

## Pretreatment of Lignocellulosic Biomass

One of the challenges in second generation fuels and chemicals is to find an economic way to break down the chemical bonds between the main components of lignocellulosic biomass and to make fermentable sugars available for conversion (Kumar et al., 2009). Potential lignocellulosic biomass for second generation fuels and chemicals production include crop residues (sugarcane bagasse, corn stover, corn fibers, rice straw, wheat straw, barley straw, coconut husks, sorghum bagasse), hardwood (black locust, poplar, eucalyptus), softwood (pine, spruce), herbaceous biomass (switchgrass, Bermuda grass), cellulose wastes (newsprint, recycled paper sludge) and municipal solid wastes (Yang, 2005; Cardona and Paz, 2010). Sweet sorghum and high fiber sugarcane (a.k.a. energy cane) are energy crops with great potential in Louisiana. Energy cane and sweet sorghum can be harvested after (January - March) or before (July - September) commercial sugarcane (October – December), have similar gross structure to sugarcane, and can be handled by the traditional sugarcane harvest and delivery system. Therefore, the processing of energy crops at a sugar mill becomes an attractive idea for the production of green fuels and chemicals.

Lignocellulosic biomass is composed mostly of carbohydrate polymers (cellulose and hemicellulose) and lignin with compositions varying amongst plant materials (Table 1). Proteins, ash and oils make up for the remaining fraction (Wyman, 1994). The cellulose and hemicellulose portions make up almost two thirds of the total dry mass. Only the carbohydrates are hydrolyzed to simple sugars and eventually fermented to fuels and chemicals. A schematic representation of the lignin-carbohydrate matrix is depicted in Fig. 1.

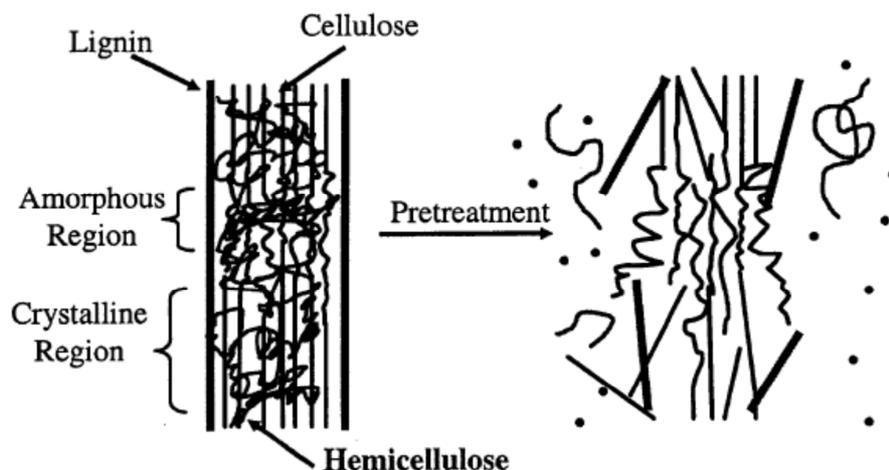


Fig. 1. Schematic Diagram of the Effect of Pretreatment on Biomass (adapted from Hsu et al., 1980).

Cellulose is composed of D-glucose monomers linearly linked by  $\beta$ - 1-4 glucosidic bonds which forms a mix of partially crystalline and partially amorphous structure (Aita and Kim, 2010). Hemicellulose is a heterogeneous combination of pentose sugars (xylose, arabinose), hexose sugars (glucose, galactose, and mannose) and sometimes uranic acids (glucuronic, methylgalacturonic and galacturonic). Lignin is another major components present in plant tissue which has a highly branched heteropolymer structure and can reduce enzyme hydrolysis efficiency by binding to the cellulose and prevent it from being productive (Aita and Kim, 2010). Hydrogen and Van-der Waals bonds link the long-chain cellulose polymers together and form cellulose microfibrils. These microfibrils are then covered by hemicellulose and lignin.

Table 1. Chemical composition of lignocellulosic biomass (percent dry basis) (Aita and Kim, 2010).

