Review

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Pretreatment: the key to unlocking low-cost cellulosic ethanol

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Abstract: New transportation fuels are badly needed to reduce our heavy dependence on imported oil and to reduce the release of greenhouse gases that cause global climate change; cellulosic biomass is the only inexpensive resource that can be used for sustainable production of the large volumes of liquid fuels that our transportation sector has historically favored. Furthermore, biological conversion of cellulosic biomass can take advantage of the power of biotechnology to take huge strides toward making biofuels cost competitive. Ethanol production is particularly well suited to marrying this combination of need, resource, and technology. In fact, major advances have already been realized to competitively position cellulosic ethanol with corn ethanol. However, although biotechnology presents important opportunities to achieve very low costs, pretreatment of naturally resistant cellulosic materials is essential if we are to achieve high yields from biological operations; this operation is projected to be the single, most expensive processing step, representing about 20% of the total cost. In addition, pretreatment has pervasive impacts on all other major operations in the overall conversion scheme from choice of feedstock through to size reduction, hydrolysis, and fermentation, and on to product recovery, residue processing, and co-product potential. A number of different pretreatments involving biological, chemical, physical, and thermal approaches have been investigated over the years, but only those that employ chemicals currently offer the high yields and low costs vital to economic success. Among the most promising are pretreatments using dilute acid, sulfur dioxide, near-neutral pH control, ammonia expansion, aqueous ammonia, and lime, with significant differences among the sugar-release patterns. Although projected costs for these options are similar when applied to corn stover, a key need now is to dramatically improve our knowledge of these systems with the goal of advancing pretreatment to substantially reduce costs and to accelerate commercial applications. © 2007 Society of Chemical Industry and John Wiley & Sons, Ltd

Keywords: lignocellulosic biomass; cellulosic ethanol; pretreatment; enzymatic hydrolysis; cellulose; hemicellulose; lignin

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Introduction

The world is in desperate need of new sources of liquid transportation fuels to address vital strategic, economic, and environmental problems. Although petroleum provides more energy (about 40% of the total) for the United States than any other resource, domestic petroleum reserves and production are very limited, with the result that imports continue to grow and have reached over 70% of total US petroleum consumption.[1] Furthermore, there is growing evidence that global conventional oil use is nearing the point where half of the accessible reserves have been depleted, pointing toward the real possibility that production will not be able to keep up with demand in the near future.[2] This situation is compounded by the tremendous growth in oil demand by China, as well as India and other developing countries.[3,4] Our growing dependence on petroleum imported from politically volatile countries also makes us strategically vulnerable to disruptions and price hikes that produce economic chaos. In the meantime, imported oil is the largest contributor to the US trade deficit, with its continued growth only making a bad situation worse.[1] On top of all these issues, petroleum is the largest contributor to emissions of carbon dioxide, which in turn has by far the largest influence on global climate change.[1]

In light of this list of vital strategic, economic, and environmental issues that continue to grow, petroleum consumption must finally be reduced.[5] Because the data clearly shows that the largest fraction of oil used, about two-thirds, goes to transportation, we have three options if we hope to succeed: 1) drive less, 2) use more efficient vehicles, and 3) switch to fuels that are not derived from petroleum. Although the first two approaches are certainly desirable, non-petroleum-based fuels are essential if we are to ultimately address the impending crises to which petroleum use will surely lead. Furthermore, any new fuel we develop should be sustainable if we are to dramatically cut greenhouse gas emissions. The overwhelming dominance of gasoline, diesel, and jet fuels for transportation also clearly shows our preference for liquid fuels.[1] When we examine the spectrum of sustainable resources and fuels that may be derived from them, biomass clearly represents the only sustainable, low-cost resource that can be converted into liquid transportation fuels on a large enough scale to have a meaningful impact on petroleum use in the near term and perhaps well beyond.[6–9] Thus, development of biofuels is virtually mandatory if we are at all serious about impacting petroleum use in a meaningful way.

Several biofuels routes to production of liquid fuels have been pursued over the years:

1. Gasification of biomass to syngas for conversion to synthetic diesel fuel.
2. Pyrolysis of biomass to oils.
3. Direct liquefaction.
4. Conversion of plant oils to biodiesel.
5. Release of sugars for fermentation to ethanol.

