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Evaluation of Different Biomass Materials as Feedstock for Fermentable Sugar Production

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Abstract

Saline crops and autoclaved municipal organic solid wastes were evaluated for their potential to be used as feedstock for fermentable sugar production through dilute acid pretreatment and enzymatic hydrolysis. The saline crops included two woods, athel (*Tamarix aphylla* L) and eucalyptus (*Eucalyptus camaldulensis*), and two grasses, Jose tall wheatgrass (*Agropyron elongatum*), and creeping wild rye (*Leymus triticoides*). Each of the biomass materials was first treated with dilute sulfuric acid under selected conditions (acid concentration = 1.4% (w/w), temperature = 165°C, and time = 8 min) and then treated with the enzymes (cellulases and β-glucosidase). The chemical composition (cellulose, hemicellulose, and lignin contents) of each biomass material and the yield of total and different types of sugars after the acid and enzyme treatment were determined. The results showed that among the saline crops evaluated, the two grasses (creeping wild rye and Jose tall wheatgrass) had the highest glucose yield (87% of total cellulose hydrolyzed) and fastest reaction rate during the enzyme treatment. The autoclaved municipal organic solid wastes showed reasonable glucose yield (64%). Of the two wood species evaluated, Athel has higher glucose yield (60% conversion of cellulose) than eucalyptus (38% conversion of cellulose).

Index Entries: Dilute acid pretreatment; enzymatic hydrolysis; ethanol potential; municipal solid waste; saline crops; fermentable sugar.

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Introduction

Production of fermentable sugars from biomass materials is an important step for biobased, chemical, and biofuel production. For lignocellulosic materials, sugars are primarily derived from hemicellulose and cellulose components. Naturally, sugar derived from cellulose—glucose—is more easily fermentable, especially for the production of ethanol, than the sugars derived from hemicellulose, which are more complex and mainly include five-carbon sugars, such as xylose. To convert cellulose to glucose, the hydrolysis is a necessary step. Hydrolysis is normally carried out using concentrated acid or enzymes. Compared with concentrated acid hydrolysis, enzymatic hydrolysis is more specific and milder, and it does not cause sugar degradation; however, it requires proper pretreatment of the biomass material to improve cellulose accessibility to enzymes by removing hemicellulose, lignin and/or reducing crystallinity of cellulose *(1)*.

Pretreatment technologies, such as dilute acid, steam-explosion, comminution, ammonia fiber explosion, alkaline, and super critical CO_2–SO_2 have been extensively investigated *(2–9)*. Herein, dilute sulfuric acid pretreatment has been widely studied because it is considered to be relatively inexpensive and effective for treatment of different biomass species. In addition, dilute sulfuric acid pretreatment can also effectively hydrolyze hemicelluloses into sugars, including monomeric sugars (xylose, arabinose, galactose, glucose, and mannose) and oligomers. With the recent advent of new microorganisms that are capable of fermenting pentoses as well as hexoses to ethanol, dilute acid pretreatment becomes a more viable step in the hydrolysis of lignocellulosic materials *(10)*.

Much previous reported research about the pretreatment of biomass materials with dilute sulfuric acid is mainly related to softwood, hardwood, grass, and agricultural residues. Acid concentration, reaction temperature, and time are the major parameters that influence the treatment effectiveness. For most types of biomass materials, dilute acid pretreatment can release more than 80% of the sugars associated with the hemicellulose fraction and allow for enzymatic conversion of 80% of the cellulose to glucose (Table 1). The amount and type of sugars that can be produced and the conditions required to achieve the optimum production during acid pretreatment and enzymatic treatment are largely dependent on the chemical composition and structure of biomass materials.

This study was focused on the evaluation of saline biomass crops and thermally pretreated municipal organic waste as the feedstock for fermentable sugar production. The saline crops include the two wood species (athel and eucalyptus) and the two grass species (creeping wild rye [CWR], and Jose tall wheatgrass [JTW]). They were planted as experimental crops on the farms located in the San Joaquin valley of California to help mitigate the salt problem in soil and drainage water. The problem caused by using fertilizers and irrigation water exists across agricultural

Table 1
Literature Review of Selected Dilute Sulfuric Acid Pretreatment and Enzymatic Hydrolysis Research

