taherzadeh, M. J., Eklund, R., Gustafsson, L., Niklasson, C., & Liden, G. (1997). Characterization and fermentation of dilute-acid hydrolyzates from wood. *Industrial & Engineering Chemistry Research*, 36(11), 4659-4665.

- Tantirungkij, M., Nakashima, N., Seki, T., & Yoshida, T. (1993). Construction of Xylose-Assimilating *Saccharomyces-Cerevisiae*. *Journal of Fermentation and Bioengineering*, 75(2), 83-88.
- Tao, F., Miao, J. Y., Shi, G. Y., & Zhang, K. C. (2005). Ethanol fermentation by an acid-tolerant *Zymomonas mobilis* under non-sterilized condition. *Process Biochemistry*, 40(1), 183-187.
- Teymouri, F., Laureano-Perez, L., Alizadeh, H., & Dale, B. E. (2005). Optimization of the ammonia fiber explosion (AFEX) treatment parameters for enzymatic hydrolysis of corn stover. *Bioresource Technology*, 96(18), 2014-2018.
- Thompson, D. R., & Grethlein, H. E. (1979). Design and Evaluation of a Plug Flow Reactor for Acid-Hydrolysis of Cellulose. *Industrial & Engineering Chemistry Product Research and Development*, 18(3), 166-169.
- Toon, S. T., Philippidis, G. P., Ho, N. W. Y., Chen, Z. D., Brainard, A., Lumpkin, R. E., et al. (1997). Enhanced cofermentation of glucose and xylose by recombinant *Saccharomyces* yeast strains in batch and continuous operating modes. *Applied Biochemistry and Biotechnology*, 63-5, 243-255.
- U.S. Grains Supply and Distribution: Wheat, Corn, Sorghum, Barley, Oats, Rye, and Rice. (2006). U.S. Department of Agriculture.
- U.S. Sales of Distillate Fuel Oil by End Use. (2005). U.S. Department of Energy - Energy Information Administration.
- Van Gerpen, J. (2005). Biodiesel processing and production. *Fuel Processing Technology*, 86(10), 1097-1107.
- Vij, A. (2003, November). A Perspective on VOC Control Technology in the Industry. *Ethanol Producer Magazine*.
- Walfridsson, M., Anderlund, M., Bao, X., & HahnHagerdal, B. (1997). Expression of different levels of enzymes from the *Pichia stipitis* XYL1 and XYL2 genes in *Saccharomyces cerevisiae* and its effects on product formation during xylose utilisation. *Applied Microbiology and Biotechnology*, 48(2), 218-224.
- Wang, L., Dale, B. E., Yurttas, L., & Goldwasser, I. (1998). Cost estimates and sensitivity analyses for the ammonia fiber explosion process. *Applied Biochemistry and Biotechnology*, 70-2, 51-66.
- Wankat, P. C. (1988). Introduction to Column Distillation. In *Equilibrium-Staged Separations*. New York, NY: Prentice Hall PTR.
- Watson, S. A. (1987). Structure and Composition. In S. A. Watson, and Ramstad, Paul E. (Ed.), *Corn: Chemistry and Technology* (pp. 53-82). Minneapolis, MN: American Association of Cereal Chemists.
- Weil, J., Brewer, M., Hendrickson, R., Sarikaya, A., & Ladisch, M. R. (1998). Continuous pH monitoring during pretreatment of yellow poplar wood sawdust by pressure cooking in water. *Applied Biochemistry and Biotechnology*, 70-2, 99-111.
- Westgate, P., Lee, J. Y., & Ladisch, M. R. (1992). Modeling of Equilibrium Sorption of Water-Vapor on Starch Materials. *Transactions of the Asae*, 35(1), 213-219.
- Whitney, M. H., Shurson, G. C., Johnston, L. J., Wulf, D. M., & Shanks, B. C. (2006). Growth performance and carcass characteristics of grower-finisher pigs fed high-quality corn distillers dried grain with solubles originating from a modern Midwestern ethanol plant. *Journal of Animal Science*, 84(12), 3356-3363.

- Wood, T. M., & Saddler, J. N. (1988). Increasing the Availability of Cellulose in Biomass Materials. *Methods in Enzymology*, 160, 3-11.
- Xue, X. D., & Ho, N. W. Y. (1990). Xylulokinase Activity in Various Yeasts Including *Saccharomyces-Cerevisiae* Containing the Cloned Xylulokinase Gene. *Applied Biochemistry and Biotechnology*, 24-5, 193-199.
- Zaldivar, J., Nielsen, J., & Olsson, L. (2001). Fuel ethanol production from lignocellulose: a challenge for metabolic engineering and process integration. *Applied Microbiology and Biotechnology*, 56(1-2), 17-34.
- Zhang, M., Franden, M. A., Newman, M., McMillan, J., Finkelstein, M., & Picataggio, S. (1995). Promising Ethanologens for Xylose Fermentation - Scientific Note. *Applied Biochemistry and Biotechnology*, 51-2, 527-536.