Feedstock	Cellulose (glucan)	Hemicellulose (xylan)	Lignin
Softwoods			
Douglas fir	50.0	2.4-3.4	28.3
Pine	44.6	5.3-8.8	27.7
Spruce	45	6.6	27.9
Hardwoods			
Black locust	41.6	17.7	26.7
Hybrid poplar	44.7	18.6	26.4
Eucalyptus	49.5	13.1	27.7
Populus tristis	40-49.9	13-17.4	18.1-20
Crop Residues			
Corn cobs	45.0	35.0	15.0
Corn fiber	14.3	16.8-35.0	8.4
Corn stover	36.8-39	14.8-25.0	15.1-23.1
Cotton gin trash	20.0	4.6	17.6
Grasses (sugarcane, sorghum bagasse)	25-50	25-50	10-30
Rice straw	35-41	25-14.8	9.9-12.0
Rice hulls	36.1	14.0	19.4
Wheat straw	30-38	21-50	20-23.4
Herbaceous Materials			
Bermuda grass	25.0	35.7	6.4
Switchgrass	31-32	20.4-25.2	14.5-18.1
Cellulose Wastes			
Newsprint	40-64.4	4.6-40	18.3-21
Paper	85-99	0.0	0-15

Pretreatment of lignocellulosic biomass is required to make sugar polymers accessible to enzymes for their conversion into fermentable sugars and further bioconversion to fuels and chemicals (Kumar et al., 2009). Numerous pretreatment methods have been suggested in the past decades and are generally categorized as mechanical, physicochemical, chemical, and biological processes or combinations of these approaches. Advantages and disadvantages of some pretreatment methods are summarized in Table 2.

Table 2. Advantages and disadvantages of some pretreatment technologies (Alvira et al., 2010).

Pretreatment	Advantages	Disadvantages
<b>Biological</b>	degrades lignin and hemicellulose low energy consumption	low rate hydrolysis
<b>Milling</b>	reduces cellulose crystallinity	high power and energy consumption
<b>Steam Explosion</b>	causes lignin transformation and hemicellulose solubilization cost effective higher yield of glucose and hemicellulose in the two-step method	generation of toxic compounds partial hemicellulose degradation
<b>AFEX</b>	increase accessible surface area low formation of inhibitors	not efficient for raw material with high lignin content high cost of large amount of ammonia
<b>CO<sub>2</sub> Explosion</b>	increase accessible surface area cost effective does not imply generation of toxic compounds	does not affect lignin and hemicellulose very high pressure requirement
<b>wet Oxidation</b>	efficient removal of lignin low formation of inhibitors minimizes the energy demand	high cost of oxygen and alkaline demand
<b>Ozonolysis</b>	reduces lignin content does not imply generation of toxic compounds	high cost of large amount of ozone needed
<b>Organosolv</b>	causes lignin and hemicellulose hydrolysis	high cost solvent needed to be drained and recycled
<b>Concentrated Acid</b>	high glucose yield ambient temperatures	high cost of acid and need to be recovered reactor corrosion problems formation of inhibitors
<b>Diluted Acid</b>	less corrosion problems than concentrated acids less formation of inhibitors	generation of degradation products low sugar concentration in exit stream

The purpose of pretreatment is to reduce cellulose crystallinity, remove lignin and/or hemicellulose and to increase biomass porosity. Table 3 summarizes the effect of pretreatment on the structure of biomass. An effective pretreatment favors the release of sugars or enhances their breakdown by enzyme hydrolysis, minimizes the loss of sugars and the formation of byproducts that are inhibitory to hydrolysis and fermentation processes, reduces energy demands and is cost-effective (Sun and Cheng, 2002).

Table 3. Effect of pretreatment technologies on the structure of lignocellulosic biomass (Alvira et al., 2010).

	Milling	Steam Explosion	LHW	Acid	Alkaline	Oxidative	AFEX	ARP	Lime	CO <sub>2</sub> Explosion
Increase accessible surface area	H	H	H	H	H	H	H	H	H	H
Cellulose decrystallization	H	-	n.d	-	-	n.d	H	H	n.d	-
Hemicellulose solubilization	-	H	H	H	L	-	M	M	M	H
Lignin removal	-	M	L	M	M	M	H	H	H	-
Generation of toxic compounds	-	H	L	H	L	L	L	M	M	-
Lignin structure alteration	-	H	M	H	H	H	H	H	H	-

H= high effect; M= moderate effect; L= low effect; n.d.= not determined; LHW= liquid hot water; ARP= ammonia recycle percolation; AFEX = ammonia fiber expansion.

