Appendix B

Overview of Cellulose-Ethanol Production Technology

Ethanol is an alcohol made from the fermentation of the carbohydrate or sugar fraction in biomass (plant) materials. The most common substrate used for ethanol production in the United States today is starch from agricultural crops, primarily corn. Only a portion of the corn kernel (starch fraction) is used to produce ethanol. The other components of the kernel, such as the germ and hull, are processed into animal feed, corn oil, or other food and industrial products.

Over 95 percent of the ethanol produced in the United States today is manufactured by dry or wet milling processes. These are relatively simple processes: corn grinding and starch separation, saccharification, fermentation and distillation. Cellulose ethanol requires more advanced pretreatment methods because the sugar carbon components – cellulose and hemicellulose – are much more difficult to hydrolyze economically into fermentable sugars. After the cellulose and hemicellulose have been saccharified, the remainder of the ethanol production process is similar to grain-ethanol.

Several organizations are working on technologies to advance pretreatment and conversion processes to improve the rate of conversion, yield and efficiency for more economical ethanol production from cellulosic feedstocks.

Pretreatment

Pretreatment refers to the solubilization and separation of one or more of the four major components of biomass – hemicellulose, cellulose, lignin, and extractives – to make the remaining solid biomass more accessible to further chemical or biological treatment. Hydrolysis (saccharification) breaks down the hydrogen bonds in the hemicellulose and cellulose fractions into their sugar components: pentoses and hexoses. These sugars can then be fermented into ethanol.

There are numerous pretreatment methods or combinations of pretreatment methods available. Physical pretreatment breaks down the feedstock size by milling or aqueous/steam processing. The physical pretreatment commonly used by the corn-ethanol producers is milling, which reduces the size of the corn kernel, opening it up for enzymatic hydrolysis. Methods used for cellulosic materials require much more intense physical pretreatments such as steam explosion.

The most common chemical pretreatment methods used for cellulosic feedstocks are dilute acid, alkaline, organic solvent, ammonia, sulfur dioxide, carbon dioxide or other chemicals to make the biomass more digestible by the enzymes. Biological pretreatments are sometimes used in combination with chemical treatments to solubilize the lignin in order to make cellulose more accessible to hydrolysis and fermentation.

Each type of feedstock, whether softwoods, corn stover or bagasse, requires a particular combination of pretreatment methods to optimize the yields of that feedstock, minimize the degradation of the substrate, and maximize the sugar yield. Pretreatment of cellulosic biomass in a cost-effective manner is a major challenge of cellulose-ethanol technology research and development.

Hydrolysis

After the pretreatment process, there are two types of processes to hydrolyze the feedstocks for fermentation into ethanol. The hydrolysis methods most commonly used are acid (dilute and concentrated) and enzymatic.

1. Acid Hydrolysis

There are two acid hydrolysis processes commonly used: dilute acid and concentrated acid. The dilute acid process is conducted under high temperature and pressure, and has a reaction time in the range of seconds or minutes, which facilitates continuous processing. The concentrated acid process uses relatively mild temperatures, and the only pressures involved are those created by pumping materials from vessel to vessel. Reaction times are typically much longer than for dilute acid.

Dilute Acid Hydrolysis. This is the oldest technology for converting cellulose biomass to ethanol. The dilute acid process involves a solution of about 1-percent sulfuric acid concentration in a continuous flow reactor at a high temperature (about 215°C). The sugar conversion efficiency with this method is about 50 percent.

The dilute acid process involves two reactions. First, the cellulosic materials are converted to sugar. However, if the reaction continues, these sugars will convert into other chemicals. The conditions that cause the first reaction are also the right conditions for the second to occur. Once the cellulosic molecules are broken down, the reaction proceeds rapidly to convert the sugars into other products – typically furfural. The sugar degradation not only reduces the sugar yield, but the furfural and other by-products can inhibit the fermentation process.

Because hemicellulose (5-carbon) sugars degrade more rapidly than cellulose (6-carbon) sugars, one way to decrease sugar degradation is to implement a two-stage process. The first stage is conducted under mild process conditions to recover the 5-carbon sugars while the second stage is conducted under harsher conditions to recover the 6-carbon sugars. Both of these hydrolyzed solutions are then fermented to alcohol. Lime is used to neutralize residual acids before the fermentation stage. Sugar degradation still occurs; therefore, theoretical yields are limited to around 80 gallons of ethanol per ton of dry wood. The residual cellulose and lignin are used as boiler fuel for electricity or steam production.

BCI will use a dilute acid hydrolysis to convert bagasse and rice hulls to sugars and then a patented genetically engineered organism will ferment the 5-carbon sugars to ethanol. A yeast will convert the 6-carbon sugars to ethanol. BCI also plans to use the dilute acid process for its rice straw ethanol project in Gridley, California. However, BCI may eventually use a dilute acid (1^{st} stage) followed by enzymatic hydrolysis process. This process may also be used for pretreating and hydrolyzing softwood feedstocks at its Collins Pine project in Chester, California.

Tembec in Canada and Georgia Pacific in Washington use dilute acid hydrolysis processes to dissolve the hemicellulose and lignin from wood at their sulfite pulp mills. While this method is used to produce specialty cellulose pulp, the resulting byproduct – hexose sugars – are fermented to ethanol. The lignin is typically burned for process steam.

