Process Review of Lignocellulose Biochemical Conversion to Fuel Ethanol

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I. Introduction

The United States is in transition. Each year we use 140 billion gallons of gasoline to fuel our cars. Over 50% is imported and transportation accounts for ½ of U.S. greenhouse gas emissions. Increasingly worrisome headlines related to energy security and economic interests and strident warnings from climate scientists all suggest the time has come to reduce oil usage and its related CO_2 emissions.

Currently, three routes are available to reduce imported oil and greenhouse gas emissions associated with transportation: conserve, switch to plug in hydrids, and rely on renewable biofuels. Quite likely, all three will be needed to reduce gasoline consumption in a meaningful manner. Coal liquefaction dates back to W.W. II and can reduce or even eliminate oil imports but will increase, possibly even doubling, CO_2 emissions.

Renewable fuels are defined as liquid fuels produced from biomass. The concept is simple; plants recycle the CO_2 released from combustion and the plants are in turn are harvested and converted to fuels. It is actually more complex because fossil fuels are used to plant, grow, harvest, and process the biomass into fuels. Despite this, lifecycle analysis indicates biofuels, especially from lignocellulose biomass, can greatly reduce CO_2 emissions and very efficiently reduce net gasoline usage(Farrell et al. 2006).

Major sources of biofuels are ethanol and to a much lesser extent biodiesel. Last year, 5 billion gallons of ethanol (95% from corn) was produced and production is expected to grow to 10-12 billion gallons in the next few years (Renewable Fuel

Association). After that any further growth in biofuel production will need to rely on lignocellulosic feedstocks because of competing demands for corn from food, industrial, and export uses(Westcott 2007).

Lignocellulose includes agricultural residues, forest industry wastes, and (potentially) perennial energy crops. Agricultural residues include corn stover (e.g. stalks and cobs), wheat straw, etc. Forest industry wastes include lumber scraps, pulping waste, as well as urban generated cellulosic wastes. Perennial energy crops include warm season grasses (e.g. switchgrass) and fast-growing trees (e.g. loblolly pine or poplar hybrids). Currently no crops are grown for energy but warm season grasses are grown as forages and trees (of course) for pulping and to make lumber. Estimated availability of each is in the 100s of million ton range (FIG 1) and all summed together could theoretically meet 20% of our total liquid transportation fuels by 2017(Perlack et al. 2005).

Recently, the Department of Energy announced they will help to fund six commercialization efforts for converting biomass to biofuels, most of which are related to production of ethanol. While ethanol is the leading candidate for a renewable generated liquid fuel, there are other alternates, some dating back nearly as far back as ethanol. These include butanol produced by acetone-butanol-ethanol (ABE) fermentation of biomass, synthetic gasoline gasification produced by gasifying biomass to syngas followed by Fisher Tropsch reformation, ethanol produced by fermentation of biomass derived syngas, and biodiesel produced from algae grown in huge salt-water ponds. Algae are the exception in relying on a new “crop” and are also by far the earliest in

development. Each is being actively pursued for commercialization; however, limited space prevents further discussion of these alternatives.

II. Chemical composition of biomass and theoretical ethanol yields

Carbohydrates are the only portion of the plant that can be fermented to ethanol. In fibrous biomass, carbohydrates are mostly present in the plant cell walls and are in the form of cellulose and hemicellulose. Cellulose can be converted to glucose and hemicellulose to a mixture of sugars, the composition of which varies with the source of biomass. Herbaceous hemicellulose contains mostly xylose and significant amounts of arabinose and glucoronic acids(Dien et al. 2005). Other more minor sugars include galactose and ribose. While glucose is a hexose and has 6 carbons, arabinose and xylose contain 5 carbons and are termed pentoses. The major significance of this is that distillers yeast (*Saccharomyces*) can not ferment pentose sugars. This precludes the use of commercial yeast strains from being used for converting lignocellulose to ethanol.

