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APPLICATIONS OF CORN STOVER AND FIBER

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I. INTRODUCTION

As a low-cost raw material vital to many important food, feed, chemical, and fuel applications, corn is the leading agricultural commodity in the United States. Most of these products made from corn value the starch, oils, and protein contained within the corn kernel. Unfortunately, the virtually equal weight of corn residues – that is, the stalks, leaves, husk, and cob - are largely underutilized. Furthermore, corn fiber, the fibrous shell surrounding the kernel that is typically incorporated in animal feed and other corn products, presents an opportunity for generating new products because of its availability at the process facility. The challenge is to utilize these abundant biomass materials to extend the impact and revenues of corn processing and realize even greater benefits from corn.

This chapter presents an overview of the availability and composition of corn biomass, the lignocellulosic portion of the corn plant that includes the cobs, stalks, leaves, husks, and fiber. Then, emerging technologies are described for deriving low-cost sugars from these fractions that could support the emergence of major new markets and greatly expand the use of agricultural products; create new rural agricultural and manufacturing employment; and improve the environment, economy, and national security. These technologies also provide important synergies with existing production and processing operations. Finally, selected traditional uses of corn residues are briefly summarized.

II. THE RESOURCE

Corn stover is defined here as the above ground portions of the corn plant to include the stalks, cobs, leaves, and husks but excluding the corn kernels themselves. The corn plant is a structural marvel in that these components support, nourish, and protect a virtually equal weight of kernels. Thus, about 225 million dry tons of stover result annually from the production of the nearly 10 billion bushels (about 250 million tons) of corn recently recorded for the United States (USDA, 2001). If all of this stover could be collected, on the order of 150 million tons of sugar and 75 million tons of boiler fuel could be produced. This quantity of sugars could be used to make well over 100 million tons of chemicals or about 20 billion gallons of ethanol, the latter equivalent to about 10% of the gasoline market on an energy-equivalent basis (US DOE, 2000). In addition, depending on the moisture content and power cycle employed, on the order of 35 billion kWh of electricity could be made annually from nonfermentable solids, equal to about 1.0% of the U.S. power market (US DOE, 2000). Obviously, not all of this material can be collected and converted to products because some must remain in the field to build the soil and some has limited accessibility. Nonetheless, the shear magnitude of corn stover generation makes it vital to seriously consider alternative uses.

A study entitled "Biobased Industrial Products: Priorities for Research and Commercialization" focused on corn stover as a promising feedstock for bioethanol production (National Research Council, 1999). A supply curve for corn stover showed limited value for fertilizer and erosion control at use levels up to about 62.5 million dry tons per year. However, the value then increases to about $35 per dry ton for consumption up to about 100 million dry tons per year based on the competing market for animal feed and 1994 hay prices. They estimate that about 7.5 billion gallons of ethanol could be made annually at this feedstock cost. For lower levels of use, corn stover can be obtained for its harvest costs, estimated by the NRC to be about $16.50 per dry ton. For larger plants, defined as processing 2.9 million tons annually to make 350 million gallons per year of ethanol, the NRC estimated a total corn stover cost of $20.00 to $21.50 per dry ton to harvest and haul all the corn stover needed from within a 50-mile radius.

Chief Executive Assistance (CEA) also studied the cost and availability of corn stover (www.ceassist.com, 2000) and estimated that approximately 80 million dry tons of stover could be collected annually in a good harvest year, enough to make 6 billion gallons of ethanol at modest yields of 75 gallons/dry ton. Working with farmers, CEA determined the cost of stover collection as about $31.60 to $35.70 per dry ton, about twice the cost projected in the NRC report. This pricing indicates that farmers may not be willing to provide corn stover at lower prices, even with the limited competing markets noted in the NRC estimates. With these contradictory prices, firm contracts are important to be sure of feedstock costs.

The CEA study identified a simple two-step operation of baling and bale collection and delivery to the processor to replace the former five-step procedure of raking, baling, field loading, hauling, and unloading. By turning off the spreader on the corn combine, a windrow is left behind that can be more readily baled, resulting in a collection of about 1.5 to 2.0 dry tons per acre of stover that could be increased to 2.5 to 3.5 dry tons per acre by adding a rake in front of the baler. They studied collection in round and square bales with target weights of 1,200 dry lbs per round bale and large square (4'x4'x8') bales and 650 dry tons for intermediate-sized square bales. Round bales were wrapped with three layers of plastic net to insure they did not break apart when collected and handled. The bales were loaded onto a "load-and-go" trailer that can safely traverse corn fields, including crossing ditches, and still travel at highway speeds of up to 60 mph for transport to the unloading center. Overall, this approach reduced stover costs from about $42 to $60 per dry ton to around $31.60.

In an alternate approach in the late 1980s, QO Chemical ran a "Cob Saver Program" with the Anderson Brothers of Columbus, OH to collect both corncobs and kernels from the field. The combines were outfitted with a sieve plate with larger diameter holes than typically used so that both the cobs and kernels passed through the holes, while the stalks and husk were returned to the field. During unloading, the cobs were separated from the kernels and stored in piles for conversion to furfural and other products. Excellent cob recovery was claimed with little damage to the kernels (QO Chemical, 1988), but the low bulk packing density could hurt transportation costs.

For the CEA study, all bales were stored in the open. High-moisture bales and uncovered square bales were processed first, and normal moisture round bales, protected by their plastic wrap were processed later. Although open-field storage of wrapped round bales is common for straw, hay, and similar materials, it may be possible to store stover less expensively in piles. For example, properly managed piles of corn cobs are said to be maintained for up to five years to support year round operation of furfural plants. It is, however, unclear if the lower bulk packing density of corn stover will lend itself to this approach.

Although stover is far more abundant, corn fiber is also of potential interest for conversion to ethanol and chemicals, as pointed out by Wyman (1993). Because over 10% of the kernel is typically fiber and about 2 billion bushels of corn are processed each year, about 5 million tons per year of this material are potentially available. Furthermore, its availability at a processing facility eliminates significant costs for gathering and transportation. On the other hand, because fiber contains some protein and provides a valuable solid carrier for animal feed, such as distillers dried grains with solubles (DDGS) and corn gluten feed, conversion to other products could create problems for feed handling. But because feed markets are nearing saturation and revenue from feed sales is essential to maintaining ethanol prices, other uses for fiber may become essential if significantly more corn is to be processed to ethanol and other products (Gulati et al, 1996).

III. CORN BIOMASS

Corn biomass (stalks, cobs, and fiber) is part of a larger class of plant material known as lignocellulosic biomass which includes other agricultural residues (e.g., wheat straw), forestry wastes (e.g., sawdust), major portions of municipal solid waste (e.g., waste paper), and hardwoods (e.g., aspenwood) and herbaceous crops (e.g., switchgrass) grown for processing. Such plant matter is often simply referred to as "biomass." Although quite distinctive in outward appearance, all forms of biomass generally share a complex structure that lends itself to processing to a diverse array of products (Holtzapple, 1993; Wyman, 1996). About 40 to 50% of such abundant materials is cellulose, long chains of glucose covalently bonded together in a primarily crystalline structure. Cellulose fibers can be used for their strength in paper, wood, or other materials. Alternatively, acids or enzymes called cellulase will breakdown these fibers by hydrolysis reactions to form glucose that, in turn, can be fermented to various chemicals and fuels, or reacted chemically to various products. About another 25 to 30% of biomass is hemicellulose, long chains of the sugars arabinose, galactose, glucose, mannose, and xylose, plus lesser amounts of other components such as acetic acid. Hemicellulose is not crystalline and is more readily hydrolyzed to its component sugars by acids than is cellulose; enzymes will also break down hemicellulose. The heterogeneous composition of hemicellulose offers promise for a number of products, although little attention has been given to capitalizing on this opportunity beyond making furfural and more recently ethanol. About 15 to 20% of biomass is lignin, a complex phenyl-propene material that can be converted into aromatic compounds or burned to take advantage of its relatively high energy content. Many forms of biomass also contain significant amounts of protein that can be recovered for use in food and feed, although little attention has been devoted to such a possibility. In addition, lower quantities of compounds known as extractives are generally contained in biomass that could command high value for various specialty products. Even the mineral matter in biomass, ash, could be recovered for applications such as road pavement and soil buildup.

Some elementary features of sugar chemistry and nomenclature for biomass materials must be understood to estimate yields and potential revenue implications and avoid confusion. As mentioned, hemicellulose is the heterogeneous portion of biomass that reacts with water in a hydrolysis reaction to release arabinose, galactose, glucose, mannose, and xylose sugars in addition to other components such as acetic acid. Prior to hydrolysis, these sugar polymers are referred to as arabinan, galactan, glucan, mannan, and xylan. Because cellulose produces only glucose when hydrolyzed, it also contributes to the glucan content. Glucan, galactan, and mannan each contain six carbon, ten hydrogen, and five oxygen atoms. Because one molecule of water is added to each such unit during hydrolysis, the sugar yield is 11.1% greater than the fraction of any of these components. For example, biomass containing 40.0% glucan could release up to 44.4 lb of glucose for every 100 lb of dry feedstock after hydrolysis. On the other hand, five carbon, eight hydrogen, and four oxygen atoms comprise each repeating unit of arabinan and xylan, and the addition of one water molecule to each releases sugar. Consequently, the sugar yield is up to 13.6% greater than the fraction of the prehydrolysis portions, and a feedstock containing 20.0% xylan would yield as much as 22.7 pounds of xylose for every 100 pounds of dry biomass used. Understanding of these relationships is vital because the nomenclature for biomass composition varies considerably, and many incorrectly treat terms such as hemicellulose, xylan, and xylose as though they are interchangeable.

Corn stover has a representative composition by weight on a moisture-free basis of 40.9% glucan, 21.5% xylan, 1.8% arabinan, 1.0% galactan, undetectable amounts of mannan, 16.7% lignin, 6.3% ash, and 1.9% acetyl groups (Torget et al, 1991). By comparison, corncobs by themselves are richer in hemicellulose with about 28.4% xylan, 3.6% arabinan, 1.1% galactan, undetectable levels of mannan, 17.5% lignin, 2.7% ash, 1.9% acetyl groups and 39.4% glucan (Torget et al, 1991). These analyses appear not to differentiate protein and other components from lignin, with others reporting that stover contains about 11% lignin, 8% protein, and 1.3% fat, and that cobs contain about 7% lignin, 3.2% protein, and 0.7% fat (Subcommittee on Feed Composition, 1982). Table 1 summarizes the composition of corn stover and corncobs including heat of combustion data for each.

Table 1. Representative composition of corn stover, cobs, and fiber.

Component	Corn stover	Corn cobs	Corn fiber[2]
Glucan	40.9%[1]	39.4%[1]	37.2%[2]
Xylan	21.5%[1]	28.4%[1]	17.6%[2]
Arabinan	1.8%[1]	3.6%[1]	11.2%[2]
Galactan	1.0%[1]	1.1%[1]	3.6%[2]
Mannan	ND[1]	ND[1]	NR[2]
Lignin	11.0%[3]	7.0%[3]	7.8%[2]
Ash	7.2%[3]	1.7%[3]	0.6%[2]
Protein	8.0-8.9%[3]	3.2%[3]	11.0%[2]
Crude fat	1.3%[4]	0.7%[4]	2.5%[2]
Gross heat of combustion	17,650 J/g[5]	18,770 J/g[5]	
Net heat of combustion	16,520 J/g[5]	17,580 J/g[5]	

ND – Not detected
NR – Not reported
1 – Reference by Torget et al, 1991.
2 – Reference by Saha et al, 1998.
3 – Reference by Subcommittee on Feed Composition, 1982.
4 – Measured as ether extract via Subcommittee on Feed Composition, 1982.
5 – Reference by Domalski et al, 1987.

