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Summary of findings from the Biomass Refining Consortium for Applied Fundamentals and Innovation (CAFI): corn stover pretreatment

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Abstract The Biomass Refining Consortium for Applied Fundamentals and Innovation, with members from 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, has developed comparative data on the conversion of corn stover to sugars by several leading pretreatment technologies. These technologies include ammonia fiber expansion pretreatment, ammonia recycle percolation pretreatment, dilute sulfuric acid pretreatment, flowthrough pretreatment (hot water or dilute acid), lime pretreatment, controlled pH hot water pretreatment, and sulfur dioxide steam explosion pretreatment. Over the course of two separate USDA- and DOE-funded projects, these pretreatment technologies were applied to two different corn stover batches, followed by enzymatic hydrolysis of the remaining solids from each pretreatment technology using identical enzyme preparations, enzyme loadings, and enzymatic hydrolysis assays. Identical analytical methods and a consistent material balance methodology were employed to develop comparative sugar yield data for each pretreatment and subsequent enzymatic hydrolysis. Although there were differences in the profiles of sugar release, with the more acidic pretreatments releasing more xylose directly in the pretreatment step than the alkaline pretreatments, the overall glucose and xylose yields (monomers + oligomers) from combined pretreatment and enzymatic hydrolysis process steps were very similar for all of these leading pretreatment technologies. Some of the water-only and alkaline pretreatment technologies resulted in significant amounts of residual xylose oligomers still remaining after enzymatic hydrolysis that may require specialized enzyme preparations to fully convert xylose oligomers to monomers.

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Introduction

The biochemical conversion of corn stover to fuels and chemicals via soluble sugar intermediates requires a pretreatment step prior to the biological steps of enzymatic saccharification and fermentation in order to adequately access the sugars contained in plant cell wall structural carbohydrates and to obtain the conversion rates and yields from those carbohydrates required for an economically viable process (Mosier et al. 2005a). This pretreatment operation helps to overcome the natural resistance of corn stover and other lignocellulosic feedstocks to microbial degradation and reduces the recalcitrance of cellulose and hemicellulose to better enable conversion of these structural carbohydrates to soluble sugars. The pretreatment step has been projected to be the most expensive capital investment in the biochemical conversion route of lignocellulosic feedstocks to ethanol (Wooley et al. 1999) and can significantly impact the cost of other conversion steps (Wyman 2007).

The pretreatment operation can have high required capital investment associated with it due to the possible need to have high pressure-rated reactors to withstand the required reaction pressures and/or temperatures, the corrosive nature of pretreatment chemicals used in some pretreatment processes, or the need to incorporate complex or expensive pretreatment catalyst recovery and recycle schemes in pretreatment processes that use large amounts of costly pretreatment catalysts (Eggeman and Elander 2005). Energy and water usage in pretreatment must also be considered, as low solids pretreatment processes (those conducted at a solids loading of less than 20% insoluble solids) can require excessive energy to operate at required temperatures and can produce overly dilute process streams in subsequent conversion steps (Mosier et al. 2005a).

The enhancement of sugar yields by proper selection and optimization of the pretreatment operation can also impact the effectiveness and cost of the other process conversion steps. Pretreatment directly influences the amount and types of enzymes needed to further saccharify all plant cell wall structural carbohydrates to monomeric sugars, as some pretreatment approaches can hydrolyze virtually all of the hemicellulose directly to monomeric xylose while other pretreatment approaches largely leave the hemicellulose intact, or only achieve partial hydrolysis to oligomeric sugars (Mosier et al. 2005a). Additionally, the type and severity of the pretreatment process can influence the amount, type and concentration of potential inhibitory compounds that can be released in soluble form directly from the biomass itself (such as acetic acid from acetylated hemicellulose or phenolics or other organic compounds released from lignin) or those produced as products from sugar degradation reactions (such as aldehydes and organic acids most commonly associated with dilute acid pretreatment). These inhibitory products can impact the fermentation performance in subsequent biological conversion steps and can require that hydrolyzate detoxification or conditioning operations be conducted, especially from high solids pretreatments where the concentration of such compounds can be relatively high.

