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Conversion of Lignocellulosics Pretreated with Liquid Hot Water to Ethanol

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ABSTRACT

Lignocellulosic materials pretreated using liquid hot water (LHW) (220°C, 5 MPa, 120 s) were fermented to ethanol by batch simultaneous saccharification and fermentation (SSF) using *Saccharomyces cerevisiae* in the presence of *Trichoderma reesei* cellulase. SSF of sugarcane bagasse (as received), aspen chips (smallest dimension 3 mm), and mixed hardwood flour (–60 +70 mesh) resulted in 90% conversion to ethanol in 2–5 d at enzyme loadings of 15–30 FPU/g. In most cases, 90% of the final conversion was achieved within 75 h of inoculation. Comminution of the pretreated substrates did not affect the conversion to ethanol. The hydrolysate produced from the LHW pretreatment showed slight inhibition of batch growth of *S. cerevisiae*. Solids pretreated at a concentration of 100 g/L were as reactive as those pretreated at a lower concentration, provided that the temperature was maintained at 220°C.

Index Entries: Liquid hot water; pretreatment; SSF; inhibition; particle size reduction.

INTRODUCTION

In order to harness biological processes to convert plant biomass into fuels on a scale large enough to displace conventional fuels significantly, lignocellulosic materials must be utilized *(1)*. Conversion of naturally occuring lignocellulosic materials to ethanol requires a pretreatment of some kind *(2)*: that is, processing to make biomass sufficiently accessible and reactive to allow high rates and yields on enzymatic hydrolysis. Unfortunately, pretreatment is one of the most expensive *(3,4)*, and poorly understood *(2,5–7)* unit operations in the conversion of biomass to ethanol.

Determinants of Pretreatment Efficacy

From an applied perspective, the principal determinants of pretreatment efficacy are the degree of fiber reactivity, recovery of pentosans, hydrolysate

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inhibition of microbial growth, extent of size reduction required, cost of associated equipment and utilities, and waste production. These metrics are discussed in the following.

Fiber Reactivity

Increased fiber reactivity is accomplished through a variety of mechanisms: solubilization of hemicellulose (5); removal of lignin (8); reduction of particle size (2); and alteration of the cellulose characteristics, such as degree of polymerization, abundance of cellulose chain ends (2), and crystallinity (9,10). Effective pretreatments in general approach or exceed 80% of theoretical cellulose conversion on subsequent hydrolysis of a representative hardwood feedstock (e.g., poplar) using moderate (e.g., 10–15 FPU/g cellulose) cellulase loadings. Such conversions are achieved in a period on the order of 5 d, although this is highly feedstock dependent (1).

Pentosan Recovery

For the production of a commodity fuel such as ethanol, product yield has a strong influence on process economics (11,12). With the increasing availability of organisms capable of converting pentoses to ethanol (13–16), high recovery of pentoses is a key feature of leading pretreatment technologies. A recovery of 80% of theoretical is representative of dilute-acid hydrolysis. (17).

Extent of Hydrolysate Inhibition

The organisms used for the fermentation of ethanol are often inhibited by the degradation products produced during pretreatment. Inhibitory compounds originate from (6) the hydrolysis of extractive components, organic and sugar acids esterified to hemicellulose (e.g., acetic, formic, glucuronic, galacturonic), and solubilized phenolic lignin derivatives; the degradation of solubilized compounds (e.g., furfural, hydroxymethyl furfural); and the release of corrosion products (e.g., metal ions). The production of inhibitors has been documented for dilute-acid (6), steam-explosion (18,19), and acid-hydrolysis (20,21) pretreatments.

Extent of Size Reduction Required

Different pretreatment processes differ widely in the extent of size reduction required. Costs and energy requirements for particle size reduction increase geometrically with decreasing particle size and can be very significant (5). For dilute-acid pretreatment, grinding down to a particle size on the order of 1 mm is required (17). This can account for one-third of the power requirements of the entire process (17).

Cost Associated with Equipment and Waste Production

Reactor size is determined by residence time and solids concentration. High reactivity and solids concentration reduce the equipment size and, thus, the investment necessary. Materials requirements are primarily a function of the corrosivity of the process conditions and, secondarily, of the pressure at which the process operates. Process residues arise principally from neutralization of acids, e.g., formation of gypsum when limestone is used to neutralize sulfuric acid. Such residues are largely, if not entirely inert, but they do require disposal, most likely via landfill.