Most of the xylose, arabinose, galactose, mannose (if present), and acetic acid can be released in a hydrolysis reaction along with some of the glucan (about 10% of the total glucan) and lignin, but a portion of the sugars will degrade for chemically catalyzed hydrolysis reactions (McMillan, 1994; Hsu, 1996). If conditions are chosen to react most of the hemicellulose without substantial degradation of hemicellulose sugars, most of the glucose along with almost all of the lignin and ash will remain in the solids and can be released by hydrolysis in a second stage by acids or enzymes. Corn stover and cobs lend themselves to biomass hydrolysis better than most biomass types (Torget et al, 1991).

Corn fiber was recognized as having important potential for conversion to ethanol and other products because of its availability at a process facility, avoiding transportation and handling costs (Wyman, 1993). Corn fiber is richer in hemicellulose than stover, lower in cellulose, and much higher in protein with a typical composition reported as 37.2% glucan, 17.6% xylan, 11.2% arabinan, 3.6% galactan, and no measurable mannan (Saha et al, 1998). Fiber also contains appreciable amounts of protein and fat as well as some lignin and minor amounts of ash. Some concerns expressed about converting fiber to ethanol and other products are potential loss of protein and fat and of the solid carrier for corn gluten feed (Wyman, 1993), considerations that could limit the availability of fiber. Table 1 summarizes data on corn fiber composition.

IV. OVERVIEW OF BIOLOGICAL PROCESSING OF CORN BIOMASS

To provide a context of the steps typically needed, this section summarizes a processing sequence that is built around enzymes and organisms to convert biomass to ethanol. These biological conversion steps are often favored because they achieve high yields vital to economic success and have low cost potential (Wright, 1988; Hinman et al, 1992; Wyman, 1999; Wooley et al, 1999; Lynd et al, 1996). However, a number of different operations are being developed and explored for converting corn residues and other forms of biomass into sugars, fermenting those sugars to a variety of products, and recovering the products formed, with some of these being closer to commercial use than others (Bozell and Landucci, 1993; Landucci et al, 1994; Fairley, 2001). The steps through fermentation can be the same regardless of the product targeted, although different purification operations will be often be needed. The reader is referred to the Section V for a summary of many of the processing and product alternatives that could be applied.

In the process illustrated in Figure 1, biomass is pretreated by dilute acid to release sugars from the hemicellulose fraction, cellulase enzymes are produced from some of the pretreated material, cellulose is enzymatically hydrolyzed to glucose, the sugars are fermented to ethanol, the ethanol is recovered, and residual lignin and other solids are burned for power generation (Wright, 1988; Hinman et al, 1992; Wyman, 1999; Wooley et al, 1999). Corn stover would be harvested, baled, transported to the plant site, and stored. When ready for use, stover would likely be washed to remove rocks and other debris that could reduce equipment lifetime or interrupt operations and conveyed to dilute acid pretreatment reactors. The stover is mixed with dilute acid and steam to achieve a temperature of around 170°C, conditions under which most of the hemicellulose reacts to soluble sugars while leaving most of the cellulose and lignin in the insoluble solid (Torget et al, 1991). This mixture is then cooled to stop sugar degradation reactions, and the hemicellulose sugar is separated from the cellulose/lignin solids. Next, the sugar stream is conditioned with lime and ion exchange, if necessary, to achieve high yields in subsequent fermentations (Wooley et al, 1999).

Some of the washed solids and possibly liquid hydrolyzate (about 4 to 9% total depending on enzyme loading requirements) supports production of cellulase enzyme that is added back to the bulk of the pretreated solids to hydrolyze cellulose to glucose. A fermentative organism able to ferment all five sugars is also added to the same vessel along with conditioned liquid hydrolyzate in a process designated simultaneous saccharification and co fermentation (SSCF) (Wooley et al, 1999), and the product recovered from the fermentation broth by distillation and dehydration. The solids are centrifuged and dried to about 10% moisture and burned to generate all the heat and electricity needed for the plant with the excess being sold. The solubles in the recovered liquid are anaerobically digested to methane that is fed to the boiler, and a portion of the remaining liquid is recycled while the rest is treated for proper disposal.

V. BIOLOGICAL PROCESSING OPTIONS

Enzymatic hydrolysis of cellulose to glucose is attractive for making ethanol because nearly theoretical yields are possible, costs have been reduced by about a factor of four, and additional opportunities have been identified to make the technology competitive (Wright et al, 1987; Hinman et al, 1992; Wyman, 1995; Lynd et al, 1996; Wyman, 1999; Wooley et al, 1999). This section summarizes many options for enzymatically converting biomass to fuels and chemicals with emphasis on hemicellulose and cellulose hydrolysis because these expensive steps offer the greatest opportunities for improvement. Only a few of the many possible references can be included, and the reader should consult the literature for more in-depth information.

1.0 Feedstock Storage and Handling

Storing biomass, delivering it to the plant site, and feeding it to the process are important and generally unappreciated steps in biomass conversion that must receive careful attention to avoid problems that could cripple a project. Because biomass conversion to fuels and chemicals is capital intensive, products must be manufactured year-round to achieve competitive costs. On the other hand, corn stover can only be collected during a limited harvest season, and either the stover must be stored or supplemental feedstocks utilized to continuously feed a plant. However, biomass will degrade under some conditions, reducing product yields from a given resource base, and storage strategies must be developed to insure feedstock preservation (Agblevor et al, 1996). As mentioned before, stover wrapped in bales retains much its integrity over time. Alternatively, corn cobs for furfural production have been stored in managed piles with limited degradation.

Another key to competitive biomass conversion is transport to the processing facility, a factor that is impacted by the mode of transport, load limitations, and loading and unloading considerations. Various projections have been offered of such factors and their cost implications (e.g., Marrison and Larson, 1995), but in the final analysis, cost is determined by signed contracts with shipping companies or internal costs for transport by a dedicated plant fleet.

Conveying corn biomass at the process facility must also be fully understood. Each type of biomass has unique features that influence material handling, and success with one type is no guarantee of success with another. On this basis, corn stalks behave differently from corn cobs, which differ from mixtures of the two, which differ from corn fiber. Important considerations include particle size, angle of repose, and bulk packing density, each of which presents important challenges, and established experience with handling these materials is essential to effectively implement corn biomass processing.

2.0 Biomass Pretreatment

Pretreatment is essential to obtain high glucose yields from enzymatic hydrolysis of cellulose because a complex hemicellulose-lignin shield limits enzyme accessibility to cellulose

(McMillan, 1994; Hsu, 1996). Technoeconomic evaluations show that pretreatment is the most expensive process step in making biomass ethanol, representing about one-third of the total cost. In addition, pretreatment substantially affects the cost of all the steps associated with converting cellulose into fermentable sugars, which collectively represent about 30% of the total, depending on how these costs are assigned (Hinman et al, 1992; Lynd et al, 1996; Wooley et al, 1999). Thus, pretreatment technology presents important opportunities for improvements to reduce sugar costs to the point that competitive manufacture of a wide range of products is feasible (Lynd et al, 1996). Key aspects of pretreatment are summarized here to help the reader understand this technology and its options.

Uncatalyzed steam explosion and dilute-acid pretreatment achieve high hemicellulose removal and good cellulose digestibility (McMillan, 1994; Hsu, 1996; Torget et al, 1991; Abatzoglou et al, 1992; Ramos et al, 1992). Dilute-acid hydrolysis tends to be favored because over 80% of the hemicellulose sugars are recovered versus less than 65% for uncatalyzed pretreatment (Hinman et al, 1992; Wooley et al, 1999). Such high yields are vital to low costs (Wright et al, 1987; Hinman et al, 1992; Wyman, 1995; Wooley et al, 1999). Despite being a frontrunner for commercial use, however, dilute sulfuric acid has important limitations including a corrosive environment that mandates expensive materials of construction, the need to neutralize acid, formation of gypsum with problematic reverse solubility characteristics when neutralized with inexpensive calcium hydroxide, formation of degradation products that must be removed before biological processing, and disposal costs for neutralization salts (Mes-Hartree and Saddler, 1983; Hinman et al, 1992; McMillan, 1994; Hsu, 1996). Additionally, about a 7-day reaction time with expensive cellulase loadings of up to 20 IFPU/g cellulose are needed to realize good yields in subsequent enzymatic hydrolysis of the pretreated cellulose (Wright et al, 1987; Hinman et al, 1992; Wooley et al, 1999). Grinding to 1 mm particle size may also be needed, accounting for 33% of the power requirements of the entire process (Hinman et al, 1992). An interesting paper provides an important perspective on dilute-acid cellulose and hemicellulose hydrolysis (Lee et al, 1999).

Various pretreatment technologies have been investigated in an effort to reduce costs and realize high sugar yields from both cellulose and hemicellulose, but performance was poor and costs were high for physical approaches such as comminution (e.g., milling) and irradiation (Tassinari et al, 1982; Khan et al, 1987). Although biological pretreatment offers conceptual advantages, such as low chemical and energy use, no effective system has been demonstrated (Fan et al, 1982). Nitric acid reduces containment costs relative to sulfuric (Brink, 1994), but the acid cost is higher. Alkaline agents delignify biomass and realize good yields (Millett et al, 1976), but chemical costs are relatively expensive. Lime provides an alternative base with lower cost that has good potential (Kaar and Holtzapple, 2000). Organosolv processes apply solvents, such as ethanol, to remove lignin effectively but are too expensive except for recovery of high-value products (Chum et al, 1988; Tirtowidjojo, 1988). Improvements in the ammonia fiber explosion (AFEX) process make it an effective route for biomass pretreatment (Holtzapple et al, 1991). Sulfur dioxide improves the yields of hemicellulose sugars from steam explosion (Mes-Hartree and Saddler, 1983) but presents safety concerns and has similar costs to dilute sulfuric acid (Schell, 1991). Carbon dioxide improves cellulose digestibility in some studies (Zheng et al, 1988) but apparently not others (McMillan, 1994; Hsu, 1996); however, the high pressures required raise questions about containment costs in any event. High yields have been achieved at near neutral pH in an approach termed "pressure cooking at controlled pH" (Weil et al, 1998), and optimization of this approach could provide a new pretreatment option. Passing hot water without chemicals (Bobleter et al, 1976; Bobleter, 1994; Allen et al, 1996; van Walsum et al, 1996) or with very low acid (about 0.07% versus the 0.7 to 3.0% typical for conventional dilute acid technology) (Torget et al, 1996; Torget et al, 1998) through biomass at high temperatures achieves virtual to total elimination of added acid, near theoretical hemicellulose sugar yields, highly digestible cellulose, liquid hydrolyzate that is more compatible with fermentative organisms, lower cost materials of construction, less neutralization and conditioning chemicals, and less size reduction requirements (Lynd et al, 1996). Unfortunately, the large amount of water used dilutes sugar streams, resulting in low sugar concentrations, and increases energy requirements for pretreatment and product recovery. Complex configurations are another drawback for this otherwise promising approach. Nonetheless, their unique attributes could point the way to lower costs for biologically processing biomass (Lynd et al, 1996).

3.0 Cellulase Production

As mentioned, cellulase enzymes catalyze the hydrolysis of cellulose to glucose with theoretical yields possible from well-pretreated materials, and various organisms release these enzymes to allow them to metabolize the sugars in cellulose. A fungus, now designated as *Trichoderma reesei* in honor of Elwyn Reese who made many of the important initial discoveries about it, was found to degrade canvas and other cotton products during World War II. Initial attention was devoted to finding ways to overcome its destructive effects at that time. However, during the "Energy Crisis" of the 1970s, its ability to release glucose from cellulose with high yields was realized to present a promising path to renewable production of fuels and chemicals that could reduce our dependence on vulnerable supplies of petroleum (Reese, 1976). Other organisms including various bacteria also make cellulase, but *T. reesei* has received considerably more attention than other options (Himmel et al, 1996; Esterbauer et al, 1991).

