Review

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Methods for Pretreatment of Lignocellulosic Biomass for Efficient Hydrolysis and Biofuel Production

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Biofuels produced from various lignocellulosic materials, such as wood, agricultural, or forest residues, have the potential to be a valuable substitute for, or complement to, gasoline. Many physicochemical structural and compositional factors hinder the hydrolysis of cellulose present in biomass to sugars and other organic compounds that can later be converted to fuels. The goal of pretreatment is to make the cellulose accessible to hydrolysis for conversion to fuels. Various pretreatment techniques change the physical and chemical structure of the lignocellulosic biomass and improve hydrolysis rates. During the past few years a large number of pretreatment methods have been developed, including alkali treatment, ammonia explosion, and others. Many methods have been shown to result in high sugar yields, above 90% of the theoretical yield for lignocellulosic biomasses such as woods, grasses, corn, and so on. In this review, we discuss the various pretreatment process methods and the recent literature that has reported on the use of these technologies for pretreatment of various lignocellulosic biomasses.

1. Introduction

Long-term economic and environmental concerns have resulted in a great amount of research in the past couple of decades on renewable sources of liquid fuels to replace fossil fuels. Burning fossil fuels such as coal and oil releases CO_2, which is a major cause of global warming.[1] With only 4.5% of the world's population, the United States is responsible for about 25% of global energy consumption and 25% of global CO_2 emissions.[1] The average price of gasoline in 2005 was $2.56 per gallon, which was $0.67 higher than the average price of gasoline in the previous year.[1] Yet, in June 2008, the average price of gasoline in the United States reached $4.10 per gallon.[2]

Conversion of abundant lignocellulosic biomass to biofuels as transportation fuels presents a viable option for improving energy security and reducing greenhouse emissions.[3] Unlike fossil fuels, which come from plants that grew millions of years ago, biofuels are produced from plants grown today. They are cleaner-burning than fossil fuels, and the short cycle of growing plants and burning fuel made from them does not add CO_2 to the atmosphere. It has been reported that cellulosic ethanol and ethanol produced from other biomass resources have the potential to cut greenhouse gas emissions by 86%.[4] Lignocellulosic materials such as agricultural residues (e.g., wheat straw, sugarcane bagasse, corn stover), forest products (hardwood and softwood), and dedicated crops (switchgrass, salix) are renewable sources of energy. These raw materials are sufficiently abundant and generate very low net greenhouse emissions. Approximately 90% of the dry weight of most plant materials is stored in the form of cellulose, hemicellulose, lignin, and pectin.[1] The presence of lignin in lignocelluloses leads to a protective barrier that prevents plant cell destruction by fungi and bacteria for conversion to fuel. For the conversion of biomass to fuel, the cellulose and hemicellulose must be broken down into their corresponding monomers (sugars), so that microorganisms can utilize them. Three major hydrolysis processes are typically used to produce a variety of sugars suitable for ethanol production: dilute acid, concentrated acid, and enzymatic hydrolysis.[5] Hemicellulose can be readily hydrolyzed by dilute acids under moderate conditions, but much more extreme conditions are needed for cellulose hydrolysis. In the dilute-acid process, the reaction is carried out at high temperature and pressure, and because of low yields of glucose from cellulose in the hydrolysis step, the ethanol yield is low. The use of concentrated acid in the hydrolysis process can yield higher quantities of ethanol because of the approximately 100% conversion to glucose from cellulose. The dilute-acid hydrolysis process uses high temperatures (160−230 °C) and pressures (∼10 atm).[6] The acid concentration in the dilute-acid hydrolysis process is in the range of 2−5%.[5,7] The acid concentration used in the concentrated-acid hydrolysis process is in the range of 10−30%.[5] Lower operating temperatures (<50 °C) and atmospheric pressures are required during the concentrated-acid hydrolysis process. The concentrated-acid hydrolysis involves longer retention times and results in higher ethanol yields than the dilute-acid hydrolysis process.[5] Enzymes produced by a variety of microorganisms are also capable of breaking down lignocellulosic materials to sugars but require longer retention times. Enzymatic hydrolysis is the most common method of producing ethanol from lignocellulosic biomasses.

The digestibility of cellulose present in lignocellulosic biomass is hindered by many physicochemical, structural, and compositional factors. In the conversion of lignocellulosic biomass to fuel, the biomass needs to be treated so that the cellulose in the plant fibers is exposed. Pretreatment uses various techniques, including ammonia fiber explosion, chemical treatment, biological treatment, and steam explosion, to alter the structure of cellulosic biomass to make cellulose more accessible.[8] Then, acids or enzymes can be used to break down the cellulose into its constituent sugars. Enzyme hydrolysis is widely used to break down cellulose into its constituent sugars. The goal of pretreatment in biomass-to-biofuels conversion is depicted in Figure 1.

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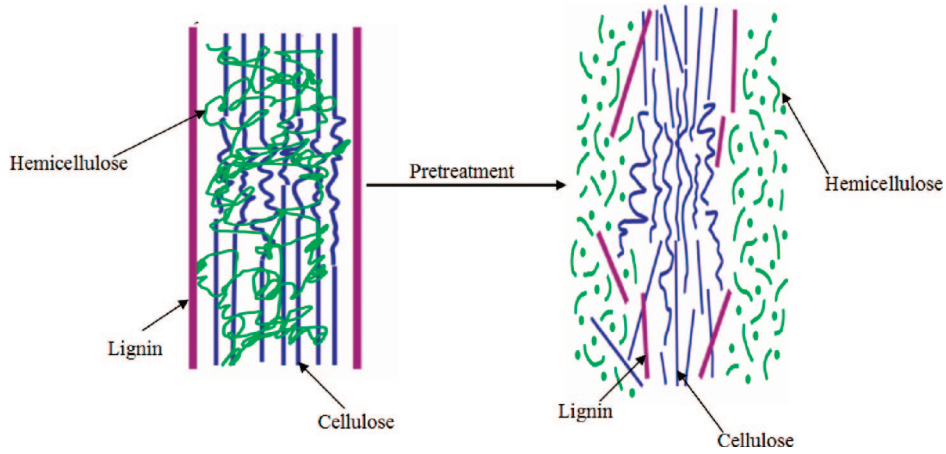

Figure 1. Schematic of the role of pretreatment in the conversion of biomass to fuel. (Adapted from ref 8.)

Table 1. Cellulose, Hemicellulose, and Lignin Contents in Common Agricultural Residues and Wastes[a]

lignocellulosic material	cellulose (%)	hemicellulose (%)	lignin (%)
hardwood stems	40−55	24−40	18−25
softwood stems	45−50	25−35	25−35
nut shells	25−30	25−30	30−40
corn cobs	45	35	15
grasses	25−40	35−50	10−30
paper	85−99	0	0−15
wheat straw	30	50	15
sorted refuse	60	20	20
leaves	15−20	80−85	0
cotton seed hairs	80−95	5−20	0
newspaper	40−55	25−40	18−30
waste papers from chemical pulps	60−70	10−20	5−10
primary wastewater solids	8−15		
solid cattle manure	1.6−4.7	1.4−3.3	2.7−5.7
coastal bermudagrass	25	35.7	6.4
switchgrass	45	31.4	12
swine waste	6.0	28	na

[a] Adapted from ref 14.

The goal of the pretreatment process is to break down the lignin structure and disrupt the crystalline structure of cellulose, so that the acids or enzymes can easily access and hydrolyze the cellulose.[9] Pretreatment can be the most expensive process in biomass-to-fuels conversion but it has great potential for improvements in efficiency and lowering of costs through further research and development.[9−13] Pretreatment is an important tool for biomass-to-biofuels conversion processes and is the subject of this review article.

2. Structure of Lignocellulosic Biomass

Lignocellulose is the primary building block of plant cell walls. Plant biomass is mainly composed of cellulose, hemicellulose, and lignin, along with smaller amounts of pectin, protein, extractives (soluble nonstructural materials such as nonstructural sugars, nitrogenous material, chlorophyll, and waxes), and ash.[14] The composition of these constituents can vary from one plant species to another. For example, hardwood has greater amounts of cellulose, whereas wheat straw and leaves have more hemicellulose (Table 1).[15] In addition, the ratios between various constituents within a single plant vary with age, stage of growth, and other conditions.[16]

Cellulose is the main structural constituent in plant cell walls and is found in an organized fibrous structure. The structure of cellulose is shown in Figure 2. This linear polymer consists of D-glucose subunits linked to each other by β-(1,4)-glycosidic

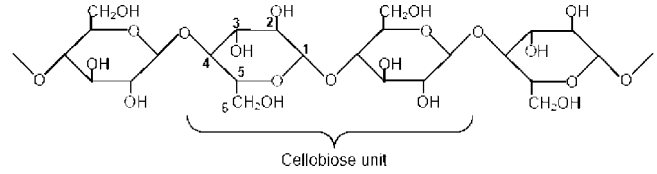

Figure 2. Illustration of a cellulose chain.

bonds. Cellobiose is the repeat unit established through this linkage, and it constitutes cellulose chains. The long-chain cellulose polymers are linked together by hydrogen and van der Waals bonds, which cause the cellulose to be packed into microfibrils. Hemicelluloses and lignin cover the microfibrils. Fermentable D-glucose can be produced from cellulose through the action of either acid or enzymes breaking the β-(1,4)-glycosidic linkages. Cellulose in biomass is present in both crystalline and amorphous forms. Crystalline cellulose comprises the major proportion of cellulose, whereas a small percentage of unorganized cellulose chains form amorphous cellulose. Cellulose is more susceptible to enzymatic degradation in its amorphous form.[17]

The main feature that differentiates hemicellulose from cellulose is that hemicellulose has branches with short lateral chains consisting of different sugars. These monosaccharides include pentoses (xylose, rhamnose, and arabinose), hexoses (glucose, mannose, and galactose), and uronic acids (e.g., 4-*o*-methylglucuronic, D-glucuronic, and D-galactouronic acids). The backbone of hemicellulose is either a homopolymer or a heteropolymer with short branches linked by β-(1,4)-glycosidic bonds and occasionally β-(1,3)-glycosidic bonds.[18] Also, hemicelluloses can have some degree of acetylation, for example, in heteroxylan. In contrast to cellulose, the polymers present in hemicelluloses are easily hydrolyzable. These polymers do not aggregate, even when they cocrystallize with cellulose chains.

Lignin is a complex, large molecular structure containing cross-linked polymers of phenolic monomers. It is present in the primary cell wall, imparting structural support, impermeability, and resistance against microbial attack.[16] Three phenyl propionic alcohols exist as monomers of lignin: coniferyl alcohol (guaiacyl propanol), coumaryl alcohol (*p*-hydroxyphenyl propanol), and sinapyl alcohol (syringyl alcohol). Alkyl−aryl, alkyl−alkyl, and aryl−aryl ether bonds link these phenolic monomers together. In general, herbaceous plants such as grasses have the lowest contents of lignin, whereas softwoods have the highest lignin contents (Table 1).

