Comparative Sugar Recovery and Fermentation Data Following Pretreatment of Poplar Wood by Leading Technologies

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Through a Biomass Refining Consortium for Applied Fundamentals and Innovation among Auburn University, Dartmouth College, Michigan State University, the National Renewable Energy Laboratory, Purdue University, Texas A&M University, the University of British Columbia, and the University of California at Riverside, leading pretreatment technologies based on ammonia fiber expansion, aqueous ammonia recycle, dilute sulfuric acid, lime, neutral pH, and sulfur dioxide were applied to a single source of poplar wood, and the remaining solids from each technology were hydrolyzed to sugars using the same enzymes. Identical analytical methods and a consistent material balance methodology were employed to develop comparative performance data for each combination of pretreatment and enzymes. Overall, compared to data with corn stover employed previously, the results showed that poplar was more recalcitrant to conversion to sugars and that sugar yields from the combined operations of pretreatment and enzymatic hydrolysis varied more among pretreatments. However, application of more severe pretreatment conditions gave good yields from sulfur dioxide and lime, and a recombinant yeast strain fermented the mixed stream of glucose and xylose sugars released by enzymatic hydrolysis of water washed solids from all pretreatments to ethanol with similarly high yields. An Agricultural and Industrial Advisory Board followed progress and helped steer the research to meet scientific and commercial needs. © 2009 American Institute of Chemical Engineers *Biotechnol. Prog.,* 25: 333–339, 2009

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Introduction

Cellulosic or plant biomass in such forms as agricultural and forestry residues, substantial portions of municipal solid waste, and herbaceous and woody crops grown to support energy production can provide a unique, low cost, and

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powerful resource for large scale sustainable production of fuels and chemicals that cannot be otherwise met.[1] Liquid fuels made from cellulosic materials will become even more vital as supplies of petroleum, currently the world's largest single energy source, become stretched through declining proven reserves, growth of economies in China, India, and other countries as well as continued increases in demand in developed nations.[2] Furthermore, politically unstable countries control most conventional petroleum resources, making all strategically vulnerable.[3] About two thirds of the oil used in the United States powers transportation, a sector that in turn is almost totally dependent on this resource (>96%). To compound matters, transportation is the largest contributor to release of carbon dioxide, a powerful greenhouse gas, in the United States, and other resources that could be used for making liquid transportation fuels at a large scale such as oil shale, tar sands, and coal emit far more carbon dioxide than petroleum as these resource conversion systems are currently configured. Concerns about these strategic, economic, and environmental challenges should drive us to rapidly develop and deploy technologies for conversion of cellulosic biomass to transportation fuels.[4]

Cellulosic biomass at about $50/dry ton is competitive in price with oil at about $17/barrel, and the key to competitiveness is low cost processing to liquid fuels, with high yields being a necessary but not sufficient condition.[4,5] In this regard, processes based on enzymes are meritorious because of their promise to simplify and improve key process steps through application of modern biotechnology and the potential to realize nearly theoretical yields. In fact, estimates of cash costs for making ethanol from cellulosics have been reduced to be similar or less than for making ethanol from corn, with the key to commercial use being to overcome the perceived risk of financing the first commercial facilities. In addition, continued technology advances will enhance returns and help compensate first practitioners.[4]

Although biological conversion offers significant technical and economic potential, cellulosic biomass has developed natural resistance to microbial attack. Consequently, a pretreatment operation must be introduced prior to the biological steps to overcome this natural recalcitrance and give the high yields vital to economic success.[6] However, pretreatment has been projected to be the most expensive single step in biological processing of cellulosic biomass to ethanol[7] and also has a major impact on the cost of other operations.[8] Although pretreatment is costly in the biological processing sequence, the low yields that result if pretreatment is eliminated drive up all other costs more than the amount saved. This conundrum has led to the statement that "the only operation more expensive than pretreatment is no pretreatment,"[8] and the key is to reduce pretreatment costs while still achieving high sugar yields from both pretreatment and enzymatic hydrolysis. Enhancing yields via improved pretreatment can reduce all other unit costs as well, and it is desirable that pretreatment not produce large amounts of degradation products that can inhibit downstream biological operations. Pretreatment must also minimize energy inputs and have low net costs for chemicals or other additives. Because low capital costs are also important, pretreatments are favored that require modest to low pressure and do not need costly materials of construction to handle very corrosive chemicals.[6]