## Pretreatment Technologies

### Mechanical Pretreatment

The objective of mechanical pretreatment is to breakdown the lignocellulosic biomass into small particles. Chopping, grinding or milling (e.g. ball milling, hammer milling, colloid milling) reduces cellulose crystallinity, increases the degree of polymerization and also the specific surface area of the lignocellulosic biomass thus increasing the total hydrolysis yield by 5-25% (Hendricks and Zeeman, 2009). Mechanical pretreatment is often used in combination with other technologies. The high energy requirement of this pretreatment is a major drawback and it is almost unlikely to be economically viable at a commercial scale.

### Physico-Chemical Pretreatment

#### *Steam Explosion*

Biomass is treated with high pressure saturated steam and the pressure is instantly reduced which causes an explosive decomposition of biomass. Typical temperatures for this method are 160 °C to 260°C which corresponds to 0.69 to 4.83 MPa pressure (Sun and Cheng, 2002). Acetic acid is produced from the acetyl groups in hemicellulose. Acetic acids and other weak acids (e.g. formic and levulinic) released during pretreatment may further catalyze hydrolysis and sugar degradation. Some lignin is removed by this pretreatment but it is redistributed on the fiber surfaces as result of the melting and depolymerization/repolymerization reactions (Li et al., 2007). Cellulose is mostly preserved in the solid fraction.

#### *Ammonia Fiber Expansion (AFEX)*

AFEX process is very similar to steam explosion. AFEX is usually conducted at temperatures of 60°C to 110°C for 5 to 30 min with a liquid ammonia dosage of 1 to 2 Kg/Kg dry biomass,

followed by an explosive pressure release (Sun and Cheng, 2002). During pretreatment, some lignin and hemicellulose are removed while decrystallizing the cellulose. The structure of the material is changed resulting in increased water holding capacity and higher digestibility (Galbe and Zacchi, 1997). Compared to acid pretreatment, AFEX has no significant effect on solubilization of hemicellulose (Vlasenko et al., 1997).

### *Supercritical Fluids*

It refers to a fluid that is in a gaseous form but it is compressed at temperatures above its critical point to a liquid like density but below the pressure required to condensate it into a solid (Jessop and Leitner, 1999). Carbon dioxide (CO<sub>2</sub>), water and propane are the most studied supercritical fluids. The high pressure facilitates the faster penetration of CO<sub>2</sub> molecules into the lignocellulosic structure. Under these conditions, carbon dioxide forms carbonic acid when in water, resulting in the hydrolysis of cellulose and hemicellulose fractions (Puri and Mamers, 1983). The hydrolyzed monomers can be further converted to furfurals, HMF, and other toxic byproducts under increasing temperatures and reaction times. The explosive release of the carbon dioxide pressure enhances substrate hydrolysis by disrupting the cellulosic structure.

### *Liquid Hot Water*

In this process water is used under pressure and at elevated temperatures to remain in the liquid state (Bobleter et al., 1981; Mok et al., 1992). This pretreatment takes place at temperatures in the range of 140°C to 300°C with a 15 min residence time, which results in the removal of 4 to 22% cellulose, 35% to 60% lignin and most of the hemicellulose. Liquid hot water cleaves the acetyl and uronic acid groups in hemicellulose generating acetic and other organic acids. The release of these acids helps catalyze the formation and removal of oligosaccharides. However, depending on the pretreatment conditions, polysaccharides can be further hydrolyzed to monomeric sugars and be partially degraded to furfurals and HMF (Palmqvist and Hahn-Hagerdal, 2000).

### *Irradiation*

Irradiation by  $\gamma$  rays, electron beam and microwave are some other physical ways to reduce the cristallinity of lignocellulosic material. However, these technologies remain expensive at full scale (Taherzadeh and Karimi, 2008).

## Chemical pretreatment

### *Ozonolysis*

Ozone is used to degrade lignin and hemicellulose. Degradation occurs more effectively to lignin and less effectively to hemicellulose and cellulose (Sun and Cheng, 2002). Although this

pretreatment requires large amounts of ozone which adds to the processing costs, it does not produce toxic compounds and it does not require high pressure and temperature (Vidal and Molinier, 1988).