Pretreatment Processes

The most thoroughly characterized pretreatment process options include dilute-acid pretreatment *(22–25)*, steam explosion *(26–31)* including acid-catalyzed steam explosion *(32,33)*, ammonia fiber explosion *(34–36)*, and treatment with organic solvents *(37,38)*. Additional processes that have been proposed, but have received less attention to date include use of milling *(39)*, supercritical fluids *(5)*, irradiation *(40)*, biological delignification *(10)*, oxidizing agents *(23)*, alkali *(41)*, and liquid hot water (LHW) (*see* "LHW Pretreatment" below).

Both steam-explosion and dilute-acid pretreatments have been the object of over a decade of research and development, much of which has been specifically targeted at fuel production from biomass. Steam explosion has been studied on a pilot scale, and a pilot plant for dilute-acid pretreatment has recently been constructed *(42)*. Steam explosion offers the advantage of less corrosive operating conditions. The chief advantage of dilute-acid hydrolysis relative to steam explosion is higher recoveries of hemicellulose sugars. Specifically, a pentosan recovery following hardwood pretreatment of 80% is representative of dilute-acid pretreatment *(20)*, whereas we know of no study on steam explosion reporting recoveries higher than that of 65% observed by Heitz et al. *(28)*.

In the context of fuel production, cost estimates for ammonia fiber explosion (AFEX) *(36)* and catalytic pretreatment with organic solvents (organosolv), (R. Katzen, personal communication) support the view that in the absence of future breakthroughs, these two processes are somewhat more costly than dilute-acid pretreatment. Milling and other purely mechanical pretreatment processes are considered not to be cost-competitive and are not under active consideration to our knowledge.

LHW Pretreatment

A much smaller body of literature exists with respect to the use of LHW to fractionate biomass, with but a portion of this literature directed toward pretreatment. Table 1 summarizes results for biomass fractionation using LHW. In general, this approach results in very high and often complete solubilization of hemicellulose, significant solubilization of both lignin and overall biomass, and rather low solubilization of cellulose. Recent work by Antal and coworkers *(43,49)* is differentiated from previous work by pentosan recovery that is higher than reported values and, in most cases, by the use of much shorter reaction times. Apart from the authors, only two of the groups represented in Table 1 have examined the use of LHW as a pretreatment: those of Ladisch and Bobleter *(10,50,51)*. Using a 30-min pretreatment reaction time, Hormeyer et al. obtained enzymatic hydrolysis yields of about 40% theoretical from poplar and straw *(50)*, with significant hydrolysis of the same materials also achieved when mediated by *Clostridium thermocellum* in a direct microbial conversion (DMC) system *(51)*. Enzymatic hydrolysis of hemicellulose from beech bark and bagasse using various xylanases was also reported *(47)*. Kohlmann et al. *(10)* employed a reaction time of 50–60 min in the presence of added caustic to pretreat rapeseed stems and soybean hulls, with near-theoretical yields reported on subsequent enzymatic hydrolysis.

Based on the results just summarized, the thermochemical aspects of LHW accord it outstanding potential as a pretreatment process. Relative to steam explosion, LHW pretreatment offers the potential advantage of high pentosan recovery.

Table 1
Solublization Profiles for Aqueous Fractionation of Biomass[a]

System type	Feedstock	Pretreatment conditions T, oC	t, min	Log Ro[b]	Solubilized material, wt.% Biomass	Lignin	Cellulose	Hemicellulose	% Pentosan recovery	Ref.
Batch	10 Different	200–230	1-15		40–60	35–60	4–22	100	90	(*43*)
Batch	Aspen	230	2	4.1	47	37	5	100	88	(*43*)
Batch	Cane bagasse	230	2	4.1	41	38	5	100	99	(*43*)
Short percolation	Cane bagasse	220	2	3.8	43	65	7	98	84	(c)
Short flow	Aspen	235	2	4.3	32	<15	6	>90	67	(*44*)
Long percolation	Cane bagasse	195	65	4.6	50	68	nd	100	nd	(*45*)
Long percolation	Wheat straw	195	25	4.2	50	<60	nd	100	nd	(*46*)
Long batch	Aspen	170	120	4.1	29	25	21	83	nd	(*47*)
Long batch	Rapeseed stems	180	50–60	4.1	nd	nd	nd	nd	nd	(*10*)
Long batch	Soybean hull	180	50–60	4.1	nd	nd	nd	nd	nd	(*10*)
Steam explosion	Aspen	230	2	4.1	27	<10	<10	90	55	(*21*)
Steam explosion	Cane bagasse	210	2	3.5	28	25	0	85	62	(*28*)
Steam explosion	Wheat straw	230	2	4.1	54	104	12	88	37	(*29*)

[a] nd, Not determined.
[b] Log Ro refers to the severity parameter defined by Overend and Chornet *(48)*.
[c] R. Katzer (personal communication).