Although numerous pretreatment approaches have been developed and published over many years, it has been difficult to compare the relative merits of these various approaches on a sound basis. Such studies have used varied biomass feedstocks, analytical techniques, and data reporting methodologies, which causes comparative analysis to be difficult or impossible. Also, the great majority of these studies do not include a comprehensive techno economic analysis that quantifies the economic and commercial potential of a given pretreatment approach based upon reported process conversion data. Despite this, there is some consensus that only a select number of pretreatment approaches may actually be commercially viable in a commercial context (Hsu 1996; Johnson and Elander 2007; McMillan 1994; Mosier et al. 2005a).

To further address the limited availability of comparative pretreatment data, 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 the Biomass Refining Consortium for Applied Fundamentals and Innovation (CAFI) in 2000 to collaboratively develop data on pretreatment options that each research institution was developing. In the same year, via a competitive solicitation, the US Department of Agriculture Initiative for Future Agriculture and Food Systems (IFAFS) Program awarded a research grant to the CAFI team, allowing it to conduct a comprehensive project where consistent feedstocks, enzymes, analytical methods, and methodologies for pretreatment and subsequent enzymatic hydrolysis of corn stover were utilized. Additionally, the Office of the Biomass Program of the US Department of Energy funded NREL to conduct comparative process economic analysis and to provide logistical support to the team for that project. At the conclusion of that project, the CAFI team summarized the results of that project in a series of papers that was published in a special issue of *BioResource Technology*, where the pretreatment processes, the subsequent enzymatic hydrolysis and fermentation of the pretreatment hydrolyzates using consistent enzyme types, enzyme loadings, fermentation microorganisms, chemical analysis methods, comparative process yields, and economic analyses were reported (Eggeman and Elander 2005; Kim and Holtzapple; 2005; Kim and Lee 2005; Liu and Wyman 2005; Lloyd and Wyman 2005; Mosier et al. 2005b; Teymouri et al. 2005; Wyman et al. 2005a, b).

More recently, the US Department of Energy's Office of the Biomass Program selected the existing CAFI team to perform a second comparative pretreatment project. During that project, the principal investigator for the CAFI project moved to the University of California, Riverside from Dartmouth College. While much of that project has focused on a hardwood feedstock (hybrid poplar), some additional work was conducted using a different variety of corn stover. Additionally, another pretreatment technology (sulfur dioxide-catalyzed steam explosion by the University of British Columbia, whose work in this project was funded by Natural Resources Canada) was included in this project, along with supply of advanced enzyme preparations by Genencor Inc. via in-kind support. In this paper, the key features and results from the CAFI team's work on corn stover pretreatment and resulting impacts on subsequent biochemical process conversion steps and overall process economics are summarized.

Materials and methods

One of the founding principles of the CAFI was that consistent and comparative materials and methodologies would be followed by all team members. To that end, the individual CAFI research groups used the same raw materials, analytical procedures, and data analysis approaches wherever possible (Wyman et al. 2005a).