Over the years, many approaches have been applied for pretreatment, but only a few offer low enough costs and good enough performance to be viable.[6,9,10] Among those, it is challenging to compare attributes because of differences in feedstocks, methodologies, and material balance approaches employed. To address this problem, a team of researchers from Auburn University, Dartmouth College, Michigan State University, the National Renewable Energy Laboratory (NREL), Purdue University, and Texas A&M University formed a Biomass Refining Consortium for Applied Fundamentals and Innovation (CAFI) in 2000 to cooperate on the leading pretreatment options that these participants were developing. The US Department of Agriculture Initiative for Future Agriculture and Food Systems (IFAFS) Program selected the CAFI team for funding through a competitive solicitation that year, making it possible for them to apply consistent feedstocks, enzymes, analytical methods, and methodologies to pretreatment and subsequent enzymatic hydrolysis of corn stover, and the Office of the Biomass Program of the US Department of Energy funded NREL to provide logistical support to the team for that project. The team has published their results with corn stover widely, including publication of articles by each member of the CAFI team in a special issue of *BioResource Technology* at the completion of the project that provided an overview of biological processing, the pretreatments studied, the approach to chemical analyses and material balances, cost estimates, and details about the feedstocks used.[11–19]

More recently, the DOE Office of the Biomass Program selected the CAFI team to develop comparative data for the pretreatment of poplar wood by the leading technologies of ammonia fiber expansion (AFEX), aqueous ammonia recycle (ARP), dilute sulfuric acid, lime, and neutral pH methods that had been employed in the corn stover project. In addition, Natural Resources Canada funded the University of British Columbia to incorporate sulfur dioxide steam explosion technology into this effort, and Genencor International was integrated into the CAFI team to provide advanced enzymes that they were developing through DOE support. During that period, the principal investigator for the CAFI project moved to the University of California, Riverside from Dartmouth College. Articles documenting results from that project, often designated as CAFI 2, are presented in this volume of *BioTechnology Progress*, with this article providing an update of the some of the common methods and sources of information to support these articles beyond that reported previously in the *BioResource Technology* volume. We also summarize the key results from this effort with details on the pretreatment technologies and results available in the articles for each pretreatment in this volume.

Materials and Methods

The research groups involved in this project all used the same raw materials, analytical procedures, and data analysis approaches wherever possible to promote easier comparison among pretreatment methods, and because most of these common approaches have been described previously,[11] our focus here, is on new aspects pertinent to this project. Materials and methods unique to particular pretreatments are covered in the appropriate articles.

Materials

Feedstocks. The poplar wood used for this project was provided through Adam Wiese of the USDA Northern

Research Station in Rhinelander, Wisconsin. The baseline material for most of the study was obtained from a farm in Alexandria, Minnesota where it was planted in the spring of 1994 and harvested and shipped in August 2004. This poplar was comprised of 43.8% glucan, 14.9% xylan, 0.6% arabinan, 3.9% mannan, 1.0% galactan, 29.1% Klason lignin, 3.6% acetyl groups, 3.6% extractives, and 1.1% ash, as determined by NREL using their standard laboratory methods.[20] The team also used a second source of poplar for some of the research that had the following composition: 45.1% glucan, 17.8% xylan, 0.5% arabinan, 1.7% mannan, 1.5% galactan, 21.4% Klason lignin, 5.7% acetyl groups, 3.4% extractives, and 0.8% ash. This feedstock was planted in 1995, most likely in the spring but possibly in the fall, at an Arlington, Wisconsin site in very rich, loamy soil and demonstrated some of best growth rates. This poplar was harvested and shipped in February 17, 2004. Both materials were sent to NREL where they were debarked, chipped, and milled in a Mitts and Merrill Model 10 × 12 knife mill (Saginaw, MI) to pass through a $^{1}/_{4}$ inch screen. The resulting material was subdivided into sealed five gallon pails, stored frozen at -20°C, and distributed to CAFI member institutions when requested.