Feedstock	*T* (°C)	*t* (min)	H_2SO_4 (w/w, %)	Reactor	Hemicellulose conversion (%)	Enzyme loading (FPU/CBU)	Enzymatic digestibility (%)	References
Mixed wood (10% birch and 90% maple)	230	0.12	1.17	Flow	–	–/–	95	*11*
Wheat straw and aspen wood	140	60	0.5 (v/v)	Batch	80	26/–33/33	>80%	*12,13*
Three hardwoods (poplar hybrid NE388, poplar hybrid N11, and sweetgum) and three herbaceous crops (switchgrass, weeping lovegrass, and *Sericea lespedeza*)	160	10	0.45–0.5 (v/v)	Batch	94	42/4.9	90–100	*14*
Corn residues (corn stovers and corn cobs) and three short rotation hardwoods (silver maple, sycamore, and black locust)	160	10	0.45–0.5 (v/v)	Batch	90	42/4.9	85	*15*
Douglas fir	200–230	1–5	0.4	Batch	90–95	25–60/–	90	*16*
White fir and ponderosa pine	200–230	1–5	0.4	Two-stage	95–100	60/–	>90	*17*
Corn stover	121	30–120	2	Batch	90	40/8	80	*18*
Rye straw and bermudagrass	121	90	1.5	Batch	55–66	25/75	52–83	*1*

lands in the San Joaquin valley. One of the approaches for solving the salinity problem is to use an integrated on-farm drainage management system, which uses the plantation of different crops and plants sequentially with increasing the salt tolerance levels to utilize drainage water. This has been practiced at Red Rock Ranch (RRR), a farm located near Five Points, California in a 640-acre demonstration project where several salt-tolerant crops, including Athel, eucalyptus, JTW, and CWR, have been planted. The saline biomass production through the integrated on-farm drainage management systems not only helps with transpiration of water and concentration of salt but also serves to capture solar energy through photosynthesis for further use. The produced crops could be used as raw materials for producing bioenergy and biochemicals, such as biogas and sugars, which can be further converted to ethanol and/or other chemicals.

This study was designed to investigate the utilization of the saline crops as feedstock for production of valuable products, such as fermentable sugar, which is important for developing a sustainable agricultural system for their continued production. This study was not focused on addressing the effect of salt contents in saline crops on fermentable sugar production step, although the salt content may influence the fermentation process. The autoclaved municipal organic waste (AMSW) was obtained from the City of San Francisco, CA, and was pretreated with an autoclave process first to partially break down the fibers and reduce the particle size, before it was tested with the hydrolysis experiments. It contained approx 30% food and 25% paper. Currently the municipal solid wastes (MSW) are largely disposed off in landfills. This study also evaluated the feasibility of separating the organic fraction (food and paper) from the MSW and converting it into sugars as valuable products. Although many lignocellulosic biomass materials have been investigated as potential feedstock for sugar and/or ethanol production, little research has been done on the hydrolysis of saline crops and AMSW with high food content. The objectives of this study were to compare different saline crops and AMSW for their potential to be used as feedstock for fermentable sugar production through dilute acid and enzymatic hydrolysis, and determine the amount and types of sugars that can be produced from these biomass materials.

Materials and Methods

Biomass Preparations

The biomass materials tested in this study include four saline crops and one AMSW. The following is detailed information about the saline crops:

1. Athel pine, *T. aphylla L*, softwood.
2. Eucalyptus, *E. camaldulensis*, hardwood.
3. CWR, *L. triticoides.*
4. JTW, *A. elongatum.*

Athel and eucalyptus from 8-yr old trees were harvested in May 2004 and then cut into approx 40 cm long logs using a chainsaw. The diameter of logs used in this study ranged from 3 to 25.4 cm. The logs were further debarked with chisel and then reduced into 5–10 cm long chips with a Dosko brush chipper (Model 1400-12; Dosko Co., Sacramento, CA). The chips were air-dried to approx 8% moisture content and sealed in plastic bags for storage in the Biomass Laboratory at the University of California, Davis for future use. The JTW and CWR were harvested from the same location as the woods, which was RRR at five points in California. They were cut, field-dried, and baled with an average straw length of 50 cm in September 2004 and in May 2005, respectively. Bales were stored indoors at ambient temperature.

The saline crops were milled into particles using a laboratory hammer mill (Model C269OYB, Franklin Co. Inc., Buffton, IN) equipped with a 0.32-cm rejection screen. After milling, the fiber particles were classified into three groups based on the particle size, more than 0.38, 0.38–0.23, and less than 0.23 mm, using a sieve shaker (RO TAP, The W. S. Tyler Company, Cleveland, OH) with corresponding sieves (Newark Wire Cloth Co., Clifton, NJ). The 0.23–0.38 mm particles were used for this sugar production study. They were stored in sealed 2-gal zip-locked plastic bags at 4°C.

