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A Techno-Economic Assessment of the Pretreatment and Fractionation Steps of a Biomass-to-Ethanol Process

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ABSTRACT

It is generally recognized that the front-end (pretreatment, fractionation, enzymatic hydrolysis) steps of a lignocellulose-to-ethanol process are both technologically immature and represent a large component (~60%) of the total product cost. In the past, we have tried to itemize the process steps and equipment for a complete plant. It was evident that, owing to the complexity and interrelated nature of this process, it was difficult to determine the influence of even minor changes to the process on the overall production cost of the product. We had originally developed a techno-economic model, based on spreadsheets, as a computational and assessment tool. However, our more recent work, which has looked at various process options such as hardwood vs softwoods, SO_2 pretreatment of softwoods, and enzyme recycling, indicated that the model required greater flexibility if it was to assess a "generic" biomass-to-ethanol process. The model is currently being modified to address both the flexibility issues, through the incorporation of flowsheeting concepts, as well as including the most recent work on the various process options. In this article, we have described some of the pretreatment and fractionation issues that are being addressed in the updated model.

Index Entries: Techno-economic modeling; biomass-to-ethanol process; lignocellulose-to-ethanol process.

INTRODUCTION

In the past, we *(1)* and other groups *(2,3)* have used techno-economic models to assess the costs of both the individual component steps and the final products from potential biomass-to-ethanol processes based on enzymatic hydrolysis. The individual component steps of the process that represent the largest portion (~60%) of the total product cost are the front-end, i.e., pretreatment, fractionation, and hydrolysis *(1)*. As a result, much of our recent research and modelling effort has concentrated on these process steps. Much of the past techno-economic analyses have emphasized the importance of recovering all of the major lignocellulosic components to offset the high feedstock cost and lower the final product cost.

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Generally, it has been assumed that a uniform feedstock, such as waste paper *(4)*, energy crops, such as willow *(5)*, or wood residues from sawmilling or wood-processing operations *(1)* would be used as the substrate for a bioconversion plant. However, a more likely scenario is one that is analogous to a typical North American pulp and paper mill where softwoods and hardwoods are simultaneously processed in parallel pulp lines or, alternatively, are sequentially processed in the one line at different times of the month. Similarly, agricultural residues tend to be seasonal in their production and vary in their ability to withstand longer-term storage. Thus, it is essential that, when an integrated bioconversion process is designed, it has the capacity to process a variety of feedstocks. In the past, we have found that steam pretreatment (SO_2-catalyzed steam explosion) and a sequential fractionation (water-alkali-peroxide wash) can be used to increase the flexibility of the bioconversion process and ensure maximum utilization of all of the components of the various feedstocks.

Pretreatment is an essential step for the efficient downstream conversion of lignocellulosic feedstocks to ethanol. Although there are many types of pretreatment, past research has shown that the pretreatment methods that are most effective for primarily cellulose recovery and conversion have generally been physicochemical in nature *(6)*. The reaction kinetics and chemical structure of the three main components of lignocellulosics, i.e., cellulose, hemicellulose, and lignin, differ to such an extent that complete recovery of the components is unlikely. However, as previously mentioned, it is economically imperative that recovery is maximized.

Steam explosion has generally been recognized as one of the most effective methods for pretreating and fractionating lignocellulosic feedstocks *(6–11)*. A range of times, temperatures/pressures, and acid catalysts have been used by researchers on various feedstocks (Table 1). A number of these researchers concentrated on maximizing the cellulose recovery and failed to recognize the importance of also recovering the hemicellulosic and lignin components. Although most of the lignocellulosic feedstocks are currently considered to be of low or negative value, it is highly likely that, as the process attains commercial scale, the feedstock price will rise. Most lignocellulosic feedstocks are sold on a weight basis, and the cellulose component represents only about a half of the original dry weight. Thus, recent modeling studies have started to recognize the importance of optimizing the pretreatment recovery of both the more labile hemicellulose component and the lignin fraction, as well as enhancing cellulose hydrolysis *(27)*.

Various groups have tried to determine the relative importance of lignin and hemicellulose in the enzymatic hydrolysis of pretreated cellulosic substrates *(28,29)*. Some researchers have linked the removal of hemicellulose to an improvement in enzyme digestibility of the pretreated wood *(28,30)*, and it has been suggested that the release of hemicellulose produces an increase in the accessible pore volume and the specific surface area *(31)*. However, we have found that, even after virtually all of the pentosan of aspen wood has been solubilized or destroyed, further steam treatment continues to improve the subsequent rate and extent of enzymatic hydrolysis *(18)*. Therefore, it appears that pentosan removal is just one of a number of factors involved in the improvement in enzyme digestibility of pretreated wood.

Contradictory results have also been reported for delignification. Results indicating beneficial *(29,32)*, little *(33)*, or no effect *(25)* on digestibility have all been described in the literature. It has also been shown that effective pretreatment can

Table 1
Pretreatment Conditions for Various Feedstocks

Material	Catalyst	Temperature, °C	Time, s	Refs.
Agricultural residues				
Wheat straw	—	247	45	*12*
	—	200–230	30–600	*13*
	35 m*M* H_2SO_4	200	120	*14*
Sugar cane bagasse	—	200	60–1200	*15*
	—	200–250	80–360	*16*
	1.2% H_2SO_4	170–250	2–90	*16*
Corn stover	—	215	120	*14*
Hardwoods				
Aspen	—	190–240	20–6000	*17*
	—	240	20–240	*18,19*
	0.2% H_2SO_4	220	10–80	*18,19*
	0.58% H_2SO_4	200	80	*20*
	1.6% SO_2	210–227	120	*20*
	1.6% SO_2	190–220	100	*21*
Eucalyptus	—	200	300	*22*
	—	220–240	120	*23*
	1% SO_2	200–220	50–150	*23*
Willow	—	180–220	600	*24*
	0.6–1.5% H_2SO_4	170–210	600	*24*
	3.0% SO_2	160–200	600	*24*
	1% SO_2	180–230	600	*24*
Softwoods				
Spruce	0.5–5% SO_2	190–220	50–250	*25*
Radiata pine	0.5–12% SO_2	182–248	30–1080	*26*

occur in the absence of hemicellulosic acetic acid, and that short steaming times can be used to produce *in situ* hemicellulose hydrolysis and good sugar recoveries *(34)*.