Relative to dilute-acid pretreatment, LHW offers advantages with respect to materials of construction and waste production. For the production of ethanol, some of the largest cost impacts of the choice of pretreatment technology are manifested in the biologically mediated process operations downstream of the pretreatment step itself *(4)*. The objective of this study was to determine the suitability of lignocellulosic materials pretreated by the process developed by Antal et al. *(43,49)* as substrates for the biologically mediated processes of enzymatic hydrolysis and fermentation in a simultaneous saccharification and fermentation (SSF) system. The effects of enzyme loading, post-pretreatment size reduction, pretreatment solids concentration, and inhibition on the conversion to ethanol were evaluated.

MATERIALS AND METHODS

Feed Materials

Fresh sugar-cane bagasse was obtained from Hawaiian Commercial & Sugar Company (Puunene, HI). The bagasse was stored at <0°C and screened (+14 mesh retained) prior to use. Aspen chips (smallest dimension 3 mm) were supplied by the US Forest Products Laboratory (Madison, WI) and also stored at <0°C. Mixed hardwood flour was stored at ambient conditions and screened (+70 mesh retained) prior to use.

LHW Pretreatment

The lignocellulosic feed materials were pretreated with LHW at the University of Hawaii using a custom-built 250-mL immersed percolation reactor *(43)*. Pretreatment was typically a single-stage processing of 10–15 g (oven dry basis) of feed material at 220°C for 120 s. In the case of aspen, three such stages were employed consecutively. The reaction pressure (5 MPa) was always high enough to prevent formation of a vapor phase, resulting in an immersed percolation. The ability to displace either hot or cold liquid water into the preheated reactor also resulted in rapid heating/cooling. A detailed description of the LHW pretreatment process is given elsewhere *(43)*.

Pretreatments were conducted at both low and high solids concentrations, with solids concentration defined as the dry mass of feed material pretreated per unit volume of liquid product. Subsequent flushing of the reactor with cold water cooled the lignocellulosic residue and removed any residual solubilized material.

Two types of operating cycles were used, batch and continuous. During a batch cycle, the displacement of hot water into the reactor was stopped on filling of this vessel. In a continuous cycle, flow was maintained at 0.5 L/min throughout the reaction time. For this study, the continuous cycle was used for the aspen and low concentration (10 g/L) bagasse. The batch cycle was used for the high solids concentration pretreatment and for the hardwood flour.

Fermentation

Conversion to ethanol was done by simultaneous SSF carried out in 250-mL serum vials filled to a working volume of 75–100 mL. The vials were incubated at 37°C and agitated at 100 rpm on an orbital shaker. Growth was allowed to continue to completion before the experiments were terminated. *Trichoderma reesei* cellulase (Genencor Cytolase) was used for hydrolysis of the substrate and was augmented

with β-glucosidase (Novozyme 188) in a ratio of 5 U of β-glucosidase/FPU of cellulase. The fermenting organism was *Saccharomyces cerevisiae* strain D_5A supplied by the National Renewable Energy Laboratory (NREL), Golden, CO.

Pretreatment hydrolysate inhibition was tested growing *S. cerevisiae* on glucose. These experiments were carried out in sealed anaerobic test tubes (20 mL filled to 10 mL) or serum vials (125 mL filled to 50 mL) and incubated at 37°C. The test tubes were not agitated. The vials were agitated at 100 rpm on an orbital shaker.