AMSW was prepared from MSW obtained from a waste management company in San Francisco, CA. The MSW was first thermally treated with a process called CR^3, which autoclaved the MSW with steam for 2–3 h, at temperature of 127°C and pressure of 172 kPa. AMSW was then screened to separate organic fraction from inorganic fraction. The separated organic fraction was used in this study for the hydrolysis treatment.

Dilute Sulfuric Acid Pretreatment

Biomass was pretreated with dilute sulfuric acid solutions (1.4%, w/w) in a 1-L reactor (Carpenter 20 Cb-3, Parr Co., Moline, IL), equipped with impeller mixers and a pressurized injection device. In this study, a 10% (w/w) solid slurry sample (65 g solids) was chosen to allow for proper mixing. Samples were reacted at 165°C for 8 min, based on results of preliminary tests conducted at different temperatures, durations, and acid concentrations. The duration was clocked from when the mixture of biomass, water, and sulfuric acid in the reactor reached the desired reaction temperature. The warming step before 165°C took approx 30 min.

After dilute sulfuric acid pretreatment was completed, the reaction was terminated by immersing the reactor in ice-water until the reactor pressure became 101 kPa. The reactor was opened slowly and the residues were recovered by washing the reactor with deionized water. The mixture of solid residues and washing water was stored in sealed bottles at 4°C. The pretreated biomass was thoroughly washed with hot deionized water (85°C) and filtered under vacuum to remove water-soluble compounds from solids. During the washing and filtration process, the pH of the filtrate was measured with a pH meter (AR20; Fisher Scientific Inc., Hampton, NH)

and washing was stopped when the pH reached 4.5 or the reducing sugar (RS) concentration reached 0.06 g/L.

A portion of the washed pretreated solid was stored at −20°C for subsequent enzymatic hydrolysis. The remaining solid was dried at 45°C in an oven for subsequent chemical composition analysis. The total volume of wash water was measured and recorded. Then a 20-mL sample was taken from the wash water, neutralized with $CaCO_3$, and filtered with glass filter paper of 0.20-μm pore size. The filtered water was stored at 4°C for analysis of total RS and individual sugars.

Enzymatic Hydrolysis

Enzymatic hydrolysis was carried out in 125-mL screw-capped Erlenmeyer flasks. The enzymes used in the study were cellulase (Novozymes, Celluclast 1.5L, available from Sigma-Aldrich Corp. [St. Louis, MO], Cat. No. C2730) supplemented with extra β-glucosidase (Novozyme188, available from Sigma, Cat. No. C6105). Both enzymes were provided by Novozymes Inc. (Davis, CA). The activities of the two enzyme stocks were determined to be 90 filter paper units (FPU)/mL and 250 cellobiose units (CBU)/mL, respectively. The cellulase and β-glucosidase contained 47.1 and 49.7 mg protein/mL, respectively, as measured by Bio-rad protein assay (Bio-rad Laboratory Hercules, CA). The enzymatic hydrolysis was performed at pH 4.8 with cellulase loading of 15 FPU/g-cellulose (7.9 mg protein/g-cellulose) and β-glucosidase loading of 52.5 CBU/g-cellulose (10.4 mg protein/g-cellulose) in prewarmed (50°C) 50-mL reaction slurry that contained solids equivalent to 2% cellulose, 0.05 *M* sodium citrate buffer (pH 4.8), and 0.3% (w/v) sodium azide. Sodium azide was used to inhibit microbial growth during the enzymatic hydrolysis *(1)*. Before loading of enzymes, the pH of slurry was adjusted with 6 *N* HCl or 6 *N* NaOH to 4.8 as needed. Duplicate 125-mL Erlenmeyer reaction flasks were incubated in a shaking incubator at 50°C with an agitation speed at 140 rpm.

During the enzymatic hydrolysis, 1-mL samples were periodically removed from each flask (0, 2, 8, 18, 24, 72, 120, and 168 h). Each sample was centrifuged for 10 min at 13,500*g*, and 500-μL supernatant was then removed and placed into a 1.5-mL Eppendorf tube, which contained the stop buffer (512 m*M* Na_2CO_3 and 288 m*M* $NaHCO_3$; pH 10.0) *(9)*. The buffered samples were stored at 4°C for subsequent glucose measurement.