Materials

Feedstocks

Whole corn stover (*Zea mays*) was used in both CAFI projects, without any attempt to differentiate or preferentially select any specific anatomical fractions of the corn plant. In the first CAFI project, the source of the corn stover was from Bio/Mass Agri-Products (B/MAP) in Harlan, IA. This corn stover was part of a large scale corn stover collection activity that was operated over several years. Collected corn stover was stored in large round bales and prior to being provided to the CAFI team, the bales were broken, tub ground, washed to remove excess soil, and dried to a moisture content of less than 12% moisture. Approximately 100 kg of this corn stover was supplied to the NREL and was used as a single, consistent feedstock by all research groups in the first CAFI project. Upon receipt at NREL, it was further washed, air-dried, and milled in a Mitts and Merrill Model 10 × 12 knife mill (Saginaw, MI) to pass through a 6.4 mm (¼ inch) screen and distributed to CAFI research teams. In the second CAFI project, a new source of corn stover was used (Pioneer variety 33A14, from the Kramer farm in Wray, CO). This corn stover was collected during a second-pass harvest using a high rake setting to reduce collection of extraneous soil, so no washing of this feedstock was performed. Once received at NREL, this corn stover was milled in an identical manner as described above and was provided to all CAFI research teams. The composition of the corn stover from both of these batches is shown in Table 1. This data shows that the composition of the two corn stover batches was fairly

Table 1 Compositional analysis of corn stover batches used in the first and second CAFI projects

	Component mass fraction (wt%-dry basis)	
	1st CAFI Project	2nd CAFI Project
Sucrose	1.2	2.2
Glucan	36.1	34.4
Xylan	21.4	22.8
Arabinan	3.5	4.2
Mannan	1.8	0.6
Galactan	2.5	1.4
Lignin	17.2	11.0
Protein	4	2.3
Acetyl	3.2	5.6
Ash	7.1	6.1
Uronic acids	3.6	3.8

similar, with the stover from the second CAFI project having slightly lower glucan, lignin, protein, and ash content and slightly higher xylan, arabinan, and acetyl content.

Enzymes

Enzymes were generously provided by Genencor Inc. for both CAFI projects. In the first CAFI project, the Genencor Spezyme CP cellulase preparation (Lot 301-00348-257), was distributed to CAFI members by NREL. The average activity of the enzyme, as determined by NREL, was 31.2 Filter Paper Units (FPU)/mL. Beta-glucosidase (Novozyme® 188), a supplementary enzyme used with Spezyme CP, was purchased from Sigma Chemical Company (Cat. No. C6150, Lot No. 11K1088). Beta-glucosidase supplementation was used by some CAFI research teams to test whether its addition would be beneficial, but all CAFI research teams utilized only Spezyme CP at a standardized loading level as described in the Methods section of this paper. In the second CAFI project, Genencor Inc. provided two baseline cellulase preparations. The first preparation was Spezyme CP, but from a newer lot (Lot 301-04075-034) than the Spezyme CP preparation used in the first CAFI project. This newer lot had a filter paper activity of 59 FPU/mL and a protein concentration of 123 mg protein/mL, as measured by Genencor. A different cellulase product from Genencor, known as GC-220 (lot 301-04232-162) was also used in the second CAFI project and had corresponding values of 89 FPU/mL and 184 mg protein/mL. The other enzymes supplied by Genencor in the second CAFI project included β-glucosidase (32 mg protein/mL), Multifect Xylanase (lot 301-04021-015, 41 mg protein/mL), and β-xylosidase (85 mg protein/mL). The baseline enzyme formulation was a combination of β-glucosidase and cellulase at a cellobiase unit (CBU)/FPU ratio of 2.0.

Methods

Pretreatment

Pretreatments were performed by the individual CAFI research teams using specific equipment, conditions, and procedures described in the referenced papers (Kim and Holtzapple 2005 [Lime pretreatment]; Kim and Lee 2005 [Ammonia recycled percolation pretreatment]; Liu and Wyman 2005 [Hot water flowthrough pretreatment]; Lloyd and Wyman 2005 [Dilute sulfuric acid pretreatment]; Mosier et al. 2005b [Controlled pH hot water pretreatment]; Teymouri et al. 2005 [Ammonia fiber expansion (AFEX) pretreatment]). Although these papers specifically describe pretreatment procedures used in the first CAFI project, the same general pretreatment systems and methods were also used for corn stover pretreatments conducted in the second CAFI project. Generally, pretreatments were conducted in bench-scale pretreatment reactor systems specifically designed or modified by each CAFI team for their particular pretreatment approach.