Although fungi typically consume biomass while exposed to air, technology has been developed to grow it submerged in water to facilitate large-scale production (Esterbauer et al, 1991). Air must be sparged into the liquid, however, for the organism to grow and make enzyme. Furthermore, the native organism only releases enzyme in large amounts when an enzyme inducer, such as cellulose or lactose, is available and more easily utilized sugars are unavailable (Esterbauer et al, 1991; Kadam, 1996). Growing the organism on biomass is particularly desirable to keep substrate costs low. Unfortunately, release of cellulase is slow in this case, and power inputs are high to aerate and mix the viscous broth containing solid biomass and growing filamentous fungus (Himmel et al, 1999; Wooley et al, 1999). Fermentors must be cooled, often with chilled water, to remove the heat released as the fungus grows and makes cellulase and keep the temperature from rising too high for the organism to function. About 4 to 9% of the biomass fed to a cellulase-based process is needed to make enzyme (Esterbauer et al, 1991; Hinman et al, 1992; Himmel et al, 1999; Wooley et al, 1999).

Many strains of *T. reesei* have been derived from the parent to enhance cellulase production rates and diversify the substrates on which it will make cellulase with high yields (Esterbauer et al, 1991; Kadam, 1996; Himmel et al, 1996). In addition, researchers have sought other strains of fungi and bacteria for less expensive enzyme production and to serve as a source of genes with which to transform organisms to enhance cellulase production (Himmel et al, 1996). Of the organisms that are publicly available, however, various mutants of *T. reesei* are currently favored, such as Rut C30, L-27, CL-847, and RLP-37, as pointed out in one reference (Kadam, 1996). Enzyme manufacturers are said to have developed strains with high productivity, but the enzymes target specialty applications, such as stone-washed jeans, that benefit from properties different from those needed for making fuels and chemicals from biomass.

Cellulase is actually a complex mixture of enzyme activities that synergistically breakdown cellulose into glucose (Mandals and Reese, 1964; Himmel et al, 1996). In simplistic terms, a portion designated as endoglucanase can be pictured to break cellulose chains to generate free ends. Then, another fraction known as exoglucanase or cellobiohydrolase attacks these ends to releases cellobiose molecules made up of two linked glucose molecules. Finally, a third enzyme fraction, β-glucosidase, breaks apart cellobiose to release simple glucose sugar. Glucose and cellobiose in particular are powerful inhibitors of enzyme action, and their accumulation substantially slows and even stops continued hydrolysis, restricting the rates, yields, and concentrations of glucose (Takagi, 1984).

At this time, cellulase is a specialty product that commands prices that are far too high for producing fuels and commodity chemicals (ceassist.com, 2000). On-site manufacture reduces costs considerably, and approaches have been developed to improve the rate of production through fed-batch fermentation (Hendy et al, 1984) and using mixed hydrolyzate from pretreatment (Mohagheghi et al, 1987; Mohagheghi et al, 1990). More recent studies also have been undertaken to improve the productivity of cellulase production (Velkovska et al, 1997; Hayward et al, 2000), and enzyme production performance has been demonstrated at laboratory and pilot plant scales with various media (Esterbauer et al, 1991; Ballerini et al, 1994). Yet, the price is still projected to be too great to realize the low costs needed to compete in commodity markets (Wooley et al, 1999; ceassist.com, 2000), and the U.S. Department of Energy recently contracted with cellulase producers to lower cellulase costs substantially (Fairley, 2000). Pretreatment also has a significant impact on cellulase dosage and glucose release rates, and advanced pretreatment technology provides a complementary path to lower enzyme use and cost.

4.0 Enzymatic Cellulose Hydrolysis

The rates, yields, and concentrations of sugars from cellulose hydrolysis can be impacted by 1) the condition of the pretreated cellulose, 2) the nature of the cellulase enzyme, and 3) the process configuration. For the first, several chemical and physical features are thought to be important including lignin, hemicellulose, and acetyl content and cellulose crystallinity (Converse, 1993; McMillan, 1994; Hsu, 1996; Chang and Holtzapple, 2000). However, the complexity of biomass confounds our understanding, and reducing one barrier to digestion can enhance or disguise the importance of others. For example, cellulose hydrolysis improves with increasing lignin removal (Gharpuray et al, 1983; Gould, 1984; Converse, 1993; Chang and Holtzapple, 2000), although differences are reported in the degree of lignin removal needed and hemicellulose alteration can confound the role of the two components (Gould, 1984; Chum et al, 1987; Thompson et al, 1990; Yoon et al, 1995). Several studies also showed a direct relationship between cellulose digestion and hemicellulose removal, with some concluding that lignin removal is not necessary for good cellulose conversion (Grohmann et al, 1986). Some substrates require higher temperatures for hemicellulose removal to be effective, however, suggesting that hemicellulose is not the only factor impacting digestibility, and some evidence does not support a role for hemicellulose in changing cellulose digestibility (Millett et al, 1976; Fan et al, 1982; Tsao et al, 1978). Removal of acetyl groups can double to triple cellulose digestibility, with some differences reported in the degree of removal needed (Grohmann et al, 1989; Kong et al, 1992). Although crystallinity is reasoned to impede enzymes, rates slow with increasing crystallinity in some studies (Lin et al, 1981; Sinitsyn, 1991) but increase in others (Grethlein, 1985). Crystallinity seems less important than lignin removal and impact rates more than yields (Chang and Holtzapple, 2000). Efforts also have been made to explain cellulose digestibility by the accessibility of cellulose to enzymes, and correlations were developed to relate rates to pore volume and accessible surface area (Grethlein, 1985; Grous et al, 1985). But, the complex shape of cellulases could have difficulty penetrating such pores, and concerns have been raised about substrate changes during these measurements (Converse, 1993). The consumption of free-chain ends generated by pretreatment has been suggested to explain the slowing of rates over time, but hydrolysis rates also decline with increasing surface coverage by enzyme, leading to the possibility that steric hindrance of enzymes by other enzymes, multilayer adsorption, enzyme binding to lignin, and cellulase stuck in dead-end alleys could reduce rates (Converse, 1993).

The nature of the enzyme also influences the rates, yields, and concentrations of glucose release from cellulose. First, cellulase must have the proper balance of the three activities noted, with a high ratio of exoglucanase to endoglucanase shown to be important to keep rates high (Karlsson et al, 1999). Furthermore, accumulation of glucose and particularly its two-unit dimer cellobiose slows cellulase action (Takagi, 1984), and high levels of β-glucosidase speed cellobiose conversion, improving the rate of enzyme action (Spindler et al, 1988). Nonetheless, the inhibitory effects of glucose limit sugar concentrations to only a few percent for currently available cellulase formulations. Lignin also adsorbs cellulase, reducing the amount available for cellulose hydrolysis (Tatsumoto et al, 1988). Finally, the nature of exoglucanase action on crystalline cellulose is slow, limiting glucose release rates (Himmel et al, 1996). Improved pretreatments that reduce crystallinity, lignin, hemicellulose, and acetyl levels and better enzymes with faster action on cellulose, less binding to lignin, and less sugar inhibition offer important benefits for improving enzymatic hydrolysis.

The process itself has a significant influence on enzymatic hydrolysis of cellulose. For example, immediate fermentation of glucose to ethanol in a so-called simultaneous saccharification and fermentation (SSF) route increases rates, yields, and ethanol concentrations compared to making glucose and sequentially fermenting it to ethanol. These benefits result from reducing the concentration of glucose, a strong inhibitor of cellulase activity (Gauss et al, 1976; Wyman et al, 1986). The SSF configuration also reduces equipment costs by combining hydrolysis and fermentation operations in a single set of vessels which clearly reduces bioethanol production costs (Wright et al, 1987). Similarly, employing fermentative organisms that ferment cellobiose improves performance even more by accelerating the removal rate of this even more powerful inhibitor (Spindler et al, 1992). Performance can be improved further by adding whole-cell broth from enzyme production because some cellulase activity is trapped in the fungal cells (Schell et al, 1990). Removal of both hemicellulose and lignin during pretreatment also improves performance by making the cellulose more accessible and preventing lignin from binding expensive enzyme, although no process has been yet developed to accomplish this at low cost.

5.0 Sugar Fermentation to Ethanol

Humans have fermented glucose to ethanol for thousands of years, and organisms have been developed to achieve high rates, yields, and concentrations of this product. However, only glucose and related sugars are released from starch and sugar crops used for ethanol production in traditional beverage and other applications, whereas five different sugars result from biomass hydrolysis: arabinose, galactose, glucose, mannose, and xylose (Wyman, 1996). Although biomass is low in cost, all of these sugars, including those present in smaller concentrations, must be converted to ethanol at high yields for the overall process to be economically viable, and failure to achieve this end badly hampers the commercial viability of biological biomass conversion technologies (Hinman et al, 1988). Unfortunately, no organism has been discovered that naturally ferments the two five-carbon sugars arabinose and xylose to ethanol with high yields, and no coproducts derived from these sugars have markets compatible in size with ethanol production (Parker et al, 1983). Until recently, this shortcoming severely limited the prospects for commercializing bioethanol technology.

Following years of efforts by a number of groups, one team successfully applied modern genetic engineering tools to develop novel organisms that ferment all five sugars in biomass to ethanol with yields as high or higher than those achieved by yeast on glucose (Ingram et al, 1987). In this breakthrough discovery, recognized by the award of U.S. Patent 5,000,000 (Ingram et al, 1991), two genes from the bacterium *Zymomonas mobilis* are inserted into any one of a number of bacteria, such as *Escherichia coli* and *Klebsiella oxytoca*, to make enzymes that divert an internal cellular compound known as pyruvate to ethanol instead of the products they normally make such as acetic and succinic acids. Because these bacteria naturally convert all five sugars to pyruvate, yield is not restricted by limitations in appetite. In addition, the two enzymes made by these genes remove pyruvate so rapidly that very little of this intermediate material is available for its usual products, and some of the natural pathways are removed to improve yields even more. The net result is that very high ethanol yields are realized from all the sugars in biomass. This approach has been successfully applied to ferment hemicellulose hydrolyzates from corn stover and fiber to ethanol with high yield (Asghari et al, 1996). Furthermore, various strains have the ability to consume oligomeric sugars, such as cellobiose, cellotriose, and xylobiose, or genes can be inserted into many of the strains to impart this capability (Wood and Ingram, 1992). These features make it possible for the genetically modified strains to remove powerful cellulase inhibitors and also ferment five- and six-carbon sugars to ethanol, resulting in superior performance for SSCF applications. Genes also have been inserted into these host organisms for producing endoglucanase, further reducing enzyme loading requirements with significant cost advantages.

While Ingram and his colleagues genetically engineered organisms that normally consume all sugars to make ethanol at high yields, others later introduced traits into organisms that naturally make ethanol to allow them to consume additional sugars. One of these teams introduced five genes from the bacteria *E. coli* into *Zymomonas mobilis* to allow the latter to take up xylose in addition to the glucose it naturally consumes (Zhang et al, 1995). Subsequently, two more genes were introduced to ferment arabinose in addition to xylose and glucose (Deanda et al, 1996). Another group genetically engineered the yeast *Saccharomyces cerevisiae* to ferment xylose to ethanol, in addition to the six-carbon sugars it naturally utilizes (Ho et al, 1998), and is also working to include arabinose fermentation (Sedlak and Ho, 2001). Approaches are also being sought to improve the yield of the yeast *Pichia stipitis* for fermenting xylose as well as glucose (Shi and Jeffries, 1998).

6.0 Sugar Fermentation to Chemicals and Materials

Although much attention has been devoted to making ethanol from biomass, numerous other products can be made from the low-cost biosugars (Wyman and Goodman, 1993a; Bozell and Landucci, 1993; Landucci et al, 1994). Thus, just as petroleum refining grew from primarily making kerosene to manufacturing a wide array of products that take advantage of the complex composition of petroleum (Yergin, 1991), biomass processing can evolve to making a wide range of chemicals and fuels as well as power, food, and feed from this sustainable resource, as illustrated in Figure 2 (Wyman, 1990). The petrochemical industry derives these numerous end products from only a few building blocks such as benzene, ethylene, methane, propylene, toluene, and xylene plus a family of four-carbon compounds including butanes, butadiene, and butlyenes. By analogy, producing a few intermediates from biomass could be an effective route to driving a large industry that makes a much broader range of biobased products than is now considered. The challenge is to focus on compounds that could drive this important evolution for sustainable biomass-based processing.