The glucose yield was calculated as:

$$\text{Glucose yield (\%)} = \frac{\text{Glucose (mg/mL)}}{\text{Cellulose added (mg/mL)} \times 1.11} \quad (1)$$

where the factor 1.11 is used to adjust the weight gained in hydrolyzing cellulose to glucose.

Analytical Methods

The moisture content of biomass was measured according to the American Society of Testing and Materials standard method D4444-92 *(19)*. Total RS was measured with the dinitrosalicylic acid method using glucose as the standard *(20)*. The activities of cellulase and β-glucosidase were measured as FPU and CBU, respectively *(21)*. Glucose content was measured with anthrone colorimetric assay *(22,23)*. Xylose, arabinose, galactose, and mannose were measured by analyzing the alditol acetate derivatives synthesized with gas chromatograph as described by Blakeney et al. *(24)* following hydrolysis with 2 *N* trifluoroacetic acid (TFA) for 1 h at 121°C *(25)*. Ash, acid insoluble lignin (Klason lignin), and acid soluble lignin were determined by standard National Renewable Energy Laboratory's laboratory procedures *(26–28)*.

Solubilized monosaccharides in dilute sulfuric acid hydrolysates, including glucose, xylose, arabinose, galactose, and mannose, were determined using gas chromatography *(24,25)*. The glucose concentrations in the samples obtained from the enzymatic hydrolysis were measured using glucose assay kit purchased from Bioassay Systems (Quantichrom™ Glucose Assay Kit, DIGL-200, CA). All measurements were conducted in duplicate trials and average results were reported.

Results and Discussion

Chemical Composition of Biomass Materials

The chemical compositions of biomass are shown in Table 2. Glucose was the major component followed by lignin (acid insoluble and soluble lignin) and xylose, and various minor components. Herein, galactose, arabinose, and mannose were minor components. The galactose, arabinose, xylose, and mannose are the major components of hemicellulose matrix *(10)*. In addition, the woody samples (athel and eucalyptus) also contained mannose as part of their xylose. Our results are consistent with the results of Grohmann et al. *(29)*, Torget et al. *(14)*, and Brigham et al. *(30)* who reported that the composition of hemicellulose varied with biomass species and woody xylose contained mannose. Therefore, it can be stated that the hemicellulose of all the biomass used in this study mainly included arabinose, galactose, xylose, and/or mannose with xylose as the dominant carbohydrate. Even though hemicellulose contained some glucose, it was difficult to distinguish the glucose source, because some glucose might come from cellulose during the chemical composition measurement. In addition, AMSW had considerably lower hemicellulose content (5.4%), compared with the saline crops.

Dilute Acid Pretreatment

Weight loss was used to evaluate the effect of dilute acid pretreatment on composition removal for different biomass materials, because the

Table 2
Chemical Composition of Untreated Biomass (dry basis [wt%])

	Athel	Eucalyptus	CWR	JTW	AMSW
Glucose	49.34	44.45	34.03	31.09	43.95
Xylose	11.82	10.53	16.48	16.90	2.43
Arabinose	0.68	0.82	3.28	2.83	0.48
Galactose	0.46	2.24	0.78	0.66	0.43
Mannose	0.27	0.28	ND[a]	ND	1.98
Acid-insoluble lignin[b]	25.97	32.73	20.85	17.70	22.20
Acid-soluble lignin[b]	4.45	2.45	3.51	2.70	1.66
Ash	5.43	2.14	7.37	8.61	9.89
Other	1.58	4.36	13.70	19.51	16.98

[a]ND, not detected.
[b]Lignin contents were measured using unextracted biomass.

Table 3
Removal of Components From Biomass Solids by Dilute Acid Pretreatment (%)[a]

	Athel	Eucalyptus	CWR	JTW	AMSW
Glucose	19	16	12	9	12
Xylose	99	98	97	99	99
Arabinose	100	100	100	100	100
Galactose	100	100	100	100	100
Mannose	100	100	–	–	100
Acid-insoluble lignin[b]	22.01	29.61	13.30	27.09	18.19
Acid-soluble lignin[b]	85.08	82.07	88.69	90.31	98.63

[a]The removal of components (%) = (components in raw solid-components in pretreated solid)/components in raw solid × 100%.
[b]Lignin contents were measured using unextracted biomass.

removal mechanism involves the hydrolysis of the various cell wall components *(14)*. Dry weight losses varied from 59% (JTW) to 35% (AMSW) (Fig. 1). As shown in Table 3, the sugars released from the acid pretreatment included arabinose, galactose, glucose, xylose, and mannose, but no mannose was found from grasses and AMSW. Most of the acid soluble lignin was removed from the solids (82.07–98.63%), but only a relatively small fraction of the acid insoluble lignin was removed (13.30–29.61%). The hydrolysis of cellulose was low (16–19% for athel and eucalyptus and 9–12% for CWR, JTW, and AMSW). Dilute acid pretreatment was equally effective at removing hemicellulose from the woods, grasses, and AMSW.

