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Relative Fermentability of Lignocellulosic Dilute-Acid Prehydrolysates

Application of a *Pichia stipitis*-based Toxicity Assay†

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ABSTRACT

The relative toxicity of the combined nonxylose components in prehydrolysates derived from three different lignocellulosic biomass feedstocks was determined. One woody (poplar) and two herbaceous (corn stover and switchgrass) feedstocks were dilute-acid pretreated under temperature and acid conditions chosen to optimize xylose recovery in the liquid prehydrolysate; xylose yields averaged 96, 89, and 87% of theoretical for switchgrass, corn stover, and poplar, respectively. Prehydrolysates from each feedstock were neutralized, adjusted to equivalent xylose concentrations, and bioassayed for toxicity, using a standardized fermentation protocol with *Pichia stipitis* NRRL 11545. Full time-courses for ethanol production (30–60 h) clearly illustrate the distinct inhibitory effects of prehydrolysates from different feedstocks. The relative toxicity of the prehydrolysates, ranked in order of decreasing toxicity, is poplar-derived prehydrolysates > switchgrass-derived prehydrolysates > corn stover-derived prehydrolysates. The inhibition of ethanol production appeared to be the result of a general slowdown of yeast metabolism, rather than the result of the production of alternative, nonethanol end products. Ethanol yields averaged 74, 83, and 88% of control values for poplar, switchgrass, and corn stover prehydrolysates,

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respectively. Volumetric ethanol productivities (g ethanol L/h) averaged 32, 70, and 102% of control values for poplar, switchgrass, and corn stover prehydrolysates, respectively. Ethanol productivities correlated closely with acetate concentrations in the prehydrolysates; however, regression lines correlating acetate concentrations and ethanol productivities were found to be feedstock-dependent.

Index Entries: Pichia stipitis; Prehydrolysate; Switchgrass; Corn Stover; Poplar.

INTRODUCTION

A widely studied process for converting lignocellulosic biomass to ethanol involves a pretreatment of the lignocellulosic biomass with dilute acid (around 1.0 wt%) at high temperatures (over 140°C) to catalyze the hydrolysis of lignocellulosic biomass hemicellulose *(1)*. Dilute-acid pretreatment yields a solids stream consisting of mostly lignin and cellulose. *Saccharomyces cerevisiae,* when used in conjunction with fungal cellulases, can ferment the cellulose fraction of the solids stream to ethanol *(2)*. Dilute-acid pretreatment also yields an aqueous prehydrolysate stream containing hemicellulose-derived xylose, lesser amounts of other sugars, acetic acid, degradation products of lignin and carbohydrates, and other endogenous compounds *(3)*.

Xylose constitutes a large fraction of the dry weight of most lignocellulosic biomass *(4)*, and, thus, fermentation of prehydrolysate xylose is essential for the economic viability of industrial biomass-to-ethanol processes *(5)*. Yeast, fungi, and bacteria are capable of fermenting xylose to ethanol *(6)*. *Pichia stipitis* is a yeast that does so with high ethanol yield (g ethanol produced per g sugar) and relatively high volumetric ethanol productivity (g ethanol produced per liter per hour), when compared to other xylose-fermenting yeast *(6,7)*. Several papers describing ethanol yields from *Pichia* fermentation of lignocellulosic biomass prehydrolysates have been published, including studies with prehydrolysate derived from aspen wood *(8,9)*, pine *(9,10)*, corn stover *(10)*, red oak *(11)*, sugar cane bagasse *(12)*, corn cob *(13)*, mixed wood chips *(14)*, and eucalyptus *(15)*. Toxic compounds in these lignocellulosic biomass prehydrolysates tend to inhibit *Pichia* fermentations, causing lower ethanol yields and lower ethanol productivities, compared to those attained in control fermentations. Relatively low ethanol yields and productivities are associated with prehydrolysate fermentations using other microorganisms, as well *(3)*. *Pichia* fermentations can be improved by conditioning the yeast to the particular prehydrolysate, and by removing inhibitors. For a review of the fermentation of prehydrolysates and detoxification, *see* Olsson and Hahn-Hägerdahl *(3)*.