As an example of the evolution to biobased chemicals, a Cargill-Dow Chemical joint venture is applying new lactic acid fermentation and recovery technologies in a large corn processing plant followed by polymerization to biodegradable plastics (Fairley, 2001). Similarly, Dupont and Genencor teamed to develop an improved organism for fermenting glucose to 1,3-propanediol, and a joint venture between Dupont and Tate and Lyle Citric Acid is demonstrating this approach in a 200,000 lb/year pilot plant (Fairley, 2001). Both technologies would certainly lend themselves to cellulose-derived glucose if the sugar can be made at competitive prices and appropriate quality.

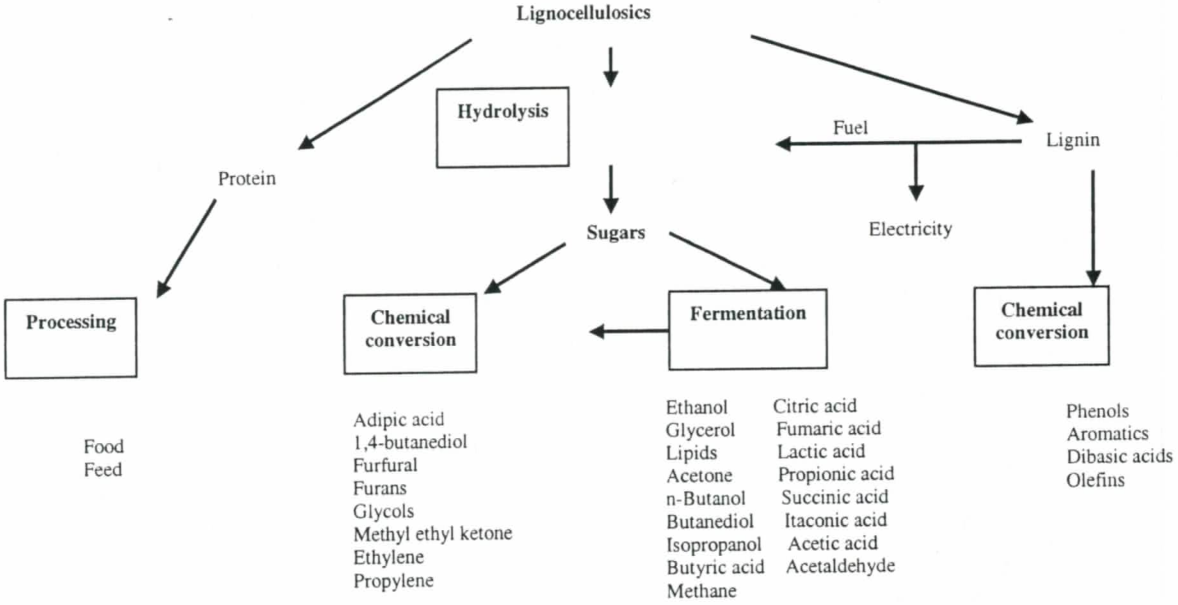

Figure 2: A biorefinery concept for making a range of products from lignocellulosic biomass (Wyman 1990).

Succinic acid has been identified as a promising four-carbon building block analogous to the C4 family for petrochemicals (Bozell and Landucci, 1993; Landucci et al, 1994). Succinic acid is now generally manufactured by hydrogenating maleic anhydride to succinic anhydride followed by hydration to succinic acid, but the high cost of such processes limits its markets. If its selling price were reduced, its versatile four-carbon dicarboxylic acid could be used both directly and as a C4 intermediate for numerous applications in agriculture, food, medicine, plastics, cosmetics, textiles, plating, and waste-gas scrubbing. For example, catalytic processes have been recently developed to convert succinic acid into a number of industrially important chemicals including 1,4-butanediol, tetrahydrofuran, gamma-butyrolactone, adipic acid, and maleic anhydride, and it can be easily esterified to dimethylsuccinate, an environmentally friendly solvent. Thus, while the market for some chemical products is insufficient to consume all the sugars from just one modest-sized biomass processing plant, the growth potential for succinic acid could use all the sugars from many plants provided low enough costs can be achieved. Assessments have also shown succinic acid to be well positioned for near-term applications based on cost of feedstock, current yields, and competition with petrochemicals (Landucci et al, 1994). Furthermore, technology developed for succinic acid will have wide application to all organic acids, supporting construction of a large number of biorefineries. In line with this, organisms have been genetically modified to improve succinic acid production (Donnelly et al, 1998) and better fermentation and separation operations devised to reduce production and recovery costs (Glassner et al, 1995; Nghiem et al, 1997).

Other fermentation chemicals receiving attention include vanillin (Li and Frost, 1998), shikimic acid (Draths et al, 1999), gallic acid (Kambourakis et al, 2000), and 1,2-propandiol (Altaras et al, 2001). Such products can be made from the glucose from corn or cellulose. These are just a few examples, with more technologies being developed to produce such products than can be addressed here, and the reader is referred to other references for additional information on such possibilities (Wyman and Goodman, 1993a; National Research Council, 1999; Lynd et al, 1999).

7.0 Lignin Utilization

Because all of the heat and electricity needed to power a bioethanol production process can be generated by burning lignin and other solids remaining after biological conversion of carbohydrates, no fossil energy is needed, thus, minimizing, if not eliminating, emissions of carbon dioxide, a powerful greenhouse gas. Furthermore, a significant amount of excess energy is available to export for extra revenue (Hinman et al, 1992; Wooley et al, 1999). Even without power sales, the avoided costs of purchasing heat and electricity and waste disposal justify the capital costs for power production in a biological process, making it possible to sell the excess power into even highly competitive deregulated electricity markets that have been the bane of many biomass power facilities. In addition, power provides one of the few markets compatible in size with large-scale ethanol production, and biorefineries can provide baseload grid power that is not readily available from other renewable resources. Alternatively, lignin could be converted into other products that could serve a similar role as benzene/toluene/xylene from petroleum refining.

VI. ECONOMICS OF BIOLOGICAL CONVERSION NOW AND FOR THE FUTURE

Substantial progress has been realized in advancing the technology for converting biomass to ethanol by enzyme-based technology with the projected cost dropping from almost \$5.00/gal 20 years ago to the point where it is now competitive in the current blending market (Wright, 1988; Hinman et al, 1992; Wooley et al, 1999; Wyman, 1999). Developing bacteria that ferment all five sugars in biomass to ethanol with high yields was critical to reaching these lower costs. Advances in overcoming the recalcitrance of biomass through improving dilute-acid pretreatment technology and enzymes, however, were also vital to this progress. In addition, integrating cellulose hydrolysis with sugar fermentation in the SSF and then SSCF configurations reduced costs by improving the rates, yields, and concentrations of ethanol production (Wright et al, 1987; Wooley et al, 1999). Also, enzymes with higher levels of β-glucosidase and organisms that utilize cellobiose had important impact. The emergence of modern biotechnology tools was vital to most of these technical advances. Several enterprising companies are now working to build the first commercial bioethanol plants (McCoy, 1998).

Coproduction of chemicals, bioethanol, and electricity can be synergistic. The higher price commanded by many chemicals, particularly at lower volumes, can support smaller biorefineries for introducing biomass conversion technology. On the other hand, bioethanol production will make economies of scale possible for latter plants that will reduce sugar costs, facilitating profitable production of chemicals at a scale that will have significant impact in expanding biomass markets, reducing petroleum demand, and reducing greenhouse gas emissions. Ethanol also can support the plant while building chemical sales to commodity levels. As mentioned before, heat and electricity production are often the most economical option for disposal of lignin and wastes and to meet the process energy requirements. Consequently, electricity can be sold at a lower cost in an integrated biorefinery than is possible in a dedicated biomass power plant.

Although this four-fold reduction in projected ethanol production costs is impressive, further cost reductions by about a factor of two are desirable to make ethanol competitive as a pure fuel without subsidies. Several studies have shown such improvements to be imminently achievable (Hinman et al, 1992; Wyman, 1995; Lynd et al, 1996). The key, however, is to develop advanced process configurations for hemicellulose and cellulose hydrolysis that simplify and combine steps and reduce operating and capital costs (Lynd et al, 1996). For example, significant advantages would result if cellulase production and fermentation of the sugars in biomass could be accomplished by a single anaerobic organism, thus, eliminating the need for a separate aerobic cellulase production step that requires high energy inputs. Although biotechnology provides a powerful tool to meet this need, improvements also are essential in biomass pretreatment to reduce materials of construction costs, enhance yields, reduce chemical costs for pretreatment, and avoid expensive downstream operations to remove inhibitors and other problematic chemicals resulting from pretreatment.

VII. BENEFITS

1.0 Farm Income

Conversing cellulosic biomass to ethanol is becoming economically feasible as a result of years of sustained research despite a very turbulent funding history (Wyman, 1999; Wyman, 2001). As a result, projects are now in progress to apply the technology commercially to niche opportunities based on retrofitting of existing plants, use of low-cost residues, low-cost debt financing, and other cost-cutting measures (Wyman and Goodman, 1993b; McCoy, 1998). Once such processing facilities are operational, a succession of similar projects will likely follow, increasing the demand for biomass. Furthermore, because biomass provides a unique resource for sustainable production of organic products, corn stover and other forms of biomass will be vital raw materials for the production of such products when the need for sustainable routes to making organic materials is finally recognized, increasing demand even more.

As efficiencies of biomass conversion improve through continued research and experience with commercial processes, the importance of feedstock costs will decline, and higher prices can be paid for raw material, expanding the market and margins for the agricultural sector. For example, if we take current yields as being about 76 gallons of ethanol per dry ton of feedstock with a raw material costing $25/dry ton, the contribution of biomass to the overall selling price of $1.16/gallon is about $0.33/gallon of ethanol made, as projected in one study for best-available current technology (Wooley et al, 1999). On the other hand, continual improvements could make it possible to increase yields to about 100 gallons/ton of dry feedstock, and at this level, a feedstock costing $40/dry ton at the plant gate would contribute about $0.40/gallon of ethanol. Although this unit cost is higher than that described in the study (Wooley et al, 1999), it can be expected that conversion costs will be reduced to less than one-third of the total cost once the technology matures. At this point, bioethanol could be manufactured for a total cost of about \$0.60/gallon, a price competitive with gasoline selling for about \$0.75/gallon at the plant gate (Wyman, 1995). In addition, coproduction of electricity and other coproducts coupled with leap-forward technology advances will reduce the required selling price of bioethanol, so a cost of as little as \$0.50/gallon, or less, is possible (Lynd et al, 1996). Thus, biomass conversion can provide farmers with a new market for biomass residues and crops at a good price to make a product that has a huge market at a competitive cost without subsidies.

Biomass processing will also create rural manufacturing jobs. Because biomass transport has a significant effect on the cost of raw material, a biomass processing facility must be located in an agricultural region of the country to have affordable access to the large amounts of corn stover needed. For example, about 65 relatively high-paying, skilled, manufacturing jobs would be created for a single modest-sized process facility, providing opportunities for well-trained chemists, biologists, operators, and maintenance and supervisory personnel in rural America (Wooley et al, 1999). In addition, new jobs are created on farms to collect corn stover, and a biorefinery would create trucking and railroad employment to ship corn stover, chemicals, nutrients, and other supplies to the plant and to transport products to the market. Numerous jobs spill over as well into the local community through increased demand for housing, schools, and stores, etc. The overall impact would thus be to create more than 200 new jobs for a single biomass processing plant, revitalizing rural economies.