Concentrated Acid Hydrolysis. This method uses concentrated sulfuric acid followed by a dilution with water to dissolve and hydrolyze or convert the substrate into sugar. This process provides a complete and rapid conversion of cellulose to glucose and hemicellulose to 5-carbon sugars with little degradation. The critical factors needed to make this process economically viable are to optimize sugar recovery and cost effectively recover the acid for recycling.

The concentrated acid process uses a 70-percent sulfuric acid concentration solution at 100ºF for 2 to 6 hours in a hemicellulose hydrolysis reactor. The low temperatures and pressures minimize the degradation of sugars. The hydrolyzed material in the reactor is then soaked in water and drained several times to recover the sugars.

The next step is to hydrolyze the cellulose. The solid residue from the first stage is dewatered and soaked in a 30 to 40 percent concentration of sulfuric acid for 1 to 4 hours as a pre-cellulose hydrolysis step. The solution is again dewatered and dried, increasing the acid concentration to about 70 percent. After reacting in another vessel for 1 to 4 hours at low temperatures, the contents are separated to recover the sugar and acid. The sugar/acid solution from the second stage is recycled to the first stage to provide the acid for the first stage hydrolysis.

The primary advantage of the concentrated process is the potential for high sugar recovery efficiency, about 90 percent of both hemicellulose and cellulose sugars. The acid and sugar are separated via ion exchange and then acid is re-concentrated via multiple effect evaporators. The low temperatures and pressures employed allow the use of relatively low cost materials such as fiberglass tanks and piping.

There are two companies planning to use concentrated acid hydrolysis processes in their proposed ethanol projects. Arkenol will use its patented process in a rice straw ethanol plant in Sacramento County. Masada Resource Group will use concentrated acid hydrolysis in its proposed Middletown, New York, municipal solid waste ethanol facility.

2. Enzyme Hydrolysis

Biological (vs. chemical) approaches to cellulose hydrolysis have the potential to improve conversion efficiencies and production economics. There are two technological developments: enzymatic and direct microbial conversion. Enzymatic hydrolysis will be the major part of the discussion in this section because it is much closer to commercial application.

Direct microbial conversion (DMC) is a method of converting cellulosic biomass to ethanol in which both ethanol and all required enzymes are produced by a single microorganism. The potential advantage of DMC is that a dedicated process step for production of cellulase enzyme is not necessary. Cellulase enzyme production (or purchase) is a significant cost in enzymatic hydrolysis processes under development. However, DMC is not considered the leading process alternative today because there are no organisms available that both produce cellulase and other enzymes at the required high levels and also produce ethanol at the required high concentrations and yields.

Our understanding of enzymatic hydrolysis of cellulose began in the South Pacific during World War II where a fungus that broke down cotton clothing and tents was discovered. This organism, *trichoderma reesei*, was found to produce cellulase enzymes. Since then, generations of cellulases have been developed through genetic modifications of the fungus strain.

For enzymes to work efficiently, they must obtain access to the molecules to be hydrolyzed. This requires some kind of pretreatment process to remove hemicellulose and break down the crystalline structure of the cellulose or removal of the lignin to expose the cellulose and hemicellulose molecules.

Various physical and chemical pretreatment methods are used to break down feedstock components. Purely physical pretreatments are typically not adequate however. High temperature/pressure and milling are examples of physical methods discussed earlier. The chemical method uses a solvent, such as a dilute sulfuric acid. The dilute acid pretreatment may require the slurry to be detoxified for removal of materials poisonous to the fermentation microorganisms.

The first application of enzymatic hydrolysis was used in separate hydrolysis and fermentation steps. The substrate was pretreated and then subsequently hydrolyzed. More recent applications integrated the saccharification and fermentation steps to improve production economics. Simultaneous saccharification and fermentation (SSF) combined the cellulase enzymes and fermenting microbes in one vessel. This enabled a one-step process of sugar production and fermentation into ethanol. The disadvantage, however, was that the cellulase enzyme and fermentation organism had to operate under the same conditions, decreasing the sugar and ethanol yields.

An alternative enzymatic hydrolysis replaces the SSF technology with sequential hydrolysis and fermentation (SHF). The separation of hydrolysis and fermentation offers various processing advantages and opportunities. It enables enzymes to operate at higher temperatures for increased performance and fermentation organisms to operate at moderate temperatures, optimizing the utilization of sugars.

Fermentation

Near-term fermentation using genetically engineered yeast or bacteria will utilize all five of the major biomass sugars – glucose, xylose, mannose, galactose and arabinose. Mid- to long-term technology will improve the fermentation efficiency of the organism, producing higher yields in less time, and a heartier organism requiring less detoxification of the hydrolysate.

The National Renewable Energy Laboratory (NREL) recently developed an advanced strain of its patented Z*ymomonas mobilis* bacterium that could lead to more efficient fermentation of cellulosic materials. The bacterium is able to ferment both five- and six-carbon sugars simultaneously, expanding the amount of biomass material that can be converted into ethanol by up to 40 percent. A license agreement with NREL will allow Arkenol to use NREL’s microorganism specifically engineered for rice straw hydrolysate at its proposed ethanol facility in Sacramento.