Plants are approximately 60% w/w carbohydrates of which hemicellulose accounts for about a third (Wiselogel et al. 1996). When fermented to ethanol, one CO_2 is produced for each ethanol, so, the theoretical yield for neutral sugars is 0.51 g of ethanol per g of sugar. A very important point, too often ignored, is that the theoretical thermodynamic yield is much higher than the C-yield, 0.98 J/J. For herbaceous biomass, the theoretical yield of ethanol is 100-110 gal per dry ton; which compares to 124 gal/ton for corn (for conversion calculator see: www1.eere.energy.gov/biomass/ ethanol_yield_calculator.html). As a practical matter, most experts use conversion

factors of 60-90 gal ethanol per dry ton of biomass, with the lower range having been demonstrated and the upper limit extrapolated from current research.

III. Biochemical Conversion

Many processes have been conceptualized for converting fibrous biomass to ethanol. All have common aspects, so, I will first discuss a conceptual design for a dilute-acid pretreatment(Aden et al. 2002). Dry biomass that arrives at the facility is first cleaned and milled. The biomass is mixed with a dilute mineral acid solution to a solids consistency of 30-40%w/w. The biomass is conveyed to a steam explosion reactor where it is heated to 180-220ºC for 0.5-5 min before being quickly (and explosively!) cooled by the sudden release of the reactor pressure(Schell et al. 2003). Treating in this manner physically reduces particle sizes and changes the consistency of the product from a damp fiber to"sludge". It also breaks down the plant cell wall, removing the hemicellulose to the syrup, displacing the lignin, and swelling the tightly (highly crystalline) arrayed cellulose fibers. To recap, following the steam explosion, the solids consist largely of lignin and cellulose and the syrup contains most of the hemicellulose carbohydrates, water-extractables, a little glucose, and minor amounts of released lignin products.

Following pretreatment, the solids are recovered and washed, possibly using a press. The syrup and wash water are mixed and the residual sulfuric acid neutralized by adding lime. Pretreatment produces a wide variety of soluble side-products, some of which are quite toxic to microbes. Therefore, the syrup often needs to be conditioned to reduce its toxicity prior to fermentation. Following this, the syrup is re-mixed with the solids. At this point the biomass is too thick to ferment directly, so, enzymes are added to

thin the slurry and to begin saccharifying the cellulose to glucose. For current commercial enzymes, the temperature is held at 50-55ºC for 18-24 hr. Next, the biocatalyst is added, which begins to ferment the released sugars to ethanol. The fermentation temperature will generally be lower than 50ºC, but its specific set-point will depend upon the choice of microorganism. At the same time the fermentation is occurring, the enzymes continue to release sugars for fermentation. As mentioned above, a special microbe needs to be used that is capable of fermenting the pentose sugars in addition to the glucose. A number of microbes are now available that ferment either xylose or both xylose and L-arabinose in addition to glucose. The fermentation could theoretically last up to 7 days, but is usually ended after 3 days. The ethanol is stripped out of the beer, distilled, and finished by passing through a molecular sieve to remove the last of the water. For the stillage, the solids are centrifuged out. These which largely consist of lignin are used to generate steam for the overall process. The recovered liquid is (hopefully!) treated and recycled in the process.

There are many process variations for converting biomass to ethanol. In a simultaneous saccharification and fermentation (SSF), the microbe is co-added with the enzyme (Takagi et al. 1977; Emert & Katzen 1980). The key advantage of co-adding them is that the microbe ferments glucose immediately to ethanol, thereby, avoiding any build up of glucose in the culture. This has the advantage of avoiding end-product inhibition of the enzyme and helps to minimize the risk of contamination. Fermentations are run as open processes and contamination is always a concern. Detailed techno-economic models for SSF of poplar wood and corn stover have been developed by NREL (Aden et al. 2002; Wooley et al. 1999). Enzymes are a major cost of processing biomass