2.0 Energy Security and Trade Deficit

Because fossil resources are ultimately finite, they provide only a temporary raw material for producing fuels and commodity chemicals, and their economic lifetime is minor compared to the length of human existence. The only control we have on the duration of the supply is how we use these resources. Furthermore, it can be argued that burning fossil fuels squanders a resource that is much more valuable and uniquely suited for making chemicals because many other options could be developed for fuels. Although the United States and many other governments initiated major programs to develop new energy sources in response to tightening petroleum supplies and skyrocketing energy costs in the “Energy Crises” of the mid to late 1970s, interest in developing new sources waned as prices fell later (Wyman, 2001). Because developing competitively priced energy sources requires time, particularly when funded lightly and sporadically, petroleum remains the largest and still growing source of energy in the United States, providing about 40% of over 95 quads of total energy (quadrillion BTUs or 10^{15} BTU) (US DOE, 2000). Consequently, chemical and liquid fuel production almost totally depends on petroleum, whereas other energy sectors are diversified, and the transportation sector uses more petroleum than any other (US DOE, 2000). About 60% of our petroleum is imported, making the United States vulnerable to periodic price hikes and supply disruptions. As a result, interrupted oil supplies would damage these sectors, causing long gasoline lines and dramatic increases in chemical prices similar to the experience of the oil crises of the 1970s.

As noted in the introduction, enough ethanol could be made from corn biomass to significantly reduce gasoline use. Furthermore, the total supply of biomass from which fuels, chemicals, and power could be made is substantial (Lynd et al, 1991; Lynd, 1996). Although some uncertainty and controversy surrounds the magnitude of the resource and the possible conflict its use could create with the demand for food, there is little doubt that enough biomass could be obtained as wastes and dedicated energy crops to make a significant dent in the huge amount of gasoline consumed in the United States (Lynd et al, 1991; Lynd, 1996). Furthermore, there is more than enough biomass to displace all petroleum for manufacturing chemicals

(Wyman and Goodman, 1993a). Thus, a biorefinery could target reductions in petroleum imports that are the single largest contributor to the U.S. trade deficit and reduce our vulnerable dependence on imported oil (Lugar and Woolsey, 1999). Furthermore, co producing electricity would be an effective way to provide baseload power to complement more cyclical renewables, such as wind. It is also possible to recover protein from many biomass sources and use it for animal feed, thereby achieving dual use of productive land.

Sustainable production of fuels and chemicals will become even more important as developing countries improve their standard of living and seek to gain greater mobility (Beck, 1997). Maintaining the status quo of energy sources would strain petroleum supplies even more. Then, the challenge will not be how to reduce petroleum use but, instead, how to meet a growing demand that supports improved lives of more and more people around the world. Biomass conversion to ethanol offers a promising path. An added benefit is that biomass fuels and chemicals can be produced in countries, including the United States, that have limited petroleum resources, helping them to reduce their trade deficit and grow their economies.

3.0 Greenhouse Gas Reduction

Perhaps the most unique and powerful attribute of biomass conversion to fuels, chemicals, and power is very low greenhouse gas emissions, particularly when compared to other transportation fuel, chemical, and power manufacturing options. Because burning unconverted solids left after making the targeted biological products can provide all the heat and electricity to run the process, no fossil fuel is required to operate the conversion plant (Hinman et al, 1992; Wooley et al, 1999). In addition, low levels of fertilizer and cultivation are adequate for many lignocellulosic crops, minimizing energy inputs for biomass production (Lynd et al, 1991). The result is that most of the carbon dioxide released during manufacture and use is recaptured to grow new biomass to replace that harvested, and the net release of carbon dioxide in a cradle-to-grave (often called a lifecycle) analysis is less than 10% of that for gasoline (Tyson, 1993; Interlaboratory Working Group, 1997). If credit is taken for exporting excess electricity that displaces power generation by fossil fuels such as coal, more carbon dioxide can be taken up than is released back into the atmosphere (Wyman, 1994). Just using the amount of corn stover viewed as available by CEA would reduce overall U.S. carbon emissions by around 1.5 to 2.0%, a big step toward lowering greenhouse gas emissions.

The impact of bioethanol can be particularly significant in the United States because the transportation sector is a major contributor to greenhouse gas emissions, accounting for about one third of the total. Furthermore, the United States accounts for about 25% of global carbon dioxide emissions, with petroleum the largest single source (42%) of that total (US DOE, 2000). As part of a Presidential Advisory Committee on reducing greenhouse gas emissions from personal vehicles, a survey of experts in the field clearly showed that most alternatives to petroleum (e.g., hydrogen production from solar energy) required significant changes in transportation infrastructure, whereas others that could be more readily used (e.g., methanol production from coal or natural gas) would have little impact on reducing greenhouse gas emissions (Clinton and Gore, 1993). On the other hand, ethanol is a versatile liquid fuel, currently produced from corn and other starch crops, that is widely accepted for blending and is added to about 10% of gasoline sold in the United States. Vehicles that can use high-level ethanol blends (E85, a blend of 85% ethanol in gasoline) are now being sold throughout the United States as well, and because bioethanol production could be commercialized in a few years, extended timeframes are not required to realize its benefits.

4.0 Solid Waste Disposal

Disposal of many waste materials is becoming more and more important. For example, farmers are being asked to reduce the amount of rice straw they burn following a harvest in northern California to reduce air pollution. In British Columbia, phase-in of similar restrictions is raising concerns about what to do with wood wastes that have been historically burned. Suppressing natural forest fires has resulted in dense forests that cause more damage to the soil and mature trees because hotter fires result when they finally rage beyond control, and many are seeking to thin the forests to restore them to their natural plant density. Biorefineries that process biomass wastes into fuels, chemicals, and power would provide a unique solution to these growing dilemmas, an underappreciated attribute for biomass conversion.

5.0 Air and Water Pollution

In addition to augmenting the fuel supply, blending ethanol with gasoline increases octane and provides oxygen to promote more complete combustion, particularly in older vehicles (Lynd et al, 1991; Bailey, 1996). The former property reduces the need for additives, such as benzene or tetra ethyl lead, that are toxic and often carcinogenic, and the latter reduces tailpipe emissions of carbon monoxide and unburned hydrocarbons. Carbon monoxide is a serious problem in many urban areas (particularly high-altitude cities in winter months), and ethanol use reduces carbon monoxide tailpipe emissions (Putsche and Sandor, 1996). Oxygenates, such as ethanol, also reduce tailpipe emissions of unburned hydrocarbons that form ground-level ozone, resulting in serious health effects. On the other hand, although ethanol has a much lower vapor pressure than gasoline, blending the two initially increases vapor pressure, promoting evaporation of gasoline components that increase ozone formation and resulting in considerable controversy about the efficacy of ethanol for ozone mitigation. Substituting lower vapor-pressure gasoline would compensate for the higher blend vapor pressure, but this change may be costly to the consumer. Instead, because methyl tertiary butyl ether (MTBE) is vapor pressure neutral, it has been widely blended into gasoline to reduce formation of ozone forming compounds as well as carbon monoxide. A related compound made from ethanol, ethyl tertiary butyl ether (ETBE), actually reduces the vapor pressure of blends, having even greater benefit as regulators continue to mandate lower and lower vapor pressure gasoline to combat air pollution. However, MTBE use is being curtailed because of concerns about its persistence in the environment and health effects, and ETBE would likely suffer from the same concerns. It is important to note that direct ethanol use for blending or neat fuels does not pose these problems because ethanol is readily metabolized as evidenced by its widespread consumption as a beverage.

Neat ethanol provides the greatest benefits with respect to both air and water pollution (Bailey, 1996). Its low vapor pressure (about one-quarter that of gasoline) coupled with its low photochemical reactivity reduces its ozone-forming potential. Furthermore, ethanol is biodegradable and readily dispersed in water, limiting damage from spills compared to immiscible and much more toxic hydrocarbon-based fuels. Although ethanol has about two-thirds of the volumetric energy density of gasoline, engines tuned to take advantage of its superior fuel properties (e.g., high octane and high heat of vaporization) can actually achieve about 80% of the range on the same volume of fuel (Wyman and Hinman, 1990). Until such engines are widely available, flexible fueled vehicles now being offered by Ford and Chrysler use any mixture of ethanol and gasoline above 15% gasoline and will facilitate transition to high-performance ethanol engines. Ultimately, use of ethanol in fuel cells promises to achieve very high efficiencies with very low emissions.

VIII. OTHER USES OF CORN BIOMASS

In addition to the emerging production of fuels and chemicals from corn biomass, other uses are also possible, with a few summarized here. An important factor that affects production of meat and milk in cattle and sheep is forage digestibility, which is impacted by lignin content (Ralph and Helm, 1993). Forage quality has been traditionally manipulated by managing the harvest time to balance overall yield against lignin content (Cherney et al, 1991), but corn biomass can be pretreated by many of the approaches described above to improve its digestibility as an animal feed. For example, steam treatment was applied to improve the nutritive value of corn stover and other biomass types (Oji and Mowat, 1978). Another study employed ammonia and/or calcium hydroxide to enhance the digestibility of corn stover (Oliveros, 1993). A solvent delignification approach similar to organosolv pretreatment was applied to increase forage digestibility for mature grasses (Sewalt et al, 1997). In another case, sulfur dioxide and ammonia were compared for improving the accessibility of corn stover (Innocenti et al, 1989). Corn stover hydrolyzate also was used to grow single-cell protein for possible use as food or feed (Gonzalezzvaldes and Mooyoung, 1981). These examples are but a sampling of the many approaches for enhancing the value of corn stover for feed and illustrate the parallel between pretreatment for enzymatic conversion and improving the digestibility of stover for animals.

Another application for corn stover is co-firing with coal to reduce sulfur emissions, with the value largely determined by displacing coal use and lowering emissions (Abdallah and Hitzhusen, 1979). Another possibility is to mix corn stover with agents, such as polymeric methylene diisocynate, to produce fiber mats as is done with wheat straw, although some controversy has developed regarding the environmental merits of this formulation (McCoy, 2001).

IX. SUMMARY

Corn biomass is the most abundant crop residue in the United States and could contribute meaningfully to the transition to sustainable manufacture of fuels and chemicals. Of course, stover collection must be controlled to insure sound soil management, but various studies project that significant amounts of this material could be available at low enough costs to be attractive for biological processing without interfering with land quality. Furthermore, the vast demand for fuels and commodity chemicals could provide a major new agricultural market. The value of stover and other biomass residues and crops will increase as conversion costs drop due to learning curve effects and technology advancements. Overall, such processes could give farmers major new revenue streams and create rural agricultural and manufacturing employment.

Biological processing of stover involves several operations needed to hydrolyze the major fractions of cellulose and hemicellulose to release sugars that can be fermented to ethanol or various chemicals. A number of options have been developed to accomplish these steps, and technology selection requires careful consideration of these alternatives. Through sustained research and development, significant progress has been made in reducing the cost of biological processing to the point it is now competitive for ethanol production in the blending market, and genetic engineering of bacteria to ferment all the sugars in biomass to ethanol at high yields was critical to this success. Sugar production from biomass is applicable to making fermentation chemicals in addition to ethanol, and a number of new organisms are being engineered to expand the range of products possible. Opportunities also have been identified to advance biomass pretreatment and cellulose conversion further so that fermentation products from biomass can compete in large commodity markets, thus increasing the demand for biomass supplies.

Biological conversion of corn stover to fuels and chemicals offers a number of important economic, environmental, and strategic benefits. The technology could create jobs in agriculture and manufacturing that are important for rural development and that reduce dependence on vulnerable supplies of imported oil while improving the balance of trade. Particularly important is the unique role biomass could play in providing a resource for sustainably producing organic fuels and chemicals that can dramatically reduce greenhouse gas emissions. The emergence of biomass conversion technologies at competitive costs in such a vast market would thus provide a major growth opportunity for corn biomass revenues.

Corn stover can also be processed to improve its digestibility as cattle feed using pretreatment technologies similar to those used for producing fuels and chemicals. Other options, such as blending with coal in boilers and conversion to particle board, could provide additional new revenues for corn growers.