When comparing the feasibility of different biomass sources for biomass-to-ethanol processes, it is important to consider the relative toxicity of the respective prehydrolysates, since this will ultimately influence the ability of microbes to produce ethanol from hemicellulose. In general, literature comparisons of the relative toxicity of prehydrolysates derived from different biomass sources are difficult, because most papers concerning prehydrolysate fermentation focus on a single feedstock, and there is little consistency between studies with respect to fermentation protocols. When comparing data from different papers, one finds that feedstock pretreatment conditions often do not correlate, and fermentation parameters may vary considerably, including differences in yeast species (and strains) and inoculum levels.

The objective of this study was twofold. The first objective was to determine the relative toxicity of three important prehydrolysates (obtained from a hybrid poplar, corn stover, and switchgrass), using a standardized fermentation bioassay. A *Pichia*-based bioassay was chosen for this work because of the well-documented susceptibility of this yeast to prehydrolysate inhibition *(3,6,8–15);* this is a physiological characteristic expected to correlate with enhanced sensitivity in a bioassay. The second objective was to provide information on *Pichia* fermentation of prehydrolysates obtained from dilute-acid pretreated switchgrass *(Panicum virgatum L.).* Switchgrass is of interest because it is currently considered a leading candidate for use as a biomass-to-ethanol crop *(16,17),* and yet, to the authors' knowledge, representative fermentations of dilute-acid-prepared switchgrass prehydrolysates have not been published. Information of this nature is obviously necessary when evaluating the economic potential of switchgrass-to-ethanol processes.

MATERIALS AND METHODS

Media, Organism, and Seed Culture Preparation

YM broth was prepared by adding 3 g yeast extract (Difco, Detroit, MI), 3 g malt extract (Sigma, St. Louis, MO), 5 g peptone (Difco), and 10 g glucose (Sigma) to 1 L distilled H_2O, and autoclaving for 30 min at 121°C. YM agar was prepared in the same manner as the YM broth, but with the addition of 20 g agar (Difco).

P. stipitis NRRL 11545 was supplied by the National Renewable Energy Laboratory (Golden, CO) as a lyophilized powder. The powder was dissolved in YM broth, then spread on YM agar. An isolated colony obtained from the plate was used to inoculate 200 mL of filter-sterilized 30 g/L xylose (Sigma) and 10 g/L yeast extract (Difco) solution in a baffled 500-mL flask. After 24 h of incubation at 25°C and 200 rpm, the cells were centrifuged at 4000g, resuspended in yeast-extract solution, and glycerol

Table 1
Percent Composition of Three Lignocellulosic Feedstocks on a Dry-Wt Basis

Component	Feed stock		
	Corn stover	Switchgrass	Poplar
Glucan	36.0[a] (0.4)[b]	32.2 (0.3)	39.8 (0.01)
Xylan	19.8 (0.3)	20.3 (0.3)	14.8 (0.03)
Galactan	1.3 (0.1)	–	–
Arabinan	2.8 (0.1)	3.7 (0.04)	1.2 (0.02)
Mannan	–	0.4 (0.02)	2.4 (0.04)
Total glycan	59.9	56.6	58.2
Klason lignin	26.9 (0.1)	19.5 (0.3)	26.9 (0.3)
Acid-soluble lignin	1.9 (0.03)	3.7 (0.03)	2.2 (0.06)
Ash	7.2 (0.01)	7.1 (0.06)	1.3 (0.02)
Uronic acid	nd	1.1 (0.1)	2.4 (0.1)
Acetyl groups	1.38 (0.1)	2.35 (0.1)	2.93 (0.1)
Other	2.7	9.7	6.1
Total	100	100	100

[a] All values are weight percents on a dry basis.
[b] Values in parentheses are standard errors of the means.

was added to a final concentration of 10% (w/v). The stock cultures were divided into 1-mL aliquots, and stored at −70°C.

Prehydrolysate Preparation

Poplar, corn stover, and switchgrass feedstocks were dried and milled (60-mesh) prior to shipment by the National Renewable Energy Laboratory. The composition of the original feedstocks is shown in Table 1. Feedstocks were dilute-acid pretreated at 10% solids (w/w) in a stainless-steel Parr reactor with a Pyrex liner, as described previously *(18)*. The prehydrolysate was stored at 4°C for 2 d, neutralized to pH 6.0 with $Ca(OH)_2$, and sterile-filtered through a 0.22-μm membrane prior to fermentations. No blockage of the membrane was observed. Filtration was chosen as a sterilization method, in order to avoid a second heating regimen potentially more rigorous than the original pretreatment conditions.

Toxicity Assay

The fermentation-based toxicity assay used in this study was developed at the National Renewable Energy Laboratory, and is similar to the fermentation protocol described by Boynton and McMillan *(19)*.