Thus, it is apparent that pretreatment and fractionation have a pivotal role, not only on the efficiency of hemicellulose and lignin recovery, but also as the key elements in achieving effective cellulose hydrolysis. In our current modeling efforts, we are considering the effect that a varied feedstock (i.e., hardwood, softwood, and agricultural residues) would have on each of the component steps of an integrated biomass-to-ethanol process. It was found that such factors as the use of an acid catalyst, feedstock handling, and various feedstock properties, such as chemical composition and cell-wall distribution, moisture content and feedstock/liquid relations, ultrastructure, bulk density, specific density, temperature, and purchase cost, will all influence the economics and process steps that would be used. In the work presented in this article, we have tried to identify the likely conditions and processes that would be used to maximize hemicellulose-derived sugar and lignin recoveries, while obtaining complete hydrolysis of the cellulosic residue.

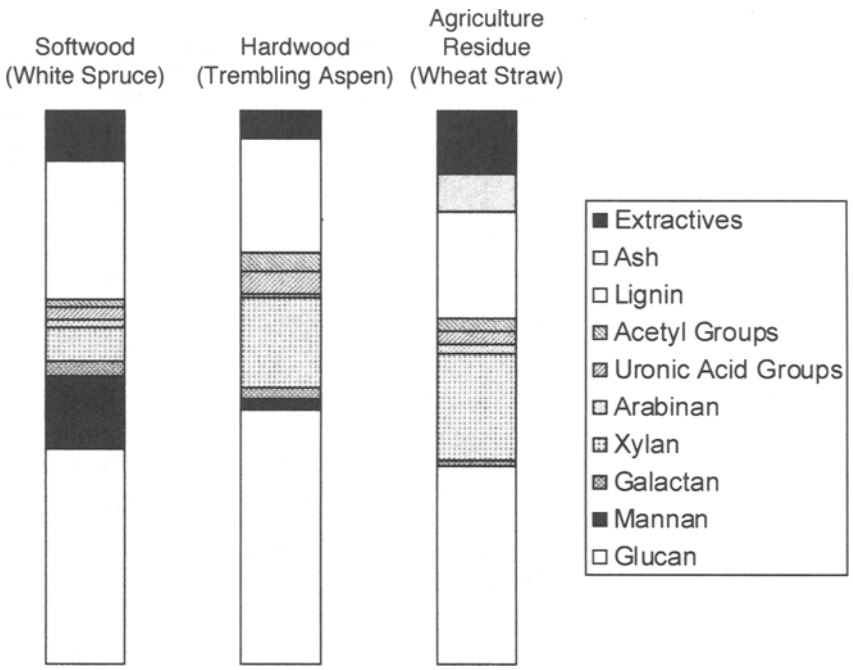

Fig. 1. Composition of representative lignocellulosic substrates: softwood (white spruce), hardwood (aspen), and agricultural residue (wheat straw).

RESULTS AND DISCUSSION

We and other research groups, associated with the International Energy Agency (IEA) "Biotechnology for the Conversion of Lignocellulosics Network," have been refining a "generic" biomass-to-ethanol process model that will be capable of processing different types of feedstocks. It was apparent that the feedstock properties of importance to the technical design and economic evaluation of any process include chemical, physical, and cost components. In the work reported in this article, we have primarily concentrated on chemical properties of the feedstock, since they tended to have a greater overall effect on the technical design of biomass-to-ethanol processes. Although the physical properties of the feedstock, such as ultrastructure, bulk density, specific density, temperature, and form (chips, wafers, sawdust, and so forth), are important, they generally impact on just the pretreatment step. Similarly, the cost of the feedstock is one parameter that is easily changed within any modeling work and did not need to be discussed further here. Thus the chemical composition and overall yield of each of the components were considered to be key variables that impacted on hemicellulose and lignin recovery and cellulose hydrolysis.

Although most of our work has concerned hardwoods *(33,34)* and softwoods *(25)*, other groups have studied the steam treatment of agricultural residues *(2)*. It is apparent (Fig. 1) that there is considerable variation in both the chemical composition of representative lignocellulosic substrates and the amount of material found in each of the cellulose, hemicellulose, lignin, and extractive fractions. The actual association of each of these components has a major impact on the efficiency

of pretreatment and fractionation. For example, although similar pretreatment conditions can be defined to maximize hemicellulose solubilization and sugar recovery, the higher concentration of extractives associated with softwood will likely lead to considerable inhibition problems when attempts are made to ferment hemicellulose-derived sugars from softwoods. Similarly, softwood lignins contain primarily guaiacyl units, whereas hardwood lignins contain both syringyl and guaiacyl components and agricultural residues, particularly the grasses, contain syringyl-, guaiacyl-, and *p*-hydroxyphenyl units. These differences in the basic building blocks combined with the three-dimensional structure within the substrate result in quite different responses to fractionation when alkali extraction and peroxide are used to remove the lignin and enhance hydrolysis of cellulosic residues *(25,34)*. In the subsequent sections, we have discussed the steam-explosion pretreatment conditions that have been used to maximize hemicellulose hydrolysis and lignin recovery while producing a cellulosic substrate that can be readily attacked by enzymes.