Enzymes. Enzymes were generously provided by Genencor International as part of the CAFI team. One cellulase enzyme, Spezyme CP, had a filter paper activity of 59 FPU/mL and a protein concentration of 123 mg protein/mL, and the other, GC-220, had corresponding values of 89 FPU/mL and 184 mg protein/mL. The other enzymes and their respective protein contents were β-glucosidase at 32 mg protein/mL, Multifect Xylanase at 41 mg protein/mL, and β-xylosidase at 85 mg protein/mL. The baseline enzyme formulation was a combination of β-glucosidase and cellulase at a CBU/FPU ratio of 2.0.

Methods

Pretreatment and Enzymatic Hydrolysis. Pretreatments were performed as indicated in the individual articles. Unless otherwise stated, enzymatic hydrolysis of the resulting solids was conducted at 1–2% glucan concentrations to minimize end product inhibition by the sugars and oligomers released in 100 mL flasks in citrate buffer plus antibiotics for a digestion time of 72 h. As a minimum, cellulase loadings corresponding to about 15 and 60 FPU/g of glucan in the raw feedstock were applied.

Calculation of Sugar Yields. Material balances for each pretreatment system were closed as described before from measurements of the composition and total mass of each liquid and solid stream leaving pretreatment.[12] Yields were also calculated as described previously based on the maximum amount of xylose and glucose that could be potentially recovered from the particular poplar wood employed. Based on the increase in mass with hydrolysis, a maximum of about 48.7 mass units of glucose and 16.9 mass units of xylose could be produced from 100 mass units of baseline poplar wood for a total maximum sugar potential of 65.6 units per 100 units of dry poplar. For the second, lower lignin poplar source, the corresponding amounts are 50.1 mass units of glucose, 20.2 mass units of xylose, and 70.3 total sugar mass units. Thus, the maximum possible glucose yield is 74.2%, and the maximum xylose yield is 25.8% for the baseline poplar source. For the lower lignin source, the corresponding values are 71.2 and 28.8%, respectively. Glucose and xylose can be released in either the pretreatment step that we designate Stage 1 or the enzymatic hydrolysis step, Stage 2. In addition, some of the sugars may be solubilized as oligomers which were accounted for as well.

Fermentations. Fermentations were run with the sugars following enzymatic hydrolysis of washed solids from each of the pretreatments. A solids loading of 200 g (dry)/L was prepared in 50 mM citrate buffer (pH 4.8), and 60 FPU/g-total glucan of Spezyme CP cellulase plus an equivalent protein mass of Multifect Xylanase were added. Hydrolysis was then performed at 50°C at 200 rpm using an orbital shaker for 168 h, and the hydrolyzate was then filtered to remove residual solids before fermentation. Next, the sugars released by enzymatic hydrolysis of the washed pretreated solids were fermented to ethanol at 28.5°C for 48 h using the glucose/xylose co-fermenting yeast strain 424A(LNH-ST) of *Sacharomyces cerevisiae* developed by Dr. Nancy Ho at Purdue University. Three enzymes were stably integrated into the yeast chromosome and feed xylose into the pentose phosphate pathway with high ethanol productivity and yield. An aliquot of 8 mL of seed culture was used to inoculate 100 mL YEPD (YEP plus 2% glucose) in a 300 mL baffled side-arm Erlenmeyer flask. The cultures were incubated in a shaker at 28.5°C and 200 rpm and grown aerobically overnight, after which a cell optical density (OD) between 450 and 500 KU was obtained. The yeast cells were harvested by centrifugation (J-21 Beckman) at 5000 rpm for 5 min at 4°C. The supernatant was discarded and the cells were transferred into a 300 mL baffled Erlenmeyer flask containing the poplar hydrolyzate from enzymatic hydrolysis. The initial cell mass concentration prior to fermentation in each experiment was about 5 g dry weight/L. The flasks were then sealed with Saran wrap to allow fermentation to be carried out under largely anaerobic conditions, and the cultures were placed in shaker and incubated at 30°C. At regular intervals 1 mL samples of the fermentation mixture was removed for monitoring the fermentation. All fermentations were run in duplicate.