1. Inoculum preparation. One mL of previously prepared and frozen seed culture was added to 200 mL of 30 g/L xylose (Sigma) and 10

g/L yeast extract (Difco) in 500-mL baffled flasks equipped with Morton closures. The pH was maintained ≥5.0 via manual addition of 1 *N* NaOH. Temperature was controlled at 30°C, and the orbital shaker speed was 200 rpm. When the culture reached a dry cell mass (DCM) of approx 5.0 g/L (approx 24 h, determined using a optical density measurement previously calibrated with actual DCM measurements), the yeast were centrifuged, the supernatant discarded, and the cells resuspended in 1 g/L yeast extract, to make the DCM approx 50 g/L.

2. Fermentation protocol. The prehydrolysate resulting from each pretreatment condition was fermented in duplicate. Sterile prehydrolysate (prepared as described above), concentrated xylose solution, yeast-extract solution, water, and concentrated *Pichia* inoculum were added to 250-mL unbaffled flasks with Morton closures, yielding 100 mL of medium with the following composition: 80% (v/v) prehydrolysate, 30 g/L xylose, 10 g/L yeast extract, and 5 g/L dry cell mass. Xylose and yeast extract were added to the prehydrolysates in order to facilitate comparisons of the impact of nonxylose prehydrolysate components on the fermentation performance of *Pichia.* When starting with equal concentrations of xylose and yeast extract, and by inclusion of the proper controls, it can be inferred that differences in fermentation parameters are caused by nonxylose compounds in the prehydrolysate, and not differences in initial carbohydrate or nutrient loads. Fermentations were conducted at 30°C, and the agitation speed was 150 rpm. The pH was initially 6.0 ± 0.1, and was maintained throughout the fermentation between 5.5 and 6.0 via manual addition of 1 *N* NaOH, which was added if the pH dropped below 5.5. Typically, NaOH additions were required at 3 and 6 h postinoculation for control fermentations; the prehydrolysate-containing fermentations did not require NaOH addition until 12 h postinoculation.
3. Fermentation controls. Duplicate control fermentations, containing only *Pichia*, xylose, yeast extract, and water, were fermented alongside each run. To independently assess the influence of acetate on *Pichia* under the fermentation conditions described above, varying amounts of acetic acid were added to 1% H_2SO_4 (w/w) solution, to simulate the aqueous prehydrolysate. Xylose and yeast extract were then added to the same concentrations as for the control fermentation above.

Analytical Methods

The composition of the feedstocks was done as described previously *(18,20);* lignin, as Klason lignin; acid-soluble lignin, as determined by

absorbance at 205 nm; glycans, as the sum of monomers resulting from acid hydrolysis; uronic acid, as acids resulting from acid hydrolysis; and acetyl groups, as acetate liberated during acid hydrolysis. In the prehydrolysate and fermentation broths, glucose, xylose, xylitol, acetate, and ethanol were measured via HPLC, using an Aminex HPX-87H column (65°C, 0.6 mL 0.01 N H_2SO_4/min) and a refractive index detector. Dry cell mass was measured gravimetrically on duplicate 10-mL samples after centrifuging, washed twice with distilled water, and dried at 90°C to constant weight.

Data Analysis

Simple linear regression analyses were performed using Microsoft Excel 5.0. Correlations were evaluated based on the corresponding *p*-values. A *t*-test was conducted to compare slope values of the different data sets, with *p*-values being used to judge the significance of differences.

RESULTS AND DISCUSSION

The different prehydrolysates were prepared by dilute-acid pretreatment, under conditions favoring maximum xylose yields within the constraints of 140–180°C, 0.6–1.2% (w/w) H_2SO_4, and 0.5–5.0 min *(18)*. Xylose yields were high; percent of theoretical xylose yields for corn stover, switchgrass, and poplar prehydrolysates averaged 89, 96, and 87%, respectively. The composition of the prehydrolysates, along with the ethanol yields and productivities resulting from *Pichia* fermentation of those prehydrolysates, are summarized in Table 2. In addition to the monomeric xylose concentrations shown in Table 2, there was an additional 2–6% soluble xylose oligomers present. The relative composition of the prehydrolysates are in general agreement with that which would be predicted, based on the composition of the feedstocks (Table 1). The corn stover prehydrolysates contained the most xylose and the least acetic acid; the poplar prehydrolysates had the least xylose and most acetic acid. All measurements were made after neutralization to 6.0 with $Ca(OH)_2$ and subsequent sterile filtration (0.22 μm). Neutralization with $Ca(OH)_2$ has been shown to have no effect on the acetate or furfural content of a pine prehydrolysate, but lowers the monomeric and polymeric phenolic concentrations by approx 25% *(21)*.