Hemicellulose Recovery

The conditions shown in Table 1 reflect various researchers' definitions of optimized pretreatment conditions. Optimization has usually been solely based on the maximum cellulose hydrolysis recovery that can be achieved with a disregard for the hemicellulose or lignin recoveries. The kinetics and structures of the lignocellulosic components differ to such an extent that there must be a compromise in the pretreatment conditions used. As mentioned previously, lignocellulosic feedstocks are purchased on a weight basis and, as a consequence, all components have a value. If possible, they should be converted to marketable or process usable forms. Near-theoretical fermentation yields can be attained from the hemicellulosic fraction *(35–37)* when the oligomeric components have been subsequently hydrolyzed prior to fermentation. However, it is preferable if the hemicellulose-derived sugar stream can be produced in a monomeric form by the pretreatment process itself and contain minimal breakdown products. Generally, it has been recognized that the use of high temperatures and short cooking times produces pretreatment conditions that both soften the wood components and take into account the solubilization kinetics of hemicellulose *(38)*. Breakdown products that are inhibitory to the fermentation organisms will be produced where more severe pretreatment conditions are used. Research efforts to address the production of inhibitors generally have pursued two main courses of action. A preventative course, i.e., adjustment of the kinetics of the pretreatment via acid catalysts, which will be discussed in greater detail in a later section, and a reactive approach that identifies the inhibitory compounds and develops methods to remove or detoxify the compounds before fermentation. Past efforts have looked at the effects of hardwood hemicellulose breakdown products, i.e., furfural and acetic acid on fermentation. There are also some inhibitory substances, such as wood extractives, that are naturally associated with the substrate (Fig. 1). Toxicity associated with the extractive fraction has been shown *(39)* to be primarily associated with the nonvolatile components and will generally be associated with the post-pretreatment water-soluble fraction. Although the optimization conditions for maximum sugar recoveries can be predicted, the nature of the inhibitory compounds and methods to detoxify the streams in a cost-effective manner are still largely unresolved. Traditional detoxification methods, such as the addition of activated coal *(40)*, extraction with organic solvents *(41–43)*,

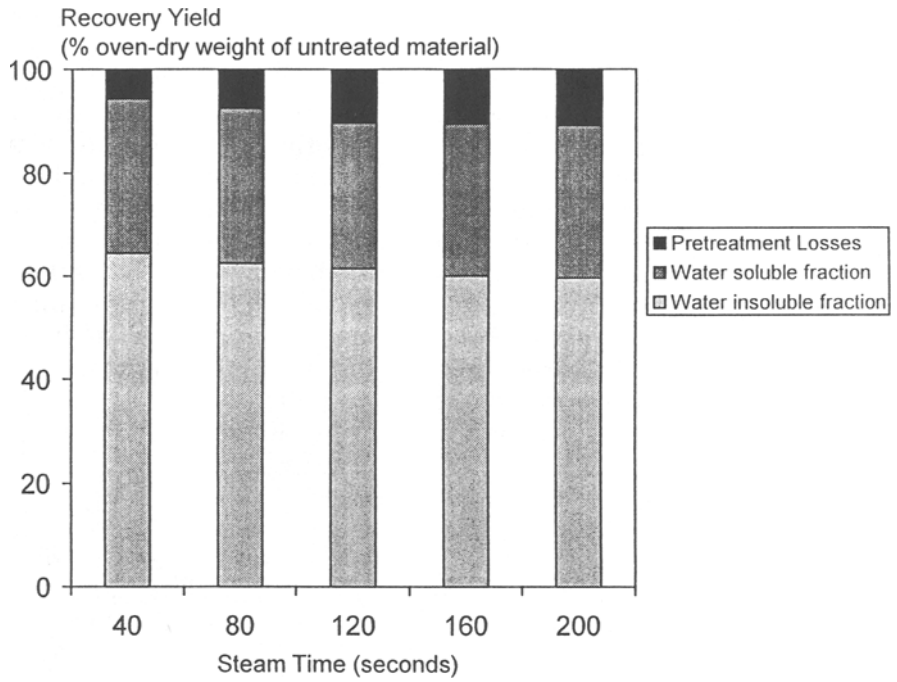

Fig. 2. Recovery yield for steam pretreatment of SO_2-impregnated hardwood (adapted from refs. *10* and *33*).

ion-exchange *(41,42,44)*, ion exclusion *(45)*, molecular sieves *(46)*, overliming *(47,48)*, and steam stripping *(49)*, have been shown to be costly or ineffective *(50)*. Although there have been some attempts to adapt the fermentation organisms to the inhibitory substances *(51)*, each feedstock change required a further adaptation period. Consequently, the detoxification step has yet to be resolved and has not been included in modeling efforts to date.

Almost all of the hemicellulose can be extracted from the steam-exploded feedstock by a subsequent water-extraction step *(52)*. The water extract contains hemicellulose-derived sugars, and any breakdown products derived from the hemicellulose and lignin components, as well as some of the extractive components. The recovery yield of fermentable sugars will primarily depend on the severity of the steam treatment and the extraction parameters. Generally, the more severe the pretreatment, the lower the hemicellulose-derived sugar yield (Fig. 2). High severity treatments are generally required to achieve good enzyme accessibility of the cellulose. However, the more severe conditions result in the generation of more hemicellulose and lignin breakdown products (Fig. 3), which are generally quite inhibitory to the fermentative organisms used to produce ethanol from the hemicellulose-derived sugars *(53)*. As a result, we have tried to define an optimal pretreatment condition as one that results in a minimum overall ethanol production cost and maximum ethanol yields, based on maximum recovery of hemicellulose-derived sugars after the pretreatment step.