Agricultural and Industrial Advisory Board

An Agricultural and Industrial Advisory Board was engaged in this project throughout its history. Meetings were held between the Board and the CAFI team twice per year, and regular updates were sent to the Board via e-mail. The Board served as an audience for the dissemination of the results to the private sector and provided important perspectives on commercial concerns and needs. They also helped to identify audiences and contacts for technologies. Membership on the Board at the time of this writing were Quang Nguyen, Abengoa Bioenergy; Jim Doncheck, Arkion Life Sciences; Gary Welch, Aventinerei; Mohammed Moniruzzaman, BioEnergy International; Paris Tsobanakis, Cargill; James Hettenhaus, CEA; Steve Thomas, CERES; Lyman Young, ChevronTexaco; Mike Knauf, Codexis; Julie Friend, DuPont; Jack Huttner, Genencor; Don Johnson, GPC (Retired); Jeff Gross, Hercules; Peter Finamore, John Deere; Glen Austin, Lallemand Ethanol Technology; Kendall Pye, Lignol; Wei Huang, LS9; James Flatt, Mascoma; Farzaneh Teymouri, MBI; James Zhang, Mendel; Richard Glass, NCGA; James Jia, NorFalco Sales; Joel Cherry, Novozymes; Mark Stowers, Poet; Ron Reinsfelder, Shell; Paul Roessler, Synthetic Genomics; Carmela Bailey, USDA; Don Riemenschneider, USDA; Kevin Gray, Verenium; and Chundakkadu Krishna, Weyerhaeuser.

Table 1. Sugar Yields from Baseline Poplar Wood for a Cellulase Loading of 15 FPU/g Glucan in the Raw Poplar Wood Before Pretreatment

	Xylose Yields			Glucose Yields			Total Sugar Monomers		
Pretreatment System	Stage 1	Stage 2	Total Xylose	Stage 1	Stage 2	Total Glucose	Stage 1	Stage 2	Combined Total
Maximum possible	25.8	25.8	25.8	74.2	74.2	74.2	100,0	100.0	100.0
Dilute acid (Sunds)	16.1	2.4	18.5	17.7	46.6	64.3	33.8	49.0	82.8
SO_2 steam explosion	19.2/14.0	2.4	21.6/16.4	2.3	72.0	74.3	21.6/16.3	74.4	95.9/90.7
Controlled pH	15.0/1.0	9.7	24.7/10.7	1.5/0.1	40.0	41.5/40.1	16.5/1.1	49.7	66.2/50.8
AFEX	0.0	13.4	13.4	0.0	39.4	39.4	0.0	52.8	52.8
ARP	9.6/0.0	8.2/8.0	17.7/8.0	0.4/0.0	36.3	36.6/36.3	10.0/0.0	44.5/44.3	54.5/44.3
Lime	1.2/0.0	18.8/16.8	20.0/16.8	0.2/0.0	71.0/67.2	71.2/67.2	1.5/0.0	89.8/84	91.3/84

Single values are for monomers. When two values are given, the first is the total yield of solubilized sugars and the second entry is the yield of just monomers with the difference being the yield of oligomers.