### *Organosolv Process*

Solvents evaluated include those with low boiling points (methanol and ethanol), high boiling points (ethylene glycol, glycerol), ethers, ketones and phenols (Thring et al., 1990). Methanol and ethanol are mostly favored. For most organosolv solvents, pretreatment is conducted at temperatures below 180°C with the addition of acids as catalysts (sulfuric, phosphoric, hydrochloric, formic, oxalic, acetylsalicylic, salicylic) to dissolve the hemicellulose fraction and increase xylose yields (Sun and Chen, 2002).

### *Alkaline Hydrolysis*

Alkaline pretreatment processes can be divided into two major groups depending on the catalyst used, metal based (calcium, sodium, potassium) and ammonia based catalysts. Unlike acid processes, alkaline pretreatments are highly effective in removing lignin exhibiting minor cellulose and slightly higher hemicellulose solubilization (Pandey et al., 2000; Chang and Hotzapple, 2000). By this process, lignocellulose biomass can be fractionated into soluble lignin, hemicellulose and solid residue (mostly cellulose). Nevertheless, the effectiveness of this process depends on the lignin content of the biomass.

### *Acid Hydrolysis*

The purpose of acid pretreatment is to solubilize the hemicellulose fraction and to enhance cellulose digestibility in the residual solids. This type of pretreatment can be performed with concentrated or diluted acids and it has been tried on a wide range of feedstocks ranging from hardwood to grasses to agricultural residues. Sulfuric acid is the most studied acid. Others include hydrochloric acid, nitric acid, phosphoric acid, peracetic acid and organic acids such as fumaric and maleic. Diluted acid pretreatment favors the solubilization of hemicellulose, xylan in particular, but it also breaks down the solubilized hemicellulose to fermentable sugars. Depending on the operational conditions, decomposition products from pentoses (furfural) and hexoses (HMF) sugars, generation of acetic acids from the acetyl groups linked to hemicellulose, and aromatic lignin degradation compounds can be found in the liquid phase of the hydrolysates. Diluted acid pretreatments generate lower degradation products than concentrated acid pretreatments (Alvira et al., 2010).

### *Oxidative Pretreatment*

An oxidative pretreatment employs the addition of an oxidizing agent such as hydrogen peroxide, air or oxygen (wet oxidation). The objective is to remove lignin and hemicellulose with minimal sugar degradation and toxic compound formation. In many cases the oxidant used

is non-selective and losses in cellulose and hemicellulose can occur (Hendricks and Zeeman, 2009).

### *Ionic Liquids*

Ionic liquids (ILs) are thermally stable, non-toxic organic salts with potential application as “green solvents” (Sheldon et al., 2002). ILs can reduce the crystallinity of cellulose and partially remove hemicellulose (Qiu et al., 2012). Pretreatment with ILs are less energy demanding, easier to handle and more environmentally friendly than other pretreatment methods such as mechanical milling, steam explosion, acid, base, or organic solvent processes (Rogers and Seddon, 2003). ILs used during pretreatment contain anion of chloride, formate, acetate or alkylphosphonate which form strong hydrogen-bonds with cellulose and other carbohydrates (Zhao et al., 2009).

### Biological Pretreatment

In biological pretreatment processes, microorganisms such as white, brown and soft-rot fungi are used in the degradation of lignocellulosic biomass. Brown-rots attack cellulose, whereas white and soft-rots attack both cellulose and lignin. White-rot fungi degrade lignin through the action of peroxidases and laccases.

*A survey on pretreatment technologies (biochemical platform) available for the conversion of lignocellulosic sugars from energy cane and sorghum bagasse is presented in Tables 4 and 5. The survey compiles information on crop variety, pretreatment technology, chemical composition of biomass, and sugar yields.*

Table 4. Effect of pretreatment technologies on the chemical composition and sugar yields of sorghum.