The process design and economics of the hemicellulose extraction step is dominated by the objectives of high extraction yields, high dissolved solids concentration in the extract, and low solvent loadings. High extraction yields of

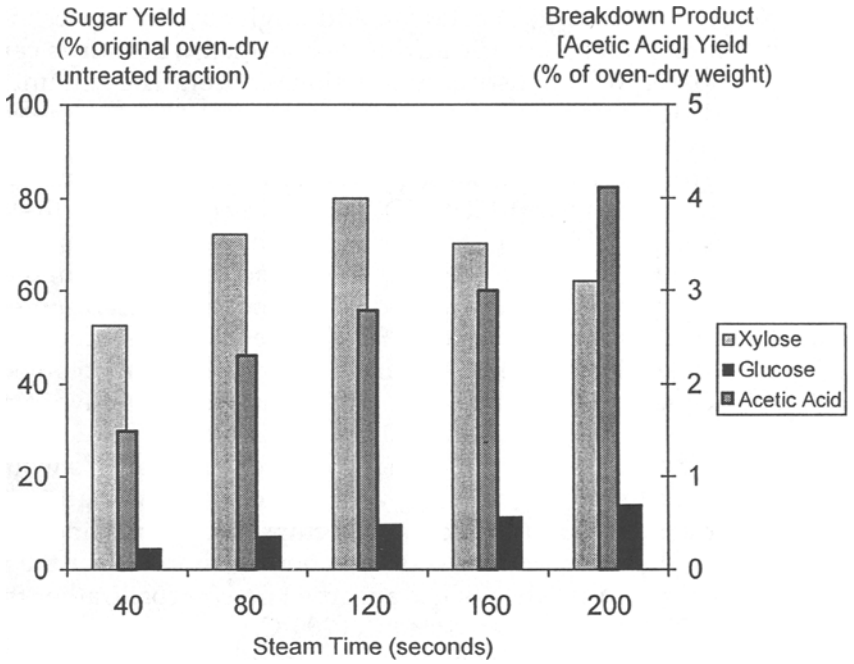

Fig. 3. Water-soluble fraction yield from steam-pretreated SO_2-impregnated hardwood (adapted from refs. *10* and *33*).

hemicellulose can provide higher ethanol returns, and both savings in capital costs (through a reduction in the total volume of the cellulose hydrolysis fermentors) and operating costs (through reduction in the required mixing energy). Reduced capital and energy costs, in the subsequent bioconversion of the hemicellulose extract to ethanol, are the main incentive for having high dissolved solids concentration in the extract. Lower water usage lowers input costs and also concentrates the hemicellulose sugars. However, it also concentrates many of the wood extractives or sugar degradation products that are inhibitory to hemicellulose fermenting microorganisms *(20)*.

If a steam-explosion-based process is to recover adequately all three components for a wide variety of feedstocks, the use of an acid catalyst will be required *(33)*. Past research has concentrated on primarily two acid catalysts, sulfuric acid (H_2SO_4) and sulfur dioxide (SO_2) *(54,79)*. These acid catalysts have been shown to provide higher hemicellulose and cellulose recoveries in both hardwoods and softwoods. Enhanced cellulose hydrolysis rates are also found in all substrates. When the two acid catalysts were compared on an equal severity basis, they both enhanced the survival of pentose sugars. However, the alkali lignin extraction from the water-washed exploded substrates was lower with H_2SO_4 *(20,55,79)*, the gaseous sulfur dioxide was easier and faster to introduce, and the sulfuric acid resulted in a greater steam consumption.

Our past work has also helped elucidate the catalytic mechanism of sulfur dioxide addition during steam explosion *(55)*. When aspenwood chips were impregnated with SO_2 and subjected to steam at 200°C, much of the SO_2 in the chips was converted to sulfuric acid within 20 s. This sulfuric acid (and not sulfurous acid

or lignosulfonic acid) is the actual catalyst. Although air, when present, may be involved in this oxidation, sulfuric acid is also formed when air is carefully excluded by N_2, apparently by a disproportionation reaction. The amount of sulfuric acid produced increases with increased SO_2 impregnation, but increases less than proportionately.

Formation of sulfuric acid and subsequent catalysis were demonstrated by impregnating aspenwood chips with 1.6% SO_2 and treatment for 20 s with steam at 200°C to produce sulfuric acid. The treatment was stopped after this time, and unused SO_2 was carefully and completely vaporized and removed from the chips and vessel, without removing the sulfuric acid. Treatment was then resumed with fresh steam for 80 s at 200°C in the absence of SO_2. The resulting well-cooked product, from this sulfuric-acid-catalyzed steaming, was similar to that obtained from a 100-s cook with SO_2. Control cooks for 100 s at 200°C without SO_2 gave relatively uncooked products, as did cooks for only 20 s with SO_2. If sulfuric acid had not formed during the first 20 s of steaming, the second steaming for 80 s would not have been catalyzed, and the product would have been under-cooked like the controls.

The conversion of SO_2 to sulfuric acid in wood chips is apparently independent of the composition of the chips, which act only as porous supports for the SO_2. A very similar conversion occurs when SO_2 is adsorbed on charcoal (rather than on wood chips) and is subjected to saturated steam at 200°C. In both cases, equilibrium conditions were not established. The conversion occurs too rapidly for the SO_2 to be driven out of the wood or charcoal, by the rapidly rising temperature, at least when the high-pressure steam is quickly admitted.

The equilibrium vapor pressure of SO_2 above aqueous SO_2 solutions is quite significant, even at low temperatures, and the solubility of SO_2 decreases with increasing temperature. Nevertheless, there appears to be little or no exchange of SO_2 between the chips during steam treatment as shown by the following experiment. The lower half of a thin-walled loosely covered canister was filled with commercial wood chips impregnated with 1.6% of SO_2 (dry wood basis), and the upper half (separated only by a wire screen) was filled with unimpregnated chips. The canister was then lowered quickly into the pressure vessel, and steam was admitted within 2 s. After 100 s in 200°C steam, only the chips in the lower half of the canister (i.e., those originally impregnated with SO_2) were thoroughly cooked. Those in the upper half (i.e., no SO_2) were virtually uncooked and resembled those from control cooks in the absence of all SO_2.