Medium Preparation

For most of the SSF experiments, the concentration of solids in the batch bottles was set to give 20 g/L cellulose. The pretreated solids were stored frozen or refrigerated until ready for use. The pretreated solids were used either as delivered or with the particle size reduced through comminuting for several minutes in a domestic blender set at high speed. For hydrolysate inhibition experiments, the carbon source used was 20 g/L glucose in varying dilutions of pretreatment hydrolysate (0–100% hydrolysate). For all experiments, the medium also included: 10 g/L yeast extract and 20 g/L peptone (both Difco). When necessary, the pH was adjusted to between 4.0 and 4.5 with sulfuric acid or potassium hydroxide. Prior to adding cellulase, the medium was sterilized by autoclaving for 45 min. The cellulase mixture was sterilized through a 0.2-μm filter (Gelman). The inoculum was 2 mL (2% v/v) of yeast broth grown on yeast extract, peptone, and 20 g/L glucose.

Sampling

Samples were taken throughout the fermentations using a sterile syringe through the top of the pressure bottles. CO_2 accumulation in the bottle head space was released at the time of sample extraction. The reaction was allowed to continue until gas production ceased.

Analysis

Dry weights of samples were determined by drying small portions of the samples at 72°C. The cellulose content of solids was determined by quantitative saccharification via acid hydrolysis (72% H_2SO_4 at 30°C for 60 min followed by 2.5% H_2SO_4 at 121°C for 60 min) and then an enzymatic glucose assay on the resulting hydrolysate (Sigma [St. Louis, MO] # 16-UV). Samples of known cellulose content (avicel or hardwood flour) were hydrolyzed alongside each batch of samples to correct for minimal glucose degradation that occurred during the two-step hydrolysis. High-performance liquid chromatography (HPLC) (Bio-Rad [Hercules, CA] HP-87H column) was used to measure ethanol and glucose concentrations in the fermentation broth. Equations (1) and (2) were used to calculate the conversion based on final ethanol concentration and on final residual cellulose, respectively.

$$\text{Conversion to ethanol} = [(\text{ethanol g/L})_{\text{final}} - (\text{ethanol g/L})_{\text{initial}} / (\text{initial cellulose g/L}) \times 0.51] \times 100 \quad (1)$$

$$\text{Cellulose conversion} = 100 - [(\text{final cellulose g/L}) / (\text{initial cellulose g/L})] \times 100 \quad (2)$$

In Eq. (1), the number 0.51 in the denominator results from the stoichiometry of the hydrolysis and fermentation reactions. One mole of a cellulose mono-

Table 2
Solubilization of Different LHW-Pretreated Lignocellulosic Substrates

Substrate	Solids concentration, g/L	Temp, °C	Time, s	Solubilization, %
Bagasse	10	220	120	40
Aspen[a]	10	220	120	32
Hardwood flour	6	220	120	40
Bagasse	80	210	120	33
Bagasse	100	220	120	32

[a] The aspen samples were pretreated three times at the given conditions.

mer hydrolyzes to glucose and is then fermented to ethanol, CO_2, and cell mass according to:

$$1\ C_6H_{10}O_5 + 1\ H_2O \rightarrow 1\ C_6H_{12}O_6 \rightarrow 2\ C_2H_5OH + 2\ CO_2 + \text{cells} \quad (3)$$

The value of carbohydrate allocated to cell production was taken as 0.2 g dry cell material produced/g ethanol produced.

RESULTS

LHW Pretreatment

Table 2 lists results from the pretreatment of three different lignocellulosic substrates. These pretreatments were done at low (<10 g/L) solids concentration. Solubilization of the solids was extensive and gave nearly complete hemicellulose solubilization. Pentosan recovery was consistently above 80%, whereas solubilization of cellulose was low, consistently <10% *(43)*.

SSF Conversion

When pretreated at low solids concentration, all the substrates tested reached a final conversion of about 90% based on cellulose concentrations of the initial and final solids. Figure 1 shows conversion to ethanol vs time for different substrates: LHW-pretreated hardwood flour, bagasse, and aspen chips, as well as raw bagasse that was not pretreated. It can be seen that all of the pretreated materials represented in Fig. 1 achieve 90% of the final conversion within 75 h, except for the unpretreated bagasse, which is minimally reactive, and the bagasse pretreated at high solids concentration, but low temperature (*see* Effect of Pretreatment Solids Concentration on Conversion). The similarity between the conversion curves testifies to the broad applicability of LHW pretreatment. For most of the experiments undertaken for this study, 90% of the final conversion was achieved in 75 h. Table 3 lists the results of those experiments plus additional experiments on comminuted bagasse at varying enzyme loadings and comminuted aspen. It can be seen from Table 3 that conversion calculated with the final ethanol concentration is usually higher than conversion based on the final residual cellulose. This may have been owing to one or a combination of low measurements for initial cellulose content in the solids, low values for the glucose content in the enzyme mixture, or too high an estimate for carbohydrate