Enzymatic hydrolysis

Enzymatic hydrolysis of the pretreated solids (typically on water-washed solids in the pretreatments that release soluble sugars) was conducted at a solids loading corresponding to a 1 wt % or 2 wt % glucan concentration to keep soluble sugar concentrations low in order to minimize end product inhibition effects of sugars or oligomers. Enzymatic hydrolysis assays were conducted in 100 mL shake flasks in citrate buffer (pH 4.8) plus antibiotics for a total digestion time of 72 h. As a minimum, cellulase loadings corresponding to 15 and 60 FPU/g of glucan in the raw feedstock were tested, although some individual CAFI research groups also conducted enzymatic hydrolysis experiments using other enzyme loadings and other enzyme preparations and combinations. Other specific conditions and methods used in the enzymatic hydrolysis assays are outlined in a specific NREL Laboratory Analytical Procedure (Dowe and McMillan 2008).

Chemical analysis

Chemical analysis of feedstocks, pretreated solids and liquids, and enzymatically digested solids and liquids were performed by the individual CAFI research teams following the standard NREL Laboratory Analytical Procedures (LAPs) that were appropriate for each type of analysis (NREL 2008).

Calculation of sugar yields

Yields of the primary corn stover-derived sugars, glucose and xylose, were calculated as described previously (Wyman et al. 2005b) based on the maximum amount of xylose and glucose that could be potentially recovered from the specific corn stover feedstock, considering its measured glucan and xylan content. The maximum potential yields for glucose and xylose were determined such that the sum total yield of the two sugars is 100%. Thus, in the case of the corn stover used in the first CAFI project, the maximum yield of glucose is 62.3% and the maximum yield of xylose is 37.7%, for a sum total of 100%. Similarly, for the corn stover used in the second CAFI project, the maximum yield of glucose is 59.6% and the maximum yield of xylose is 40.4%, as there is slightly less glucan and more xylan in this corn stover sample. Sugar yields of each sugar were determined after the pretreatment step and after the subsequent standardized enzymatic hydrolysis step, as there are different sugar release profiles depending on the pretreatment process.

Results and discussion

In both CAFI projects that studied the pretreatment and subsequent enzymatic hydrolysis of corn stover, a number of pretreatment processes were employed and comparative conversion data was generated. The general features and specific operational details for these pretreatment approaches are discussed elsewhere (Mosier et al. 2005a; Wyman et al. 2005a).

In Table 2, some of the representative conditions for each of the CAFI pretreatment technologies are presented. There are significant differences across many of the operational parameters for the various pretreatment approaches. With regard to chemical usage, the “no-catalyst” processes (water-only flow-through pretreatment and the pH controlled hot water pretreatment) use no added chemicals. The sulfuric acid and SO_2-catalyzed pretreatment processes use

Table 2 Pretreatment technologies and ranges of representative reaction conditions used on corn stover in CAFI projects

Pretreatment technology	Chemicals used	Temperature (°C)	Pressure (atm absolute)	Reaction times (minutes)	Concentrations of solids (wt%)
Dilute sulfuric acid	0.5–3.0% Sulfuric acid	130–200	3–15	2–30	10–40
SO_2 catalyzed steam explosion	0.5–3.0% SO_2	170–210	8–18	5–10	20–40
Flowthrough pretreatment	Sulfuric acid	190–200	20–24	12–24	2–4
pH controlled water pretreatment	Water or stillage	160–190	6–14	10–30	5–30
AFEX	100% (1 g ammonia:1 g dry biomass) anhydrous ammonia	70–90	15–20	<5	60–90
ARP	10–15 wt% ammonia	150–170	9–17	10–20	2–12
Lime	0.05–0.15 g Ca(OH)2/g biomass	70–130	1–6	1–6 h	5–20
Lime + air	0.05–0.15 g Ca(OH)2/g biomass	25–60	1	2 weeks–2 months	10–20

Information compiled from Wyman et al. (2005a) relatively low loadings of inexpensive catalysts and thus do not require recovery and re-use of the pretreatment catalyst. The alkaline pretreatment processes often use higher catalyst loadings and are likely to require recovery and re-use of the pretreatment catalyst in a commercial process context. This can add significant cost and complexity to the overall process design and operation (Eggeman and Elander 2005).