We have also carried out a sulfur balance (Fig. 4) in order to determine further the mode of action of the SO_2 and the fate of the sulfur *(55)*. Approximately 50% of the input SO_2 remained in the exploded substrate following explosive decompression and air drying at room temperature of the resulting exploded wood, indicating that, at the level of SO_2 impregnation used in this work (1.6% of OD wood input), half of the SO_2 was acting as sulfurous acid or was present in the vapor phase in the void volume of the gun. Water washing removed the large majority (34.4%) of the retained sulfur with some 7% of the original sulfur remaining in the washed substrate. The sulfur located in the water solubles is nonvolatile, since freeze-drying of the wash liquors did not result in a total loss of sulfur. Approximately one-third of the total original sulfur remains in the dry water solubles. The water-soluble sulfur was not inorganic sulfite resulting from reaction with the wood ash. Although lignosulfonates were not isolated from the water-soluble material, it was probable that the sulfur was bound to lignin fragments rather than to any soluble or insoluble carbohydrate.

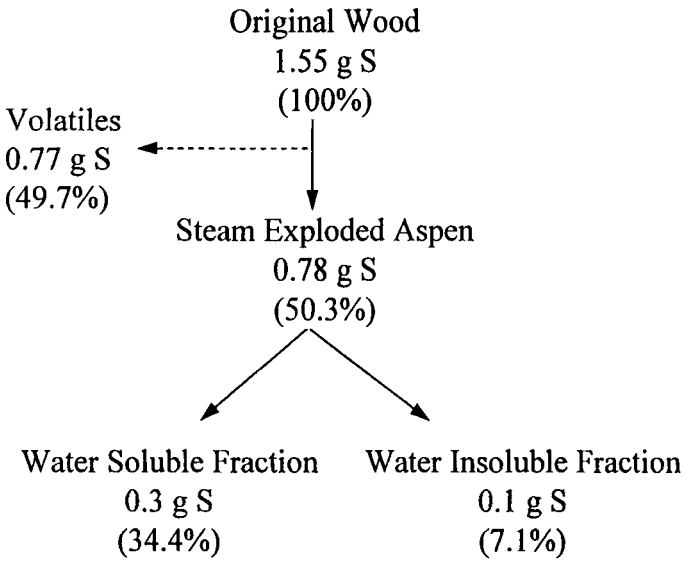

Fig. 4. Sulfur balance for typical SO_2-impregnated hardwood sample *(20)*.

Sulfur dioxide impregnation prior to steam pretreatment particularly at lower temperatures and pressures is the preferred catalytic procedure. It reduces the wastage of SO_2, less is converted to H_2SO_4, it reduces the corrosion rate of the steam pretreatment reactor, since SO_2 under the milder conditions is less corrosive, and it provides the opportunity to recycle the unconverted SO_2 more easily and prevent its release into the environment. Previous work, using impregnation before steam pretreatment, determined that 1% of SO_2, or less, would be satisfactory, if the SO_2 was converted to H_2SO_4 in high yield within the wood *(55)*. We are currently in the process of reviewing the methods and costs of implementing SO_2 usage, and will be using the model to provide a cost–benefit analysis.

Feedstock compositional differences have been shown to influence the effectiveness of the fractionation and consequently the level of product yield attained for the so-called "optimized" process. Past research has concentrated primarily on hardwood species, such as aspen, willow, birch, and eucalyptus, and has shown that each of these species requires a slightly different pretreatment condition (Table 1) to optimize the recovery of the hemicellulose sugars. The optimal conditions (Figs. 2 and 3) for a representative hardwood were determined to be 1.6% SO_2 for 120 s at 210°C, which produces an 80% hemicellulose recovery of xylan as xylose. Agricultural residues, such as wheat straw, sugar cane bagasse, and corn stover, have also been studied and react in a manner similar to hardwoods. Softwood species, such as spruce *(25)* or radiata pine *(26)*, require substantially different pretreatment conditions, i.e., longer residence times (Figs. 5 and 6) and higher catalyst concentrations (Table 1). Softwood hemicellulose-derived monomers are primarily mannose, glucose, and galactose with minor amounts of xylose and arabinose (Fig. 1). Softwood pretreatment optimization focused on the mannose and glucose recoveries, rather than the xylose, and these hemicellulose-derived hexoses could be combined with the glucose stream coming from the cellulose recovery. However, the potential capital and operating cost benefits that could be anticipated by combining the fermentation of both the cellulose and hemicellulose streams may be offset by

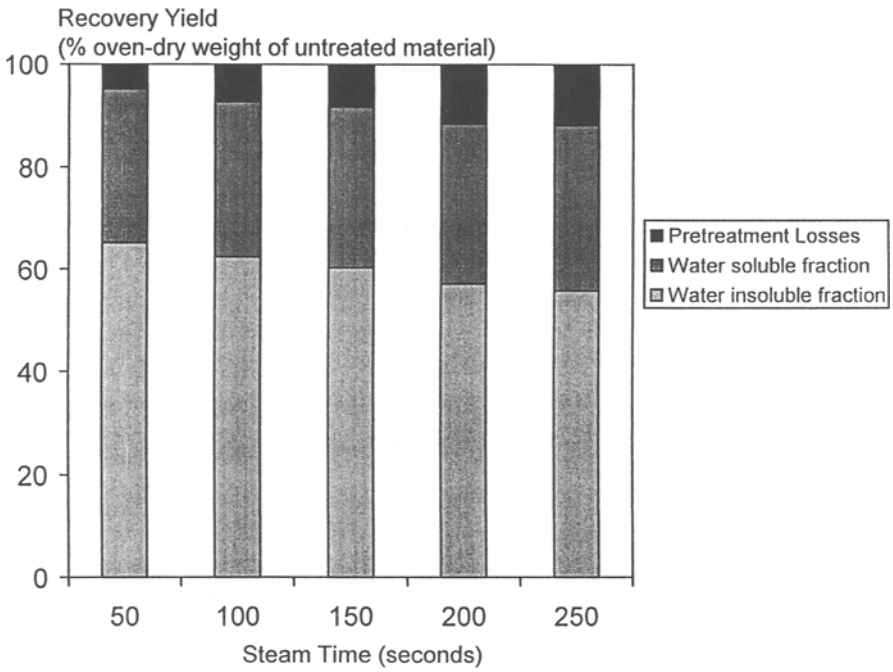

Fig. 5. Recovery yield for steam pretreatment of SO_2-impregnated softwood (adapted from ref. *25*).

the higher wood extractives concentration that can be anticipated in the hemicellulose stream from softwood substrates.