Pretreatment	Variety	Biomass Composition	Pretreatment Conditions	Solid Loading	Enzyme Loading	Fermentation	Cellulose Digestibility		Hemicellulose Digestibility		Lignin		Ethanol yield	Chemical Composition after Pretreatment
											AIL % w/w	ASL % w/w		
SO <sub>2</sub> Catalyzed Steam pretreatment, (Shen et al., 2011)	Sweet sorghum, variety Liaotian 1	Glucan 35.1 Xylan 19.4 Galactan 1.5 Arabinan 1.4 Mannan 0.9 AIL 18.4 ASL 0.2 Ash 1.9 Extractives 21.2	Run#	10%	Enzyme loading of Spezyme-CP and Novzymes188 was 20 FPU/g glucan and 40 CBU/g glucan	<i>Sacharomyces cerevisiae</i> 5 g/L	N/A	N/A					g/100 g biomass	
			190 °C, 5 min, 0% SO <sub>2</sub>	10%			N/A	N/A	24.7	1.9	WSF 1.7 WIF 6.8	Glucan 54.2 Xylan 14.9 Arabinan 0.7		
			210 °C, 10 min, 0% SO <sub>2</sub>	10%			N/A	N/A	30.2	1.7	WSF 0.1 WIF 12.2	Glucan 61.8 Xylan 5.3 Arabinan 3		
			190 °C, 10 min, 0% SO <sub>2</sub>	10%			N/A	N/A	30	1.6	WSF 0 WIF 12.1	Glucan 60.8 Xylan 5.7 Arabinan 0.2		
			210 °C, 10 min, 0% SO <sub>2</sub>	10%			N/A	N/A	34	1.7	WSF 0 WIF 13	Glucan 62.7 Xylan		

											1.7	
			190 °C, 5 min, 5% SO <sub>2</sub>	10%			N/A	N/A	25.8	1.9	WSF 2.1 WIF 10.4	Arabinan 0.1
			210 °C, 5 min, 5% SO <sub>2</sub>	10%			N/A	N/A	39.3	1.6	WSF 0 WIF 10.2	Glucan 59.6 Xylan 9.4 Arabinan n.d.
			190 °C, 10min, 5% SO <sub>2</sub>	10%			N/A	N/A	32.7	1.7	WSF 2.1 WIF 10.9	Glucan 64.5 Xylan 3.7 Arabinan n.d.
			200 °C, 7.5 min, 2.5% SO <sub>2</sub> ( <b>best ethanol yield</b> )	10%			N/A	N/A	30.8	1.7	WSF 2.2 WIF 13	Glucan 63.6 Xylan n.d. Arabinan n.d.
Steam pretreatment with SO <sub>2</sub> , (Shen et al., 2011)	Sweet sorghum cultivar Liaotian I	Glucan 35.1 Xylan 19.4 Galactan 1.5 Arabinan 1.4 Mannan 0.9 AIL 18.4	190 °C, 5min.	10%	cellulase, β- glucosidase, Xylanase	N/A	N/A	N/A	N/A	N/A	N/A	Glucan 54.3 Xylan 9.8 Galactan 0.8 Arabinan 0.6 Mannan 1.2 AIL 25.8

		ASL 0.2 Ash 1.9									ASL 1.9 Ash 2.3
Steam pretreatment with SO <sub>2</sub> , (Shen et al., 2011)	Sweet sorghum cultivar Liaotian I	Glucan 35.1 Xylan 19.4 Galactan 1.5 Arabinan 1.4 Mannan 0.9 AIL 18.4 ASL 0.2 Ash 1.9	190 °C, 5min.	10%	cellulase and β-glucosidase and Xylanase	N/A	N/A	N/A	N/A	N/A	Glucan 55.7 Xylan 12.9 Galactan 0.2 Arabinan 0.7 Mannan 1.0 AIL 24.7 ASL 1.9 Ash 2.7
Steam pretreatment with H <sub>2</sub> SO <sub>4</sub> , (Yu, et al. 2010)	Sweet sorghum Rio	Glucose 3.0 Fructose 4.5 Sucrose 8.0 Glucan 4.6 Xylan 2.6 g/100g stem	H <sub>2</sub> SO <sub>4</sub> (0.25 g/g dry matter), 100 °C, 60 min.	10%	Cellulose (60 FPU/g DM)	<i>Saccharomyces cerevisiae</i>	N/A	N/A	N/A	44.5 g/L by SSF	N/A
Sulfuric acid (Xu et al., 2011)	PS sorghum (1990/AC791)	Glucan 32.5 Xylan 19.8 Lignin 11.7% Soluble sugar 17.5%	50 mM acetate buffer, 0.5 g yeast/L, 38 °C	9%	Accellerase 1500@ and β-glucosidase (0.5 ml/g glucan)	N/A	82.2% glucose recovery	N/A	N/A	0.21 g ethanol/g dry biomass by SSF	N/A
Microwave irradiation, (Su et al., 2010)	Kinmen Sorghum liquor waste	Glucan 17.2% Xylan 19% Lignin 18.5%	Washed and pH adjusted to 5, 6, 7 and 9 for different samples, dried at 60°C, deionized water added to 10% (w/v), microwaved at 800w for 3 min.	4%	Cellulase 25 FPU/g on dry weight base and cellobiase 30 IU/g dry weight base	<i>Saccharomyces cerevisiae</i>	mg/g dry weight base  212 for pH 5	mg/g dry weight base  30 for pH 5	N/A	mg/g dry weight base  0.13 for pH 5	N/A