The acidic and non-catalyzed pretreatment processes generally operate at relatively high temperatures, while the alkaline processes operate at significantly lower temperatures. This is inversely related to pretreatment catalyst loading, with high temperature processes using lower amounts of pretreatment catalyst and low temperature processes using greater catalyst loadings. An exception to this is the ammonia recycled percolation (ARP) process, which is performed at temperatures at or above 150 °C while still using significant catalyst loadings. While many of the alkaline processes operate at lower pressures corresponding to their lower operating temperatures, the AFEX process operates at high pressures, as shown in Table 2. This is due to the high loading of ammonia catalyst in this process and the resulting high pressure due to the high vapor pressure of ammonia.

Reaction times for the various pretreatment processes generally range from a few minutes to less than 30 min. The primary exception to this is the lime pretreatment process, where residence times range from a few hours in the higher temperature version of this process to as long as several weeks in the low temperature "pile" version of this process that also utilizes air to facilitate oxidative removal of lignin during pretreatment.

There is also a wide range of solids concentrations employed across the various pretreatment processes, and in some cases, within a specific pretreatment approach. For example, dilute acid pretreatment can be conducted in a low solids environment in stirred pressure vessels or in a high solids environment using feedstock that is pre-impregnated with the sulfuric acid catalyst and is then loaded into tubular reactors or steam explosion-type reactors with no internal mixing. The "flowthrough" or "percolation" pretreatment approaches are practiced at low solids, as a relatively large volume of liquid is passed through a packed bed of feedstock, with collection of the exiting free liquid fraction. AFEX is often referred to as a "dry" pretreatment process, as the anhydrous ammonia catalyst effectively penetrates biomass pores without a free liquid phase and only small amounts of steam are necessary to achieve the relatively low pretreatment temperatures of the AFEX process.

In Table 3, sugar yields for both xylose and glucose are presented for the corn stover batch used in the first CAFI project. Pretreatments are listed from top to bottom in approximate order of increasing pH. In the left portion of Table 3, xylose yields are presented after the pretreatment step and after the subsequent enzymatic hydrolysis step at an enzyme loading of 15 FPU/g glucan in the untreated corn stover. Often, enzyme loadings are specified based on the glucan content in the pretreated solids, but since the various CAFI pretreatment processes achieve different degrees of hemicellulose and lignin solubilization, specifying the enzyme loading based on the glucan content in the untreated feedstock allows for a clearer basis for comparison.

For the more acidic pretreatments and in the low or no catalyst flowthrough and pH controlled liquid hot water pretreatments, a significant amount of xylose (based upon a maximum possible xylose yield of 37.7%) is released during the pretreatment step. A large proportion of released xylose is in monomer form for dilute acid pretreatment while most of the released xylose is in oligomeric form for the low or no catalyst flowthrough and pH controlled liquid hot water pretreatments. AFEX is a "dry" pretreatment process and results in no solubilization of xylan to either oligomeric or monomeric xylose after the pretreatment step. The ARP process results in release of about half of the xylan to monomeric xylose after pretreatment, while about one-fourth of the xylan is released upon lime pretreatment, primarily to oligomeric xylose. After subsequent enzymatic hydrolysis using the Spezyme CP enzyme preparation, small additional amounts of xylose are formed from the acidic and flowthrough pretreated solids, with a somewhat larger amount from the controlled pH liquid hot water pretreated solids. In the alkaline pretreated solids, significant amounts of xylan is hydrolyzed in the enzymatic hydrolysis step and in the case of AFEX, high yields of monomeric xylose are achieved. This is a significant finding, as this performance was obtained using only a commercial