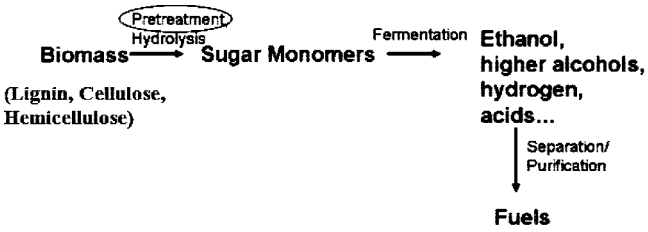

Figure 3. Schematic of the conversion of lignocellulosic biomass to fuel.

3. Overview of the Conversion of Biomass to Fuel

Action of microorganisms and enzymes on biological sources can lead to the production of mostly ethanol and, less commonly, propanol and butanol. These agents carry out the fermentation of sugar, starch, hemicellulose, or cellulose, with cellulose fermentation being the most difficult. Biobutanol, which is also called biogasoline, is often claimed to provide a direct replacement for gasoline, because it can be used directly in a gasoline engine similarly to the way in which biodiesel can be used in diesel engines. There has been extensive research on the conversion of lignocellulosic materials to fuels, especially ethanol, in the past few decades.

A schematic for the conversion of biomass to fuel is shown in Figure 3. The conversion includes the hydrolysis of various components in the lignocellulosic materials to fermentable reducing sugars and the fermentation of the sugars to fuels such as ethanol and butanol. The pretreatment step is mainly required for efficient hydrolysis of cellulose to its constituent sugars. The hydrolysis is usually catalyzed by acids or cellulase enzymes, and the fermentation is carried out by yeasts or bacteria. The factors affecting the hydrolysis of cellulose include porosity (accessible surface area) of the biomass materials, cellulose fiber crystallinity, and content of both lignin and hemicellulose.[19] The presence of lignin and hemicellulose makes the accessibility of cellulase enzymes and acids to cellulose more difficult, thus reducing the efficiency of the hydrolysis process. Pretreatment is required to alter the size and structure of the biomass, as well as its chemical composition, so that the hydrolysis of the carbohydrate fraction to monomeric sugars can be achieved rapidly and with greater yields. The hydrolysis process can be significantly improved by removal of lignin and hemicellulose, reduction of cellulose crystallinity, and increase of porosity through pretreatment processes.[19]

In the hydrolysis process, the sugars are released by breaking down the carbohydrate chains, before they are fermented for alcohol production. The cellulose hydrolysis processes include (1) acid hydrolysis and (2) an enzymatic hydrolysis. In traditional methods developed in the 19th and early-20th centuries, hydrolysis is performed by reacting the cellulose with an acid. Dilute acid can be used under conditions of both high temperature and pressure, or concentrated acid can be used at lower temperatures and atmospheric pressure. The decrystallized cellulosic mixture of acid and sugars reacts in the presence of water to release individual sugar molecules. The dilute-acid process is a harsh process that leads to the formation of toxic degradation products that can interfere with fermentation. Cellulose chains can also be broken down into individual glucose sugar molecules by enzymes known as cellulase. Cellulase refers to a class of enzymes produced chiefly by fungi, bacteria, and protozoans that catalyze the hydrolysis of cellulose. However, there are also cellulases produced by plants and animals. The reaction occurs at body temperature in the stomachs of ruminants such as cows and sheep, where the enzymes are produced by intestinal bacteria. Lignocellulosic materials can similarly be enzymatically hydrolyzed under relatively mild conditions (50 °C and pH ∼5), enabling effective cellulose breakdown without the formation of byproducts that would otherwise inhibit enzyme activity.

The six-carbon sugars, or hexoses, glucose, galactose, and mannose are readily fermented to ethanol by many naturally occurring organisms.[9] Baker's yeast, or *Saccharomyces cerevisiae*, has been traditionally used in the brewing industry to produce ethanol from hexoses. Because of the complex nature of the carbohydrates present in lignocellulosic biomasses, five-carbon sugars such as xylose and arabinose, derived from the hemicellulose portion of the lignocellulose, are also present in the hydrolysate. For example, the hydrolysate of corn stover contains approximately 30% of the total fermentable sugars as xylose. As a result, the ability of the fermenting microorganisms to utilize the whole range of sugars available from the hydrolysate is vital to increasing the economic competitiveness of cellulosic ethanol and potentially biobased chemicals. In recent years, metabolic engineering of microorganisms used in fuel ethanol production has shown significant progress.[20] Microorganisms such as *Zymomonas mobiliz* and *Escherichia coli*, in addition to *Saccharomyces cerevisiae*, have been targeted through metabolic engineering for cellulosic ethanol production. Recently, engineered yeasts have been reported to efficiently ferment xylose[21] and arabinose,[22] as well as mixtures of xylose and arabinose.[23]

The recovery of fuels from the fermentation broth is achieved by distillation or a combination of distillation and adsorption. The other components, including residual lignin, unreacted cellulose and hemicellulose, and enzymes, accumulate at the bottom of the distillation column.

In the following sections, different techniques used for the pretreatment of lignocellulosic biomass are summarized. The effects of pretreatments on the composition of lignocellulosic biomass and structural changes are also discussed in detail.

4. Pretreatment of Lignocellulosic Materials

Goals of Pretreatment. The beneficial effects of pretreatment of lignocellulosic materials have been recognized for a long time.[19] The goal of the pretreatment process is to remove lignin and hemicellulose, reduce the crystallinity of cellulose, and increase the porosity of the lignocellulosic materials. Pretreatment must meet the following requirements: (1) improve the formation of sugars or the ability to subsequently form sugars by hydrolysis, (2) avoid the degradation or loss of carbohydrate, (3) avoid the formation of byproducts that are inhibitory to the subsequent hydrolysis and fermentation processes, and (4) be cost-effective.

Pretreatment methods can be roughly divided into different categories: physical (milling and grinding), physicochemical (steam pretreatment/autohydrolysis, hydrothermolysis, and wet oxidation), chemical (alkali, dilute acid, oxidizing agents, and organic solvents), biological, electrical, or a combination of these. The following pretreatment technologies have promise for cost-effective pretreatment of lignocellulosic biomass for biological conversion to fuels and chemicals.

4.1. Physical Pretreatment. 4.1.1. Mechanical Comminution. Comminution of lignocellulosic materials through a combination of chipping, grinding, and/or milling can be applied to reduce cellulose crystallinity. The size of the materials is usually 10−30 mm after chipping and 0.2−2 mm after milling or grinding.[15] Vibratory ball milling was found to be more effective than ordinary ball milling in reducing cellulose

crystallinity of spruce and aspen chips and in improving their digestibility.[24] The final particle size and biomass characteristics determine the power requirement for mechanical comminution of agricultural materials.[25] The energy consumption for size reduction of hardwoods and agricultural wastes as a function of final particle size and comminution ratio (size reduction) was quantified by Cadoche et al.[25] It was proposed that, if the final particle size is held to the range of 3−6 mm, the energy input for comminution can be kept below 30 kWh per ton of biomass. The energy consumption is higher than the theoretical energy content available in the biomass in most cases. Irradiation of cellulose by γ-rays, which leads to cleavage of β-1,4-glycosidic bonds and gives a larger surface area and a lower crystallinity, has also been tested.[26] This method is far too expensive, however, to be used in a full-scale process.[13]

4.1.2. Pyrolysis. Pyrolysis has also been used for the pretreatment of lignocellulosic materials. Cellulose rapidly decomposes to gaseous products and residual char when biomass is treated at temperatures greater than 300 °C.[27,28] At lower temperatures, the decomposition is much slower, and the products formed are less volatile. Fan et al. reported that mild acid hydrolysis (1 N H_2SO_4, 97 °C, 2.5 h) of the products from pyrolysis pretreatment resulted in 80−85% conversion of cellulose to reducing sugars with more than 50% glucose.[29] The pyrolysis process is enhanced when carried out in the presence of oxygen.[28] Zwart et al. reported production of transportation fuels from biomass via a so-called biomass-to-liquids (BtL) route, in which biomass is converted to syngas from which high-quality Fischer−Tropsch (FT) fuels are synthesized.[30] Chipping, pelletization, torrefecation, and pyrolysis have been studied as pretreatment processes for biomass-to-FT-fuel conversion. Pretreatment by torrefecation was found to be far more attractive than pyrolysis.

4.2. Physicochemical Pretreatment. 4.2.1. Steam Explosion. Steam explosion is the most commonly used method for the pretreatment of lignocellulosic materials.[19] In this method, biomass is treated with high-pressure saturated steam, and then the pressure is suddenly reduced, which makes the materials undergo an explosive decompression. Steam explosion is typically initiated at a temperature of 160−260 °C (corresponding pressure, 0.69−4.83 MPa) for several seconds to a few minutes before the material is exposed to atmospheric pressure.[15] The biomass/steam mixture is held for a period of time to promote hemicellulose hydrolysis, and the process is terminated by an explosive decompression. The process causes hemicellulose degradation and lignin transformation due to high temperature, thus increasing the potential of cellulose hydrolysis. Hemicellulose is thought to be hydrolyzed by acetic and other acids released during steam-explosion pretreatment. Grous et al. reported that 90% efficiency of enzymatic hydrolysis was achieved in 24 h for poplar chips pretreated by steam explosion, compared to only 15% hydrolysis of untreated chips.[31] Removal of hemicelluloses from the microfibrils is believed to expose the cellulose surface and increase enzyme accessibility to the cellulose microfibrils.[32] Lignin is removed only to a limited extent during the pretreatment but is redistributed on the fiber surfaces as a result of melting and depolymerization/repolymerization reactions.[33] The removal and redistribution of hemicellulose and lignin increase the volume of the pretreated sample. Rapid flashing to atmospheric pressure and turbulent flow of the material cause fragmentation of the material, thereby increasing the accessible surface area.[34] Depending on the severity of the pretreatment, some degradation of the cellulose to glucose can take place.[14]

Water acts as an acid at high temperatures.[35−37] Addition of H_2SO_4 (or SO_2) or CO_2 [typically 0.3−3% (w/w)] in steam explosion can decrease time and temperature, effectively improve hydrolysis, decrease the production of inhibitory compounds, and lead to complete removal of hemicellulose.[38,39] For pretreatment of softwoods, the addition of an acid catalyst is a prerequisite to make the substrate accessible to enzymes.[14,34,39] Steam provides an effective vehicle to rapidly heat cellulosics to the target temperature without excessive dilution of the resulting sugars. Rapid pressure release reduces the temperature and quenches the reaction at the end of the pretreatment. The rapid thermal expansion used to terminate the reaction opens up the particulate structure of the biomass, but enhancement of the digestibility of the cellulose in the pretreated solid is only weakly correlated with this physical effect.[14]