Coefficients of variance for the sugar and ethanol concentrations of the duplicate fermentations were calculated, and ranged from 2 to 9%. Ethanol yields (g ethanol/g sugar) were calculated at the point of maximum ethanol concentration (Table 2). As a point of reference, 0.51 g ethanol/g xylose is most commonly considered a maximum theoretical yield *(22)*. However, yields are known to be strongly dependent on fer-

Table 2
Pretreatment Conditions, Composition, and *P. stipitis* Fermentation Results of Dilute-Acid Prehydrolysate, Prepared from Lignocellulosic Biomass

Label	Feed stock	Time (min)	Weight % H_2SO_4	Temp. (°C)	Glucose (g/L)	Xylose (g/L)	Acetate (g/L)	Ethanol yield (g/g)[a]	Ethanol productivity (g/L/h)[a]	Final Dry Cell Mass (percent relative to control)[b]
CS 1	Corn stover	2.1	1.1	170	3.5	20.8	1.2	0.41	0.80	+6
CS 2	Corn stover	1.4	0.9	180	3.5	20.7	1.2	0.41	0.85	+5
CS 3	Corn stover	1.1	1.0	180	3.9	23.3	1.4	0.39	0.74	+6
CS 4	Corn stover	3.9	1.2	160	3.7	23.3	1.4	0.41	0.75	+4
POP 1	Poplar	1.1	1.1	170	2.2	16.6	3.0	0.35	0.25	−10
POP 2	Poplar	1.1	1.2	170	2.4	17.4	3.2	0.31	0.20	−17
POP 3	Poplar	0.5	1.0	180	1.9	14.3	2.5	0.38	0.32	−18
POP 4	Poplar	0.5	1.1	180	2.3	15.6	2.9	0.32	0.22	−15
SG 1	Switchgrass	0.5	1.1	180	5.7	24.7	2.3	0.37	0.47	+7
SG 2	Switchgrass	0.5	1.0	180	5.7	25.1	2.1	0.36	0.46	+4
SG 3	Switchgrass	0.5	1.0	180	5.8	22.6	1.7	0.42	0.68	+9
Average of controls (n = 5)		na[c]	na[c]	na[c]	0	30.65 (0.47)[d]	0	0.46 (0.004)[d]	0.77 (0.02)[d]	na[c]

[a] Calculated at maximum ethanol concentration. *See* Methods for fermentation parameters; xylose added to all prehydrolysates to a final concentration of 30 g/L.

[b] Values were calculated using the formula = 100* (final DCM-control final DCM)/control DCM.

[c] na, Not applicable.

[d] Values in parentheses are standard errors of the mean.

mentation conditions. The control samples in this study, which are based on the use of an optimum semidefined medium, had average ethanol yields equal to approx 90% of theoretical (using 0.51 g ethanol/g sugar as a reference value). Ethanol yields from all of the prehydrolysates were a minimum of 10% below that of the control samples. Ethanol yields from poplar prehydrolysates were the lowest, averaging ~67% of theoretical; those from corn stover prehydrolysate were the highest, averaging ~80% of theoretical. Yield calculations are based on total sugar in the prehydrolysate, which includes xylose, glucose, and arabinose. Arabinose is included in these calculations by default, because arabinose co-elutes with xylose on the column typically employed for fermentation media analyses. Arabinose in a prehydrolysate will lower apparent ethanol yields, since, under microaerobic conditions, *Pichia* does not assimilate arabinose until xylose and glucose are consumed, and then it does so concurrently with ethanol *(23)*. Arabinose measurements on prehydrolysates from each feedstock indicate that the ethanol yield was lowered approx 6, 8, and 2% for corn stover, switchgrass, and poplar, respectively.