Of these, the first technology has been applied during war and is used in South Africa, albeit with coal, as a result of its development to overcome petroleum embargoes resulting from apartheid. Commercial conversion of plant oils to biodiesel is growing, although ultimate market penetration will be limited by oil availability and prices.[10,11] Brazil and the USA produce over 5 billion gallons per year of both ethanol from cane sugar and starch from corn and other grains, but neither resource is sufficient to make a major impact on world petroleum use. Only cellulosic biomass, such as agricultural and forestry residues and herbaceous and woody energy crops, offers the volumes and environmental attributes that can support production of biofuels on a sufficient scale to have a major impact on petroleum use. Biomass at about $40/ton costs about the same as petroleum at about $13/barrel on an equivalent energy basis.[12] Furthermore, release of sugars from the cellulose and hemicellulose fractions that typically make up more than two-thirds of such materials followed by their fermentation to ethanol can take advantage of the power of modern biotechnology to significantly reduce costs and also capitalize on the unique environmental and fuel attributes of ethanol as a fuel.[6]

Despite sporadic and limited funding, the production cost of cellulosic ethanol has been reduced significantly over the last two decades, with advances in pretreatment, sugar fermentation, enzyme production, and enzymatic hydrolysis having the greatest impacts.[13] Furthermore, opportunities have been identified for sufficiently cutting the costs to be competitive with gasoline from oil at about $25/barrel.[14]

Economic analyses point out that the greatest fraction of projected costs, almost 40%, is associated with releasing sugars from hemicellulose and cellulose by the combined operations of pretreatment, enzyme production, and enzymatic hydrolysis, with pretreatment responsible for almost half of this total.[15–17] The US Department of Energy recently funded three major centers to focus on advancing plant and process biotechnology to reduce costs (http://www.energy.gov), and BP Corporation established a new center for advancing biotechnology for making biofuels (http://www.bp.com). In this review, we will point out the importance of pretreatment, discuss the need to advance this technology to produce low-cost fuels by biologically processing of biomass, and describe leading pretreatment technologies.

The role of pretreatment in biological processing of cellulosic biomass

In the context of biological processing of cellulosic biomass to sugars for fermentation to ethanol and other products, pretreatment generally refers to the disruption of the naturally resistant carbohydrate-lignin shield that limits the accessibility of enzymes to cellulose and hemicellulose.[18–20] However, the choice of pretreatment technology is not trivial and must take into account sugar-release patterns and solid concentrations for each pretreatment in conjunction with their compatibility with the overall process, feedstock, enzymes, and organisms to be applied.[15,17] Given its significant impact on process economics, most if not all of the following key attributes should be targeted for low-cost, advanced pretreatment processes.[14,18,21–23]

- The need for chemicals in pretreatment and subsequent neutralization and prefermentation conditioning should be minimal and inexpensive.
- Because milling of biomass to small particle sizes is energy-intensive and costly, pretreatment technologies that require limited size reduction are desirable.
- High yields of fermentable hemicellulose sugars of close to 100% should be achieved through pretreatment.
- The concentration of sugars from the coupled operations of pretreatment and enzymatic hydrolysis should be above 10% to ensure that ethanol concentrations are adequate to keep recovery and other downstream costs manageable.
- Pretreatment reactors should be low in cost through minimizing their volume, not requiring exotic materials of construction due to highly corrosive chemical environments, and keeping operating pressures reasonable.
- The liquid hydrolyzate from pretreatment must be fermentable following a low-cost, high-yield conditioning step. However, it is highly desirable to eliminate conditioning to reduce costs and to reduce yield losses.
- The chemicals formed during hydrolyzate conditioning in preparation for subsequent biological steps should not present processing or disposal challenges (e.g., gypsum).
- Cellulose from pretreatment should be highly digestible with yields of greater than 90% in less than five and preferably less than three days with low cellulase loadings of less than 10 FPU/gram cellulose.
- Lignin and other constituents should be recovered for conversion to valuable co-products and to simplify downstream processing.
- The distribution of sugar recovery between pretreatment and subsequent enzymatic hydrolysis should be compatible with the choice of organisms to ferment the five sugars in hemicellulose.
- The heat and power demands for pretreatment should be low and/or be compatible for being thermally integrated with the rest of the process.

Pervasiveness of pretreatment

Pretreatment is not only costly in its own right but has a pervasive impact on the cost of virtually all other biological processing operations, including those preceding pretreatment, the handling of the liquid stream generated, the processing of the solids from pretreatment, waste treatment, and potential production of co-products (Fig.1).