The factors that affect steam-explosion pretreatment are residence time, temperature, chip size, and moisture content.[34,40] Optimal hemicellulose solubilization and hydrolysis can be achieved by either high temperature and short residence time (270 °C, 1 min) or lower temperature and longer residence time (190 °C, 10 min).[34] The advantages of steam-explosion pretreatment include the low energy requirement compared to mechanical comminution and no recycling or environmental costs. The conventional mechanical methods require 70% more energy than steam explosion to achieve the same particle size reduction.[41] Steam pretreatment with addition of a catalyst is the technology that has been claimed to be closest to commercialization.[41] The pretreatment has been tested extensively for a large number of different lignocellulosic feedstocks. The technology has been scaled-up and operated at the pilot-plant scale at the Iogen demonstration plant in Canada.[14] Steam explosion is recognized as one of the most cost-effective pretreatment processes for hardwoods and agricultural residues, but it is less effective for softwoods.[15]

Kobayashi et al. extended the use of steam-explosion technology, primarily by applying it to improving the fermentation process for the conversion of bamboo into methane.[42] Digestion sludge obtained from a sewage treatment plant was used as an original microbial seed for the methane fermentation process. Methane could not be produced from raw bamboo, but methane production was enhanced by steam explosion. The maximum amount of methane produced, about 215 mL, was obtained from 1 g of exploded bamboo at a steam pressure of 3.53 MPa and steam application for 5 min. A negative correlation between the amount of methane produced and the amount of Klason lignin (i.e., high-molecular-weight lignin) was observed in the methane fermentation of steam-exploded bamboo. Ballestros et al.[43] evaluated the effect of particle size on the steam-explosion pretreatment of herbaceous lignocellulosic biomass. Chipped *B. carinata* biomass (5% moisture) was used in this study. The parameters tested were particle size (2−5, 5−8, and 8−12 mm), temperature (190 and 210 °C), and residence time (4 and 8 min). Higher cellulose recoveries were observed at larger particle size (8−12 mm) compared to small particle sizes for all pretreatment conditions tested. After pretreatment, the water-insoluble fiber was enzymatically hydrolyzed to determine the maximum obtainable sugar yield. Cellulase (Celluclast 1.5 L) enzyme loading was 15 filter paper units (FPU) per gram of substrate. Enzymatic hydrolysis was performed at 50 °C on a rotary shaker at 150 rpm for 72 h and at 2% (w/v) substrate concentration. Enzymatic hydrolysis yields of 70% were obtained for biomass samples steam pretreated at lower temperatures (190 °C) and residence times of 4 and 8 min. Higher enzymatic hydrolysis yields of about 99% were obtained for samples pretreated at

210 °C. In a recent study, Viola et al.[44] reported steam-explosion treatment of wheat, barley, and oat straws. The steam-explosion treatment was optimized at the batch scale on the basis of carbohydrate recovery. The yields of fodder, lignin, and hemicellulose were found to be dependent on the nature of the starting straw. The yield was expressed as weight of dry product/starting weight of dry straw. Delignified fodder (insoluble fraction) was produced with yields of 0.64, 0.59, and 0.55 from wheat, barley, and oat straw, respectively. Samples of feeds and products were analyzed for dry matter by being dried at 60 °C for 16 h to calculate the digestibility coefficients as (feed − undigested product)/(feed). Steam explosion improved the digestibility of the straw by 25%. Cara and co-workers[45] studied the production of fuel ethanol from olive-tree pruning. Olive-tree-pruning biomass was subjected to steam-explosion pretreatment in the temperature range of 190−240 °C, with and without previous impregnation by water or sulfuric acid solution. The influence of both pretreatment temperature and impregnation conditions on sugar and ethanol yields was investigated by enzymatic hydrolysis and simultaneous saccharification and fermentation (SSF) on the pretreated solids. The results showed that the maximum ethanol yield (7.2 g of ethanol/100 g of raw material) was obtained from water-impregnated residue that was steam pretreated at 240 °C. SSF performance was evaluated by SSF yields, expressed as a percentage of the maximum theoretical ethanol yield (0.51 g of ethanol/g of glucose), considering that all of the glucose in the pretreated material is available for fermentation.

Limitations of steam explosion include destruction of a portion of the xylan fraction, incomplete disruption of the lignin−carbohydrate matrix, and generation of compounds that might be inhibitory to microorganisms used in downstream processes.[46] Because of the formation of degradation products that are inhibitory to microbial growth, enzymatic hydrolysis, and fermentation, pretreated biomass needs to be washed with water to remove the inhibitory materials along with water-soluble hemicellulose.[19] The water wash decreases the overall saccharification yield through the removal of soluble sugars, such as those generated by hydrolysis of hemicellulose.

Pretreatment using liquid water is also used occasionally. Water pretreatments use pressure to maintain the water in the liquid state at elevated temperatures.[9,47,48] Flow-through processes pass water maintained in the liquid state at elevated temperatures through lignocellulosics. This type of pretreatment has been termed hydrothermolysis,[47] aqueous or steam/aqueous fractionation,[49] uncatalyzed solvolysis,[48] and aquasolv.[50] The residence time for this process is usually ∼15 min at temperatures in the range of 200−230 °C. Approximately 40−60% of the total biomass is dissolved in this process, with 4−22% of the cellulose, 35−60% of the lignin, and all of the hemicellulose being removed. Three types of liquid hot water reactor configurations are used, namely, cocurrent, counter-current, and flow-through. In cocurrent pretreatment, water and lignocellulose move in the same direction, and the slurry of biomass and water is heated to the desired temperature and held at the pretreatment conditions for the desired residence time before being cooled. In counter-current pretreatment, water and lignocellulose move in opposite directions through the pretreatment reactor. In a flow-through reactor, hot water is made to pass over a stationary bed of lignocellulose. For liquid hot water pretreatment, size reduction of the biomass is not needed because the lignocellulose particles break apart when cooked in water.[51]

Gonzalez et al.[52] studied pretreatment of olive-tree-pruning biomass, by either liquid hot water or steam explosion, which was used as a substrate for enzymatic hydrolysis. Glucose (2.8 g/100 g of raw material) and 1.3 g of hemicellulosic sugars/100 g were recovered in liquids from liquid hot water pretreatment. Steam explosion resulted in sugar recoveries in the liquid fraction of 5.4 g of glucose and 5.4 g of hemicellulosic sugars per 100 g of raw material. When steam-explosion pretreatment was applied, 76.5% of total sugars was obtained, mainly as oligomers. In contrast, 45.5% of the oligomers were released from liquid hot water pretreatment. Perez et al.[53] evaluated the effect of liquid hot water process parameters, i.e., temperature (170 and 200 °C), residence time (0 and 40 min), solid concentration [5% and 10% (w/v)] and overpressure applied in the reactor (30 bar), on pretreatment of wheat straw. Pretreatment effectiveness was evaluated based on the compositions of the solid and liquid fractions obtained after filtration of pretreated material and the susceptibility of the solid fraction to enzymatic hydrolysis using commercial cellulases. The authors concluded that the effect of pretreatment time in hemicellulose-derived sugar recovery in the prehydrolyzate depends on temperature; enzyme hydrolysis yield was enhanced as both temperature and time were increased. Maximum enzyme hydrolysis yield was reported to be ∼96 g of glucose per 100 g of potential glucose in the pretreated residue. Xylan and acetyl group content remaining in the solid residue after pretreatment had a marked effect on substrate degradability.

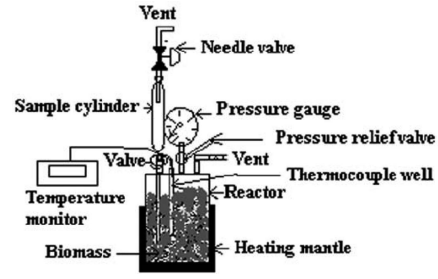

Figure 4. Schematic diagram of laboratory AFEX apparatus. (Reproduced from ref 58.)

4.2.2. Ammonia Fiber Explosion (AFEX). Ammonia fiber explosion is a physicochemical pretreatment process in which lignocellulosic biomass is exposed to liquid ammonia at high temperature and pressure for a period of time, and then the pressure is suddenly reduced. The AFEX process is very similar to steam explosion. In a typical AFEX process, the dosage of liquid ammonia is 1−2 kg of ammonia/kg of dry biomass, the temperature is 90 °C, and the residence time is 30 min. A schematic apparatus for laboratory AFEX pretreatment of biomass is shown in Figure 4.[54] AFEX pretreatment can significantly improve the fermentation rate of various herbaceous crops and grasses. The AFEX technology has been used for the pretreatment of many lignocellulosic materials including alfalfa, wheat straw, and wheat chaff.[55] During pretreatment only a small amount of the solid material is solubilized; that is, almost no hemicellulose or lignin is removed. The hemicellulose is degraded to oligomeric sugars and deacetylated,[56] which is most likely the reason that the hemicellulose is not soluble. The structure of the material is changed, resulting in increased water holding capacity and higher digestibility.[13] Over 90% hydrolysis of cellulose and hemicellulose was obtained after AFEX pretreatment of bermudagrass (approximately 5% lignin) and bagasse (15% lignin).[57] However, the AFEX process was not very effective for biomass with higher lignin content such as newspaper and aspen chips (25% lignin). Hydrolysis yields of AFEX-pretreated newspaper and aspen chips were reported as only 40% and below 50%, respectively.[19] Thus, AFEX is not a

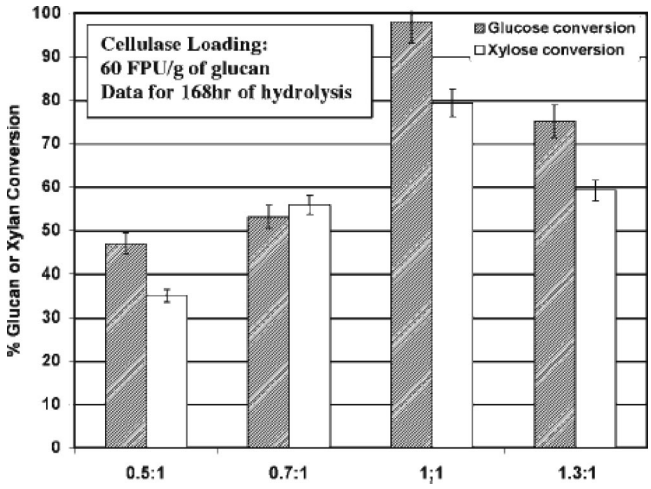

Figure 5. Effects of ammonia loading (grams of NH_3 per gram of dry biomass) on the enzymatic conversion of glucan and xylan for AFEX treatment of corn stover at 90 °C and 60% moisture content. (Reproduced from ref 48.)

very efficient technology for lignocellulosic biomass with relatively high lignin content such as woods and nut shells.