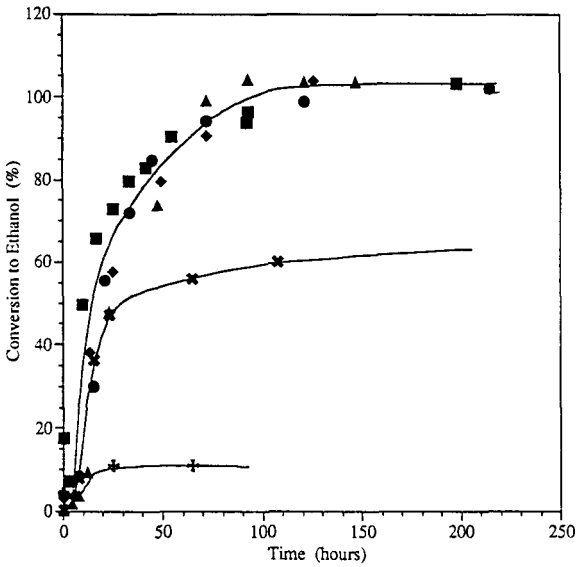

Fig. 1. Percent of theoretical conversion to ethanol in batch SSF of different LHW-pretreated materials (pretreatment: 5–10 g/L, 220°C unless noted otherwise): (■) bagasse, 20 g cellulose/L at 15 FPU/g cellulose; (▲) mixed hardwood flour, 35 g cellulose/L at 20 FPU/g; (●) Aspen chips, 22 g cellulose/L at 14 FPU/g; (✚) raw bagasse (unpretreated), 20 g cellulose/L at 17.5 FPU/g; (◆) bagasse (pretreatment 100 g solids/L, 220°C), 28 g cellulose/L at 13 FPU/g; (✖) bagasse (pretreatment: 80 g solids/L, 210°C), 20 g cellulose/L at 15 FPU/g. Bagasse and Aspen symbols each represent averages of duplicate experiments.

allocated to cell production. Note that the conversion data represented in Fig. 1 are based on ethanol concentrations.

Effect of Enzyme Loading

Figure 2 shows the ethanol conversion obtained from batch SSF experiments on comminuted bagasse at different cellulase loadings of 7, 15, and 30 FPU cellulase/g of cellulose. For all three experiments, the cellulase was supplemented with β-glucosidase. The rate is seen to increase with increasing cellulase content, and at 7 FPU/g, the time to reach 90% of final conversion is up toward 100 h. Results from experiments carried out on bagasse with and without supplemental β-glucosidase show that the absence of supplement β-glucosidase slows down the initial conversion rate, but does not substantially diminish the final conversion (data not shown).

Effect of Post-Pretreatment Particle Size Reduction

Side-by-side experiments were carried out on both aspen and bagasse to compare the effect of post-pretreatment comminution on conversion. Both the bagasse and the aspen showed no difference in reaction rate or extent as a result of comminution. Table 3 lists final conversion numbers for these experiments. Particle size after comminution was not measured, but for both aspen and bagasse, the change was from a substrate with large, obvious particles (chips up to 1.5 cm for aspen, or coarse fibers up to several cm long for bagasse) down to a homogeneous watery mash.

Table 3
Conversion in Batch SSF of LHW Pretreated Lignocellulosic Materials

Sample pretreatment conditions	Enzyme load, IU/g	Additional size reduction	Cellulose before rxn, g/L	Cellulose after rxn, g/L	Final EtOH, g/L	Conversion% By cellulose	Conversion% By EtOH
Hard wood flour							
10 g/L at 220°C	20	no	35	—	18	—	100.8
Bagasse							
10 g/L at 220°C	15	no	20	2.55	9.94	87.25	99.4
10g/L at220°C	7	yes	20	2.23	8.5	88	85
10g/L at 220°C	15	yes	20	1.73	10.1	91.35	101
10 g/L at 220°C	30	yes	20	1.53	10.7	92.4	107
Not pretreated	17.6	no	20	—	1.07	—	10.7
10 g/L at 220°C	15[a]	no	20	—	9.73	—	97
80g/L at 210°C	15	no	20	1.18	7.6	41	76.3
80 g/L at 210°C	15	yes	20	1.19	7.6	40.5	76
100 g/L at 220°C	13	no	28	1.94	14.6	93	103.8
Aspen chips							
	14	no	22	1.69	11.5	92.3	104.5
	14	yes	22	1.97	11.4	91.0	103.6

[a] No supplemental β-glucosidase added.