LIGNIN RECOVERY

Lignin can be readily extracted from the water-insoluble exploded hardwoods using dilute sodium hydroxide *(56)*. The lignin extraction yield is dependent on factors, such as pretreatment effectiveness, caustic concentration, extraction method used, and the use of further extraction steps, e.g., peroxide wash. Lignin extraction yield is enhanced when a catalyst, such as SO_2, is added or more severe pretreatment conditions (Fig. 7) are used. Concentrations of caustic over 5% also increase the lignin extractability, although the chemical cost is extremely high and two consecutive washes are required to attain over 90% recovery (Fig. 8) of the extractables in batch extraction *(55)*. Preliminary modeling has shown the importance of optimizing the concentration of alkali and reducing the water volumes *(57)*.

The alkali wash will be more fully concentrated only if the majority of the lignin is readily alkali-soluble, as occurs in the case of the hardwoods, and agricultural residues. With hardwoods, it is possible to recover close to 90% of the original lignin by following steam explosion and water washing by a subsequent alkali wash (Fig. 8). However, with softwoods, only 50% of the original lignin could be removed by alkali extraction (Fig. 9). As discussed later in this article, the extraction was also shown to greatly reduce the efficiency of hydrolysis of the cellulosic residue. A subsequent peroxide treatment greatly increased the degree of cellulose hydrolysis (Fig. 10), while only removing a further 30% of the original lignin. The benefits

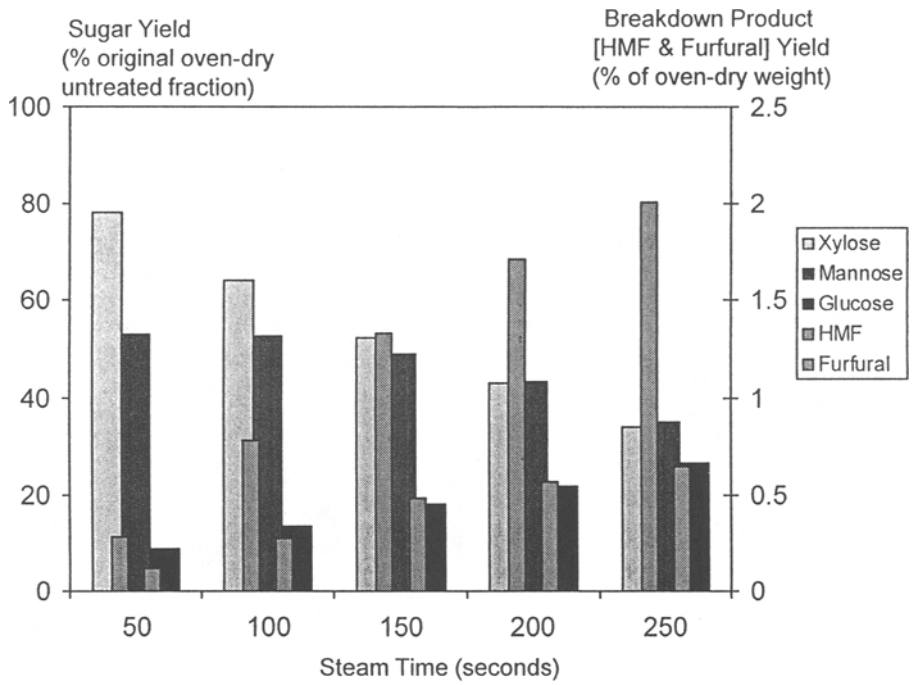

Fig. 6. Water-soluble fraction yield from steam-pretreated SO_2-impregnated softwood (adapted from ref. 25).

of delignification include a reduction in the bulk density of the cellulosic residue, and enhanced cellulose recovery benefits by reducing the total volume of the reactors required for each of the remaining steps associated with the cellulose stream. It also reduces the required mixing energy, while concentrating the cellulose content of the hydrolysis input stream.

Cellulose Recovery

Enzymatic hydrolysis of cellulose generally has been suggested to be limited by factors that are either related to the structure of the substrate or the type and composition of the cellulase system used to carry out hydrolysis. It has been shown *(33)* that the hydrolysis rate declines logarithmically over a range of enzyme and substrate concentrations. It has been hypothesized that the gradual decline in hydrolysis rate is a reflection of the increase in substrate recalcitrance resulting from the structural impediments within the substrate that limit enzymatic hydrolysis. Each cellulosic substrate's susceptibility to enzymatic hydrolysis depends on a number of structural features. Those that have been proposed include the crystallinity *(58–61)*, degree of polymerization *(59)*, surface area available to the enzymes *(61–66)*, and the lignin content and distribution *(61)*. However, other enzyme-related factors, such as enzyme adsorption, enzyme inactivation and end-product inhibition, have also been shown to affect the overall mechanism of hydrolysis *(67)*.