Another type of process utilizing ammonia is the ammonia recycle percolation (ARP) method.[13,15] In this process, aqueous ammonia (10−15 wt %) passes through biomass at elevated temperatures (150−170 °C) with a fluid velocity of 1 cm/min and a residence time of 14 min, after which the ammonia is recovered. In the ARP method, the ammonia is separated and recycled. Under these conditions, aqueous ammonia reacts primarily with lignin and causes depolymerization of lignin and cleavage of lignin−carbohydrate linkages. The ammonia pretreatment does not produce inhibitors for the downstream biological processes, so a water wash is not necessary.[55]

Generally, AFEX and ARP processes are not differentiated in the literature, although AFEX is carried out in liquid ammonia and ARP is carried out in an aqueous ammonia solution (10−15%). The ammonia fiber explosion pretreatment simultaneously reduces lignin content and removes some hemicellulose while decrystallizing cellulose. It can have a profound effect on the rate of cellulose hydrolysis. The cost of ammonia, and especially of ammonia recovery, drives the cost of the AFEX pretreatment.[9]

Recently, various research groups have done a significant amount of research to determine the optimum conditions for ammonia pretreatment of lignocellulosics. Teymouro et al.[58] evaluated the optimum process conditions and parameters, namely, ammonia loading, moisture content of biomass, temperature, and residence time, necessary for maximum effectiveness of the ammonia fiber explosion process on corn stover (∼17% lignin). A comparison of enzymatic digestibility of corn stover pretreated with AFEX at different ratios of ammonia to biomass is shown in Figure 5. The optimal pretreatment conditions for corn stover were found to be a temperature of 90 °C, an ammonia/dry corn stover mass ratio of 1:1, a moisture content of corn stover of 60% (dry weight basis), and a residence time (holding at target temperature) of 5 min. Approximately 98% of the theoretical glucose yield was obtained during enzymatic hydrolysis of the optimally treated corn stover. The ethanol yield from the pretreated corn stover increased up to 2.2 times over that of the untreated sample. Teymouro et al. also reported that pretreatment temperature is a very important variable in the AFEX process, as it determines the amount of ammonia vaporized during the explosive flash and influences the system pressure. At higher temperatures, more ammonia vapors flash, and therefore, greater disruption of the biomass fiber structure probably occurs.

Alizadeh et al.[54] evaluated the optimum process conditions for the pretreatment of switchgrass. The optimal pretreatment conditions were found to be a temperature of ∼100 °C, an ammonia loading of 1 kg of ammonia per kilogram of dry matter, a moisture content of 80% (dry weight basis), and a residence time of 5 min. Hydrolysis results of AFEX-treated and untreated samples showed 93% versus 16% glucan conversion, respectively. The ethanol yield of optimized AFEX-treated switchgrass was measured to be about 0.2 g of ethanol/g of dry biomass, which was 2.5 times the yield of the untreated sample. In another recent article, sugar yields during enzymatic hydrolysis from AFEX-pretreated miscanthus (a tall perennial grass) were reported. Pretreatment conditions including temperature, moisture, ammonia loading, residence time, and enzyme loadings were varied to maximize hydrolysis yields. The optimal AFEX conditions determined were 160 °C, 2:1 (w/w) ammonia-to-biomass loading, 2.3 g of water per gram of biomass, and 5-min reaction time for water-soaked miscanthus. Approximately 96% glucan and 81% xylan conversions were achieved after 168 h of enzymatic hydrolysis at a 1% glucan loading using cellulase and α-glucosidase along with xylanase and Tween-80 supplementation.[59] Isci et al.[60] reported that 40−50% delignification (Klason lignin basis) was achieved when switchgrass was soaked in aqueous ammonium hydroxide (30%) with different liquid/solid ratios (5 and 10 mL/g) for either 5 or 10 days. The hemicellulose content decreased by approximately 50%. The highest delignification (47%) was achieved with soaking in ammonium hydroxide for 10 days at 10 mL/g of biomass. Lee et al. reported approximately 50% lignin removal from corn stover in 4 days with a loading of 12 mL/g of ground corn stover.[61] Kim and co-workers reported that the ARP process can remove up to 85% of lignin from corn stover.[62]

4.2.3. Carbon Dioxide Explosion. In attempts to develop improved lignocellulose pretreatment techniques, the idea of using supercritical CO_2 explosion, which would have a lower temperature than steam explosion and possibly a reduced expense compared to ammonia explosion, was developed. Supercritical fluid refers to a fluid that is in a gaseous form but is compressed at temperatures above its critical point to a liquidlike density. It was hypothesized that, because CO_2 forms carbonic acid when dissolved in water, the acid increases the hydrolysis rate. Carbon dioxide molecules are comparable in size to water and ammonia and should be able to penetrate small pores accessible to water and ammonia molecules. Carbon dioxide was suggested to be helpful in hydrolyzing hemicellulose as well as cellulose. Moreover, the low temperature prevents any appreciable decomposition of monosaccharides by the acid. Upon an explosive release of the carbon dioxide pressure, the disruption of the cellulosic structure increases the accessible surface area of the substrate to hydrolysis. Dale et al.[63] used the method to pretreat alfalfa (4 kg of CO_2/kg of fiber at a pressure of 5.62 MPa) and obtained 75% of the theoretical glucose released during 24 h of the enzymatic hydrolysis. The yields were relatively low compared to those of steam or ammonia explosion pretreatments but high compared to that of enzymatic hydrolysis without pretreatment. Zheng et al.[64] compared CO_2 explosion with steam and ammonia explosion for pretreatment of recycled paper mix, sugarcane bagasse, and repulping waste of recycled paper and found that CO_2 explosion was more cost-effective than ammonia explosion. Further, it

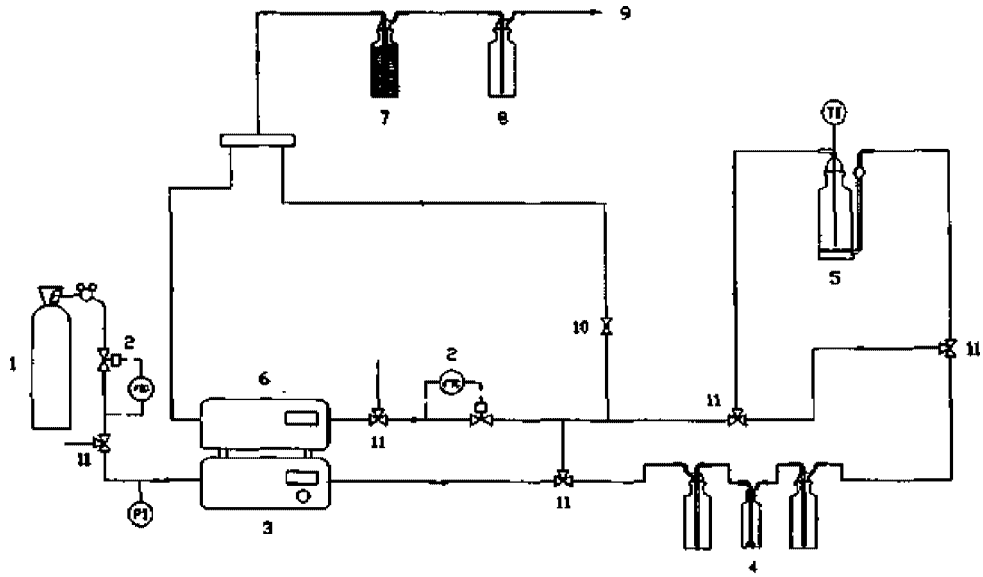

Figure 6. Experimental ozonolysis setup: (1) oxygen cylinder, (2) automatic gas flow control valve, (3) ozone generator, (4) process gas humidifier, (5) reactor, (6) ozone UV spectrophotometer, (7) ozone catalytic destroyer, (8) iodide trap to test catalyst efficiency, (9) vent, (10) pressure regulation valve, (11) three-way valve. (Reproduced from ref 68.)

did not cause the formation of inhibitory compounds that could occur in steam explosion. An increase in pressure facilitated faster penetration of CO_2 molecules into the crystalline structures, producing more glucose after the explosion. Because CO_2 explosion is operated at low temperatures, it does not cause degradation of sugars such as the degradation of sugars observed with steam explosion due to the high temperature involved.

4.3. Chemical Pretreatment. 4.3.1. Ozonolysis. Ozone treatment is one way of reducing the lignin content of lignocellulosic wastes. This results in an increase of the in vitro digestibility of the treated material, and unlike other chemical treatments, it does not produce toxic residues. Ozone can be used to degrade lignin and hemicellulose in many lignocellulosic materials such as wheat straw,[65] bagasse, green hay, peanut, pine,[66] cotton straw,[67] and poplar sawdust.[68] The degradation is mainly limited to lignin. Hemicellulose is slightly affected, but cellulose is not. A schematic diagram of a laboratory-scale ozonolysis apparatus is shown in Figure 6.[69] The rate of enzymatic hydrolysis increased by a factor of 5 following 60% removal of the lignin from wheat straw using an ozone pretreatment.[68] Enzymatic hydrolysis yield increased from 0% to 57% as the percentage of lignin decreased from 29% to 8% after ozonolysis pretreatment of poplar sawdust.[68] Ozonolysis pretreatment has an advantage that the reactions are carried out at room temperature and normal pressure. Furthermore, the fact that ozone can be easily decomposed by using a catalytic bed or increasing the temperature means that processes can be designed to minimize environmental pollution.[69] A drawback of ozonolysis is that a large amount of ozone is required, which can make the process expensive.

Most ozonation experiments have been conducted in hydrated fixed bed which leads to more effective oxidations than aqueous suspension or suspensions in 45% acetic acid.[68] Lasry et al.[70] and Euphrosine-Moy et al.[71] ozonized hydrated poplar sawdust (45% moisture) and identified oxalic and formic acids as the major products in the aqueous extract of the treated material, along with glycolic, glycoxylic, succinic, glyceric, malonic, *p*-hydroxybenzoic, fumaric, and propanoic acids. Morrison and Akin,[72] on the other hand, used ozone to oxidize herbaceous species moistened to 50% and identified caproic, levulinic, *p*-hydroxybenzoic, vanillic, azelaic, and malonic acids and aldehydes such as *p*-hydroxybenzaldehyde, vanillin, and hydroquinone in the aqueous extract.