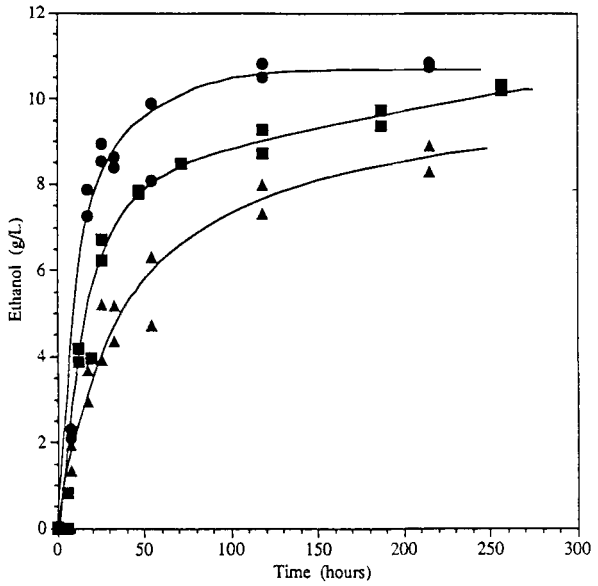

Fig. 2. Effect of enzyme loading on ethanol production in batch SSF of LHW-pretreated bagasse, 20 g cellulose/L. (▲) Enzyme loading of 7 FPU/g cellulose; (■) 15 FPU/g; (●) 30 FPU/g. Symbols each represent averages of duplicate experiments.

Effect of Pretreatment Solids Concentration on Solubilization

Table 2 lists the pretreatment conditions and solubilization results for the higher concentration substrates tested using SSF.

Effect of Pretreatment Solids Concentration on Conversion

Figure 1 shows the contrast between the SSF conversion of solids pretreated under different conditions. Bagasse treated at 220°C showed no reduction in reactivity as pretreatment concentration was increased from 10–100 g/L. However, bagasse pretreated at high concentration (80 g/L), but only 210°C reacted both more slowly and less completely than did the bagasse pretreated at high-temperature. The entries in Table 3 show that, as with the low-concentration pretreated material, there was no difference in conversion through comminution of the high-concentration pretreated material.

Hydrolysate Inhibition

Experiments were done to determine the degree of microbial growth inhibition caused by the hydrolysate produced during LHW pretreatment. Two hydrolysates were tested, both produced by the high-concentration pretreatment of bagasse: one at 210°C (80 g/L solids) and the other at 220°C (100 g/L solids). The fermentation was carried out on glucose to remove the effect of potential hydrolysis limitation to growth. The hydrolysate produced at 210°C showed no sign of growth inhibition up to a concentration of 100% hydrolysate in the fermentation medium. The hydrolysate produced at 220°C did show some inhibition. From Fig. 3 it can be seen that the inhibited culture took about twice as long as the uninhibited

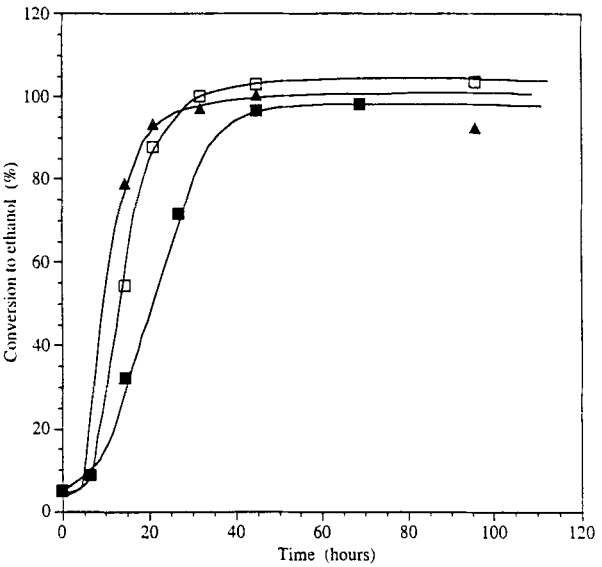

Fig. 3. Effect of presence of L,HW pretreatment hydrolysate on ethanol production in batch fermentation of 20 g/L glucose. (▲) 0% Hydrolysate; (□)100% hydrolysate from pretreatment at 210°C, 80 g solids/L; (■) 100% hydrolysate from pretreatment at 220 C, 100 g solids/L. Symbols each represent averages of duplicate experiments.

cultures to produce full conversion of the glucose feed. This inhibited growth proceeds at roughly the same rate as the SSF conversion of the pretreated lignocellulosics.