Table 3 Xylose, glucose, and total sugar yields for each CAFI pretreatment process after pretreatment, enzymatic hydrolysis, and combined process steps from corn stover feedstock used in the first CAFI project

Pretreatment system	Xylose yields			Glucose yields			Total sugar yields		
	Pretreatment	Enzymatic hydrolysis	Combined	Pretreatment	Enzymatic hydrolysis	Combined	Pretreatment	Enzymatic hydrolysis	Combined
Maximum possible	37.7	37.7	37.7	62.3	62.3	62.3	100.0	100.0	100.0
Dilute acid	32.1/31.2	3.2	35.3/34.4	3.9	53.2	57.1	36.0/35.1	56.4	92.4/91.5
Flowthrough	36.3/1.7	0.6/0.5	36.9/2.2	4.5/4.4	55.2	59.7/59.6	40.8/6.1	55.8/55.7	96.6/61.8
Partial flow pretreatment	31.5/2.8	2.6/2.4	34.1/5.2	4.3/4.2	51.2	55.5/55.4	35.8/7.0	53.8/53.6	89.6/60.6
Controlled pH	21.8/0.9	9.0	30.8/9.9	3.5/0.2	52.9	56.4/53.1	25.3/1.1	61.9	87.2/63.0
AFEX		34.6/29.3	34.6/29.3		59.8	59.8		94.4/89.1	94.4/89.1
ARP	17.8	15.5	33.3/15.5		56.1	56.1	17.8	71.6	89.4/71.6
Lime	9.2/0.3	19.6	28.8/19.9	1.0/0.3	57.0	58.0/57.3	10.2/0.6	76.6	86.8/77.2

Data compiled from Wyman et al. (2005b)

Yield values for xylose and glucose are relative to the “Maximum Possible” values for each component. Single value indicates yield as monomer sugar. When two values are present, the first value is a total (monomer + oligomer) sugar yield and the second value is monomer sugar yield "cellulase" preparation. It is apparent that this enzyme preparation has some effectiveness on AFEX-treated xylan. The combined xylose yields (after pretreatment and enzymatic hydrolysis) are quite high for most of these pretreatments, even though the bulk of the xylose yield occurred after different steps in the process (typically after pretreatment for the more acidic pretreatment conditions and after enzymatic hydrolysis for the more alkaline pretreatment conditions). In several cases, however, a large proportion of the released xylose is still in oligomeric form. It is possible that with proper identification and usage of enzyme systems with good ability to convert xylose oligomers, high monomeric xylose yields could also be achieved for these cases.

In the center portion of Table 3, glucose yields are presented after the pretreatment step and after the subsequent enzymatic hydrolysis step. As opposed to the xylose yield data, the glucose yield data portrays a much greater consistency in performance across the various pretreatment processes. After all pretreatments, only small amounts of glucan are released, either as monomeric or oligomeric glucose. High levels of glucan conversion to monomeric glucose are achieved upon enzymatic hydrolysis of the pretreated solids using the standard enzymatic hydrolysis method for all of the pretreatment methods. In all cases, glucose yields approach or exceed 90% of the theoretically available glucose and are even slightly higher when combined with the small additional glucose yields achieved directly in pretreatment for some of the pretreatment processes.

In the right portion of Table 3, the total sugar (xylose + glucose) yields are presented after the pretreatment step and after the subsequent enzymatic hydrolysis step. Although there are wide-ranging differences with regard to sugar release after the pretreatment steps or the enzymatic hydrolysis steps, when overall sugar yields are combined from both process steps, the resulting overall sugar yields are quite similar for the various pretreatment processes. As total sugars (monomer + oligomer), overall yields generally range from 85 to 95% of theoretical. For dilute acid pretreatment and AFEX pretreatment processes, overall monomer sugar yields are only slightly lower than the total sugar yields. For the other pretreatment processes, overall monomer sugar yields are significantly lower, indicating that there are significant amounts of oligomeric sugars (primarily xylose oligomers) remaining after enzymatic hydrolysis for these processes. Identification and utilization of enzyme activities that can better convert these xylose oligomers to monomers would result in higher overall monomer sugar yields from these processes.