4.3.2. Acid Hydrolysis. Concentrated acids such as H_2SO_4 and HCl have also been used to treat lignocellulosic materials. Pretreatment with acid hydrolysis can result in improvement of enzymatic hydrolysis of lignocellulosic biomasses to release fermentable sugars. Although they are powerful agents for cellulose hydrolysis, concentrated acids are toxic, corrosive, hazardous, and thus require reactors that are resistant to corrosion, which makes the pretreatment process very expensive. In addition, the concentrated acid must be recovered after hydrolysis to make the process economically feasible.[15,73]

Dilute-acid hydrolysis has been successfully developed for pretreatment of lignocellulosic materials. Sulfuric acid at concentrations usually below 4 wt %, has been of the most interest in such studies as it is inexpensive and effective. Dilute H_2SO_4 has been used to commercially manufacture furfural from cellulosic materials.[74,75] Dilute H_2SO_4 is mixed with biomass to hydrolyze hemicellulose to xylose and other sugars and then continues to break xylose down to form furfural.[9] The dilute H_2SO_4 pretreatment can achieve high reaction rates and significantly improve cellulose hydrolysis.[76] Dilute acid effectively removes and recovers most of the hemicellulose as dissolved sugars, and glucose yields from cellulose increase with hemicellulose removal to almost 100% for complete hemicellulose hydrolysis. Hemicellulose is removed when H_2SO_4 is added and this enhances digestibility of cellulose in the residual solids.[9] High temperature in the dilute-acid treatment is favorable for cellulose hydrolysis.[19] Recently developed dilute-acid hydrolysis processes use less severe conditions and achieve high xylan to xylose conversion yields. Achieving high xylan to xylose conversion yields is necessary to achieve favorable overall process economics because xylan accounts for up to one-third of the total carbohydrate in many lignocellulosic materials.[77]

Two types of dilute-acid pretreatment processes are typically used: a high-temperature ($T > 160$ °C), continuous-flow process for low solids loadings (weight of substrate/weight of reaction mixture = 5−10%)[78,79] and a low-temperature ($T < 160$ °C), batch process for high solids loadings (10−40%)[76] The most widely used and tested approaches are based on dilute sulfuric

acid. However, nitric acid,[80] hydrochloric acid,[81,82] and phosphoric acid[81] have also been tested. Numerous plant materia1s have been examined, inc1uding legume byproducts; reed canary grass; corn (husks, cobs, and stover); mixed hardwood (10% map1e and 90% birch); and hardwood bark from aspen, poplar, and sweet gum. Thompson et al.[83] used dilute H_2SO_4 to pretreat mixed hardwood and observed that the crystallinity index, although not a function of pretreatment temperature, still increased as a consequence of pretreatment. The removal of amorphous cellulose fractions, leaving a more crystalline fraction behind, could explain this observation.

Recently, acid pretreatment has been used on a wide range of feedstocks ranging from hardwoods to grasses and agricultural residues. Ishizawa et al.[84] evaluated whether porosity was one of the factors governing the overall enzymatic digestibility of the cellulose in dilute-acid-pretreated biomass. Corn stover was subjected to dilute H_2SO_4 pretreatment in a pilot-scale vertical reactor using a fixed residence time of ~1 min at temperatures ranging from 180 to 200 °C, solid loadings between 25% and 35% (w/w), and acid loadings of 0.03−0.06 g of acid/g of dry biomass. All of the pretreated samples showed higher pore volumes than untreated corn stover. The authors determined that porosity might be a factor for materials with low digestibility but that it is not so much of a factor for lignocellulosic materials with high digestibility. Lu et al.[85] carried out pretreatment of corn stover for sulfuric acid concentrations of 2%, 4%, and 6% at 80, 100, and 120 °C. The optimum conditions for corn stover pretreatment were a H_2SO_4 concentration of 2.0% and a reaction time of 43 min at 120 °C. Up to 77% xylose yield was obtained, whereas the glucose yield was only 8.4%. The corresponding solid phase showed good susceptibility toward enzymatic hydrolysis, leading to solutions containing up to 42.1 g of glucose/100 g of substrate, equivalent to a conversion yield of 70% under the optimum conditions.

The production of fermentable sugars from olive-tree biomass by dilute-acid pretreatment and further saccharification of the pretreated solid residues was studied by Cara and co-workers.[86] Pretreatment was performed at 0.2%, 0.6%, 1.0%, and 1.4% (w/w) sulfuric acid concentrations, and the temperature was varied in the range of 170−210 °C. Sugar recoveries in both the liquid fraction issued from pretreatment (prehydrolysate) and the water-insoluble solid were taken into consideration. A maximum of 83% of hemicellulosic sugars in the raw material was recovered in the prehydrolysate obtained at 170 °C and 1% H_2SO_4 concentration, but the enzyme accessibility of the corresponding pretreated solid was not very high. A maximum enzymatic hydrolysis yield of 76.5% was obtained from a pretreated solid at 210 °C and 1.4% acid concentration. Cellulose solubilization was detected, but sugar recovery in the prehydrolysate was the poorest among all of the experiments compared. To take into account the fermentable sugars generated by pretreatment and glucose released by enzymatic hydrolysis, an overall sugar yield was calculated. The maximum value of 36.3 g of sugar/100 g of raw material (75%) was obtained for the pretreatment of olive-tree biomass at 180 °C and 1% H_2SO_4 concentration. Dilute-acid pretreatment improved the enzymatic hydrolysis process compared to water pretreatment. In a recent article, Yat et al. reported dilute-acid pretreatment of four timber species (aspen, balsam fir, basswood, and red maple) and switchgrass using dilute H_2SO_4 for 50 g of dry biomass/L under similar conditions.[1] Xylose formation and degradation at various reactor temperatures (160−190 °C), sulfuric acid concentrations [0.25−1.0% (w/v)], and particle sizes (28−10/20 mesh) in a glass-lined 1-L well-mixed batch reactor were studied. Reaction rates for the generation of xylose from hemicellulose and the generation of furfural from xylose were found to depend strongly on both temperature and acid concentration. Maximum yields ranged from 70% (balsam) to 94% (switchgrass) for xylose, from 10.6% to 13.6% for glucose, and from 8.6% to 58.9% for other minor sugars. Xylose degradation varied linearly as a function of acid concentration.

Addition of very dilute sulfuric acid (about 0.1% versus the 0.7−3.0% typical for batch dilute-acid technology) in a flow-through reactor configuration is effective at acid levels lower than 0.1%. Despite achieving excellent hemicellulose sugar yields and highly digestible cellulose with low acid loadings, the equipment configurations and the high ratio of water to solids employed in flow-through systems require significant energy for pretreatment and product recovery. Although dilute-acid pretreatment can significantly improve cellulose hydrolysis, its cost is usually higher than those of physicochemical pretreatment processes such as steam explosion or AFEX. Neutralization of pH is necessary for the downstream enzymatic hydrolysis or fermentation processes. Dilute-acid pretreatment is also known to have a negative influence on the enzymatic hydrolysis of biomass. In a recent article, Selig et al.[87] reported the formation of spherical droplets on the surface of residual corn stover following dilute-acid pretreatment at high temperature. They suggested that the droplets formed were composed of lignins and possible lignin−carbohydrate complexes. It was demonstrated that these droplets were produced from corn stover during pretreatment under neutral and acidic pHs at and above 130 °C and that they can deposit onto the surface of residual biomass. The deposition of droplets produced under certain pretreatment conditions (acidic pH, $T > 150$ °C) and captured on pure cellulose was shown to have a negative effect on the enzymatic saccharification of the substrate. Therefore, it is extremely important to carefully examine the appropriate dilute-acid pretreatment of lignocellulose biomass species. It has been shown that materials that have been subjected to acid hydrolysis can be harder to ferment because of the presence of toxic substances.[13] Further, acid pretreatment results in costly materials of construction, high pressures, neutralization and conditioning of hydrolysate prior to biological steps, slow cellulose digestion by enzymes, and nonproductive binding of enzymes to lignin.[88]

4.3.3. Alkaline Hydrolysis. Some bases can be used for the pretreatment of lignocellulosic materials, and the effect of alkaline pretreatment depends on the lignin content of the materials.[19,29] Alkali pretreatment processes utilize lower temperatures and pressures than other pretreatment technologies.[9] Alkali pretreatment can be carried out at ambient conditions, but pretreatment times are on the order of hours or days rather than minutes or seconds. Compared with acid processes, alkaline processes cause less sugar degradation, and many of the caustic salts can be recovered and/or regenerated. Sodium, potassium, calcium, and ammonium hydroxides are suitable alkaline pretreatment agents. Of these four, sodium hydroxide has been studied the most.[89−92] However, calcium hydroxide (slake lime) has been shown to be an effective pretreatment agent and is the least expensive per kilogram of hydroxide. It is possible to recover calcium from an aqueous reaction system as insoluble calcium carbonate by neutralizing it with inexpensive carbon dioxide; the calcium hydroxide can subsequently be regenerated using established lime kiln technology. The apparatus for the laboratory-scale lime pretreatment of biomass is shown in Figure 7.[93] The process of lime pretreatment involves slurrying the lime with water, spraying it onto the biomass material, and storing

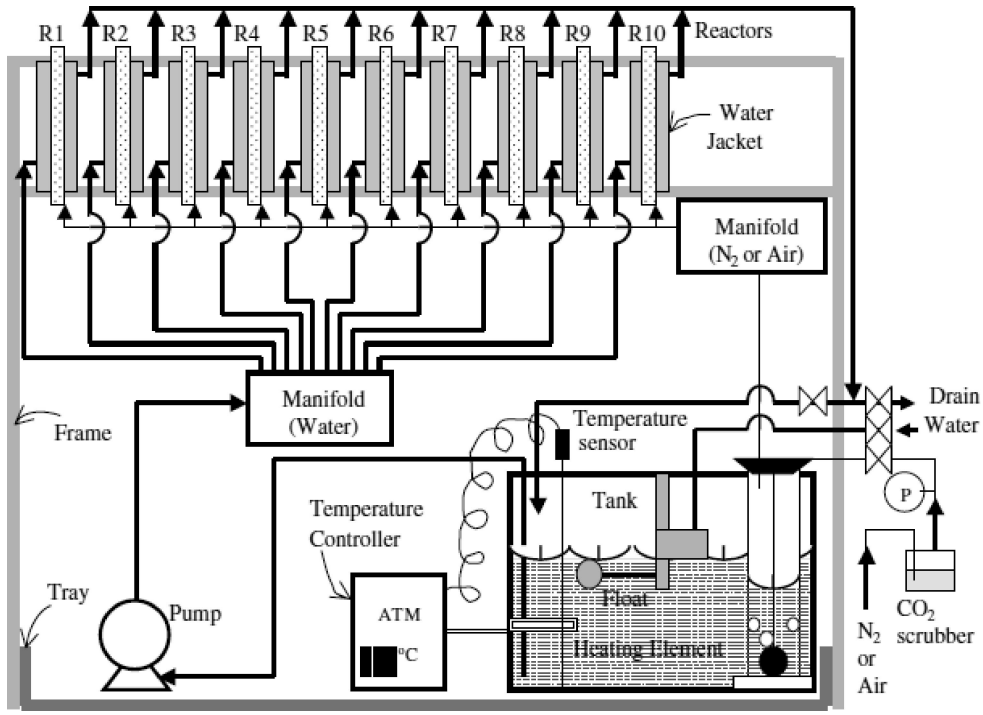

Figure 7. Schematic diagram of the jacketed reactor system for lime pretreatment under nonoxidative (N_2 supply) and oxidative (air supply) conditions. (Reproduced from ref 93.)

the material in a pile for a period of hours to weeks. The particle size of the biomass is typically 10 mm or less. Elevated temperatures reduce contact time.