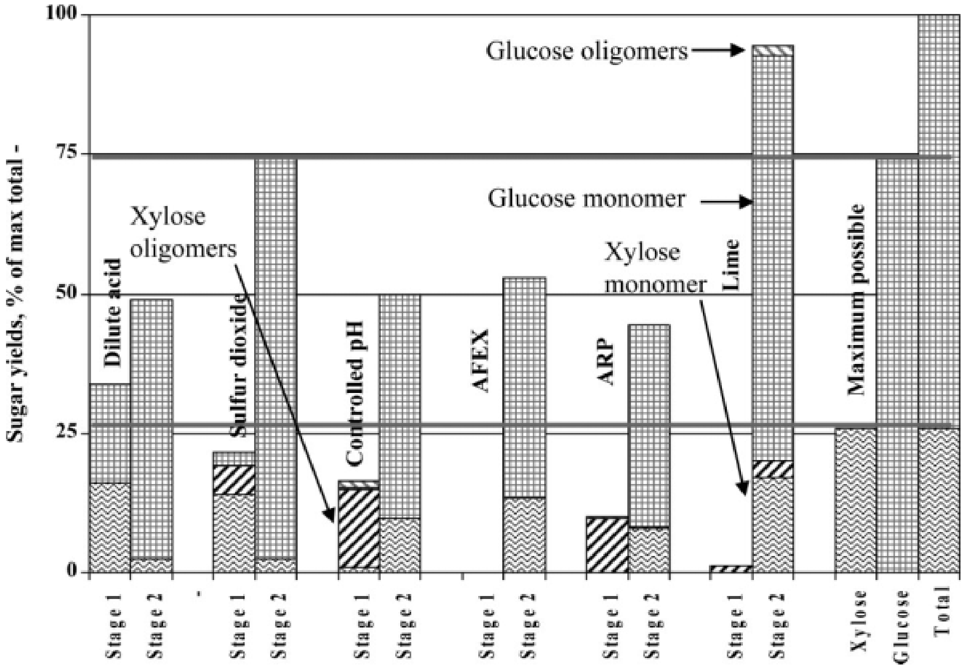

Figure 1. Distribution of glucose and xylose yields between Stage 1, pretreatment, and Stage 2, enzymatic hydrolysis, and between monomers and oligomers for the pretreatments studied and a cellulase loading of 15 FPU/g glucan in unpretreated poplar.

The upper horizontal line represents the maximum glucose yield possible and the lower horizontal line represents the maximum yield of xylose possible.

Comparative Results

Details for the pretreatments employed and their distinguishing features are available elsewhere,[11] and this section will summarize glucose and xylose yields on a common basis to facilitate comparisons. Xylose monomer and oligomers yields are presented in Table 1 for an enzyme loading of 15 FPU/g glucan in the original poplar wood. Pretreatments are listed in approximate order of increasing pH, and a much wider range of xylose yields was observed than for previous results with corn stover. However, just as for corn stover, most of the xylose was released in pretreatment, Stage 1, for dilute acid and controlled pH pretreatments, and sulfur dioxide pretreatment followed the same trend, as expected. Once again, cellulase enzymes released a large fraction of the xylose left in the solids for all pretreatments, with this outcome being particularly important for pretreatment technologies at higher pH and particularly for AFEX that left a large part of the xylose in the solids. Overall, the order of decreasing total xylose (monomer plus oligomers) yields was controlled pH, sulfur dioxide, lime, dilute acid pretreatment with the Sunds reactor, ARP, and AFEX. In terms of xylose monomers, the order of decreasing yields is dilute acid, lime, sulfur dioxide, AFEX, controlled pH, and ARP.