Direct costs

As mentioned, about 18% of the total projected cost for biological production of cellulosic ethanol can be attributed to pretreatment, more than for any other single step.[15–17] Dilute acid pretreatment was chosen as the basis for these projections because of extensive experience with its development and the high yields attained in the laboratory. However, credit was only taken for about 63% of the potential hemicellulose sugars during pretreatment based on experience with

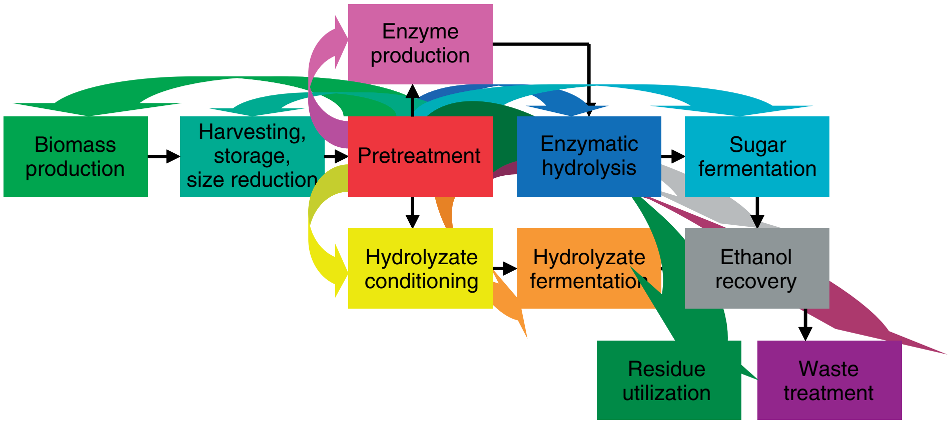

Figure 1. Simplified process flow diagram for biological conversion of cellulosic biomass to ethanol illustrating potential effects of pretreatment on other operations.

a particular pilot plant system. In addition, limitations in solids concentrations to about 30% for the feed to pretreatment, expensive construction materials, separation of the liquid hydrolyzate from pretreated solids, acid neutralization costs, costs for removal of inhibitors released from biomass or generated during pretreatment from the hydrolyzate, and yield losses during hydrolyzate conditioning all have significant impacts on these projected pretreatment costs. The loss of sugars or poor release during pretreatment and subsequent enzymatic hydrolysis is a particularly important cost consideration.

Effect on upstream operations

Such direct costs are just the proverbial tip of the iceberg for the impact of pretreatment on overall costs for biological processing of cellulosic biomass to ethanol, with pretreatment affecting virtually every other step (Fig. 1). Upstream of pretreatment, the choice of feedstock may be dictated by the selection of pretreatment or vice versa as not all types of pretreatments are equally effective on all feedstocks. For example, a number of pretreatments are effective with corn stover,[22,24] while a low-cost pretreatment has yet to be defined that achieves high sugar yields from softwoods. [25,26] Research is in progress to develop comparable data on other feedstocks including poplar wood and switchgrass. The choice of the pretreatment/substrate combination also influences upstream harvesting, storage, and size-reduction needs as aging during storage can increase resistance to some pretreatments, and temperature and chemicals used for pretreatment must reach near uniform values in times that are short relative to reaction times. [27–29]

Impact on downstream processing of liquid

Pretreatment has a major effect on downstream processing steps as well, and because of the number of such operations, the impacts may be even greater. If we first focus on the liquid fraction leaving the pretreatment step, we see that the type of pretreatment chosen determines how much of the cellulose and hemicellulose in the entering biomass dissolves and the distribution of these components among monomers and oligomers.[22] In turn, the choice of organism to ferment these sugars to ethanol is influenced by the relative concentrations of arabinose, galactose, glucose, mannose, and xylose as most will prefer glucose while delaying, and most often not completely utilizing, the others. [30] In addition, generation of significant quantities of oligomers in pretreatment presents a challenge in that most organisms cannot directly utilize these compounds, [31] with the result that an additional step must be incorporated into the process to achieve high ethanol yields [32–37]. The choice of pretreatment technology also impacts how much of the lignin, ash, and other fractions of biomass enter the solution and how they must be subsequently recovered. Pretreatment will generally release extractives and other natural products and can form degradation products, such as lignin fragments, that are inhibitory or even toxic to downstream enzymes and organisms. [38–42] On top of all of this, the solids concentration during pretreatment determines the concentration of the sugars, oligomers, inhibitory compounds, and other dissolved components in the liquid hydrolyzate which, in turn, impacts the need for hydrolyzate conditioning, ethanol concentration, the size of fermentation vessels and product-recovery systems, and energy costs for product recovery.[15–17,43] In addition to the cost of hydrolyzate-conditioning equipment, chemicals, and associated yield losses, separation of the liquid from the solids following pretreatment will generally be needed if hydrolyzate conditioning by ion exchange, overliming, or many other approaches with which solids would interfere are to be used. The type of hydrolyzate conditioning needed can introduce new impurities that present difficulties during product recovery such as the reverse solubility of gypsum formed during overliming of sulfuric acid pretreated hydrolyzate and the consequent precipitation when the fermentation broth is heated up during distillation. Further along in the process sequence, the range of compounds released by pretreatment can have a major impact on wastewater treatment and the fraction of water that can be recycled.