						200 for pH 6 100 for pH 7 125 for pH 8 150 for pH 9	21 for pH 6 12 for pH 7 13 for pH 8 14 for pH 9		0.12 for pH 6 0.05 for pH 7 0.07 for pH 7 0.08 for pH 8	
Dilute acid (Zhang et al., 2011)	sweet sorghum "chuntian No. 2"	Xylan 26.3 Glucan 45.3 Lignin 15.2	Bagasse (10% w/v) was mixed with 1% (w/v) H <sub>2</sub> SO <sub>4</sub> for 10 min at 180 °C, washed and dried at 75 °C.	20 FPU/g dry substrates	N/A	59.40%	1.80%	26.50%	N/A	N/A
Lime (Zhang et al., 2011)	Sweet sorghum "chuntian No. 2"	Xylan 26.3 Glucan 45.3 Lignin 15.2	Bagasse (10% w/v) and lime (1.5% w/v) were mixed and autoclaved at 121 °C for 1 h.	20 FPU/g dry substrates	N/A	55.20%	15.10%	26.50%	N/A	N/A
Steam-explosion pretreatment (Zhang et al., 2011)	Sweet sorghum "chuntian No. 2"	Xylan 26.3 Glucan 45.3 Lignin 15.2	160 °C for 5 min.	enzyme loading was at 20 FPU/g dry substrates	N/A	48.20%	12.50%	23.40%	N/A	N/A
Ionic Liquids pretreatment, (Zhang et al., 2011)	Sweet sorghum "chuntian No. 2"	Xylan 26.3 Glucan 45.3 Lignin 15.2	10% w/w cellulose solution composed of 5 g baggase and 45 g Cl was prepared in a 110 °C oil bath for 1 h at 120 rpm. 200 ml deionized water was added immediately, precipitated cellulose was collected	20 FPU/g dry substrates	N/A	48.80%	16.70%	25.30%	N/A	N/A
Mild acid pretreatment,	Sorghum straw	Glucan 32.4	2% H <sub>2</sub> SO <sub>4</sub> with 10% (w/v) substrate at 134 °C , 60 min	20 FPU/g dry substrates	N/A	22.0 g/l	16.9 g/l	N/A	N/A	N/A

(Jeon et al., 2010)		Xylan 27 Lignin N/A									
Dilute sulfuric acid pretreatment, (Zhong et al., 2010)	Sweet sorghum	Glucan 48.3 Xylan 23.7 Lignin 17.3	30 g sorghum fibers were added to 300 ml of 2% sulfuric acid dilution, boiled for 1 hour and dried at 80 oC in an oven.	20 FPU/g dry substrates	N/A	75%	5%	15%	N/A	N/A	
Mild alkaline oxidative pretreatment (Zhong et al., 2010)	Sweet sorghum	Glucan 48.3 Xylan 23.7 Lignin 17.3	30 g sorghum treated with 300 ml 40% sodium hydroxide solution for 12 h at 40 oC, kept for another 12 h after adding hydrogen peroxide to 1% solution, fiber were washed	20 FPU/g dry substrates	N/A	80%	10%	4%	N/A	N/A	
Steam explosion pretreatment (Zhong et al., 2010)	Sweet sorghum	Glucan 48.3 Xylan 23.7 Lignin 17.3	1.5 Mpa for 5 min, then washed and dried at 80 oC.	20 FPU/g dry substrates	N/A	65%	10%	10%	N/A	N/A	
Dilute Ammonia pretreatment, (Salvi et al., 2010)	Sorghum bicolor (L.)	Ash 0.31 Lignin 22.04 Arabinan 2.79 Xylan 27.72 Mannan 0.72 Glucan 44.52%	biomass, ammonia and water mixed at a ratio of 1:0.14:8 at 160 °C, 1 h.	10%	full strength: 30 FPU Spezymes CP/g of glucan and 32 CBU Novozyme 188/g glucan; half strength: 15 FPU Spezymes and 16 CBU Novozyme /g glucan.	<i>Sacharomyces cerevisiae</i> (D <sub>2</sub> A)	84% at full strength, 73% at half-strength	73% at full strength, 55% at half-strength	44% lignin removal	84% ethanol at full strength, 73% at half-strength by SHF	Ash 0.38 Lignin 12.36 Arabinan 1.78 Xylan 18.01 Mannan 0.52 Glucan 40.18