Table 1 also summarizes the distribution of glucose yields for the different pretreatments, and once again, a much wider range of glucose yields was observed than experienced by the team with corn stover. Only dilute acid pretreatment released a substantial amount of glucose in the first stage, while much lower amounts were released in Stage 1 for all of the other technologies, with AFEX releasing essentially none. On the other hand, enzymes were able to solubilize a large fraction of the glucose in the second stage, although to varying degrees among these technologies. Overall, the order of decreasing glucose yields as a percent of total potential glucose plus xylose for Stages 1 and 2 combined was sulfur dioxide, lime, dilute acid (Sunds), controlled pH, AFEX, and ARP, with the high being 74.3% and the lowest being 36.6%. Almost all of the dissolved glucose was as monomers with yields of low levels of oligomers increasing in the order of ARP, controlled pH, and lime pretreatments.

Total sugar yields are also presented in Table 1 for a loading of 15 FPU/g glucan in the raw biomass. As a result of

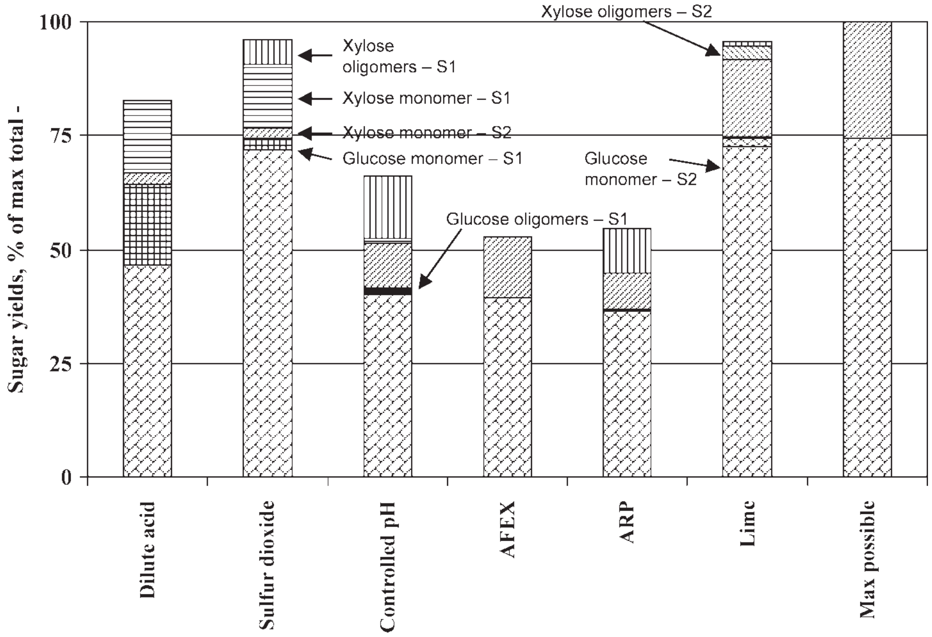

Figure 2. **Total yields irrespective of stage for glucose and xylose for combined Stage 1 (S1), pretreatment, and Stage 2 (S2), enzymatic hydrolysis, including monomers and oligomers for the pretreatments studied and a cellulase loading of 15 FPU/g glucan in unpretreated poplar wood.**

Table 2. Pretreatment Conditions Employed to Maximize Combined Recovery of Glucose and Xylose Irrespective of Release in Stage 1 or Stage 2

Pretreatment System	Temperature (°C)	Reaction Time (min)	Chemical Agent Used	Percent Chemical Used	Other Notes
Dilute acid	190	1.1	Sulfuric acid	2.0%	Acid as % dry solids in a Sunds pilot reactor
SO_2 steam explosion	190	5	Sulfur dioxide	3.0%	Soaked in SO_2 overnight
Controlled pH	200	10	None	0.0	15% Dry solids loading, solids washed with hot DI water
AFEX	180	10	Concentrated ammonia	39%	No Stage 1. All sugars in Stage 2 are available at high concentration "dry to dry" process
ARP	185	27.5	Ammonium hydroxide	15%	
Lime	160 140	120	Lime	20%	39% Solids, oxygen pressurized at 200 psig

Table 3. Glucan and Xylan Content in Washed Solids Produced by Pretreatments at the Conditions Listed in Table 2