One measure of the rate of ethanol production is volumetric ethanol productivity (g ethanol/L/h), in which the calculation is based on the time required to reach maximum ethanol concentration *(3)*. The means of the relative ethanol productivities (i.e., the productivity of the prehydrolysate-based media divided by the productivity of the control media) were corn stover (102%) > switchgrass (70%) > poplar (32%) (Table 2). These values corresponded well with relative initial rates of ethanol production (corn stover [89%] > switchgrass [61%] > poplar [34%]) when initial rate is defined as the apparent linear rate of ethanol production in the early phase of fermentation (based on the 0, 6, and 12 h time-points of Fig. 1). The complete time-courses for ethanol production (Fig. 1) clearly show that rates of ethanol production throughout the fermentation are feedstock-specific, i.e., there was little to no overlap in the time-courses corresponding to prehydrolysates derived from switchgrass, corn stover, and poplar. Prehydrolysates derived from the same feedstock fermented similarly, with only poplar prehydrolysates showing noticeable variability. The reason for the greater variability between poplar prehydrolysates is not clear. This variability is not explained by differences in acetate or furfural concentrations. Furfural concentrations were less than 0.05 g/L at the beginning of a typical fermentation, and less than 0.01 g/L 6 h after inoculation.

The time-courses for xylose consumption in each of the prehydrolysates are presented in Fig. 2. As expected, the prehydrolysates in which ethanol was produced the fastest showed the highest rates of xylose consumption. Comparison of xylose utilization rates for the three feedstocks indicates an overall slowing of xylose consumption in poplar prehydrolysate fermentations, which was reflected in the slower production

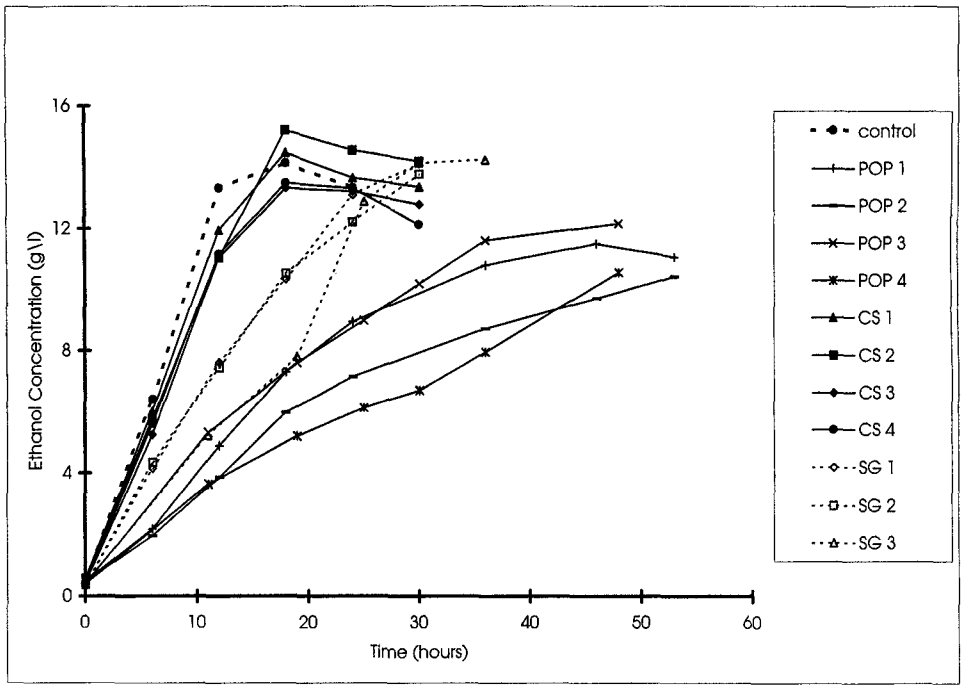

Fig. 1. Ethanol concentration vs time during *P. stipitis* fermentations of lignocellulosic prehydrolysates. *See* Table 1 for legend key.

of ethanol. The similarity in the time-courses of ethanol production and xylose uptake for a given prehydrolysate medium strongly suggests that the inhibition observed in this study is caused by a general decrease in the rate of xylose metabolism. This mechanism is distinct from one in which alternative, nonethanol end products, e.g., xylitol or glycerol, are produced. The idea that the observed inhibition in prehydrolysate-based media is caused by a general slowdown in yeast metabolism is also consistent with the dry cell mass data of Table 2, which shows that, in general, dry cell mass production was lowest in the fermentations with the lowest rates of ethanol production.