Effect on downstream processing of solids

Pretreatment also has a significant impact on the downstream operations for the exiting solids. Although pretreatment is traditionally targeted to make cellulose accessible to cellulase enzymes so that a high glucose yield results, not all of the hemicellulose sugars are released from the solids, and their recovery and conversion to ethanol should improve overall revenues even for dilute acid, sulfur dioxide, and other pretreatments that remove most of the hemicellulose sugars. Release of hemicellulose sugars during enzymatic hydrolysis is critical for pretreatments such as ammonia fiber expansion (AFEX) and others discussed below that leave much if not all of the hemicellulose in the solid fraction exiting pretreatment.[22] Cellulase enzyme formulations contain enough hemicellulase activity to release about half of the residual hemicellulose, but supplementation with xylanase, beta-xylobiase, pectinases, and other activities can release more sugars from hemicellulose while reducing total protein levels, and therefore costs. Of course, pretreatment also governs the accessibility of cellulose and hemicellulose to enzymes and the degree of crystallinity that has a significant effect on the ratios of key enzyme components needed to most effectively release sugars. [44–46] Furthermore, pretreatment affects many other key substrate features, such as degree of acetylation, and lignin removal that various studies show to govern the digestibility of pretreated cellulose.[47–49] Because lignin nonproductively ties up cellulase enzyme,[50 51] alteration of lignin to reduce its capacity for cellulase or its removal can significantly reduce enzyme costs, while some pretreatments may actually increase the capacity of lignin for nonproductive binding of enzymes.[50] The concentration of pretreated solids following pretreatment governs ethanol concentrations resulting from the coupled operations of enzymatic hydrolysis and fermentation of sugars released, again having important consequences for the size of fermentation vessels and product-recovery equipment, as well as the amount of energy needed to purify ethanol.

Implications for waste treatment

Because the operations for treating the solid and liquid residues from cellulosic ethanol production are capital intensive, they have a major impact on profit margins needed to realize acceptable rates of return on investment.[15,17] In addition, these operations benefit significantly from economies of scale, driving cellulosic operations toward large capacities to reduce costs. Soluble degradation products generated during pretreatment, such as furfural, must be removed through anaerobic digestion or other operations, increasing waste treatment costs. In addition, any natural biomass components released during pretreatment, such as acetic acid, present an additional load on waste treatment and associated costs. Wastes, such as gypsum, generated during pH adjustment and conditioning of hydrolyzates from pretreatment prior to enzymatic hydrolysis and fermentation present an additional burden for waste treatment and disposal. Current process designs typically target burning of the residual solids from ethanol production and also of the methane gas produced during anaerobic digestion to remove organics from the liquid left following ethanol recovery, but the minerals and any other insolubles from biomass or generated to adjust pretreated streams to be compatible with subsequent biological steps can present problems in boiler fouling and ash disposal. Characterization of these streams and evaluation of their impact on processing of residuals also introduces significant delays in designing and building conversion facilities.

Pretreatment and co-products

Cellulosic ethanol process designs typically assume that lignin will be used as a boiler fuel to provide all of the heat and power for a cellulosic ethanol facility with a considerable amount of excess power available for export as base-load power to the grid.[15,17] Use of lignin to drive the process in this way results in very low inputs of fossil or other external energy and a very favorable energy balance. Of even greater consequence, this use of lignin accounts for the superior greenhouse gas emission features of producing cellulosic ethanol, with export of excess electricity providing even greater benefits by reducing the need for coal to generate power 24 hours a day.[52–55] However, little attention has been given to understanding how pretreatment affects lignin fuel value in terms of the fate of lignin (e.g., does an appreciable amount end up with the gypsum or in the liquid or the solids?), changes in chemical structure, or its heating value. In addition, virtually no information is available on how pretreatment affects the ability to easily dewater lignin prior to burning and or whether chemical modifications during pretreatment impact its combustion and emission characteristics. The aromatic nature of lignin could also make it valuable for other uses, such as producing synthetic gasoline or making various chemical intermediates similar to benzene, toluene, and xylene,[56] although consideration would have to be given to the impact on the positive greenhouse gas profile of making cellulosic ethanol. Pretreatment will no doubt impact the susceptibility of lignin to such uses.