Pretreatment System	Organization	% Glucan	% Xylan
Untreated	NREL	43.8	14.9
Dilute acid	NREL/Dartmouth College	57.3	2.1
SO_2 steam explosion	U. British Columbia	55.1	2.5
Controlled pH	Purdue University	58.8	7.0
ARP	Auburn University	57.5	13.5
AFEX	Michigan State University	46.6	15.0
Lime	Texas A&M University	53.1	16.8

Table 4. Compositions of Liquids Following Enzymatic Hydrolysis of the Washed Solids from Each Pretreatment

Pretreatment System	Glucose (g/L)	Xylose (g/L)	Acetic Acid (g/L)
Dilute acid	41.4	22.3	5.1
SO_2 explosion	33.2	25.8	6.2
Controlled pH*	56.1	12	2.3
AFEX	62.3	16.2	3.5
ARP	32.7	8	n/d
Lime (O_2)	52.8	16	n/d

* Pretreated at 150 g (dry) solids/L, hydrolyzed by 15 FPU/g-total glucan Spezyme CP and 40 IU/ g-total glucan Novo188 for 120 h, no Multifect Xylanase was used.

the significant distribution in yields for glucose and xylose, the combined yield for the two spanned a much greater range than for corn stover. The highest total sugar yield was 95.9% of potential glucose plus xylose and the lowest was 52.8%, with the order of decreasing yields being sulfur dioxide, lime, dilute acid (Sunds), controlled pH, ARP, and AFEX. To help visualize these results more easily, Figure 1 shows the distribution of glucose and xylose monomer and oligomer yields released in Stages 1 and 2 graphically, and Figure 2 illustrates the overall total of the two sugars irrespective of the release point.

Table 2 lists pretreatment conditions used to obtain the maximum yields outlined in Table 1. Overall, the temperatures tend to be higher than needed for maximum recovery of sugars from corn stover, as reported previously, in that the lowest temperature used by any pretreatment for poplar was 140°C for lime vs. a low of 55°C for lime with stover. All of the other temperatures for poplar are in the range from 180 to 200°C. These harsher conditions reflect the more recalcitrant nature of poplar, which may be at least partially due to the higher lignin content compared with stover. Relative to corn stover, the times for maximum total sugar recovery were reduced to 1.1 min for dilute acid with the NREL Sunds reactor, 5 min for sulfur dioxide, 10 min for AFEX and controlled pH, 27.5 min for ARP, and 120 min for lime. The chemical inputs and other aspects of each pretreatment are listed in Table 2.

Table 5. Metabolic and Productive Yields, Xylose Consumption, and Final Ethanol Concentrations Following Fermentation of the Hydrolyzates in Table 4 to Ethanol

Pretreatment System	Metabolic Yield*	Productive Yield†	Xylose Consumption in 48 h (%)	Final Ethanol Concentration (g/L)
Dilute Acid	85.0%	81.4%	87.8%	26.4
SO2 Explosion	89.9%	86.2%	90.8%	25.9
Controlled pH	86.8%	82.7%	80.0%	28.7
AFEX	93.0%	88.6%	78.7%	35.5
ARP	98.6%	98.6%	100%	20.5
Lime (O2)	100%	100%	90.4%	39.9

* Metabolic yield = [ethanol]/[0.51 × (*consumed* glucose + *consumed* xylose)]. † Productive yield = [ethanol]/[0.51 × (*initial* glucose + *initial* xylose)].

Table 3 presents the compositions of the solids following pretreatment by each option. As with corn stover, pretreatments at low pH solubilized much of the hemicellulose while pretreatments at high pH removed much more lignin except for AFEX which removed neither one. Furthermore, low pH pretreatments produced more monomers whereas those at mid to high pH gave high fractions of oligomers, as shown previously in Table 1.