The enzymatic hydrolysis of lime-treated biomass is affected by structural features resulting from the treatment.[93] These are the extents of acetylation, lignification, and crystallization. Lime pretreatment removes amorphous substances (e.g., lignin and hemicellulose), which increases the crystallinity index. Chang et al.[94] reported correlations between enzymatic digestibility and three structural factors: lignin content, crystallinity, and acetyl content. They concluded that (1) extensive delignification is sufficient to obtain high digestibility regardless of acetyl content and crystallinity, (2) delignification and deacetylation remove parallel barriers to enzymatic hydrolysis; and (3) crystallinity significantly affects initial hydrolysis rates but has less of an effect on ultimate sugar yields. These results indicate that an effective lignocellulose treatment process should remove all of the acetyl groups and reduce the lignin content to about 10% in the treated biomass.[93] Therefore, alkaline pretreatment can play a significant role in exposing the cellulose to enzyme hydrolysis. Lignin removal increases enzyme effectiveness by eliminating nonproductive adsorption sites and by increasing access to cellulose and hemicellulose. Kim et al.[93] pretreated corn stover with excess calcium hydroxide [0.5 g of $Ca(OH)_2$/g of raw biomass] in nonoxidative (in the presence of nitrogen) and oxidative (in the presence of air) conditions at 25, 35, 45, and 55 °C. The enzymatic digestibility of lime-treated corn stover was affected by the change of structural features such as acetylation, lignification, and crystallization resulting from the treatment. Extensive delignification required oxidative treatment and additional consumption of lime [up to 0.17 g of $Ca(OH)_2$/g of biomass]. Deacetylation reached a plateau within 1 week, and there were no significant differences between nonoxidative and oxidative conditions at 55 °C; both conditions removed approximately 90% of the acetyl groups in 1 week at all temperatures studied. Delignification highly depended on temperature and the presence of oxygen. Lignin and hemicellulose were selectively removed or solubilized, but cellulose was not affected by lime pretreatment at mild temperatures (25−55 °C). The degree of crystallinity increased slightly with delignification (from 43% to 60%) because amorphous components such as lignin and hemicellulose were removed. Lee et al.[95] reported that the rate of enzymatic hydrolysis depends on enzyme adsorption and the effectiveness of the adsorbed enzymes, instead of the diffusive mass transfer of enzyme. Lignin removal increases enzyme effectiveness by eliminating nonproductive adsorption sites and by increasing access to cellulose and hemicellulose. Kong et al.[96] reported that alkalis remove acetyl groups from hemicellulose (mainly xylan), thereby reducing the steric hindrance of hydrolytic enzymes and greatly enhancing carbohydrate digestibility. They concluded that the sugar yield in enzymatic hydrolysis is directly associated with acetyl group content.

Lime has been used to pretreat wheat straw (85 °C for 3 h),[97] poplar wood (150 °C for 6 h with 14 atm of oxygen),[98] switchgrass (100 °C for 2 h),[99] and corn stover (100 °C for 13 h).[100] Karr et al.[101] showed that pretreatment with slake lime (calcium hydroxide) increased the enzymatic hydrolysis of corn stover by a factor of 9 compared to that of untreated corn stover. The effect of lime pretreatment on the enzymatic hydrolysis of corn stover is shown in Figure 8.[101] The optimal pretreatment conditions were determined to be a lime loading 0.075 g of $Ca(OH)_2$/g of dry biomass, a water loading of 5 g of H_2O/g of dry biomass, and heating for 4 h at 120 °C. It was suggested that that pretreatment with lime can lead to corn stover polysaccharide conversions approaching 100%.

Dilute NaOH treatment of lignocellulosic materials has been found to cause swelling, leading to an increase in internal surface area, a decrease in the degree of polymerization, a decrease in crystallinity, separation of structural linkages between lignin and carbohydrates, and disruption of the lignin structure.[29] The digestibility of NaOH-treated hardwood was reported to increase from 14% to 55% with a decrease of lignin content from 24−55% to 20%. However, no effect of dilute NaOH pretreat-

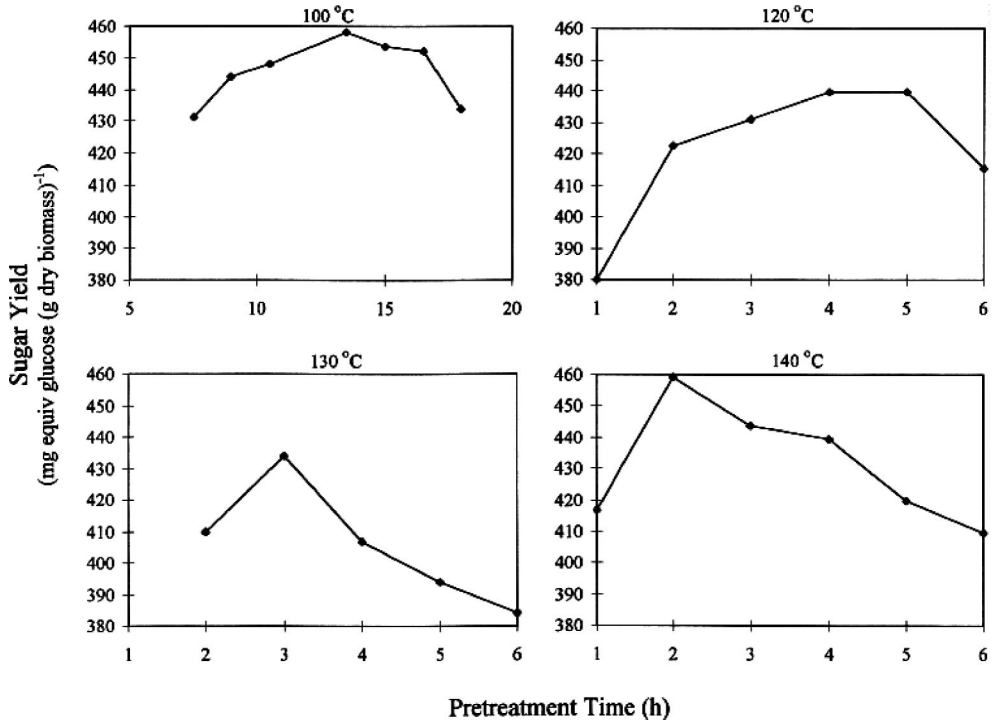

Figure 8. Sugar yields from enzymatic hydrolysis of corn stover after lime pretreatment at various times and temperatures. Pretreatment conditions: 0.1 g of $Ca(OH)_2$/g of dry biomass and 10 g of H_2O/g of dry biomass. Hydrolysis conditions: 5 FPU of cellulose/g of dry biomass for 72 h at 50 °C. (Reproduced from ref 101.)

ment was observed for softwoods with lignin content greater than 26%.[24] Dilute NaOH pretreatment was also found to be effective for the hydrolysis of straws with relatively low lignin contents of 10−18%.[102] Chosdu et al.[103] used a combination of irradiation and 2% NaOH for pretreatment of corn stalk, cassava bark, and peanut husk.[103] The glucose yield of corn stalk was 20% in untreated samples compared to 43% after treatment with electron beam irradiation at a dose of 500 kGy and 2% NaOH, but the glucose yields of cassava bark and peanut husk were only 3.5% and 2.5%, respectively.

Hu et al.[104,105] used radio-frequency- (RF-) based dielectric heating in the NaOH pretreatment of switchgrass to enhance its enzymatic digestibility. Because of the unique features of RF heating (i.e., volumetric heat transfer, deep heat penetration of the samples, etc.), switchgrass could be treated on a large scale, at high solids content, and with a uniform temperature profile. At 20% solids content, RF-assisted alkali pretreatment (at 0.1 g of NaOH/g of biomass loading and 90 °C) resulted in a higher xylose yield than the conventional heating pretreatment. The optimal particle size and alkali loading in the RF pretreatment were determined to be 0.25−0.50 mm and 0.25 g of NaOH/g of biomass, respectively. The switchgrass used in this study had a composition of 33.6% glucan, 19.3% xylan, 21.4% lignin, and 3.9% ash. Glucose and xylose are the major sugars (>90%) in switchgrass, therefore; the yields of these two types of sugars were used to evaluate the efficiency of the pretreatment process. The sugar yield was expressed as grams of sugar released per 100 g (dry weight) of original, untreated biomass (switchgrass). Based on the glucan and xylan contents, the maximum yields for glucose and xylose would be $33.6 \times 1.111 = 37.3$ g/100 g of biomass and $19.3 \times 1.136 = 21.9$ g/100 g of biomass, respectively. At alkali loadings of 0.20−0.25 g of NaOH/g of biomass, a heating temperature of 90 °C, and a solids content of 20%, the glucose, xylose, and total sugar yields from the combined RF pretreatment and enzymatic hydrolysis were 25.3, 21.2, and 46.5 g/100 g of biomass, respectively. Hu and co-workers soaked switchgrass in NaOH solutions of different concentrations and then treated the samples by microwave or conventional heating. With alkali loadings of 0.05−0.3 g of alkali/g of biomass, microwave pretreatment resulted in higher sugar yields than conventional heating, with the highest yield (90% of maximum potential sugars) being achieved at an alkali loading of 0.1 g/g. These results suggest that microwave-assisted alkali treatment is an efficient way to improve the enzymatic digestibility of switchgrass.

Ammonia has also been used as a pretreatment to remove lignin. Iyer et al. described an ammonia recycled percolation process (temperature = 170 °C, ammonia concentration = 2.5−20%, reaction time = 1 h) for the pretreatment of a corn cobs/stover mixture and switchgrass.[106] The efficiency of delignification was 60−80% for corn cobs and 65−85% for switchgrass.

4.3.4. Oxidative Delignification. Lignin biodegradation could be catalyzed by the peroxidase enzyme with the presence of H_2O_2.[107] The pretreatment of cane bagasse with hydrogen peroxide greatly enhanced its susceptibility to enzymatic hydrolysis. About 50% of the lignin and most of the hemicellulose were solubilized by 2% H_2O_2 at 30 °C within 8 h, and 95% efficiency of glucose production from cellulose was achieved in the subsequent saccharification by cellulase at 45 °C for 24 h.[107] Bjerre et al. used wet oxidation and alkaline hydrolysis of wheat straw (20 g of straw/L, 170 °C, 5−10 min) and achieved an 85% conversion yield of cellulose to glucose.[102] Wet oxidation combined with base addition readily oxidizes lignin from wheat straw, thus making the polysaccharides more susceptible to enzymatic hydrolysis. Furfural and hydroxymethylfurfural, known inhibitors of microbial growth when other pretreatment systems are applied, were not observed following the wet oxidation treatment.