Although cellulosic biomass is often characterized as if it only contained cellulose, hemicellulose, and lignin or worse yet, as just containing glucan, xylan, and lignin, its composition is actually far more complex with minerals, oils, proteins, and other potentially valuable components integral to its makeup.[6,20] Some consideration has been given to the fate of these components from the viewpoint of their release as inhibitors that interfere with subsequent biological operations.[38,39] In addition, concerns have been raised about how minerals in biomass can cause problems in power generation, with the high silica in rice straw and rice hulls being a particularly relevant example.[57,58] With few exceptions such as investigations of protein recovery from various grasses,[59–63] separation of these components for other uses is largely ignored during pretreatment and subsequent operations, and such possibilities as recovery of silica for solid state electronic applications and extractives such as taxol for healthcare merit consideration. [64–66] Once again, the choice of pretreatment and its operating conditions is likely to potentially alter the nature and fate of these natural biomass constituents and determine whether they retain value or not.

Pretreatment options

Over the years, many technologies have been considered in the quest for low-cost pretreatment approaches that realize high sugars yields from both cellulose and hemicellulose that can be categorized as 1) biological, 2) chemical, 3) physical, and 4) thermal processes.[18,21] Biological pretreatment offers some conceptually important advantages such as low chemical and energy use, but a controllable and sufficiently rapid system has not yet been found.[67–70] The performance of physical pretreatments such as biomass comminution (e.g., milling) is poor while costs are high.[71–78] Using only steam for pretreatment has the advantage of being very simple, but yields are too low to be economical in many applications.[18,21,79–84] Passing hot water through biomass at high flow rates has been shown to be effective in recovering hemicellulose sugars with high yields, removing over half of the lignin, and producing a highly digestible cellulose,[23,85–93] but the water and energy requirements are excessive and this configuration would be challenging to implement commercially.

So far, the most promising pretreatment options require addition of one or more chemicals to be effective.[18,21] However, not all chemical pretreatments appear promising. For example, although pretreatments with sodium or potassium hydroxide delignify biomass and can realize good yields, the cost of these chemicals is too high for making fuels.[19,74,94–97] Various solvents such as ethanol and methanol can also be applied to remove lignin effectively in organosolv processes, but the costs are too high to be practical for anything other than the recovery of higher value products than fuels.[98–100] Carbon dioxide, attractive because of its co-production during ethanol fermentations, has shown promise in improving the digestibility of cellulose in some studies[101,102] but not in others.[18,21,103] In any event, the pressures are so high as to raise serious questions about containment costs.

The use of ionic liquids (ILs) for pretreatment of cellulosic biomass has recently received attention as a promising green solvent for biomass fractionation and deserves research attention. ILs are nonflammable and recyclable solvents with very low volatility and high thermal stability. Carbohydrates and lignin can be simultaneously dissolved in ILs with anion activity (e.g. the 1-butyl-3-methylimidazolium caution $[\text{C4mim}]^+$) because ILs form hydrogen bonds between the non-hydrated chloride ions of the IL and the sugar hydroxyl protons in a 1:1 stoichiometry.[104,105] As a result, the intricate network of non-covalent interactions between biomass polymers of cellulose, hemicellulose, and lignin is effectively disrupted while minimizing formation of degradation products.[105] However, most data showing the effectiveness of ILs has been developed using pure crystalline cellulose, and its applicability to the more complex combination of constituents in cellulosic biomass is yet to be established. In addition, process costs are unknown, and it is not clear whether impurities will build up in these low volatility liquids or how they may be removed. Their toxicity toward enzymes and microorganisms must also be established before ILs can be considered as a real option for biomass pretreatment.[105]

Leading pretreatment technologies

A biomass refining Consortium for Applied Fundamentals and Innovation (CAFI) was formed in early 2000 to systematically compare leading pretreatment technologies. The results of the research by this team are the first comparative data based on use of the same feedstock, the same enzyme formulations, identical analytical methods, consistent material balance approaches, and a common framework for economic comparisons.[24] All of the pretreatment in this research use chemicals including dilute acid, sulfur dioxide, ammonia, and lime but span a wide range of pH values.[22,24,106] Among these options, low pH acidic pretreatments tend to remove and recover a large fraction of the hemicellulose in biomass, and high pH pretreatments using base typically remove a significant fraction of the lignin effectively.[35,107–109] The performance of CAFI pretreatments including process configurations, mass balances, and estimated ethanol costs has been published recently for applications to corn stover,[19,22,35,37,87,106–108,110] and work is in progress to report on similar data that is now being finalized for poplar wood. A summary of selected features of these technologies is provided below, with the reader referred to the literature for more details.[109]

Dilute sulfuric acid and sulfur dioxide pretreatments

Pretreatment with dilute sulfuric acid has been the subject of research for over two decades and development, particularly targeted at fuels production.[18,21,29,111–115] The National Renewable Energy Laboratory (NREL), overseers of the largest biomass ethanol development effort in the world, favors dilute acid hydrolysis,[15,17,43] primarily because 80% to 90% of hemicellulose sugars are recoverable by dilute acid technology.[29,111,116] Although little lignin is dissolved, various studies indicate that lignin is disrupted, increasing cellulose susceptibility to enzymes.[29,117–119] It has been demonstrated that explosive decompression is not needed for dilute acid to be effective.[120,121]