to ethanol. If microbes were used that produce (some) of their own enzymes, it would be possible to eliminate a large expense item. Using microbes that produce their own carbohydrolytic enzymes is the central theme of consolidated bioprocessing (CBP) (Den Haan et al. 2007; Katahira et al. 2006; Lynd et al. 2005). An alternate to SSF, would be to completely hydrolyze the carbohydrates and remove the solids prior to fermentation, which is referred to as SHF. This is the process used by Iogen Corp for their demonstration plant (Tolan 1999). It has the advantages of making the fermentation faster – because it is not enzyme limited, eliminating solids from the bioreactor, supplying a cleaner burning lignin, and may (theoretically) allow for recovering and recycling of enzymes. However, end product inhibition of the cellulases is a major concern.

IV Unit Operations

The major processing steps for converting biomass to ethanol are pretreatment, enzymatic saccharification, fermentation, and recovery. Lignocellulose contains primarily structural carbohydrates, which are highly resistant to enzymatic conversion to monosaccharides. Thermo-chemical pretreatment is needed to deconstruct the cell wall structure allowing enzymes access to the carbohydrate polymers. The cell wall has been compared to reinforced concrete, where hemicellulose is the concrete, lignin the hydrophobic sealant, and cellulose microfibrils are the reinforcing bars (Bidlack et al. 1992). Specifically, pretreatment is needed to reduce particle size, dissolve the xylan, displace the lignin, and create broken ends in and swell the cellulose microfibers. There

are numerous pretreatments available, some of which are summarized in Table 1 (reviews: (Dien et al. 2005; Mosier et al. 2005)).

There are three major categories of enzymes for converting pretreated biomass into fermentable sugars. These include cellulases, xylanases, and auxiliary enzymes for de-branching xylan. A list of these enzymes is presented in Table 2. Cellulases are by far the most important because are used to convert cellulose into glucose (Zhang & Lynd 2004). Dilute acid pretreatment converts the hemicellulose carbohydrates directly to monosaccharides and, therefore, only requires cellulase blends; though commercial blends containing xylanase activity can in some cases improve conversion efficiency. Other pretreatments will solubilize the xylan, but require additional enzymes (hemicellulases, (Saha 2003)) to saccharify it completely too fermentable sugars. Ligninases have not been widely applied to biomass bioconversion as yet.

The bioethanol industry is dependent upon *S. cerevisiae* for fermentation of glucose. Unfortunately, *S. cerevisiae* does not ferment pentose sugars and these sugars are too abundant in lignocellulose to ignore. The bacterium *Zymomonas mobilis* also selectively produces ethanol and has been offered as a substitute for *S. cerevisiae*. But like *Saccharomyces*, *Z. mobilis* does not ferment pentoses. Therefore, researchers have had to depend upon molecular methods for developing new biocatalysts for converting pentoses (and especially xylose) into ethanol. Two approaches have been taken to solving this problem: (a) engineering *S. cerevisiae* and *Z. mobilis* to ferment xylose and in the case of *Z. mobilis* also arabinose or (b) engineering Gram-negative bacteria that use a wide variety of sugars to only produce ethanol under anaerobic conditions. There has been considerable work on using the later strategy to develop Gram-positive bacteria, but

while progress is being made, full success has been elusive. Table 3 reviews the microorganism available for fermenting xylose (for reviews: (Dien et al. 2003; Jeffries & Jin 2004)).

V. Future Trends

Ethanol production from wood dates back before WWII. Modern technology has allowed the potential of much higher yields with a smaller environmental footprint. However, challenges remain and further research will be needed to make lingocellulosic ethanol cost-competitive. Efforts will continue toward producing more robust pentose fermenting microorganisms with higher productivity and more efficient, less expensive enzymes. However, more work will also be directed at understanding the cell wall and the sources of biomass recalcitrance. Simultaneously there should be increased efforts to engineer plants for easier conversion to sugars (e.g. less lignin or altered cell wall structures) or that produce some of the enzymes needed for breaking down cell walls *in situ*. The Department of Energy has recently announced that they will fund three institutions for 5 years with this goal in mind (genomicsgtl.energy.gov/centers/).