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XI. LITERATURE CITED

ABATZOGLOU, N., CHORNET, E., BELKACEMI, K., and OVEREND, R. 1992. "Phenomenological Kinetics of Complex Systems: The Development of a Generalized Severity Parameter and Its Application to Lignocellulosics Fractionation, *Chem. Eng. Science* **47**(5): 1109-22.

ABDALLAH, M., and HITZHUSEN, F.J. 1979. "Economics of Corn Stover as a Coal Supplement in Steam-Electric Power-Plants in the North Central United States," *Ohio Agricultural Research and Development Center Research Bulletin* **1112**: 3-17.

AGBLEVOR, F.A., BESLER, S., and WISELOGEL, A.E. 1996. "Production of Oxygenated Fuels from Biomass: Impact of Feedstock Storage," *Fuel Science and Technology International* **14**(4): 589-612.

ALLEN, S.G., KAM, L.C., ZEMANN, A.J., and ANTAL, M.J. 1996. "Fractionation of Sugar Cane with Hot, Compressed, Liquid Water," *Industrial Engineering Chemistry Research* **35**: 2709-15.

ALTARAS, N.E., ETZEL, M.R., and CAMERON, D.C. 2001. "Conversion of Sugars to 1,2-Propanediol by *Thermoanaerobacterium thermosaccharolyticum* HG-8," *Biotechnology Progress* **17**(1): 52-6.

ASGHARI, A., BOTHAST, R.J., DORAN, J.B., and INGRAM, L.O. 1996. "Ethanol Production from Hemicellulose Hydrolyzates of Agricultural Residues Using Genetically Engineered *Escherichia coli* KO11," *Journal of Industrial Microbiology* **16**(1): 42-7.

BAILEY, B.K. 1996. Performance of Ethanol as a Transportation Fuel, in *Handbook on Bioethanol, Production and Utilization*, Wyman CE, editor, Taylor & Francis, Washington, DC, Chapter 3.

BALLERINI, D., DESMARQUEST, J.P., POURQUIE, J., NATIVEL, F., and REBELLER, M. 1994. "Ethanol-Production from Lignocellulosics – Large-Scale Experimentation and Economics," *Bioresource Technology* **50**(1): 17-23.

BECK, R.J. 1997. *Worldwide Petroleum Industry Outlook: 1998-2002 Projections to 2007, 14th Edition.* Tulsa, Oklahoma: Pennwell Books pp. 25-8.

BOBLETER, O., NIESNER, R., and ROHR, M. 1976. "The Hydrothermal Degradation of Cellulosic Matter to Sugars and Their Fermentative Conversion to Protein," *Journal of Applied Polymer Science* **20**: 2083-93.

BOBLETER, O. 1994. "Hydrothermal Degradation of Polymers Derived from Plants," *Progress in Polymer Science* **19**: 797-841.
BOZELL, J., and LANDUCCI, R. 1993. "Alternative Feedstocks Program Technical and Economic Assessement: Thermal/Chemical and Bioprocessing Components," National Renewable Energy Laboratory, Golden, Colorado, 232 pp.
BRINK, D.L. 1994. *Method of Treating Biomass Material*, US Patent 5,366,558.
ceassist.com. 2000. "Collection Report," Chief Executive Assistance, Charlotte, NC.
CHANG, V.S., and HOLTZAPPLE, M.T. 2000. *Applied Biochemistry and Biotechnology* **84-86**: 5-37.
CHERNEY, J.H., CHERNEY, D.J.R., AKIN, D.E., and AXTELL, J.D. 1991. "Potential of Brown-Midrib, Low-Lignin Mutants for Improving Forage Quality," *Advances in Agronomics* **46**: 157-98.
CHUM, H.L., JOHNSON, D.K., BLACK, S., BAKER, J., GROHMANN, K., SARKANEN, K.V., WALLACE, K., and SCHROEDER, H.A. 1988. "Organosolv Pretreatment for Enzymatic-Hydrolysis of Poplars .1. Enzyme Hydrolysis of Cellulosic Residues," *Biotechnology and Bioengineering* **31**(7): 643-9.
CLINTON, W.J., and GORE, A. 1993. *Global Climate Change Action Plan*. Washington, DC: The White House
CONVERSE, A.O. 1993. "Substrate Factors Limiting Enzymatic Hydrolysis," in *Bioconversion of forest andAgricultural plant residues*, Saddler JN, editor, CAB International Wallingford, UK.
DEANDA, K., ZHANG, M., EDDY, C., and PICATAGGIO, S. 1996. "Development of an Arabinose-Fermenting *Zymomonas mobilis* Strain by Metabolic Pathway Engineering," *Applied and Environmental Microbiology* **62**(12): 4465-70.
DOMALSKI, E.S., JOBE, T.L. JR., and MILNE, T.A. 1982. *ThermodynamicData for Biomass Materials and Waste Components*. New York, N.Y., American Society of Mechanical Engineers.
DONNELLY, M.I., MILLARD, C.S., CLARK, D.P., CHEN, M.J., and RATHKE, J.W. 1998. "A Novel Fermentation Pathway in an *Escherichia coli* Mutant Producing Succinic Acid, Acetic Acid, and Ethanol," *Applied Biochemistry and Biotechnology* **70-72**: 187-98.
DRATHS, K.M., KNOP, D.R., and FROST, J.W. 1999. "Shikimic Acid and Quinic Acid: Replacing Isolation from Plant Sources with Recombinant Microbial Catalysis," *Journal of the American Chemical Society* **121**(7): 1603-4.
ESTERBAUER, H., STEINER, W., LABUDOVA, I., HERMANN, A., and HAYN, M. 1991. "Production of *Trichoderma* Cellulase in Laboratory, and Pilot Scale," *Bioresource Technology* **36**: 51-65.
FAIRLEY, P. 2001. "Bioprocessing Comes Alive: No Longer a Field of Dreams," *Chemical Week*, March 14.
FAN, L.T., LEE,Y., and GHARPURAY, M.M. 1982. "The Nature of Lignocellulosics and Their Pretreatment for Enzymatic Hydrolysis," *Advances in Biochemical Engineering* **23**: 157-87.
GAUSS, W.F., SUZUKI, S., and TAKAGI, M. 1976. *Manufacture of Alcohol from Cellulosic Materials Using Plural Ferments*. US Patent No. 3,990,944, November 6.
GHARPURAY, M.M., FAN, L.T., and LEE, Y. 1983. "Caustic Pretreatment Study for Enzymatic Hydrolysis of Wheat Straw," in *Wood and Agricultural Residues - Research on Use for Feed, Fuels, and Chemicals*, Soltes EJ, ed. Academic Press, New York, pp. 369-89.
GLASSNER, D.A., ELANKOVAN, P., BEACOM, D.R., and BERGLUND, K.A. 1995. "Purification Process for Succinic Acid Produced by Fermentation," *Applied Biochemistry and Biotechnology* **51/52**: 73-82.

GONZALEZVALDES, A., and MOOYOUNG, M. 1981. "Production of Yeast SCP from Corn Stover Hydrolysates," *Biotechnology Letters* **3**(3): 143-8.
GOULD, J.M. 1984. "Alkaline Peroxide Delignification of Agricultural Residues to Enhance Enzymatic Saccharification," *Biotechnology and Bioengineering* **26**: 46-52.
GRETHLEIN, H.E. 1985. "The Effect of Pore Size Distribution on the Rate of Enzymatic Hydrolysis of Cellulosic Substrates," *Bio/Technology* **3**: 155-60, February.
GROHMANN, K., TORGET, R., and HIMMEL, M. 1986. "Dilute Acid Pretreatment of Biomass at High Solids Concentrations," *Biotechnology and Bioengineering Symposium* **17**: 137-51.
GROHMANN, K., MITCHELL, D.J., HIMMEL, M.E., DALE, B.E., and SCHROEDER, H.A. 1989. "The Role of Ester Groups in Resistance of Plant Cell Wall Polysaccharides to Enzymatic Hydrolysis," *Applied Biochemistry and Biotechnology* **20/21**: 45-61.
GROUS, W.R., CONVERSE, A.O., and GRETHLEIN, H.E. 1985. "Effect of Steam Explosion Pretreatment on Pore-Size and Enzymatic-Hydrolysis of Poplar," *Enzyme and Microbial Technology* **8**: 274-80.

GULATI, M., KOHLMANN, K., LADISCH, M.R., HESPELL, R., and BOTHAST, R.J. 1996. "Assessment of Ethanol Production Options for Corn Products," *Bioresource Technology* **58**: 253-64.

HAYWARD, T.K., HAMILTON, J., THOLUDUR, A., and MCMILLAN, J.D. 2000. "Improvements in Titer, Productivity, and Yield Using Solka-Floc for Cellulase Production," *Applied Biochemistry and Biotechnology* **84-86**: 859-73.
HENDY, N., WILKE, C.R., and BLANCH, H. 1984. "Enhanced Cellulase Production in Fed-Batch Culture of *Trichoderma reesei* C30," *Enzyme and Microbiology Technology* **6**: 73-7.
HIMMEL, M.E., ADNEY, W.S., BAKER, J.O., NIEVES, R.A., and THOMAS, S.R. 1996. "Cellulases: Structure, Function, and Applcations," in *Handbook on Bioethanol, Production and Utilization*, Wyman CE, editor, Taylor & Francis, Washington, DC, Chapter 8.
HIMMEL, M.E., RUTH, M.F., and WYMAN, C.E. 1999. "Cellulase for Commodity Products from Cellulosic Biomass," *Current Opinion in Biotechnology* **10**(4): 358-64.
HINMAN, N.D., WRIGHT, J.D., HOAGLAND, W., and WYMAN, C.E. 1988. "Xylose Fermentation: An Economic Analysis," *Applied Biochemistry and Biotechnology* **20**: 391-401.
HINMAN, N.D., SCHELL, D.J., RILEY, C.J., BERGERON, P.W., and WALTER, P.J. 1992. Preliminary Estimate of the Cost of Ethanol Production for SSF Technology. *Applied Biochemistry and Biotechnology* **34/35**: 639-49.
HO, N.W.Y., CHEN, Z.D., and BRAINARD, A.P. 1998. "Genetically Engineered *Saccharomyces* Yeast Capable of Effective Cofermentation of Glucose and Xylose," *Applied and Environmental Microbiology* **64**(5): 1852-9.
HOLTZAPPLE, M.T., JUN, J., ASHOK, G., PATIBANDLA, S.L., and DALE, B.E. 1991. "The Ammonia Freeze Explosion (AFEX) Process: A Practical Lignocellulose Pretreatment," *Applied Biochemistry and Biotechnology* **28/29**: 59-74.
HOLTZAPPLE, M.T. 1993. In *Encyclopedia of Food Science, Food Technology, and Nutrition*, Macrae R, Robinson RK, Sadler MJ, eds. Academic Press, London, pp. 758-67, 2324-34, 2731-8.

HSU, T.-A. 1996. "Pretreatment of Biomass," in *Handbook on Bioethanol, Production and Utilization*, Wyman CE, ed. Taylor & Francis, Washington, DC, Chapter 10.
INGRAM, L.O., CONWAY, T., CLARK, D.P., SEWELL, G.W., and PRESTON, J.F. 1987. "Genetic Engineering of Ethanol Production in *Escherichia coli*," *Applied Environmental Microbiology* **53**: 2420-5.

INGRAM, L.O., CONWAY, T., and ALTERTHUM, F. 1991. *Ethanol Production by Escherichia coli Strains Co-expressing Zymomonas PDC and ADH Genes*. US Patent 5,000,000, March 19.

INNOCENTI, E., MOWAT, D.N., and MANDELL, I.B. 1989. "Effect of Sulfur-Dioxide and Ammonia on Chemical-Composition and Invitro Digestibility of Corn Stover and Barley Straw," *Canadian Journal of Animal Science* **69**(4): 963-72.