Despite being often considered a frontrunner, dilute sulfuric acid hydrolysis has important limitations. Its very corrosive environment mandates exotic and expensive construction materials.[43,122] In addition, reaction degradation products such as furfural, solubilized biomass constituents, such as acetic acid, and corrosion products, such as metal ions, must be removed by overliming, steam stripping, or other processes prior to fermentation.[18,21,111,123] Fermentation inhibition and conditioning processes are not well understood, and the loss of sugars in conditioning hurts process economics. Although lime is by far the least expensive option for acid neutralization and hydrolyzate conditioning, the gypsum formed has reverse solubility characteristics that cause difficulties downstream.[43] Furthermore, even low-cost sulfuric acid and lime have significant cost impacts that increase further when disposal costs are included.[15,17] Additionally, about a seven-day reaction time with expensive cellulase loadings of up to 20 FPU/gram cellulose are needed to realize good yields in subsequent enzymatic hydrolysis of cellulose pretreated with dilute sulfuric acid.[17,124] The non-productive binding of enzymes to lignin following dilute acid and many other pretreatments exacerbates high enzyme use and costs.[50,125,126] There are also challenges associated with introducing sulfuric acid at the high solids concentrations needed for low energy inputs.

Several other chemicals have been used as an approach to lower pH and hydrolyze hemicellulose in pretreatment. Sulfur dioxide has been used in steam explosion to achieve similar yields to sulfuric acid.[25,127,128] and offers the advantage of more rapid penetration than sulfuric acid and possible recovery and recycle. However, this chemical presents some safety concerns and is projected to be similar in costs to dilute sulfuric acid.[129] Nitric acid reduces containment costs compared to sulfuric,[130,131] but the acid itself costs enough more to negate much if not all of the advantage.

Controlled pH pretreatment

An alternative approach has been developed based on controlling the pH at near neutral conditions.[132] This system is being applied to release hemicellulose sugars from corn fiber for integration into a corn wet-milling operation, and data has been developed with corn stover. In this case, the goals are 1) to stop hemicellulose hydrolysis with formation of soluble oligomers and minimize break down to sugar monomers that are subject to subsequent degradation reactions, hurting yields and 2) to hydrate the crystalline structure of the cellulose and modify it to a form that is more accessible and susceptible to enzyme hydrolysis, thereby enhancing conversion of cellulose to glucose.[133] During pretreatment, organic acids can be released that dissociate into hydrogen ions and promote acid catalyzed hydrolysis of cellulose to glucose. In addition, hydrogen ion formation from water and from organic acids is important during aqueous pretreatment of lignocellulosic materials and is typically favored by higher temperatures. However, high temperatures also accelerate acid catalyzed degradation of xylose, glucose, and other sugars released during hydrolysis as well as the acid catalyzed hydrolysis of cellulose and hemicellulose. Monitoring and control of the pH of this system can minimize hydrogen ion concentrations during pretreatment and help prevent hydrolysis of cellulose and hemicellulose to oligosaccharides and monosaccharides and more importantly, the acid catalyzed degradation of monosaccharides to degradation products.[132,134]

Controlled pH pretreatment differs significantly from others utilizing water or steam in that pretreatment conditions, particularly pH, are directed to minimizing hydrolytic reactions while maximizing physical changes. Control of pH between 4 and 7 can enhance susceptibility of cellulose to enzymes and also avoids formation of the monosaccharide degradation products, furfural, and hydroxymethyl furfural, which otherwise interfere with subsequent cellulose hydrolysis or ethanol fermentation. It also appears to impart important physical changes that improve enzymatic hydrolysis of cellulose, a decrease in cellulose crystallinity, and lower association of cellulose with lignin.[96,112,135–137]