The sum of the individual sugars and amounts of solubilized RS were also measured (Fig. 2) because the RS contents in the prehydrolyzates can effectively indicate the solubilization of hemicellulose and cellulose during the pretreatment process *(1)*. JTW released the highest amount of RS and individual sugars from hemicellulose. Because of its low content of

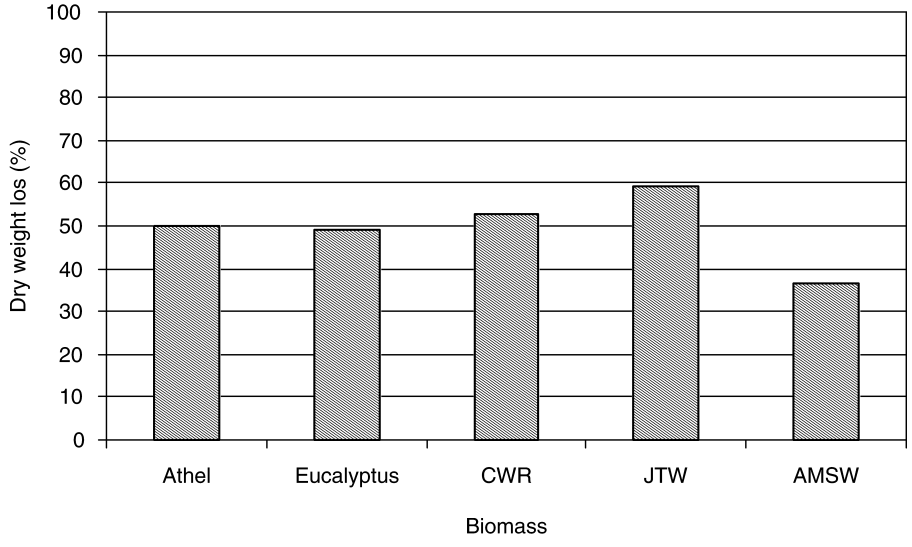

Fig. 1. Dry weight losses of different biomass after dilute acid pretreatment.

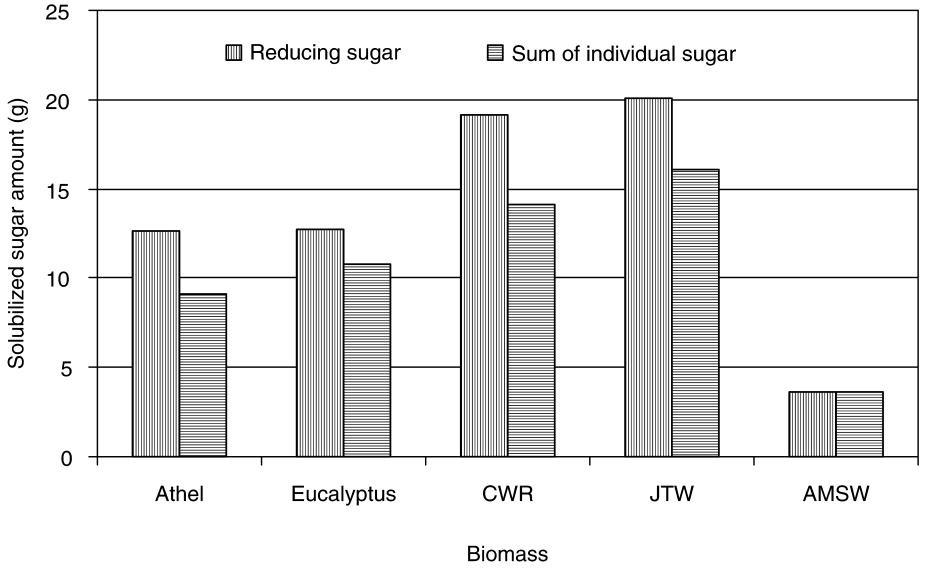

Fig. 2. Solubilized RS and sum of individual sugar released from different biomass materials after dilute acid pretreatment.

hemicellulose (5.32%), AMSW had the lowest amount of solubilized RS and individual sugars. In Fig. 2, it can be seen that the amount of RS was higher than the sum of individual sugars. The difference might be because of the presence of other RSs (such as cellobiose) and nonsugar reducing components that were measured as RSs.