In a typical control fermentation, the concentration of xylitol in the medium peaked between 0.5 and 1 g/L at 12 h and then slowly tapered off. In contrast, for corn stover and switchgrass fermentations, the xylitol concentration peaked near 4 g/L at 12–18 h, then dropped precipitously to approx 0.5 g/L within the next 6 h. For the faster-fermenting poplar prehydrolysates (poplar 1 and poplar 3), the xylitol concentration peaked near 1.4 g/L at 24 h and dropped to 0.8 g/L. For the slower-fermenting poplar prehydrolysates (poplar 2 and poplar 4), the xylitol concentration reached a maximum of 0.8 g/L at 36 h. Thus, a brief burst of xylitol in the media was associated with the faster-fermenting, prehydrolysate-containing media,

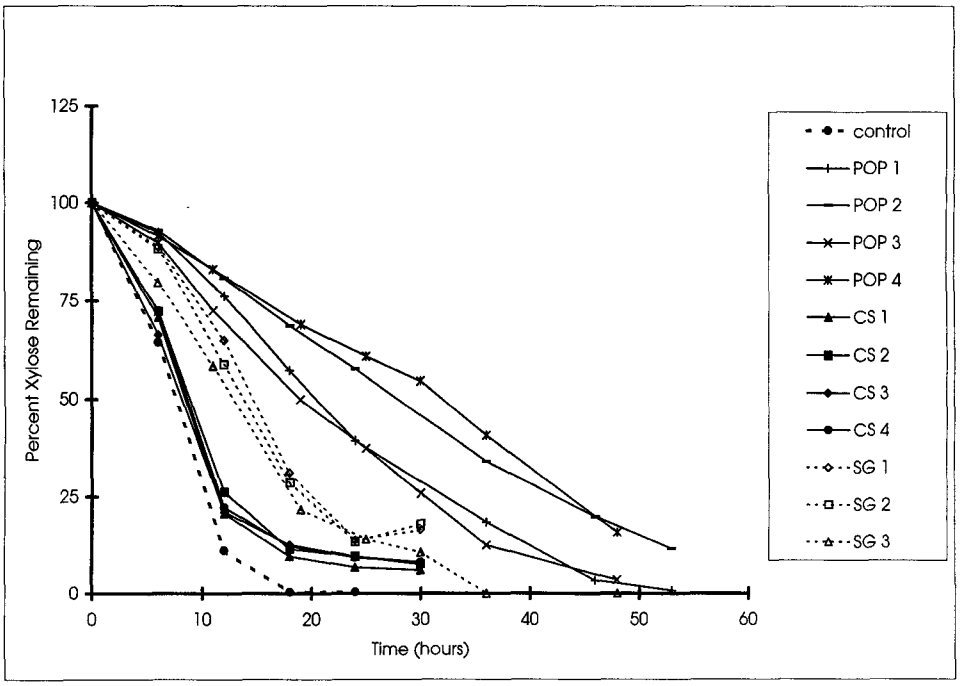

Fig. 2. Percent xylose remaining vs time during *P. stipitis* fermentations of lignocellulosic prehydrolysates. *See* Table 1 for legend key.

i.e., all herbaceous prehydrolysates and the two faster-fermenting poplar prehydrolysates. The secreted xylitol apparently was quickly assimilated and metabolized by *Pichia*, as evidenced by its rapid disappearance.

Acetate inhibition of fermentation is well-documented in *Pichia* systems *(11,15,24)*; hence, correlations between prehydrolysate acetate concentrations and corresponding ethanol productivities were determined. A plot of acetate concentration vs relative ethanol productivity for each of the prehydrolysates is shown in Fig. 3. The data were grouped into three sets. The first set corresponds to those fermentations that were done with acetate-supplemented control media. These values represent the inhibition of *Pichia* caused by acetate alone. The second and third data sets include fermentations done on the herbaceous (switchgrass and corn stover) and woody (poplar) prehydrolysates, respectively. These show the inhibition caused by all the compounds in the prehydrolysates, including acetate. The best-fit line obtained using simple linear regression is included for each of the three data sets.