INTERLABORATORY WORKING GROUP ON ENERGY-EFFICIENT AND LOW-CARBON TECHNOLOGIES. 1997. *Scenarios of U.S. Carbon Reductions, Potential Impacts of Energy Technologies by 2010 and Beyond*. Washington, DC: Office of Energy Efficiency and Renewable Energy, US Department of Energy.

KAAR, W.E., and HOLTZAPPLE, M.T. 2000. "Using Lime Pretreatment to Facilitate the Enzymatic Hydrolysis of Corn Stover," *Biomass and Bioenergy* **18**(3): 189-99.

KADAM, K. 1996. "Cellulase Production," in *Handbook on Bioethanol, Production and Utilization*, Wyman CE, editor, Taylor & Francis, Washington, DC, Chapter 11.

KAMBOURAKIS, S., DRATHS, K.M., and FROST, J.W. 2000. "Synthesis of Gallic Acid and Pyrogallol from Glucose: Replacing Natural Product Isolation with Microbial Catalysis," *Journal of the American Chemical Society* **122**(37): 9042-3.

KARLSSON, J., MEDVE, J., and TJERNELD, F. 1999. "Hydrolysis of Steam-Pretreated Lignocellulose: Synergism and Adsorption for Cellobiohydrolase I and Endoglucanase II for *Trichoderma reesei*," *Applied Biochemistry and Biotechnology* **82**: 243-58.

KHAN, A.W., LABRIE, J., and MCKEOWN, J. 1987. "Electron-Beam Irradiation Pretreatment and Enzymatic Saccharification of Used Newsprint and Paper-Mill Wastes," *Radiation Physical Chemistry* **29**(2): 117-20.

KONG, F., ENGLER, C.R., and SOLTES, E.J. 1992. "Effects of Cell-Wall Acetate, Xylan Backbone, and Lignin on Enzymatic Hydrolysis of Aspen Wood," *Applied Biochemistry and Biotechnology* **34/35**: 23-35.

LANDUCCI, R., GOODMAN, B.J., and WYMAN, C.E. 1994. "Methodology for Evaluating the Economics of Biologically Producing Chemicals and Materials from Alternative Feedstocks," *Applied Biochemistry and Biotechnology* **45/46**: 677-96.

LEE, Y.Y., IYER, P., and TORGET, R.W. 1999. "Dilute Acid Hydrolysis of Lignocellulosic Biomass," *Advances in Biochemical Engineering and Biotechnology* **65**: 93-115.

LI, K., and FROST, J.W. 1998. "Synthesis of Vanillin from Glucose," *Journal of the American Chemical Society* **120**(40): 10545-6.

LIN, K.W., LADISCH, M.R., SCHAEFER, D.M., NOLLER, C.H., LECHTENBERG, V., and TSAO, G.T. 1981. "Review of Effect of Pretreatment on Digestibility of Cellulosic Materials," *AIChE Symposium* **207**(77): 102-6.

LUGAR, R.G., and WOOLSEY, R.J. 1999. "The New Petroleum," *Foreign Affairs* **78**(1): 88-102.

LYND, L.R., CUSHMAN, J.H., NICHOLS, R.J., and WYMAN, C.E. 1991. "Fuel Ethanol from Cellulosic Biomass," *Science* **251**: 1318-23.

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 Environmment* **21**: 403-65.

LYND, L.R., ELANDER, R.T., and WYMAN, C.E. 1996. "Likely Features and Costs of Mature Biomass Ethanol Technology," *Applied Biochemistry and Biotechnology* **57/58**: 741-61.

LYND, L.R., WYMAN, C.E., and GERNGROSS, T.U. 1999. "Biocommodity Engineering," *Biotechnology Progress*, **15**: 777-93.

MANDALS, M., and REESE, E.T. 1964. "Fungal Cellulases and the Microbial Decomposition of Cellulosic Fabric," *Developments in Industrial Microbiology* **5**: 5-20.
MARRISON, C.I., and LARSON, E.D. 1995. "Cost Versus Scale for Advanced Plantation-Based Biomass Energy Systems in the U.S.," U.S. EPA Symposium on Greenhouse Gas Emissions and Mitigation Research, Washington, DC, June 27-29.
MCCOY, M. 1998. "Biomass Ethanol Inches Forward," *Chemical and Engineering News,* December 7, pp. 29-32.
MCCOY, M. 2001. "The Business Fallout of 'Ecoefficiency'," *Chemical and Engineering News,* April 2, p. 36.
MCMILLAN, J.D. 1994. "Pretreatment of Lignocellulosic Biomass," in *Enzymatic Conversion of Biomass for Fuels Production*, Himmel ME, Baker JO, Overend RP, editors. ACS Symposium Series 566, pp. 292-324. Washington, DC: American Chemical Society.
MES-HARTREE, M., and SADDLER, J.N. 1983. "The Nature of Inhibitory Materials Present in Pretreated Lignocellulosic Substrates Which Inhibit the Enzymatic-Hydrolysis of Cellulose," *Biotechnology Letters* **5**(8): 531-6.
MILLETT, M.A., BAKER, A.J., and SATTER, L.D. 1976. "Physical and Chemical Pretreatments for Enhancing Cellulose Saccharification," *Biotechnology and Bioengineering Symposium* **6**: 125-53.
MOHAGHEGHI, A., GROHMANN, K., and WYMAN, C.E. 1987. "Production of Cellulase on Mixtures of Xylose and Cellulose," *Applied Biochemistry and Biotechnology* **17**: 263-77
MOHAGHEGHI, A., GROHMANN, K., and WYMAN, C.E. 1990. "Production of Cellulase on Mixtures of Xylose and Cellulose in a Fed Batch Process," *Biotechnology and Bioengineering* **35**: 211-16.
NATIONAL RESEARCH COUNCIL. 1999. *Biobased Industrial Products: Priorities for Research and Commercialization*, National Academy Press, Washington, DC, 114 pp, July 27.
NGHIEM, N.P., DAVISON, B.H., SUTTLE, B.E., and RICHARDSON, G.R. 1997. "Production of Succinic Acid by *Anaerobiospirillum succiniciproducens*," *Applied Biochemistry and Biotechnology* **63-65**: 565-76.
OJI, U.I., and MOWAT, D.N. 1978. "Nutritive Value of Steam-Treated Corn Stover," *Canadian Journal of Animal Science* **58**(2): 177-81.
OLIVEROS, B.A., BRITTON, R.A., and KLOPFENSTEIN, T.J. 1993. "Ammonia and/or Calcium Hydroxide Treatment of Maize Stover – Intake, Digestibility, and Digestion Kinetics," *Animal Feed Science and Technology* **44**(1-2): 59-72.
PARKER, S., CALNON, M., FEINBERG, D., POWER, A., and WEISS, L. 1983. *The Value of Furfural/Ethanol Coproduction from Acid Hydrolysis Processes*. Report SERI/TR-231-2000, DE84000007. Golden, CO: Solar Energy Research Institute. August.
PUTSCHE, V., and SANDOR, D. 1996. "Strategic, Economic, and Environmental Issues for Transportation Fuels," in *Handbook on Bioethanol, Production and Utilization*, Wyman CE, ed. Taylor & Francis, Washington, DC, Chapter 2.
QO CHEMICAL. 1988. "Cob Saver Program," Video, QO Chemical, Omaha, Nebraska.
RALPH, J., and HELM, R.F. 1993. "Lignin/Hydroxycinnamic Acid/Polysaccharide Complexes: Synthetic Models for Regiochemical Characterization," in *Forage Cell Wall Structure and Digestibility*, Jung HG, Buxton RD, Hatfield RD, Ralph J, eds. ASA, CSSA, SSSA, Madison, Wisconsin, pp. 201-46.
RAMOS, L.P., BREUIL, C., and SADDLER, J.N. 1992. "Comparison of Steam Pretreatment of Eucalyptus, Aspen, and Spruce Wood Chips and Their Enzymatic Hydrolysis," *Applied*

Biochemistry and Biotechnology **34/35**: 37-48.
REESE, E.T. 1976. "History of the Cellulase Program at the U.S. Army Natick Development Center," *Biotechnology and Bioengineering Symposium* **6**: 9-20.
SAHA, B.C., DIEN, B.S., and BOTHAST, R.J. 1998. "Fuel Ethanol Production from Corn Fiber," *Applied Biochemistry and Biotechnology* **70-72**: 115-25.
SCHELL, D.J., HINMAN, N.D., and WYMAN, C.E. 1990. "Whole Broth Cellulase Production for Use in Simultaneous Saccharification and Fermentation," *Applied Biochemistry and Biotechnololgy* **24/25**: 287-97.
SCHELL, D., TORGET, R., POWER, A., WALTER, P.J., GROHMANN, K., and HINMAN, N.D. 1991. "A Technical and Economic Analysis of Acid-Catalyzed Steam Explosion and Dilute Sulfuric Acid Pretreatments Using Wheat Straw or Aspen Wood Chips," *Applied Biochemistry and Biotechnology* **28/29**: 87-97.
SEDLAK, M., and HO, N.W.Y. 2001. "Expression of *E. coli* araBAD Operon Encoding Enzymes for Metabolizing L-Arabinose in *Saccharomyces cerevisiae*," *Enzyme and Microbial Technology* **28**: 16-24.
SEWALT, V.J.H., BEAUCHEMIN, K.A., RODE, L.M., ACHARYA, S., and BARON, V.S. 1997. "Lignin Impact on Fiber Degradation 4 – Enzymatic Saccharification and In Vitro Digestibility of Alfalfa and Grasses Following Selective Solvent Delignification," *Bioresource Technology* **61**(3): 199-206.
SHI N, Q., and JEFFRIES, T.W. 1998. "Anaerobic Growth and Improved Fermentation of *Pichia stipitis* Bearing a URA1 Gene from *Saccharomyces cerevisiae*," *Applied Microbiology and Biotechnology* **50**: 339-45.
SINITSYN, A.P., GUSAKOV, A.V., and VLASENKO, E.Y. 1991. "Effect of Structural and Physiochemical Features of Cellulosic Substrates on the Efficiency of Enzymatic Hydrolysis," *Applied Biochemistry and Biotechnology* **30**: 43-59.
SPINDLER, D.D., WYMAN, C.E., GROHMANN, K., and MOHAGHEGHI, A. 1988. "Simultaneous Saccharification and Fermentation of Pretreated Wheat Straw to Ethanol with Selected Yeast Strains and β-Glucosidase Supplementation,' *Applied Biochemistry and Biotechnology* **21**: 529-40.
SPINDLER, D.D. WYMAN, C.E., GROHMANN, K., and PHILIPPIDIS, G.P. 1992. "Evaluation of the Cellobiose-Fermenting Yeast *Brettanomyces Custersii* in the Simultaneous Saccharification and Fermentation of Cellulose," *Biotechnology Letters* **14**(5): 403-7.
SUBCOMMITTEE ON FEED COMPOSITION. 1982. "United States-Canadian Tables of Feed Composition: Nutritional Data for United States and Canada, Third Revision," National Research Council, National Academy Press, Washington, DC.
TAKAGI, M. 1984. "Inhibition of Cellulase by Fermentation Products," *Biotechnology and Bioengineering* **26**: 1506-7.
TASSINARI, T., MACY, C., and SPANO, L. 1982. "Technology Advances for Continuous Compression Milling Pretreatment of Lignocellulosics for Enzymatic-Hydrolysis," *Biotechnology and Bioengineering* **22**: 1495-505.
TATSUMOTO, K., BAKER, J.O., TUCKER, M.P., OH, K.K., MOHAGHEGHI, A., GROHMANN, K., and HIMMEL. M.E. 1988. "Digestion of Pretreated Aspen Substrates: Hydrolysis Rates and Adsorptive Loss of Cellulase Enzymes," *Applied Biochemistry and Biotechnology* **18**: 159-74.
THOMPSON, D.N., CHEN, H., and GRETHLEIN, H.E. 1990. "Changes in the Rate of Enzymatic Hydrolysis and Surface Area Available to Cellulase With Pretreatment Methods," in

Biotechnology in Pulp and Paper Manufacture, Kirk TK, Chang H, eds. Butterworth-Heinemann, Boston, pp. 329-38.