Mild acid pretreatment (Vancov and McIntosh, 2012)	Sorghum straw, (bicolor var. MR Buster)	Neutral detergent fiber 63 Acid detergent fiber 36 Acid detergent lignin 2.9 Glucan 32.4 Xylan 27 AIL 7 Arabinose 3	2% H <sub>2</sub> SO <sub>4</sub> for 60 min at 121 °C	10% (w/v)	solid loading of 5% dry matter (w/v) Cellulase (NS50013), $\beta$ -glucosidase (NS50010) and xylanase (NS50030), 70 (FPU)/g	N/A	N/A	N/A	N/A	N/A	328 mg/g glucose, 179 mg/g xylose, 33 mg/g arabinose and 20 mg/g fructose
Diluted acid pretreatment, (Sajid et al., 2009)	sorghum straw (variety YSS-9)	Cellulose 35.01 ± 0.71 Hemicellulose 24.40 ± 1.06 Lignin 19 ± 2.07 Ash 7.02 ± 1.93 Moisture 4.98 ± 0.11	(121°C, 1% acid H <sub>2</sub> SO <sub>4</sub> concentration and 60 min)	20% (w/v)	1. solid load of 5% DM (w/w), 2. D105 enzyme loading in the reaction mixture was 3:1 ratio of celluloclast (10 FPU/g cellulose) and Novozyme 188, respectively 50°C and 160 rpm for 48 h	Saccharomyces cerevisiae Ethanol Red	71.98% (0.28 g/g DM) of cellulose 84.84% (0.23 g/g DM) of hemicellulose was converted	84.84% (0.23 g/g DM) of hemicellulose was converted	N/A	maximum yield was obtained at 96 h but in SHF with detoxification of inhibitors	fed batch (SSF) 120 min, ethanol yield 0.1332 g/g DM
Microwave (Dogaris et al., 2009)	N/A	Glucan 40.4% Xylan 35.5% (w/w)	210 °C for 20 min.	6.7 g /g	N/A	N/A	N/A	N/A	N/A	N/A	Glucan 58.36 Xylan 10.05
Dilute sulfuric acid (Wu et al., 2011)	Sorghum cv. <i>Kyushuko4</i>	Glucan 38.7 Xylan 22.6 AIL 15.4 Ash 2.2	0.5 % (w/w) acid, 170 °C, 5–60 min.	10%	Celluclast 1.5L and Novozyme 188 at 20 FPU, 50 CBU/g glucan	N/A	70% after 60 min	N/A	N/A	N/A	N/A

NaOH pretreatment (A) (Cao et al., 2012)	Sweet sorghum (Chongming No. 1)	N/A	10 g bagasse was mixed with 100 mL 2% (w/v) NaOH solution for 5 min, autoclaved at 121 °C for 60 min. The yield of product was $5.978 \pm 0.212$ g	10%	Celluclast 1.5L, $\beta$ -glucosidase at 20 FPU/g and 40 IU/g dry biomass	Active dry yeast	12.64 g/L	N/A	Acid detergent lignin (ADL) removal was 84.52%	5.55 g/L	Glucan 78.44 Xylan 15.09 ADL 1.68
High concentration NaOH (B) (Cao et al., 2012)	Sweet sorghum (Chongming No. 1)	N/A	10 g bagasse mixed with 100 mL 20% (w/v) sodium hydroxide solution for 5 min, and then stood for 2 h, The yield of product was $1.642 \pm 0.141$ g	10%			72.89 g/L	N/A	Acid detergent lignin (ADL) removal was 30.82%	Ethanol concentration was 5.73 g/L	Glucan 69.9 Xylan 13.94 ADL 7.5
Dilute NaOH solution autoclaving, H <sub>2</sub> O <sub>2</sub> immersing pretreatment C (Cao et al., 2012)	Sweet sorghum (Chongming No. 1)	N/A	10 g bagasse mixed with 100 mL 2% (w/v) NaOH for 5 min, 121 °C for 60 min. 5% (w/v) hydrogen peroxide was mixed into the pretreated slurry. The yield of product was $5.432 \pm 0.038$ g	10%			74.29 g/L	NA	Acid detergent lignin (ADL) removal was 91.02%	6.12 g/L	Glucan 82.08 Xylan 9.45 ADL 0.97
Alkaline peroxide pretreatment (D) (Cao et al., 2012)	Sweet sorghum (Chongming No. 1)	N/A	10 g bagasse mixed with 100 mL 2% (w/v) NaOH for 5 min, and then stood for 2 h. 5% (w/v) hydrogen peroxide was mixed into the pretreated slurry. The yield of product was $6.856 \pm 0.014$ g.	10%			Cellulose Hydrolysis yield was 62.46 g/L	N/A	Acid detergent lignin (ADL) removal was 78.84%	4.93 g/L	Glucan 72.45 Xylan 17.52 ADL 2.29