The xylose mass balance was done based on the dilute acid pretreatment system. The xylose contents were measured in original solids,

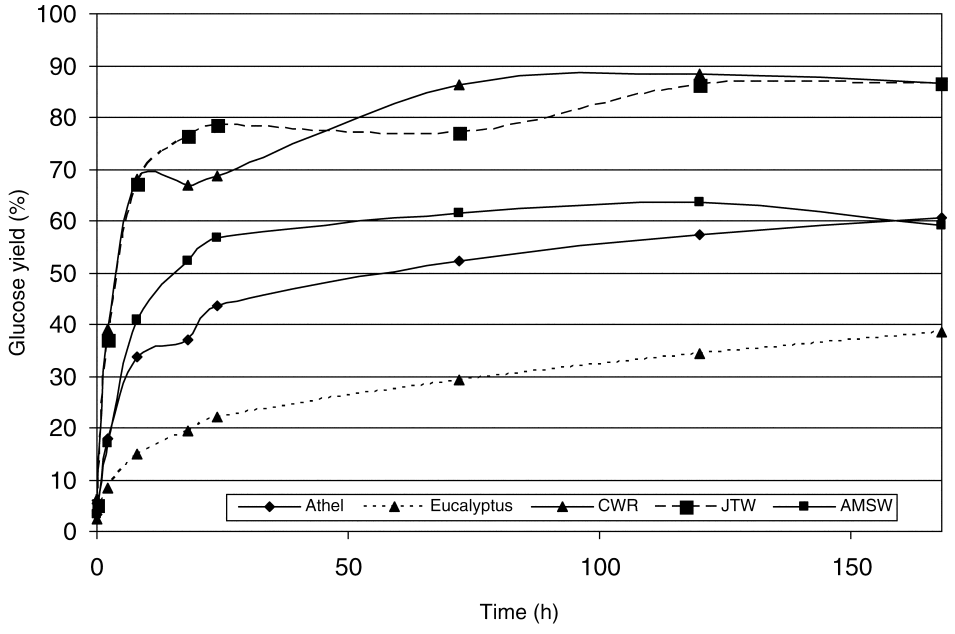

Fig. 3. Glucose yield from different acid-treated biomass materials during the enzymatic hydrolysis.

pretreated solids, and prehydrolyzate. It was found that xylose mass balance could not be closed. For example, 33% of the xylose in Athel and 20% in CWR were not recovered as monomeric xylose. These findings were similar to reported results, which indicated that some sugars, such as xylose and glucose, were solubilized in the prehydrolyzate as oligomeric sugar, furfural, hydroxymethylfurfural, and/or other byproducts during the acid pretreatment *(1,7,31)*.

Enzymatic Hydrolysis for Glucose Yield

The glucose yields from different pretreated biomass materials during the enzymatic hydrolysis are shown in Fig. 3. The two grasses, CWR and JTW, had the highest yields (87% cellulose saccharified) after the 168 h treatment. The two woods, athel and eucalyptus, had the lowest glucose yield (60 and 38% cellulose converted, respectively). The glucose yield for AMSW was 63% cellulose converted, which was higher than the glucose yield from the woods. The CWR and JTW also had the fastest rate of sugar release, two to three times faster than Athel and AMSW and five times faster than eucalyptus. In particular, the conversion of cellulose to glucose for JTW was nearly 80% completed after only 24 h of enzymatic hydrolysis. Eucalyptus had the lowest rate of released glucose and even after 168 h the glucose continued to increase in concentration.

The reason for the difference in cellulose hydrolysis rates and final glucose yields among the different biomass materials might be that cellulose

Table 4
Total Sugar Yield From Different Biomass by Dilute Acid Pretreatment and Enzymatic Hydrolysis (gram per gram of Original Dry Matter)

Biomass	Glucose	Xylose	Arabinose	Galactose	Mannose	Total sugar[a]
Athel	0.24	0.08	0.005	0.003	0.002	0.33
Eucalyptus	0.19	0.07	0.007	0.01	0.003	0.28
CWR	0.31	0.13	0.03	0.007	–	0.48
JTW	0.34	0.12	0.02	0.005	–	0.49
AMSW	0.36	0.03	0.008	0.006	0.02	0.42

The sugar is specified as monomeric sugar. For example, total glucose yield = (total monomeric glucose released by dilute acid pretreatment in liquid + total glucose yield from enzymatic hydrolysis)/(total raw dry matter loaded initially in dilute acid pretreatment step). Other sugar yield = (total sugars released by dilute acid pretreatment)/(total raw dry matter loaded initially in dilute acid pretreatment step).