4.3.5. Organosolv Process. The organosolvation method is a promising pretreatment strategy, and it has attracted much

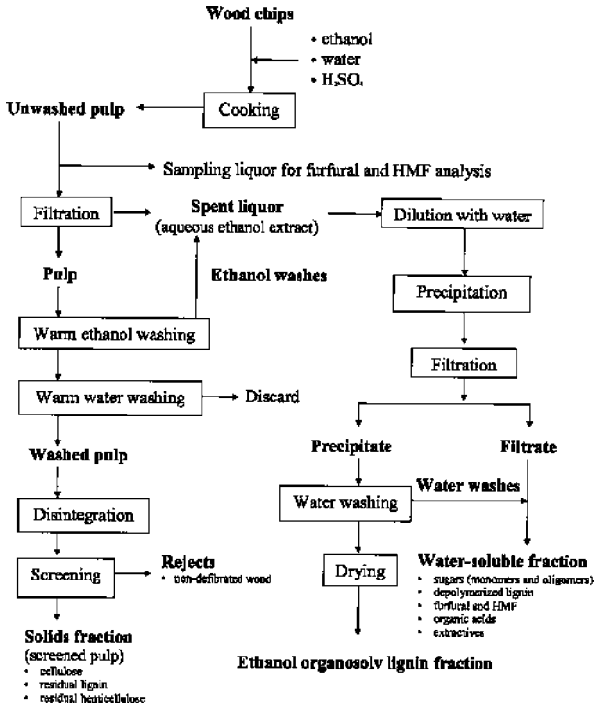

Figure 9. Flowchart of the laboratory-scale ethanol organosolv process. (Reproduced from ref 112.)

attention and demonstrated the potential for utilization in lignocellulosic pretreatment.[108] In the organosolvation process, an organic or aqueous organic solvent mixture with inorganic acid catalysts (HCl or H_2SO_4) is used to break the internal lignin and hemicellulose bonds. The solvents commonly used in the process are methanol, ethanol, acetone, ethylene glycol, triethylene glycol, and tetrahydrofurfuryl alcohol.[109,110] Organic acids such as oxalic, acetylsalicylic, and salicylic acids can also be used as catalysts in the organosolvation process.[111] In essence, the organosolv process involves simultaneous prehydrolysis and delignification of lignocellulosic biomass supported by organic solvents and, usually, dilute aqueous acid solutions. A flowchart for organosolvation pretreatment of biomass is shown in Figure 9.[112] A high yield of xylose can usually be obtained with the addition of acid.

Pulps with residual lignin ranging from 6.4% to 27.4% (w/w) have been prepared from mixed softwoods using a biorefining technology called the lignol process, which is based on an aqueous ethanol organosolvation extraction.[112] This process uses a blend of ethanol and water in the ratio of 50:50 (w/w) at ~200 °C and 400 psi to extract most of the lignin from wood chips or other lignocellulosic biomass. Lignin is recovered as a fine precipitate by flashing the pulping liquor to atmospheric pressure, followed by rapid dilution with water. Other coproducts such as hemicellulose sugars and furfural are recovered from the water soluble stream. Pan et al.[112] found that all pulps were readily hydrolyzed without further delignification and more than 90% of the cellulose in low lignin pulps (<18.4% residual lignin) was hydrolyzed to glucose in 48 h. Arato et al.[113] also reported application of the lignol process and proposed that, when woody biomass is cooked in water and ethanol liquor under selected temperature and time conditions, numerous chemical hydrolysis reactions occur, splitting the biomass into its various chemical components. The preferred conditions depend on the nature of the feedstock being processed, but will generally be in the following ranges: a cooking temperature of 180−195 °C, a cooking time of 30−90 min, an ethanol concentration of 35−70% (w/w), and a liquor-to-solids ratio ranging from 4:1 to 10:1 (w/w). The pH of the liquor might range from 2.0 to 3.8. The largest component, cellulose, is partially hydrolyzed into smaller fragments that still remain insoluble in the liquor. The second largest component, hemicellulose, is hydrolyzed mostly into soluble components, such as oligosaccharides, monosaccharides, and acetic acid. Acetic acid lowers the liquor pH, stimulating acid-catalyzed hydrolysis of the other components. Some of the pentose sugars are subsequently dehydrated under the operating conditions to form furfural. The third major polymer component, lignin, is hydrolyzed under the conditions employed in the process primarily into lower-molecular-weight fragments that dissolve in the aqueous ethanol liquor. Pan et al.[114] applied the organosolvation process using extraction with aqueous ethanol for the conversion of poplar to ethanol. The process resulted in the fractionation of poplar chips into a cellulose-rich solids fraction; an ethanol organosolvation lignin (EOL) fraction; and a water-soluble fraction containing hemicellulosic sugars, sugar breakdown products, degraded lignin, and other components.

Solvents generally used in the organosolvation process need to be drained from the reactor, evaporated, condensed, and recycled to reduce the cost. Removal of solvents from the system is necessary because the solvents might be inhibitory to the growth of microorganisms, enzymatic hydrolysis, and fermentation.

4.4. Biological Pretreatment. Most pretreatment technologies require expensive instruments or equipment that have high energy requirements, depending on the process. In particular, physical and thermochemical processes require abundant energy for biomass conversion. Biological treatment using various types of rot fungi, a safe and environmentally friendly method, is increasingly being advocated as a process that does not require high energy for lignin removal from a lignocellulosic biomass, despite extensive lignin degradation.[115] In biological pretreatment processes, microorganisms such as brown-, white-, and soft-rot fungi are used to degrade lignin and hemicellulose in waste materials.[13] Brown rots mainly attack cellulose, whereas white and soft rots attack both cellulose and lignin. Lignin degradation by white-rot fungi occurs through the action of lignin-degrading enzymes such as peroxidases and laccase.[116] These enzymes are regulated by carbon and nitrogen sources. White-rot fungi are the most effective for biological pretreatment of lignocellulosic materials.[29] Hatakka et al.[117] studied the pretreatment of wheat straw by 19 white-rot fungi and found that 35% of the straw was converted to reducing sugars by *Pleurotus ostreatus* in 5 weeks. Similar conversion was obtained in the pretreatment by *Phanerochaete sordida*[38] and *Pycnoporus cinnabarinus*[115] in 4 weeks. To prevent the loss of cellulose, a cellulase-less mutant of *Sporotrichum pulverulentum* was developed for the degradation of lignin in wood chips.[118] Akin et al.[119] also reported the delignification of bermudagrass by white-rot fungi. The biodegradation of bermudagrass stems was improved by 29−32%, after 6 weeks, using *Ceriporiopsis subvermispora* and by 63−77% using *Cyathus stercoreus*. Lee et al.[116] studied the effects of biological pretreatment on the Japanese red pine *Pinus densiflora*, after exposure to three white-rot fungi: *Ceriporia lacerata*, *Stereum hirsutum*, and *Polyporus brumalis*. Of the three white-rot fungi tested, *S. hirsutum* selectively degraded the lignin of the wood sample, rather than the holocellulose (hemicellulose + cellulose) component. After 8 weeks of pretreatment with *S. hirsutum*, the total weight loss was 10.7%, and the lignin loss was the highest among the tested samples at 14.5%. However, the holocellulose loss was lower, at 7.8%, than the losses obtained using *C. lacerata* and *P. brumalis.* Extracelluar enzymes from *S. hirsutum* showed higher

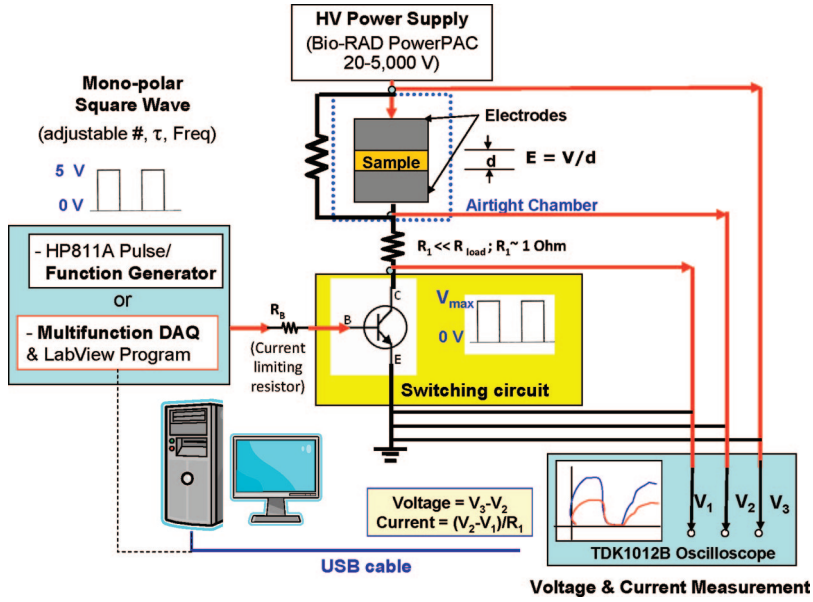

Figure 10. Schematic of pulsed-electric-field (PEF) system for the pretreatment of biomass.

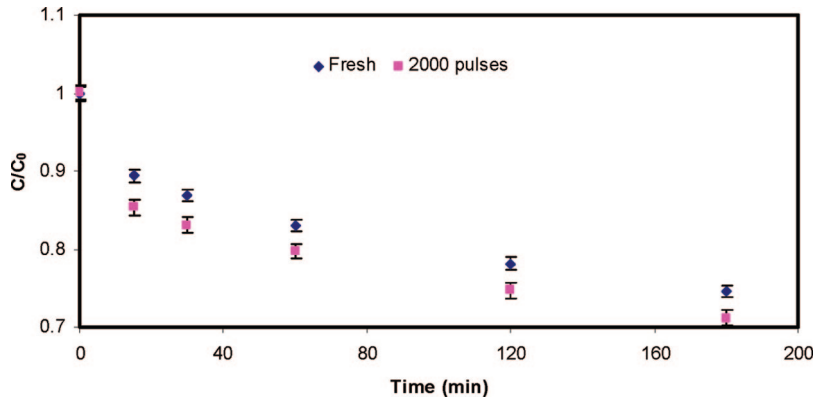

Figure 11. Neutral red uptake comparison for fresh and PEF-treated (2000 pulses at 8 kV/cm) switchgrass samples.

activity of ligninase and lower activity of cellulase than those from other white-rot fungi. *S. hirsutum* was considered as an effective potential fungus for biological pretreatment. When Japanese red pine chips treated with *S. hirsutum* were enzymatically saccharified using commercial enzymes (Celluclast 1.5 L and Novozyme 188), the sugar yield was increased to 21.0%, compared to nonpretreated control samples.

The white-rot fungus *P. chrysosporium* produces lignin-degrading enzymes, lignin peroxidases and manganese-dependent peroxidases, during secondary metabolism, in response to carbon or nitrogen limitation.[120] Both enzymes have been found in the extracellular filtrates of many white-rot fungi for the degradation of wood cell walls.[121,122] Singh et al.[123] evaluated eight bioagents, including fungi and bacteria, for their pretreatment effects on sugarcane trash. They narrowed down the C/N ratio of trash from 108:1 to a varying range from approximately 42:1 to 60:1. The maximum drop in C/N ratio of 61% was observed using *Aspergillus terreus*, followed by those obtained using *Cellulomonas uda* (52%) and *Trichoderma reesei* and *Zymomonas mobiliz* (49%). The C/N ratio is important for biomass pretreatment, because degradation of lignocellulosic material depends on the material's C/N ratio. To degrade each molecule of carbon, a definite proportion of nitrogen is required by the microorganisms, and this varies with different kinds of microflora. Fungi have a higher C/N ratio of 30:1 as compared to 10:1 for the bacteria; hence, fungi are more capable of degrading any lignocellulosic material, as their dependency on nitrogen is comparatively lower.[124] The bioagents helped in the degradation of sugarcane trash by production of cellulases, the maximum being produced by *A. terreus* (12-fold), followed by *C. uda* (10-fold), *Cellulomonas cartae* (9-fold), and *Bacillus macerans* (8-fold). The microbial pretreatment of trash rendered the sugars more accessible for enzymatic hydrolysis.