In an attempt to jump start a lignocellulose ethanol industry, the Department of Energy announced that they will fund 6 commercial efforts for up to $385 million dollars (News Release: www.energy.gov/news/4827.htm). Abengoa Bioenergy Biomass, Poet Companies, and Iogen Biorefinery will focus on biochemical conversion of herbaceous biomasses, including corn cobs and fiber, switchgrass, and wheat straw. Alico, Inc. will use a hybrid process whereby the biomass is converted to syngas and the syngas fermented to ethanol. Range Fuels will apply a strictly thermochemical approach. Blue

Fire Ethanol, Inc. will utilize the Arkenol process, which relies on strong acid hydrolysis (bluefireethanol.com). It is hoped by those working in the field that the combination of strong political and industrial interests will help to unlock lignocellulose as a commercially successful feedstock for ethanol.

Table 1. Selected Pretreatments for Lignocellulose

Acid

Base

Pretreatment	Pentoses	Inhibitors
Strong Acid	+	++
Dilute Acid	+	++
Hot Water	-	+
AFEX	-	-
Alkaline Peroxide	-	-

Table 2: Biomass related enzymes

- **Cellulases**

endo-1,4- β -D-glucanase (EC-3.2.1.4), exo-1,4- β -glucanase (exocellobiohydrolase, EC-3.2.1.91) and β -D-glucosidase (β -D-glucoside glucanhydrolase, EC-3.2.1.21).

- **Xylanases**

endo-1,4-β -D-xylanase, EC-3.2.1.8), β -xylosidase (EC-3.2.1.37)

- **Xylan Debranching Enzymes**

α-L-arabinofuranosidase (EC-3.2.1.55), β-glucuronidase (EC-3.2.1.31), acetylxylan esterase (EC- 3.1.1.72), feruloyl esterase (EC-3.1.1.73), p-coumaroyl esterase (EC-3.1.173), others

- **Ligninases**

Lignin peroxidase (LiP, EC-1.11.1.7), manganese peroxidase (MnP, EC-1.11.1.13) and laccase (EC-1.10.3.2)

Table 3. Comparison of Batch fermentations with xylose for ethanologenic strains

Strain	Host	Xylose (g/l)	Max. EtOH (g/l)	EtOH Eff[1] (%)	Reference
E. coli	K011[2]	90	41.0	89	(Yomano et al. 1998)
	FBR5	95	41.5	90	(Dien et al. 2000)
	LY01	140	63.2	88	(Yomano et al. 1998)
K. oxytoca	*M5A1(pLOI555)*	100	46.0	95	(Ohta et al. 1991)
Z. mobilis	*CP4:pZB5*	60	23.0	94	(Lawford and Rousseau, 1999)
S. cerevisiae	*TMB 3400*[3]	50	13.3	67	(Karhumaa et al. 2007; Kuyper et al. 2005)
	RWB 218[3]	20	8.4	85	(Kuyper et al. 2005)
	RE700A(pKDR)[4]	45	19	74	(Kuyper et al. 2005; Sedlak & Ho 2004)

[1]Ethanol efficiency: % yield of theoretical based upon 51 g ethanol per 100 g of xylose present
[2]On 140 g/l xylose, strain K011 produced 59.5 g/l ethanol in 120hr (Yomano et al, 1998)
[3]cultured on mineral medium
[4]cultured on rich medium, estimated from Fig. 8

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Figure 1: US land can supply 1.3 billion ton biomass for biofuels and still meet other needs (adapted from billion ton vision report).