DISCUSSION

Previous work looking at the physical and chemical performance of LHW has suggested that it would be an effective pretreatment method for the conversion of lignocellulosic material to ethanol via SSF. LHW pretreatment offers the promise of high pentosan recovery *(43,49)* without the use of added acid. This translates into less need for corrosion-resistant materials of construction and reduces solid waste disposal requirements. Other metrics for the suitability of LHW to pretreatment for conversion of biomass to ethanol have been addressed in this study.

Figure 1 shows that the LHW pretreatment is effective on a variety of lignocellulosic materials. In most experiments, the substrates tested went to 90% of their final conversion within 75 h at a cellulase loading of 15 FPU/g cellulose. This is comparable to the performance of other available pretreatments. Figure 2 illustrates that there is a definite reduction in the rate of conversion with decreasing enzyme load. However, when examining the conversion numbers (Table 3) based on cellulose concentration, it appears that the decline in final substrate utilization with declining enzyme load is relatively light. If this retention of high extent of reaction at low cellulase loading proves to be a pretreatment-specific effect, rather than a substrate-specific effect, it would be a significant advantage for LHW pretreatment. This effect may result from reduced inactivation of enzyme owing to the low lignin content of the LHW material. This point will require further investigation.

Table 4
Comparison of Pretreatment Processes

Metric of pretreatment effectiveness	Steam explosion	Dilute acid	LHW
Reactive fiber	Yes	Yes	Yes
Pentosan recovery	Low	Medium/high	High
Hydrolysate inhibitory	Yes *(18,19)*	Yes *(6)*	Slightly
Size reduction required	No	Yes	No
Materials of construction	Less costly	More costly	Less costly
Solid residue from neutralization salts	Slight	Significant	Slight

The absence of any difference between comminuted and as-received substrates in both the rate and extent of conversion suggests that LHW pretreatment may require much less by way of size reduction than acid-based pretreatment alternatives. Although steam explosion appears to be similarly robust with respect to size reduction, this process is accompanied by low recovery of hemicellulose sugars.

For a pretreatment to be useful for ethanol production, it is important that the carbohydrate concentration of the substrate medium not be too diluted. The results in Fig. 1 showing effective conversion of solids pretreated at a solids concentration of 100 g/L are encouraging to this end. The striking difference between the samples pretreated at 210°C and 220°C underlines the importance of maintaining effective control over reaction severity at high solids concentration. It has not been determined if the deficit in temperature at 210°C can be compensated for with a longer reaction time.

The data obtained in this study are the first known to us that approach the question of inhibition by pretreatment byproducts associated with LHW. The low level of inhibition by the hydrolysate tested here suggests that LHW may have a decided advantage in this category relative to both acid and steam-explosion pretreatment. The results in Fig. 3 show a marked difference between the degree of inhibition at 210°C and 220°C pretreatment. This difference seems to reflect the observed differences in reactivity of the two substrates. Further study of these two phenomena may help in understanding the mechanisms at work and optimization of this process.

Table 4 summarizes the performance of LHW, dilute-acid hydrolysis, and steam explosion with respect to the metrics that have been developed. Acknowledging that the two other pretreatment methods have been far more extensively studied at this point, including on the pilot-scale level, it can be seen that based on the results of this study, LHW compares favorably with these two other technologies on all counts.

CONCLUSION

LHW is an effective pretreatment for the conversion of lignocellulosic materials to ethanol via SSF. Lack of particle size reduction was seen to pose no disadvantage to hydrolysis. Bagasse pretreated at higher solids concentration performs compar-

ably to that pretreated at lower concentrations provided the pretreatment temperature can be maintained at 220°C. The hydrolysate produced in high-concentration pretreatment showed only slight inhibition of *S. cerevisiae*. Future work should look at performing a definitive experiment in which all of these advantages are observed simultaneously along with the scale-up of the LHW process.

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