Biological pretreatment in combination with other pretreatment technologies has also been studied. Itoh et al.[125] reported production of ethanol by simultaneous saccharification and fermentation (SSF) from beech wood chips after bio-organosolvation pretreatments by ethanolysis and white-rot fungi, *Ceriporiopsis subvermispora*, *Dichomitus squalens*, *Pleurotus ostreatus*, and *Coriolus versicolor*. The purpose of biotreatments with wood-rot fungi was to reduce the energy input required for the separation of wood components by ethanolysis. Beech wood chips were pretreated with the white-rot fungi for 2−8 weeks without addition of any nutrients. The wood chips were then subjected to ethanolysis to separate them into pulp and soluble fractions. From the pulp fraction, ethanol was produced by SSF. Among the four strains, *C. subvermispora* gave the highest yield on SSF. The yield of ethanol obtained after pretreatment with *C. subvermispora* for 8 weeks was 0.294 g/g of ethanolysis pulp (74% of theoretical) and 0.176 g/g of beech wood chips (62% of theoretical). The yield was 1.6 times higher than that obtained without the fungal treatments. The combined

Table 2. Summary of Various Processes Used for the Pretreatment of Lignocellulosic Biomass

pretreatment process	advantages	limitations and disadvantages
mechanical comminution	reduces cellulose crystallinity	power consumption usually higher than inherent biomass energy
steam explosion	causes hemicellulose degradation and lignin transformation; cost-effective	destruction of a portion of the xylan fraction; incomplete disruption of the lignin−carbohydrate matrix; generation of compounds inhibitory to microorganisms
AFEX	increases accessible surface area, removes lignin and hemicellulose to an extent; does not produce inhibitors for downstream processes	not efficient for biomass with high lignin content
CO_2 explosion	increases accessible surface area; cost-effective; does not cause formation of inhibitory compounds	does not modify lignin or hemicelluloses
ozonolysis	reduces lignin content; does not produce toxic residues	large amount of ozone required; expensive
acid hydrolysis	hydrolyzes hemicellulose to xylose and other sugars; alters lignin structure	high cost; equipment corrosion; formation of toxic substances
alkaline hydrolysis	removes hemicelluloses and lignin; increases accessible surface area	long residence times required; irrecoverable salts formed and incorporated into biomass
organosolv	hydrolyzes lignin and hemicelluloses	solvents need to be drained from the reactor, evaporated, condensed, and recycled; high cost
pyrolysis	produces gas and liquid products	high temperature; ash production
pulsed electrical field	ambient conditions; disrupts plant cells; simple equipment	process needs more research
biological	degrades lignin and hemicelluloses; low energy requirements	rate of hydrolysis is very low

process enabled the separation of lignin, cellulose, and hemicelluloses using only water, ethanol, and wood-rot fungi. The biological pretreatments saved 15% of the electricity needed for ethanolysis. In another interesting approach, Balan et al. studied the effect of fungal conditioning of rice straw followed by AFEX pretreatment and enzymatic hydrolysis.[126] They reported that treating rice straw with white-rot fungi, *Pleurotus ostreatus*, followed by AFEX gave significantly higher glucan and xylan conversions and less-severe AFEX conditions than did treating rice straw with AFEX directly.

The advantages of biological pretreatment include low energy requirements and mild environmental conditions. However, the rate of hydrolysis in most biological pretreatment processes is very low.

4.5. Pulsed-Electric-Field Pretreatment. Pulsed-electric-field (PEF) pretreatment involves application of a short burst of high voltage to a sample placed between two electrodes. When an electric field is generated between two parallel-plate electrodes, the field strength (E) is given by $E = V/d$, where V is the voltage and d is the distance between the two electrodes. PEF pretreatment can have serious effects on the structure of plant tissues. When a high-intensity, external electric field is applied, a critical electric potential is induced across the cell membrane, which leads to rapid electrical breakdown and local structural changes of the cell membrane, the cell wall, and therefore the plant tissue. The electric field results in a dramatic increase in mass permeability and, in some cases, mechanical rupture of the plant tissue. The electric field pulses most commonly applied are in the form of exponential-decay or square waves. Application of high-intensity electric field pulses from nanoseconds to microseconds in duration leads to the permeabilization of biological membranes. Based on this phenomenon, many practical applications of high electric fields for reversible or irreversible permeabilization of various biological systems have been studied in the fields of medicine and bioscience.[127−132]

The permeabilization of plant membranes to improve mass transfer of metabolites is currently of interest to the food industry. Initial efforts mainly focused on reduction of endogenous microbial load of food products.[133] More recently, there have been reports on the application of PEF pretreatment for inactivation of enzymes.[134] Pulsed electrical fields have also been used on vegetable tissues to improve mass-transfer processes such as (1) diffusion of soluble substances,[135] juice extraction,[136,137] and dehydration.[138] In applying PEF pretreatment to plant processing, the electric field strength (voltage/distance), the number of pulses, and the treatment time are the most important factors. Typically, the plant tissue is placed or transported between two electrodes, and the electric discharges are applied in the form of pulses. A typical setup consists of a pulse generator, treatment chamber, data acquisition and control system, and material-handling equipment. Field strengths applied for irreversible plant tissue permeabilization are usually above 1.0 kV/cm. Pulse durations are in the microsecond range.

In biomass-to-fuel conversion, the biomass needs to be treated so that the cellulose in the plant fibers is exposed. Pretreatment with PEFs can facilitate this process. Using high field strengths in the range of 5−20 kV/cm, plant cells can be significantly ruptured. By applying electric pulses with high field strengths, PEF pretreatment can create permanent pores in the cell membrane and hence facilitate the entry of acids or enzymes used to break down the cellulose into its constituent sugars. In the case of the chemical modification of plant tissue, particularly in lignocellulose hydrolysis, appropriate chemicals might need to be transported into the tissue to aid in cell-wall breakdown and digestion and pretreatment with PEFs is important to facilitate the process. Two advantages of PEF pretreatment are that it can be carried out at ambient conditions and energy use is low because pulse times are very short (100 μs). Furthermore, the actual PEF process itself does not involve moving parts, so that the equipment is not complex.

Kumar et al.[139] have designed and fabricated a PEF system for the treatment of woodchips (Southern pine) and switchgrass samples. A schematic of the PEF system is shown in Figure 10. The PEF apparatus consists of a high-voltage power supply, a function generator, a switching circuit, and a sample holder.

The switching circuit consists of a transistor that is driven by the function generator. The function generator feeds a pulse of desired shape and width to the switching circuit. The switching circuit is turned on when the pulse is applied to it and transfers the high voltage supplied by the power supply across the sample holder. Therefore, high-voltage pulses of desired shape and width can be applied to the sample using the function generator and switching circuit. Switchgrass samples were treated with 2000 pulses of 8 kV/cm with a pulse width of 100 μs and a frequency of 3 Hz. Neutral red dye uptake experiments were performed on fresh and PEF-treated samples to study the effects of PEF treatment. Switchgrass samples were stirred in an aqueous solution of neutral red, and the concentration of dye in water was measured as a function of time using a UV−vis spectrophotometer. An example of experimental dye-uptake results is shown in Figure 11. Switchgrass samples treated with 2000 pulses of 8 kV/cm showed faster dye uptake than fresh samples, suggesting a positive effect of PEF pretreatment on switchgrass samples. Pulsed-electrical-field-treated samples can show similar characteristics for enzymes used for hydrolysis and can increase the hydrolysis rate. Further treatments with high electric fields and higher numbers of pulses are planned to study the effects of PEF treatment on wood chip samples.

5. Summary of Biomass Pretreatment Methods

A vast array of materials are suitable for the production of biofuels. It must be emphasized that it is not always possible to transfer the results of pretreatment from one type of material to another. Further, one technology that is efficient for a particular type of biomass material might not work for another material. Various pretreatment processes for lignocellulosic biomass, and their advantages and disadvantages, are summarized in Table 2. The choice of the pretreatment technology used for a particular biomass depends on its composition and the byproducts produced as a result of pretreatment. These factors significantly affect the costs associated with a pretreatment method. There have been some reports comparing various pretreatment methods for biomass.[88,140−142] Rosgaard et al.[141] evaluated the efficacy of three different pretreatment procedures, i.e., acid or water impregnation followed by steam explosion versus hot water extraction, on barley and wheat straw. The pretreatments were compared after enzyme treatment using a cellulase enzyme system. The acid or water impregnation followed by steam explosion of barley straw was the best pretreatment in terms of the resulting glucose concentration in the liquid hydrolysate after enzymatic hydrolysis.

Silverstein et al.[142] evaluated the effectiveness of sulfuric acid, sodium hydroxide, hydrogen peroxide, and ozone pretreatments for the conversion of cotton stalks to ethanol. Solids from H_2SO_4, NaOH, and H_2O_2 pretreatments showed significant lignin degradation and/or high sugar availability and hence were hydrolyzed more readily by Celluclast 1.5 L and Novozym 188. Sulfuric acid pretreatment resulted in the highest xylan reduction (95.2% for 2% acid, 90 min, 121 °C/15 psi) but the lowest cellulose-to-glucose conversion during hydrolysis (23.9%). Sodium hydroxide pretreatment resulted in the highest level of delignification (65.6% for 2% NaOH, 90 min, 121 °C/15 psi) and cellulose conversion (60.8%). Hydrogen peroxide pretreatment resulted in significantly lower delignification (maximum of 29.5% for 2%, 30 min, 121 °C/15 psi) and cellulose conversion (49.8%) than sodium hydroxide pretreatment. Ozone did not cause any significant changes in lignin, xylan, or glucan contents over time. Wyman et al.[140] studied various pretreatment technologies for corn and emphasized that different methods yield different results, so that the choice of pretreatment technology for a particular material depends on which components of the biomass material need to be altered.

6. Conclusions

An increased use of biofuels would contribute to sustainable development by reducing greenhouse-gas emissions and the use of nonrenewable resources. Lignocellulosic biomass, including agricultural and forestry residues instead of traditional feedstocks (starch crops), could prove to be an ideally inexpensive and abundantly available source of sugar for fermentation into transportation fuels. Cellulose crystallinity, accessible surface area, protection by lignin, and sheathing by hemicellulose all contribute to the resistance of cellulose in biomass to hydrolysis. The biomass pretreatment and the intrinsic structure of the biomass itself are primarily responsible for its subsequent hydrolysis. The conditions employed in the chosen pretreatment method will affect various substrate characteristics, which, in turn, govern the susceptibility of the substrate to hydrolysis and the subsequent fermentation of the released sugars. Therefore, pretreatment of biomass is an extremely important step in the synthesis of biofuels from lignocellulosic biomasses, and there is a critical need to understand the fundamentals of various processes, which can help in making a suitable choice depending on the structure of the biomass substrate and the hydrolysis agent.

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