Simple linear regression analysis of ethanol productivity vs prehydrolysate acetate concentrations for the acetate-supplemented control media gives convincing evidence that, over the range of acetate concentrations shown, relative productivity is linearly correlated with acetate

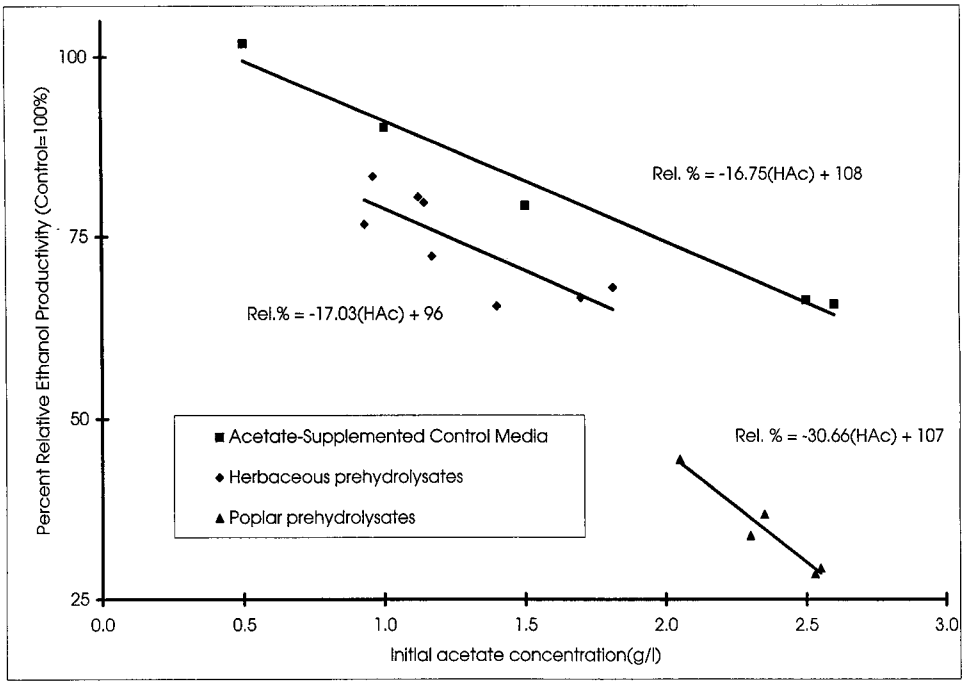

Fig. 3. Percent relative volumetric ethanol productivity at 12 h vs initial acetate concentration for *P. stipitis* fermentations of dilute-acid lignocellulosic biomass prehydrolysate media and acetate-supplemented control media solutions. Initial acetate concentrations reflect dilution by addition of inocula and yeast extract.

concentration (two-sided p-value, 0.001). Comparison of the best-fit regression lines describing the acetate-supplemented control media fermentations and the poplar prehydrolysate fermentations, which are significantly different (two-sided p-value = 0.03), strongly suggests that apparent acetate toxicity is dependent on the fermentation medium; i.e., the slopes of the lines correlating acetate concentration and ethanol productivity are different in the acetate-supplemented control media and poplar prehydrolysate media.

The slope of the regression line of ethanol productivity on initial acetate concentration for the herbaceous prehydrolysate-based media was not significantly different from the corresponding slope for acetate-supplemented control media. Comparing the regression lines of the poplar prehydrolysate data and the herbaceous prehydrolysate data suggests, although inconclusively (two-sided p-value, 0.08), that correlations between ethanol productivity and initial acetate concentration are different for woody and herbaceous feedstocks. A cursory analysis of Fig. 3 shows that nonacetate compounds in poplar prehydrolysate are associated with a higher degree of inhibition than that found in the corresponding

herbaceous prehydrolysates. In this regard, prehydrolysate compounds other than acetate, such as lignin and carbohydrate degradation products, have been shown to be toxic to *Pichia (11,25).* The paper by Delgenes et al. *(25)* illustrates that both the quantity and quality of phenolics are important factors in inhibition of *Pichia.* A linear correlation between ethanol productivities and acetate concentration may be expected for most lignocellulosic-derived prehydrolysates, since acetate liberation is one means of assessing the general severity of a dilute-acid pretreatment.

The relative toxicity of prehydrolysates derived from three important lignocellulosic feedstocks has been determined, using a standardized bioassay. The relative toxicity of the prehydrolysates, in order of decreasing toxicity, was poplar > switchgrass > corn stover. This trend agrees with work done by Parekh et al. *(9),* in which a herbaceous (corn stover) prehydrolysate was reported to be more completely fermented than were prehydrolysates prepared from woody feedstocks (pine and aspen). The differences in ethanol productivities observed in the present study are relatively large, ranging from 32% (poplar) to 102% (corn stover) of control. This broad range appears to reflect the relatively high sensitivity of the *Pichia*-based bioassay employed in this study. The diminished ethanol productivities associated with the fermentations of biomass-derived prehydrolysates were found to be closely related to prehydrolysate acetate concentrations. However, control fermentations with acetate-supplemented media indicate that inhibitors other than acetate are present in each of the prehydrolysates. Ethanol productivities, xylose utilization, nonethanol end product levels, and dry cell mass productions are all consistent with the reduction in ethanol productivity being a result of prehydrolysate inhibitors causing a general slowdown in yeast metabolism.