In Table 4, similar data is presented for the corn stover batch used in the second CAFI project. Data in Table 4 is portrayed as total (oligomer + monomer) yield in all cases. Since there is slightly more xylan

Table 4 Xylose, glucose, and total sugar yields for each CAFI pretreatment process after pretreatment, enzymatic hydrolysis, and combined process steps from corn stover feedstock used in the second CAFI project

Pretreatment system	Xylose yields			Glucose yields			Total sugar yields		
	Pretreatment	Enzymatic hydrolysis	Combined	Pretreatment	Enzymatic hydrolysis	Combined	Pretreatment	Enzymatic hydrolysis	Combined
Maximum possible	40.4	40.4	40.4	59.6	59.6	59.6	100.0	100.0	100.0
Dilute acid	32.0	1.2	33.2	5.1	49.8	54.9	37.1	51.0	88.1
SO_2 catalyzed steam explosion	15.0	22.1	37.1	2.3	54.3	56.6	17.3	76.4	93.7
Controlled pH	23.4	9.7	33.1	3.3	50.5	53.8	26.7	60.2	86.9
AFEX		32.9	32.9		61.2	61.2		94.1	94.1
ARP	19.0	15.1	34.1	0.8	52.9	53.7	19.8	68.0	87.8
Lime	9.2	19.5	28.7	0.9	57.2	58.1	10.1	76.7	86.8

Yield values for xylose and glucose are relative to the "Maximum Possible" values for each component. Values indicate total (oligomer + monomer) sugar yield and slightly less glucan than in the corn stover used in the first CAFI project, the maximum possible xylose and glucose yields are slightly different. Overall, the data in Table 4 is very similar to the data in Table 3. The difference in xylose yield after pretreatment as a function of pretreatment pH is still evident, along with the trend of good conversion of residual xylan upon enzymatic hydrolysis of the pretreated solids. The finding of very good conversion of glucan in the pretreated solids to monomeric glucose upon enzymatic hydrolysis is also evident. Overall yields from this batch of corn stover are ultimately very close to those achieved from the corn stover batch used in the first CAFI project. Table 4 also includes data for the SO_2 steam explosion pretreatment process, which was included in the second CAFI project from the University of British Columbia. In general, the SO_2 steam explosion pretreatment results in similar performance to dilute sulfuric acid pretreatment, although xylose yields were somewhat lower after pretreatment but were slightly higher after enzymatic hydrolysis.

Comparative process economic analysis using the results from the first CAFI project in Table 3 was also performed. The specific material and energy balance methods and economic modeling methodologies have been previously described (Eggeman and Elander 2005). The process economic results are reported as a Minimum Ethanol Selling Price (MESP), which is defined as the ethanol sales price required for a zero net present value when the cash flows are discounted at a 10% real after-tax basis, based upon the yields reported. Each CAFI pretreatment process was embedded in a full bioethanol commercial facility model at a nominal 2,000 dry ton/day scale. Capital costing of all process equipment was conducted, including costs associated with any pretreatment catalyst recovery and recycle operation that was deemed necessary. This was combined with an estimate of all operating costs, including estimated raw materials costs, other consumables, labor, and other fixed operating costs.