Ammonia fiber expansion (AFEX) pretreatment

Pretreatment with ammonia is effective in improving cellulose digestion with the advantage of ammonia being recyclable due to its high volatility.[138] The AFEX process treats lignocellulosic materials with liquid ammonia under pressure and then rapidly releases pressure, with the result that 1) cellulose is decrystallized, 2) hemicelluoses are prehydrolyzed, 3) lignin in the treated material is altered, 4) the fiber structure is greatly disrupted, and 5) the small amounts (1–2% of the dry weight of the cellulosic material) of ammonia left behind can serve as a nitrogen source in subsequent fermentations.[138,139] AFEX can achieve greater than 90% conversion of cellulose and hemicellulose to fermentable sugars for a variety of lignocellulosic materials including alfalfa, barley straw, corn residue, wheat straw, rice straw, corn fiber, sugarcane bagasse, switchgrass, coastal bermudagrass, and rye grass straw.[138–144] For most of these materials, it has been shown that AFEX permits essentially complete conversion of cellulose and hemicellulose to fermentable sugars at very low enzyme loadings of 1–10 FPU cellulase per gram of dry lignocellulosic material. AFEX can employ lower-cost construction materials than for dilute sulfuric acid and the hydrolyzate is compatible with fermentation organisms without conditioning. AFEX removes or loses little lignin or hemicellulose, but AFEX-treated cellulose can be hydrolyzed to glucose with high yields at low enzyme loadings. However, most of AFEX-treated hemicellulose is oligomeric, and the primary challenge is to make this stream fermentable to products. AFEX treatment is a batch process, and continuous processing in an extruder in an approach called FIBEX (fiber extrusion) significantly reduces both the time required for treatment and the ammonia levels required while giving hydrolysis results similar to those for batch AFEX processing.

Ammonia recycle percolation (ARP) pretreatment

ARP, another process based on ammonia, passes aqueous ammonia solution (5–15 wt%) through a reactor packed with biomass at elevated temperatures (80–180 °C) and then separates and recycles the ammonia in the effluent.[145,146] When incorporated into a biomass saccharification process, ARP technology almost completely fractionates biomass into the three major constituents (pentose/pentosans, cellulose, and lignin) with the treated solids being a low-lignin, short-chained cellulosic material with high glucan content. Ammonia in aqueous solution and at high temperature breaks down lignin via the ammoniolysis reaction but has virtually no effect on carbohydrates. Upon contact with lignocellulosic material, aqueous ammonia, therefore, exhibits high selectivity for delignifcation reactions over other reactions, and removing lignin increases cellulose accessibility to cellulase. In addition, solid lignin hinders bioprocessing and increases agitation power requirements. Lignin and its derivatives are also toxic to microorganisms and adsorb enzymes, reducing hydrolysis rates. As a result, lower lignin levels in the solid substrates improve microbial activity and overall enzyme efficiency, reducing enzyme dosages and costs. Early removal of lignin can also facilitate cell and enzyme recovery and recycle and simplify distillation.

ARP pretreatment of biomass was initially found to significantly enhance enzymatic digestibility of hardwoods.[145] ARP technology was subsequently extended to herbaceous biomass, corn stover, and switchgrass with good success.[147] For example, the digestibility of ARP-treated corn stover was 90% at 72 hours with 10 FPU cellulose per gram glucan of enzyme loading, a far better yield than is possible when more enzyme is used with α-cellulose. Continued work on ARP pretreatment has explored the effect of additional treatment with hydrogen peroxide[108,148] and as a supplement dilute-acid pretreatment.[149] A major challenge for ARP is to reduce liquid loadings to keep energy costs low, and an alternative configuration called Soaking in Aqueous Ammonia (SAA) is being developed with this target in mind.[150]

Lime pretreatment

Because the least expensive alkali is lime, available as either quick lime (CaO) or slaked lime ($Ca(OH)_2$), pretreatment with this chemical provides a low-cost alternative for lignin removal at higher pH values.[151,152] Lime also removes acetyl groups that have been shown to affect hydrolysis rates. Lime removes lignin and improves cellulose digestion by enzymes through opening up the structure and reducing non-productive cellulase adsorption. Although lime was used to pretreat a variety of materials in the past, such as corn stover and sorghum stalks, the vast majority of these early studies were performed at conditions typical of those used for sodium hydroxide, a well-established alkaline pretreatment agent, with resulting poorer performance for lime. A more thorough study of lime as a pretreatment agent under various conditions (i.e. over a wider range of times, temperatures, and lime and water loading) showed that lime pretreatment effectively increased enzymatic digestibility of herbaceous biomass by as much as ten times.[151–153] Example conditions for effective lime pretreatment are 100°C for 1 to 2 hours at a lime loading of 0.1 g $Ca(OH)_2$/g biomass with 5 to 15 g water /g biomass. However, lime treatment has been less effective on woody biomass than for many herbaceous plants or agricultural residues at the same process conditions because of the generally higher lignin content of wood. Work in progress is finding new process conditions that enhance performance with poplar wood.

Because the action of lime is slower than that of ammonia or more expensive bases, such as sodium hydroxide, low cost containment, such as pretreatment in piles, is needed to be cost effective.[37] In addition to its low cost, lime is relatively safe to handle and available all over the world in common limestone deposits. Lime can be recovered for recycling by washing biomass with water which can then be saturated with carbon dioxide to form a calcium carbonate precipitate that can be fed to a lime kiln to regenerate lime.