The compositions of the hydrolyzates employed for fermentations are shown in Table 4, and the yields, xylose utilization after 48 h, and final ethanol concentrations are reported in Table 5 following fermentations of these sugar solutions. In Table 5, the metabolic yield takes into account how much ethanol was produced for a given amount of sugar consumed and is defined as:

Metabolic yield = [ethanol concentration]/[0.51 × (*consumed* glucose + *consumed* xylose)]

The productive yield was also calculated to account for any sugars that were not utilized and was defined as:

Productive yield = [ethanol concentration]/[0.51 × (*initial* glucose + *initial* xylose)]

There are some challenges in developing fermentation data given differences in solids concentrations and other aspects, but this data shows that high yields were realized for all washed pretreated substrates. However, the yields tended to be somewhat higher for the higher pH pretreatments, and the removal of a significant amount of lignin might help explain this behavior for ARP and lime. More conclusive proof is needed as to whether this is the mechanism responsible.

Conclusions

This project is another important step toward providing data on the integrated operations of pretreatment and enzymatic hydrolysis on a consistent and comparative basis that can help others select technologies. Pretreatment of poplar revealed more significant differences among the pretreatment technologies being evaluated in the CAFI project than experienced with corn stover, with these differences possibly due to the higher lignin content of poplar. However, more systematic evidence is needed to draw a firm conclusion as such possibilities as different lignin compositions, the nature of the hemicellulose, or other factors could also play a role. It is also important to note that pretreatment pH *per se* did not seem to govern performance in that the highest yields were achieved for the two extremes in pH: sulfur dioxide and lime. The differences seen between the results for sulfur dioxide and dilute sulfuric acid would not be expected and may be due to operational problems with the Sunds reactor that affected residence time control or heat distribution. Just as for corn stover, there was no clear trend as to whether removal of hemicellulose or lignin was the key as preferential removal of either one resulted in the highest observed yields.

Because theoretical ethanol yields are identical for fermentation of both glucose and xylose, both sugars were treated as though they are of the same value in this study. Furthermore, no significant problems were found with fermenting all of the sugar following enzymatic hydrolysis of washed solids to ethanol with the organism employed in this research. However, yields can vary among organisms that are available for fermentation to ethanol or other products, shifting the relative desirability of different pretreatments. Furthermore, some processes may prefer only monomeric sugars whereas others may ferment oligomers of glucose, xylose, or both as well as monomers. In addition, some processes may work best with as much sugars released in pretreatment as possible whereas others may not be impacted by sugar release patterns or benefit if all sugars are released from the solids together. These and other process, equipment, and intellectual property considerations must be properly factored into analysis of this data, and ranking these pretreatments based solely on sugar yields or first cut economics would be unwise. On this basis, it is our intent to provide practitioners with data that allows them to decide which technologies would best integrate into their application and perhaps develop novel pretreatments that would work even better.

Although cellulases were reasonably effective in releasing residual xylan from pretreated solids, an important consideration is that the results reported herein are for just use of cellulase supplemented with beta-glucosidase, and the CAFI team has found benefits to supplementing the enzymes with xylanases, beta-xylosidases, and other enzyme activities that are more tailored to the composition of the solids remaining following pretreatment. However, because the appropriate cocktail varies with pretreatment technology, details on these approaches are left to articles devoted to each system that can provide the depth needed for proper consideration.

Finally, the reader should remember that this data is only applicable to the poplar species reported, and given our limited ability to predict *a priori* performance for different feedstocks, cannot safely be generalized to other substrates. In fact, the CAFI team experienced better performance with the lower lignin poplar described in this article even though it was originally thought to be genetically similar to the substrate used as our standard. The CAFI team had planned to undertake an in-depth study to gather more comprehensive data on this material with the goal of understanding what caused these differences, but funding could not be obtained for this research. However, the team is working to develop data and insight for pretreatment of a third material, switchgrass, by these same technologies through support of the DOE Office of the Biomass Program, and results from that project should provide perspective on a third class of leading cellulosic biomass materials.

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