[a]Total sugar is the summation of all the individual sugars released by dilute acid pretreatment and enzymatic hydrolysis.

fibers in pretreated grasses (CWR and JTW) were inherently more digestible than those of pretreated woods (athel and eucalyptus) and AMSW *(14)*. Another possibility could be that lignin condensation during dilute acid pretreatment contributed to the development of higher porosity for grasses than for woods and AMSW, making the cellulose in grasses more accessible by the enzymes *(14)*. More research is needed to identify the mechanism.

Total Monomeric Sugars Yield From Dilute Acid Pretreatment and Enzymatic Hydrolysis

Because of the improvement of genetic engineering, microorganisms now can ferment pentose and hexose sugars to ethanol at the same time *(32–37)*. Therefore, it has become important to measure the total sugar yield from hydrolysis of biomass. In fact, based on either the total or just glucose yield, the grasses and AMSW had higher yields than the woods (Table 4). AMSW had the highest glucose yield (0.36 g/g original dry matter) and eucalyptus had the lowest yield (0.19 g/g original dry matter). For total sugar yields, CWR and JTW had the highest values (0.48 and 0.49 g/g original dry matter, respectively). Eucalyptus had the lowest total sugar yield (0.28 g/g original dry matter). The total sugar yield of AMSW was notable because it was 0.42 g/g original dry matter, which indicated that AMSW could be a good biomass feedstock for ethanol production.

Conclusions

Athel and eucalyptus had higher cellulose and lignin contents than CWR, JTW, and AMSW. Dilute acid pretreatment was highly effective in hydrolyzing the hemicelluloses in all of the tested biomass materials (over 97%). After dilute acid pretreatment, removal of lignin from the

solids was 13–30%. Overall, the lignins in the grasses were more resistant to dilute acid pretreatment than the woody lignins. Pretreated CWR and JTW had the highest glucose yields (86% of total cellulose converted) and fastest rates of hydrolysis, but the pretreated eucalyptus had the lowest glucose yield (38%) and lowest hydrolysis rate of the samples evaluated in this study. Considering the fact that AMSW is originated from waste streams, its glucose yield of 63% makes it an attractive resource for use as a feedstock for sugar production. CWR and JTW had the highest yield of total monomeric sugars (0.48 and 0.49 g/g original dry matter). AMSW showed promising yield (0.42 g/g original dry matter) of total monomeric sugars. The future research will focus on enzymatic hydrolysis of JTW, CWR, and AMSW with lower enzyme and higher solid loadings.

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References

1. Sun, Y. and Cheng, J. J. (2005), *Bioresour. Technol.* **96,** 1599–1606.
2. Brownell, H. H. and Saddler, J. N. (1984), *Biotechnol. Bioeng. Symp.* **14,** 55–68.
3. Gould, J. M. (1984), *Biotechnol. Bioeng.* **XXVI,** 046–052.
4. Cadoche, L. and Lopez, G. D. (1989), *Biol. Wastes*, **30,** 153–157.
5. Martinez, J., Negro, M. J., Saez, F., Manero, J., Saez, R., and Martin, C. (1990), *Appl. Biochem. Biotechnol.* **24–25,** 127–133.
6. Shah, M. M., Song, S. K., Lee, Y. Y., and Torget, R. (1991), *Appl. Biochem. Biotechnol.* **28–29,** 99–109.
7. Torget, R., Himmel, M. E., and Grohmann, K. (1991), *Bioresour. Technol.* **35,** 239–246.
8. Torget, R., Himmel, M., and Grohmann, K. (1992), *Appl. Biochem. Biotechnol.* **34–35,** 115–123.
9. Vlasenko, E. Y., Ding, H., Labavitch, J. M., and Shoemaker, S. P. (1997), *Bioresour. Technol.* **59**, 109–119.
10. McMillan, J. D. (1996), In: *Handbook on Bioethanol*: *Production and Utilization*. Wyman, C. E. (ed.), Taylor & Francis, Washington, DC, pp. 287–313.
11. Grethlein, H. E., Allen, D. C., and Converse, A. O. (1984), *Biotechnol. Bioeng.* **XXVI,** 1498–1505.
12. Grohmann, K., Torget, R., and Himmel, M. (1985), *Biotechnol. Bioeng. Symp.* **15,** 59–80.
13. Grohmann, K., Torget, R., and Himmel, M. (1986), *Biotechnol. Bioeng. Symp.* **17,** 135–151.
14. Torget, R., Walter, P., Himmel, M., and Grohmann, K. (1990), *Appl. Biochem. Biotechnol.* **24–25,** 115–126.
15. Torget, R., Walter, P., Himmel, M., and Grohmann, K. (1991), *Appl. Biochem. Biotechnol.* **28–29,** 75–86.
16. Nguyen, Q. A., Tucker, A. P., Boynton, B. L., Keller, F. A., and Schell, D. J. (1998), *Appl. Biochem. Biotechnol.* **70–72,** 77–87.
17. Nguyen, Q. A., Tucker, A. P., Keller, F. A., and Eddy, F. P. (2000), *Appl. Biochem. Biotechnol.* **84–86,** 561–576.
18. Um, B. H., Karim, M. N., and Henk, L. L. (2003), *Appl. Biochem. Biotechnol.* **105–108,** 115–125.