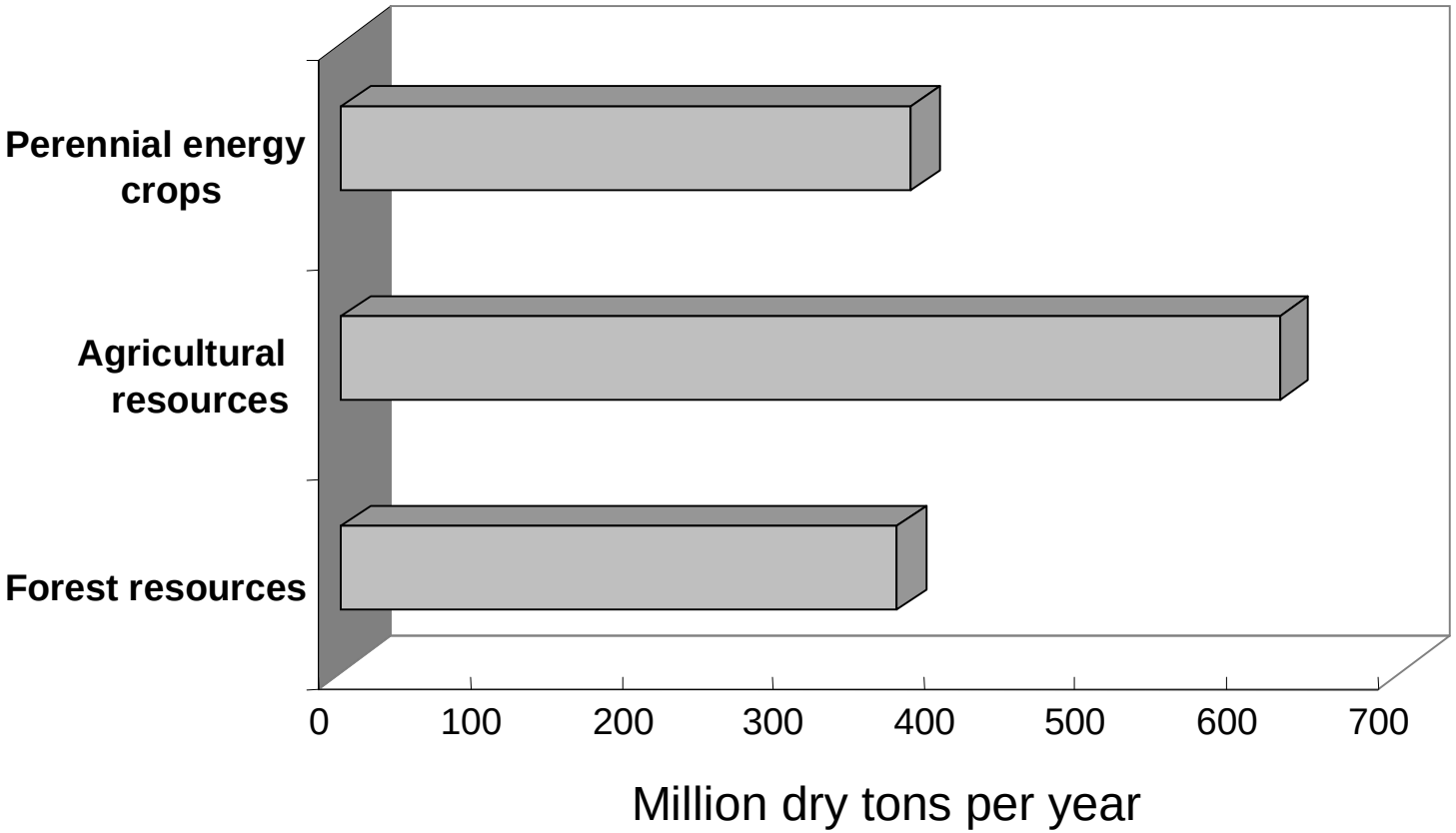

Figure 2: Conceptual flow diagram of process for converting lignocellulose to ethanol (see text for details).