TIRTOWIDJOJO, S., SARKANEN, K.V., PLA, F., and MCCARTHY, J.L. 1988. "Kinetics of Organosolv Delignification in Batch-Through and Flow-Through Reactors,"*Holzforschung* **42**: 177-83.

TORGET, R., WALTER, P., HIMMEL, M., and GROHMANN, K. 1991. "Dilute-Acid Pretreatment of Corn Residues and Short-Rotation Woody Crops," *Applied Biochemistry and Biotechnology* **28/29**: 75-86.

TORGET, R., HATZIS, C., HAYWARD, T.K., HSU, T.-A., and PHILIPPIDIS, G.P. 1996. "Optimization of Reverse-Flow, Two-Temperature, Dilute-Acid Pretreatment to Enhance Biomass Conversion to Ethanol," *Applied Biochemistry and Biotechnology* **57/58**: 85-101.

TORGET, R.W., KIDAM, K.L., HSU, T.-A., PHILIPPIDIS, G.P., and WYMAN, C.E. 1998. *Prehydrolysis of Lignocellulose*. US Patent No. 5,705,369.

TSAO, G.T., LADISCH, M., LADISCH, C., HSU, T.-A., DALE, B., and CHOU, T. 1978. "Fermentation Substrates from Cellulosic Materials: Production of Fermentable Sugars from Cellulosic Materials," *Annual Report on Fermentation Processes* **2**: 1-21.

TYSON, K.S. 1993. *Fuel Cycle Evaluations of Biomass-Ethanol and Reformulated Gasoline*, Volume I, NREL/TP-463-4950, DE94000227. Golden, CO: National Renewable Energy Laboratory, November.

U.S. DEPARTMENT OF AGRICULTURE. 2001. *Crop Production: 2001 Summary*, Report Cr Pr 2-1 (01) c. National Agriculture Statistics Service, Washington, DC, January.

U.S. DEPARTMENT OF ENERGY. 2000. *Annual Energy Review 1999*. Report DOE/EIA-0384(99). Energy Information Administration Washington, DC, July.

VAN WALSUM, G.P., ALLEN, S.G., SPENCER, M.J., LASER, M.S., ANTAL, M.J., and LYND, L.R. 1996. "Conversion of Lignocellulosics Pretreated with Liquid Hot Water to Ethanol," *Applied Biochemistry and Biotechnology* **57/58**: 157-70.

VELKOVSKA, S., MARTEN, M.R., and OLLIS, D.F. 1997. "Kinetic Model for Batch Cellulase Production by *Trichoderma reesei* RUT C30," *Journal of Biotechnology* **54**(2): 83-94.

WEIL, J., BREWER, M., HENDRICKSON, R., SARIKAYA, A., and LADISCH, M.R. 1998. "Continuous pH Monitoring During Pretreatment of Yellow Poplar Wood Sawdust by Pressure Cooking in Water," *Applied Biochemistry and Biotechnology* **70-72**: 91-111.

WOOD, B.E., and INGRAM, L.O. 1992. "Ethanol Production from Cellobiose, Amorphous Cellulose, and Crystalline Cellulose by Recombinant *Klebsiella Oxytoca* Containing Chromosomally Integrated *Zymomonas Mobilis* Genes for Ethanol Production and Plasmids Expressing Thermostable Cellulase Genes from *Clostridium Thermocellum*," *Applied and Environmental Microbiology* **58**(7): 2103-10.

WOOLEY, R., RUTH, M., GLASSNER, D., and SHEEHAN, J. 1999. "Process Design and Costing of Bioethanol Technology: A Tool for Determining the Status and Direction of Research and Development," *Biotechnology Progress* **15**: 794-803.

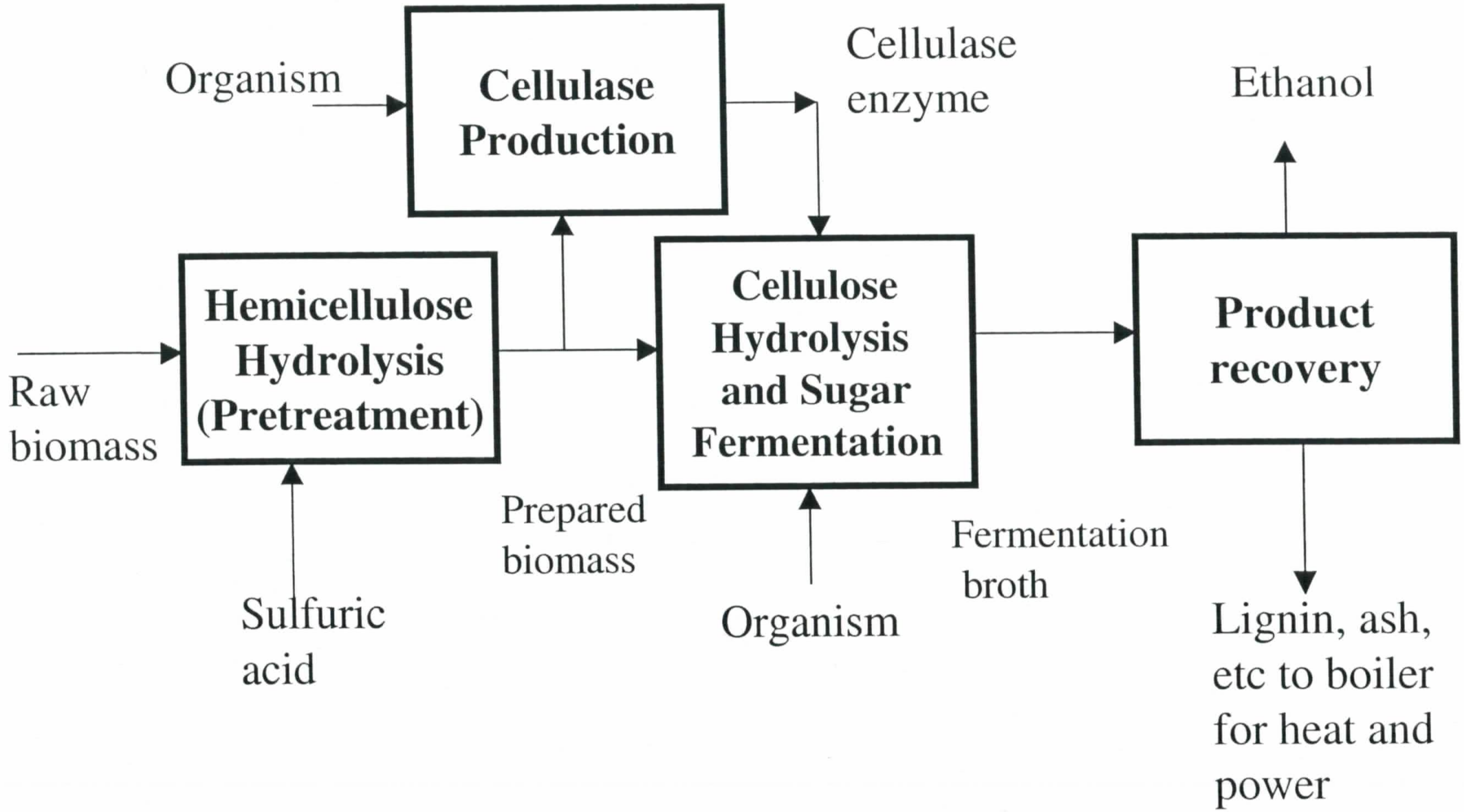
WRIGHT, J.D., WYMAN, C.E., and GROHMANN, K. 1987. "Simultaneous Saccharification and Fermentation of Lignocellulose: Process Evaluation," *Applied Biochemistry and Biotechnology* **18**: 75-90.

WRIGHT, J.D. 1988. "Ethanol from Biomass by Enzymatic Hydrolysis," *Chemical Engineering Progress* 62-74, August.

WYMAN, C.E., SPINDLER, D.D., GROHMANN, K., and LASTICK, S.M. 1986. "Simultaneous Saccharification and Fermentation of Cellulose with the Yeast *Brettanomyces clausenii*,"

Biotechnology and Bioengineering, **17**: 221-38.
WYMAN, C.E. 1990. "Biological Production of Chemicals from Renewable Feedstocks," National Meeting, American Chemical Society, Washington, DC, August 28.
WYMAN, C.E., and HINMAN, N.D. 1990. "Ethanol: Fundamentals of Production from Renewable Feedstocks and Use as a Transportation Fuel," *Applied Biochemistry and Biotechnology* **24/25**: 735-53.
WYMAN, C.E. 1993. "Cellulosic Biomass Conversion Technology and Its Application to Ethanol Production from Corn," *Fuel Reformulation*, pp. 67-74, March/April.
WYMAN, C.E., and GOODMAN, B.J. 1993a. "Biotechnology for Production of Fuels, Chemicals, and Materials," *Applied Biochemistry and Biotechnology*, **39/40**: 41-59.
WYMAN, C.E., and GOODMAN, B.J. 1993b. "Near Term Application of Biotechnology to Fuel Ethanol Production from Lignocellulosic Biomass," in *Opportunities for Innovation in Biotechnology*, ed. RM Busche, pp. 151-190. NIST GCR 93-633. Gaithersburg, MD: National Institute of Standards and Technology.
WYMAN, C.E. 1994. "Alternative Fuels from Biomass and Their Impact on Carbon Dioxide Accumulation," *Applied Biochemistry and Biotechnology* **45/46**: 897-915.
WYMAN, C.E. 1995. "Economic Fundamentals of Ethanol Production from Lignocellulosic Biomass," in *Enzymatic Degradation of Insoluble Carbohydrates*, ACS Symposium Series 618, Saddler JN, Penner MH, eds. Washington, DC: American Chemical Society, pp. 272-290.
WYMAN, C.E., ed. 1996. *Handbook on Bioethanol: Production and Utilization. Applied Energy Technology Series*. Washington, DC: Taylor & Francis. 424 pp.
WYMAN, C.E. 1999. "Biomass Ethanol: Technical Progress, Opportunities, and Commercial Challenges," *Annual Reviews of Energy and the Environment* **24**: 189-226.
WYMAN, C.E. 2001. "20 Years of Trials, Tribulations, and Research Progress on Bioethanol Technology: Selected Key Events Along the Way," *Applied Biochemistry and Biotechnology* **91-93**: 5-21.
YERGIN, D. 1991. *The Prize*. New York: Simon and Schuster.
YOON, H.H., WU, Z., and LEE, Y.Y. 1995. "Ammonia-Recycled Percolation Process for Pretreatment of Biomass Feedstock," *Applied Biochemistry and Biotechnology* **51/52**: 5-20.
ZHANG, M., EDDY, C., DEANDA, K., FINKELSTEIN, M., and PICATAGGIO, S. 1995. "Metabolic Engineering of a Pentose Metabolism Pathway in Ethanologenic *Zymomonas mobilis*," *Science* **267**: 240-3.
ZHENG, Y., LIN, H.-M., and TSAO, G.T. 1998. "Pretreatment for Cellulose Hydrolysis by Carbon Dioxide Explosion," *Biotechnology Progress* **14**: 890-6.

Figure 1. Simplified process flow diagram for biological processing of biomass to ethanol involving steps for pretreatment, enzyme production, cellulose hydrolysis by enzymes, sugar fermentation, product recovery, and power generation with lignin and other unutilized portions of the feedstock.


Organism
Cellulase Production
Cellulase enzyme
Ethanol
Raw biomass
Hemicellulose Hydrolysis (Pretreatment)
Cellulose Hydrolysis and Sugar Fermentation
Product recovery
Sulfuric acid
Prepared biomass
Organism
Fermentation broth
Lignin, ash, etc to boiler for heat and power