Several major changes in the structure of the cellulose have been demonstrated after steam explosion of cellulosic residues. Various authors have reported a gradual decrease in the degree of polymerization (DP) of cellulose and a substan-

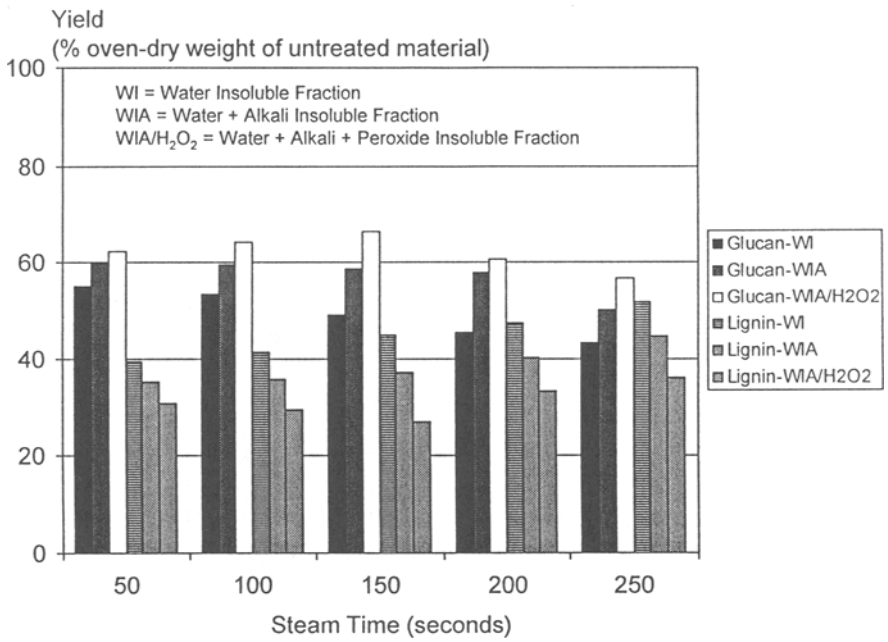

Fig. 7. Water-insoluble fraction yield from steam-pretreated SO_2-impregnated softwood (adapted from ref. *25*).

tial increase in the crystallinity index (CrI) of the substrate *(68,69)*. The apparent increase in crystallinity seems to be the result of fusion between cellulose crystallites, which were originally separated by a matrix of hemicellulose and lignin. Therefore, the gradual removal of hemicelluloses and lignin, to increase substrate surface area, appears to trigger the reorientation of the cellulose molecules, which, after pretreatment, assume a distinct crystalline form.

The initial rapid rate by which cellulose is hydrolyzed has been associated with the occurrence of more accessible regions at the substrate surface. At the fiber level, these more accessible regions are often associated with cracks or defects in the fiber, whereas at the molecular level, they are characterized by a larger pore volume and/or available surface area *(55,70)* and a lower crystallinity index (amorphous cellulose) *(71,72)*. The initial rate of hydrolysis of cellulosic substrates has been linearly correlated with the distribution of micropores, which were accessible to the enzymes *(62)*. The lower susceptibility of softwood substrates to hydrolysis, compared to hardwood substrates, was associated with a smaller increase in the distribution of these micropores during pretreatment. The development of pore volumes accessible to the enzymes has also been demonstrated to be a key factor in determining the degree of enhancement of substrate accessibility for steam-treated aspen *(73)*, poplar *(63)*, and radiata pine *(70)* chips.

It has been suggested that lignin acts as a physical barrier during the hydrolysis of steam-treated substrates and hinders the contact of the substrate by the enzymes *(74)*. Various factors, such as the irreversible adsorption and nonspecific binding of enzymes onto the lignin-containing residue, have been implicated *(75,76)*. Although alkali washing of steam-treated hardwoods usually results in a substrate

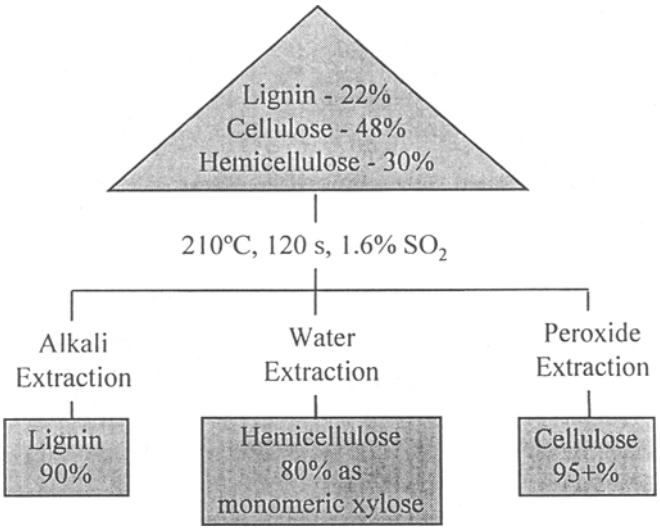

Fig. 8. Optimized conditions for maximum sugar and lignin yields from a representative hardwood.

with a lower lignin content, it is generally only as readily hydrolyzed as the water-washed substrate (Fig. 10) *(70,73,77)*. It appears that the beneficial effects of alkali extraction, such as lignin removal and cellulose swelling, are offset by other factors that have a detrimental effect on hydrolysis, such as the possible redistribution of the residual lignin and modifications in the crystalline state of the cellulose. Despite the small cellulose hydrolysis gain from delignification, the extraction of the lignin from the pretreated residues is still desirable, since it produces a substrate with a comparatively higher cellulose content from which a higher glucose yield per gram of substrate can be achieved. However, alkali washing of steam-treated softwoods generally results in substrates that are more recalcitrant to enzymatic degradation than the respective water-washed substrate (Fig. 10) *(25,70)*. This was more dramatic in the case of spruce, where alkali extraction seemed to redeposit the lignin in a fashion that greatly reduced the ease of hydrolysis *(25)*. Extraction of the residual alkali-insoluble lignin by oxidative agents, such as sodium chlorite *(77)* and hydrogen peroxide *(78)*, has been shown to increase the susceptibility to enzymatic hydrolysis of pretreated substrates derived from both hardwood and softwood residues. It was suggested that it was the redistribution of this residual alkali-insoluble lignin that limited complete hydrolysis of alkali-washed substrates. After alkali washing, this highly condensed, modified lignin appeared to reprecipitate on the surface of the substrate, causing a reduction of both the available surface area and the ability of the cellulose fibers to swell in water.