Comparisons of the leading options

All of the pretreatments included in the CAFI project achieved similar high yields of both xylose and glucose from corn stover in the combined operations of pretreatment and enzymatic hydrolysis with cellulase supplemented with beta-glucosidase.[22] However, the patterns of sugar release varied considerably among these technologies. Dilute sulfuric acid pretreatment released most of the xylose in hemicellulose as sugar monomers in pretreatment.[107] Xylose yields from sulfur dioxide pretreatment were lower than those from dilute sulfuric acid pretreatment and a larger portion of the xylose was oligomeric. Controlled pH pretreatment followed a similar pattern to dilute sulfuric acid pretreatment except that xylose yields were lower. Almost all of the xylose was released as oligomers during controlled pH pretreatment.[106] On the other hand, ARP released about half of the potential xylose in pretreatment, all as oligomers.[108] Xylose yields during lime pretreatment were half of those of ARP, and again virtually entirely in oligomeric form.[35] No sugars were released in AFEX pretreatment itself.[110]

All of the technologies removed only a small fraction of the potential glucose during pretreatment. Cellulase supplemented with beta-glucosidase was effective in solubilizing and recovering almost all of the glucose left in the pretreated solids, with small differences among the results for the different pretreatments[22] (Table 1). Furthermore, although no hemicellulases were added during the initial CAFI research with corn stover, cellulase supplemented with beta-glucosidase was still effective in recovering a large fraction of the residual xylose in hemicellulose for all pretreatments from the extreme of dilute sulfuric acid in which the lowest amount of residual xylose was left to AFEX that released no xylose in pretreatment. As a result, overall xylose yields for the combined operations of pretreatment and enzymatic hydrolysis were very similar.

Because of the high total sugar yields and low-cost chemicals employed, a first-cut economic evaluation based on CAFI data for corn stover showed little difference among the projected costs of making ethanol using any of the CAFI pretreatments assuming oligomers released from each technology can be converted to fermentable sugars at a low cost and that organisms will ferment the mixtures of sugars to ethanol with high yields.[37] Thus, with similar high yields of glucose and xylose for all these pretreatments, this data provides no simple answer as to the key need for pretreatments to be effective for corn stover. Preliminary results from research in progress reveal that poplar wood is more recalcitrant to all pretreatments except sulfur dioxide and lime pretreatments when just cellulase enzymes are employed. However, additional work is needed to understand how changes in enzyme formulations can impact yields and reach sound conclusions for this feedstock. Other pretreatments may also have similar yield and cost profiles, but comparative data is lacking to make this assessment.

Conclusion

Fuels derived from cellulosic biomass are essential in order to overcome our excessive dependence on petroleum for liquid fuels and also address the build-up of greenhouse gases that cause global climate change. Biological conversion offers the potential for radical technical advances through

Table 1. Composition of solids from pretreatment of corn stover (percent of dry weight) by CAFI leading technologies and their digestibilities after 72 hours for an enzyme loading of 15 FPU/g cellulose in the original feedstock.

Pretreatment system	Temperature, °C	Reaction time, minutes	Percent Chemical used	Percent Glucan	Percent Xylan	Percent Lignin	Percent conversion at 72 hours (15FPU/g cellulose)
Untreated Corn stover	---	---	---	36.1	21.4	17.2	23.3
Dilute acid	160	20	0.49 of sulfuric acid	59.3	9.5	22.5	91.1
Flowthrough	200	24	water only	76.1	4.8	7.1	95.5
Control pH	190	15	none	52.7	16.2	25.2	85.2
AFEX	90	15	100 of anhydrous ammonia	36.1	21.4	17.2	96.0
ARP	170	10	15 of ammonia	61.9	17.9	8.7	90.1
Lime	55	4 weeks	0.08g CaO/g biomass	52.70	16.20	25.20	93.0

application of the powerful tools of modern biotechnology to realize truly low costs. However, pretreatment is the key cost element in the biological conversion of cellulosic biomass to ethanol or other products, such as butanol, that still require low-cost sugars to be cost competitive. In addition, pretreatment can have pervasive impacts on the performance and cost of virtually all other operations. Thus, pretreatments must be advanced and carefully integrated with the rest of the process to realize the full potential of cellulosic ethanol or other biologically derived products. Although a wide range of pretreatment approaches have been trialed over the years, only a few achieve the high yields of sugars from biomass with low enough costs to be considered attractive, and all of them rely on chemical addition. Unfortunately, relatively little funding has targeted advancing either the technologies or their understanding, impeding significant breakthroughs that reduce cost and more confident commercial applications, and it is now time for far more aggressive and concerted fundamental and applied research on pretreatment. It is particularly vital to better understand its integration with the rest of the process.

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