Using the yields reported in Table 3, the resulting MESP for selected pretreatment processes from the first CAFI pretreatment project are presented in Fig. 1. When yields based upon both monomeric and oligomeric sugars are used (i.e. the “Oligomer Credit” case), there is very little difference in MESP (within a range of 10 cents/gallon of ethanol) across the various pretreatment processes, assuming that enzyme preparations needed to convert remaining oligomers do not add any additional cost to the process. But when only monomer sugar yields are included (i.e. the “w/o Oligomer Credit” case), there is substantial differentiation in MESP, with the lower yielding processes resulting in higher MESP values.

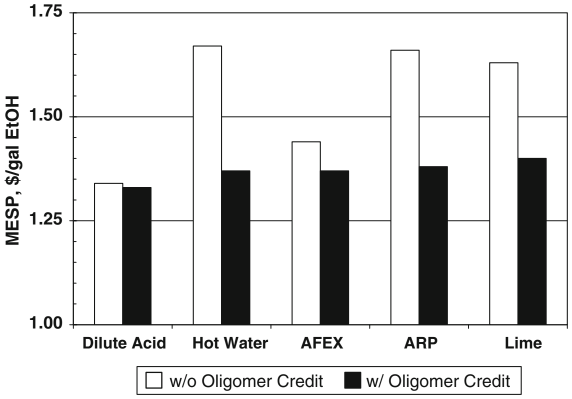

Fig. 1 Predicted minimum ethanol selling price (MESP) for a conceptual 2,000 dry ton/day bioethanol production facility for various CAFI pretreatment processes using data from first CAFI project. Source: Eggeman and Elander 2005

Conclusions

Comparative sugar yield data from two different batches of corn stover have been developed and reported for a variety of pretreatment processes being investigated within the Biomass Refining Consortium for Applied Fundamentals and Innovation. Consistent feedstocks, chemical analysis methods, enzymatic hydrolysis assays, and data reporting methodologies were utilized by all CAFI research teams for two batches of corn stover over two distinct CAFI research projects. This comparative approach across different pretreatment types facilitates meaningful technology evaluation of process options for potential lignocellulosic biomass conversion technology developers and commercializers.

Several distinct trends were observed in the corn stover conversion data from the various CAFI pretreatment technologies. In the pretreatment step itself, xylan hydrolysis to oligomeric and/or monomeric xylose occurred to a significant extent in acidic pretreatments and some non-catalyzed pretreatments but to a much lesser extent in alkaline pretreatments, with the AFEX pretreatment process resulting in no measured hydrolysis of xylan to soluble sugar products. Residual xylan in the AFEX-pretreated solids could be hydrolyzed enzymatically using a commercial cellulase preparation, although not always completely to monomeric xylose. It is likely that thorough analysis of the properties and structure of the remaining oligomeric xylose, coupled with a systematic determination of the enzyme activities necessary to break all types of covalent bonds needed to fully liberate all xylose monomers, could result in higher monomeric xylose yields from these processes, potentially at little or no added process cost.

The hydrolysis of cellulose to glucose occurred to a very limited extent in the pretreatment step itself for all of the CAFI pretreatment processes. Very good enzymatic conversion of the cellulose in pretreated solids to monomeric glucose using standard commercial cellulase preparations was achieved in all cases. These enzymatic hydrolysis assays were conducted at low solids loadings and hence low cellulose concentrations, while commercial application will likely require effective enzymatic hydrolysis performance at much higher slurry concentrations and sugar product concentrations.

Overall sugar yields of glucose and xylose from combined pretreatment and enzymatic hydrolysis process steps showed quite similar performance across both batches of corn stover tested, especially when total (monomer + oligomer) yields are considered. Several of the pretreatment approaches resulted in significant amounts of residual xylose oligomers remaining after enzymatic hydrolysis. It must be emphasized that these findings may not by applicable to all feedstock types and that there may be significant differences in overall sugar yields across various pretreatment approaches for different feedstocks. In fact, recent work conducted by the CAFI team on a hardwood feedstock (hybrid poplar) indicated that the different pretreatments give a wide range of overall sugar yields for this particular feedstock (Wyman et al. 2009).

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