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REFERENCES

1. Penner, M. H., Hashimoto, A. G., Esteghlalian, A., and Fenske, J. J. (1996), in *Agricultural Materials as Renewable Resources—Nonfood and Industrial Applications* (Fuller, G., McKeon, T. A., and Bills, D. D., eds.), Am. Chem. Soc. pp. 12–31.
2. Spindler, D. D., Wyman, C. E., and Grohmann, K. (1991), *Appl. Biochem. Biotechnol.* **28/29,** 773–786.
3. Olsson, L. and Hahn-Hägerdahl, B. (1996), *Enz. Microb. Technol.* **18,** 312–331.

4. Puls, J. and Schuseil, J. (1993) in *Hemicellulose and Hemicellulases* (Hazlewood, G. and Coughlan M., eds.), London, pp. 1–27.
5. Hinman, N. D., Wright, J. D., Hoagland, W., and Wyman, C. E. (1989), *Appl. Biochem. Biotechnol.* **20/21,** 391–401.
6. Skoog, K. and Hahn-Haegerdahl, B. (1988), *Enz. Microb. Technol.* **10,** 66–80.
7. Jeppsson, H. (1996), Doctoral Dissertation, Lund University, Lund, Sweden.
8. Wilson, J. J., Deschatelets L., and Nishikawa, N. K. (1989), *Appl. Microbiol. Biotechnol.* **31,** 592–596.
9. Parekh, S. R., Parekh, R. S., and Wayman, M. (1988), *Enz. Microb. Tech.* **10,** 660–668.
10. Qureshi, N. and Manderson, G. J. (1991), *J. Industr. Microbiol.* **7,** 117–122.
11. Tran, A. V. and Chambers, R. P. (1986), *Enz. Microb. Technol.* **8,** 439–444.
12. Roberto, I. C., Lacis, L. S., Barbosa, M. F. S., and de Mancilha, I. M. (1991), *Process Biochem.* **26,** 15–21.
13. Hahn-Haegerdahl, B., Jeppsson, H., Olsson, L., and Mohagheghi, A. (1994), *Appl. Microbiol. Biotechnol.* **41,** 62–72.
14. Perego, P., Converti, A., Palazzi E., del Borghi, M., and Ferraiolo, G. (1990), *J. Ind. Microbiol.* **6,** 157–164.
15. Ferrari, M. D., Neirotti, E., Albornoz, C., and Saucedo, E. (1992), *Biotechnol. Bioeng.* **40,** 753–759.
16. Downing, M., McLaughlin, S., and Walsh, M. J. (1995), in *Second Biomass Conference of the Americas: Energy, Environment, Agriculture, and Industry Proceedings,* National Renewable Energy Laboratory, Golden, CO.
17. Sanderson, M. A., Reed, R. L., McLaughlin, S. B., Wullschleger, S. D., Conger, B. V., Parrish, D. J., et al. (1996) *Biores. Technol.* **56,** 83–93.
18. Esteghlalian, A., Hashimoto, A. G., Fenske, J. J., and Penner, M. H. (1997), *Biores. Technol.* **59,** 129–136.
19. Boynton, B. L. and McMillan, J. D. (1994), *Appl. Biochem. Biotechnol.* **45/46,** 509–514.
20. Fenske, J. J. (1994), M.S. Thesis, Oregon State University, Corvallis, Or.
21. Frazer, F. R. and McCaskey, T. A. (1989), *Biomass* **18,** 31–42.
22. Evans, C. T. and Ratledge, C. (1984), *Arch. Microbiol.* **139,** 48–52.
23. Delgenes, J., Moletta, R., and Navarro, J. (1988), *Appl. Microbiol. Biotechnol.,* **29,** 155–161.
24. van Zyl, C., Prior, B. A., and du Preez, J. C. (1991), *Enz. Microb. Technol.* **13,** 82–86.
25. Delgenes, J. P., Moletta, R., and Navarro, J. M. (1996), *Enz. Microb. Technol.* **19,** 220–225.