Autoclaving pretreatment (E) (Cao et al., 2012)	Sweet sorghum (Chongming No. 1)	N/A	10 g bagasse mixed with 100 mL distilled water for 5 min, 121 °C for 60 min. The yield of product was 8.824 ± 0.156 g.	10%		Cellulose Hydrolysis yield was 11.7 g/L	N/A	Acid detergent lignin (ADL) removal was 1.16%	0.89 g/L	Glucan 54.40 Xylan 22.44 ADL 10.71
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ASL= acid soluble lignin; AIL= acid insoluble lignin; SHF= separate hydrolysis and fermentation; SSF= simultaneous saccharification and fermentation; WSF= water soluble; WIF= water insoluble fraction; N/A= data not available; n.d.= not detectable.

Table 5. Effect of pretreatment technologies on the chemical composition and sugar yields of energy cane.

Pretreatment	Biomass Variety	Biomass Composition	Pretreatment Conditions	Enzyme Loading	Enzyme Hydrolysis Yield	Microorganism	Ethanol Yield
Dilute acid pretreatment (Shields and Boopathy, 2011)	Energy cane (leaves) L 79-1002	Glucan 36% Xylan 28% Lignin 20% Other organics 13%	0.8 M (7.8% v/v) H <sub>2</sub> SO <sub>4</sub> , 121 °C, 20 min.	N/A	N/A	<i>K. oxytoca</i>	700 mg/L
			1.4 M (7.8% v/v) H <sub>2</sub> SO <sub>4</sub> , 121 °C, 20 min.	N/A	N/A	<i>K. oxytoca</i>	820 mg/L
			1.6 M (7.8% v/v) H <sub>2</sub> SO <sub>4</sub> , 121° C, 20 min	N/A	N/A	<i>K. oxytoca</i>	590 mg/L
Dilute ammonia pretreatment (Aita et al., 2011)	Energy cane L 79-1002	Glucan 46% Xylan 25% Lignin 14-17%	0.9-1.1 MPa pressure at 160 °C with NH <sub>4</sub> OH (28% v/v solution) and water, ratio: 1:0.5:8 for 1h	Novozyme 188 at 30 FPU/g glucan, and Spezyme CP at 32 CBU/g glucan	cellulose digestibility: 78%, hemicellulose digestibility: 39%	<i>Saccharomyces cerevisiae</i>	19 g/100 g dry biomass (cellulose only)
		same as above	same as above	Novozyme 188 at 60 FPU/g glucan, and Spezyme CP at 64 CBU/g glucan	cellulose digestibility: 88%, hemicellulose digestibility: 49%	<i>Saccharomyces cerevisiae</i>	23 g/100 g dry biomass (cellulose only)
Ionic liquid pretreatment, (Qiu et al., 2012)	Energy cane (stalks) L 79-1002	Glucan 40.9% Xylan 20.8% Lignin 24.8%	1-ethyl-3-methylimidazolium acetate, mixed with biomass at a 20:1 ratio and heated to 120 °C for 30 min.	15 FPU/g glucan and Novozyme 188 at 15 CBU/g glucan	cellulose digestibility: 68.9%, hemicellulose digestibility: 46.5%	N/A	N/A
				30 FPU/g glucan and Novozyme 188 at 30 CBU/g glucan	cellulose digestibility: 87%, hemicellulose digestibility: 64.3%	N/A	N/A

N/A= data not available.

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