19. American Society of Testing and Materials (1997), Standard test method for direct moisture content measurement of wood and wood-base materials. Designation: ASTM D4444-92, West Conshohocken, PA, pp. 517–521.
20. Miller, G. L. (1959), *Anal. Chem.* **31(3),** 426–428.
21. Ghose, T. K. (1987), *Pure Appl. Chem.* **59(2),** 257–268.
22. Dische, D. (1962), In: *Method in Carbohydrate Chemistry*. Whistler, R. L., and Wolfrom, M. L., (eds.), Academic Press, New York, pp. 480–514.
23. Ahmed, A. E. and Labavitch, J. M. (1980), *Plant Physiol.* **65,** 1009–1013.
24. Blakeney, A. B., Harris, P. J., Henry, R. J., and Stone, B. A. (1983), *Carbohyd. Res.* **113,** 291–299.
25. Albersheim, P., Nevins, D. J., English, P. D., and Karr, A. (1967), *Carbohyd. Res.* **5,** 340–345.
26. Templeton, D. and Ehrman, T. (1995), NREL Analytical Procedure No. LAP-003, National Renewable Energy Laboratory, Golden, CO.
27. Ehrman, T. (1996), NREL Analytical Procedure No. 003, National Renewable Energy Laboratory, Golden, CO.
28. Sluiter, A., Hanes, B., Ruiz, R., Scarlata, C., Sluiter, J., and Templeton, D. (2005), NREL Analytical Procedure No. 004, National Renewable Energy Laboratory, Golden, CO.
29. Grohmann, K., Himmel, M., Rivard, C., et al. (1984), *Biotechnol. Bioeng. Symp.* **14,** 137–157.
30. Brigham, J. S., Adney, W. S., and Himmel, M. E. (1996), In: *Handbook on Bioethanol: Production and Utilization*. Wyman, C. E. (ed.), Taylor & Francis, Washington, DC, pp. 119–141.
31. Prieto, S., Clausen, E. C., and Gaddy, J. L. (1986), *Biotechnol. Bioeng. Symp.* **17,** 123–133.
32. Deanda, K., Zhang, M., Eddy, C., and Picataggio, S. (1996), *Appl. Environ. Microbial.* **62,** 4465–4470.
33. Dien, B. S., Hespell, R. B., Wyckoff, H. A., and Bothast, R. J. (1998), *Enzyme Microbial. Technol.* **23,** 366–371.
34. Aristidou, A. and Penttila, M. (2000), *Curr. Opin. Biotechnol.* **11,** 187–198.
35. Bothast, R. J., Nichols, N. N., Dien, B. S., and Cotta, M. A. (2002), *Society of Industrial Microbiology Annual Meeting*, paper No. 12-1, Philadelphia, PA.
36. Sues, A., Millati, R., Edebo, L., and Taherzadeh, M. J. (2005), *FEMS Yeast Res.* **5,** 669–676.
37. Ruohonen, L., Aristidou, A., Frey, A. D., Penttila, M., and Kallio, P. T. (2006), *Enzyme Microb. Technol.* **39,** 6–14.