The role that hemicellulose and lignin play in the enzymatic hydrolysis of lignocellulosic substrates is still being debated and, from a purely economic standpoint, may be inconsequential, since the main objective of this bioconversion process should be to recover the maximum amount of all three of the lignocellulosic components. We have defined a series of process steps that provide the technical ability to attain maximum sugar recovery for representative hardwoods and soft-

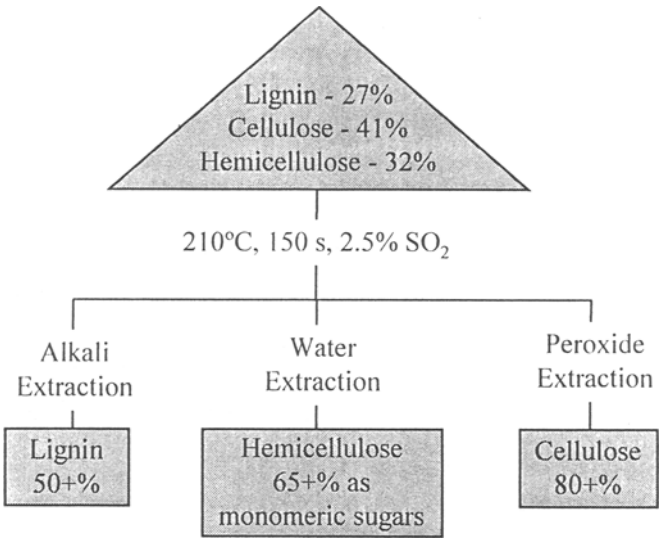

Fig. 9. Optimized conditions for maximum sugar and lignin yields from a representative softwood.

woods. We are now in the process of determining the economic cost of implementing these process steps.

CONCLUSIONS

Integrated bioconversion processes have to be designed with the capacity to process a variety of feedstocks. To ensure maximum substrate utilization, process optimization should be based on the utilization of all three of the main lignocellulosic components. With hemicellulose being the most labile of the three main lignocellulosic components, the pretreatment should ensure maximum hemicellulose solubilization and sugar recovery during the pretreatment step, as well as enhanced cellulose hydrolysis. Similarly, pretreatment and fractionation should be optimized to produce the highest degree of lignin recovery for the least amount of solvent and water usage. It is currently technically possible, through the use of SO_2-catalyzed steam pretreatment and a fractionation sequence consisting of water-alkali-peroxide wash steps, to process both hardwoods and softwoods effectively. Steam pretreatment of hardwoods using SO_2 catalysis can recover more than 80% of the hemicellulose-derived sugars as monomers, more than 90% of the lignin by alkali washing, while complete cellulose hydrolysis at high substrate concentrations and low enzyme loadings can be achieved in a relatively short period of time *(34)*. Using the same process with softwoods, it is currently possible to recover 65% of the hemicellulose-derived sugars as monomers, 80% of the lignin by a combination of both alkali and peroxide washing, and complete hydrolysis at high substrate concentrations *(25)*.

Although we have optimized pretreatment and recovery yields, certain technical and economic aspects still require refinement. For example, we still have to determine all of the inhibitory products associated with the hemicellulose-rich water-soluble stream, their distribution in the various process streams, and cost-

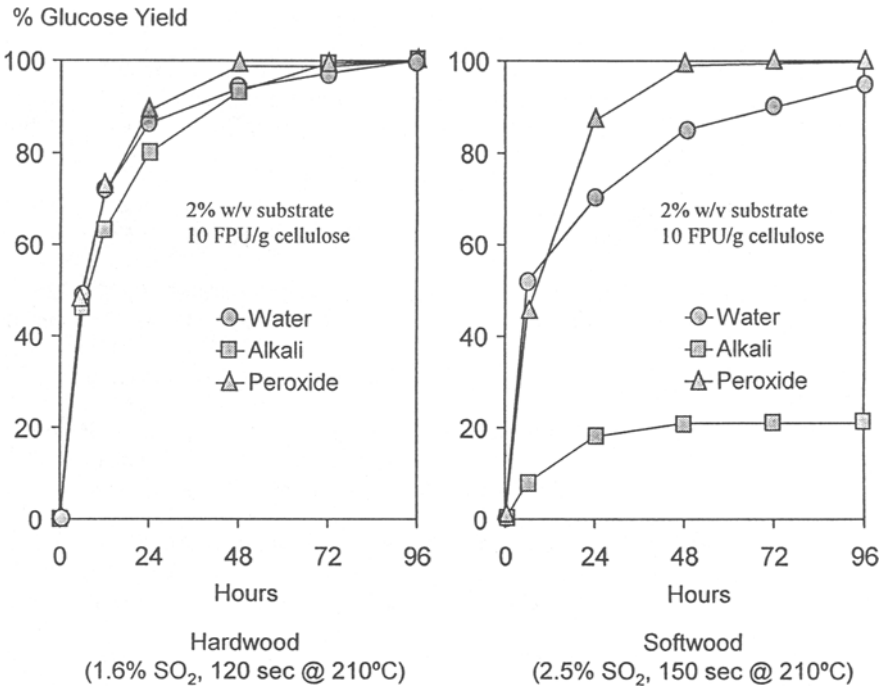

Fig. 10. Glucose yields for steam-pretreated SO_2-impregnated aspen and spruce following each of a water wash, alkali wash, and peroxide wash (adapted from refs. *19* and *21*).

effective methods to alleviate their effects on fermentation. Furthermore, peroxide washing, required to achieve effective hydrolysis of the softwood-derived cellulosic stream, has generally been viewed in the past as being too costly. However, the high cost of enzymes implies that substrates containing low levels of lignin are required if strategies, such as enzyme recycle or simultaneous saccharification and fermentation, are to be used at a commercial scale. Thus, maximum hemicellulose and lignin recoveries are required to optimize utilization of these streams, enhance cellulose hydrolysis, and reduce the cost of producing ethanol from a range of lignocellulosic substrates.

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