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Scientific Note

Simultaneous Saccharification and Fermentation of Pretreated Hardwoods

Effect of Native Lignin Content

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ABSTRACT

A series of correlations was made between the performance of 15 wood species in simultaneous saccharification and fermentation (SSF) and their respective chemical compositions. A compelling inverse trend ($p < 0.001$) was demonstrated between the percent conversion of glucan to ethanol during SSF and the Klason lignin content of the wood samples before dilute acid pretreatment. No significant relationships were found between the glucan, xylan, and ash compositions of the native wood samples and ethanol yield. This observation is unique and provides a convenient predictor of biomass conversion efficiency.

Index Entries: Lignin; sawdust; SSF; statistical analysis; hardwoods.

INTRODUCTION

The efficiency of the feedstock pretreatment, saccharification, and fermentation unit operations is key to the commercial success of lignocellulose-based simultaneous saccharification and fermentation (SSF)

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Table 1
Data Base for SSF Trend Analysis

Wood	Native wood (dry wt%)					Pretreated wood (dry wt%)		
	%EtOH	Lignin	Glucan	Xylan	Ash	Lignin	Glucan	Xylan
soft maple	93	20.7	44.9	17.3	0.61	30.5	62.6	1.6
sycamore	87	23.2	53.1	17.1	0.59	31.1	61.3	2.3
red oak	87	22.4	43.4	18.9	0.86	35.3	58.2	1.9
walnut	84	21.9	46.2	16.5	0.99	35.3	57.1	2.1
poplar	78	18.1	49.9	17.4	0.52	29.7	63.0	3.7
white oak	76	23.2	43.6	18.0	0.57	33.0	62.0	2.0
cherry	76	23.8	46.0	19.8	0.29	37.4	58.7	1.9
chestnut oak	70	24.2	44.0	20.0	0.82	32.9	59.5	3.8
basswood	64	21.5	46.7	17.5	0.81	31.7	61.4	2.5
hickory	64	24.2	49.9	19.9	1.0	32.0	63.7	1.9
beech	60	26.2	42.9	20.8	0.53	35.9	60.4	2.5
buckeye	57	23.9	52.6	14.9	0.77	31.2	60.8	3.5
ash	43	28.5	48.3	17.4	0.79	35.7	59.0	2.5
hard maple	35	25.0	42.8	16.8	1.5	35.7	56.4	3.8
white pine	21	29.0	46.4	8.8	0.35	39.6	54.6	1.0
P-value[a]	>0.2	>0.2	>0.2	0.015	>0.2	>0.2	>0.2	0.053
normality	pass	pass	pass	fail	pass	pass	pass	pass
P-value[b]				0.103				
normality				pass				

[a]Normality test based on Kolmogorov–Smirnov test where P-to-reject normality is 0.050.
[b]Pine data omitted as outlier.

processes *(1,2)*. Although a recent cost-benefit analysis of near total delignification of hardwood has shown this procedure to be beneficial for a full-scale, grass-roots N^{th} ethanol plant *(3)*, the impact of native lignin content on conventional SSF is largely unknown. Traditionally, studies of the effects of lignin content on the "digestibility" (and ultimately the fermentability) of pretreated biomass have been performed with a single feedstock subjected to chemical procedures designed to produce samples with varying degrees of delignification *(4)*. This approach not only yields correlations based on a single feedstock type, but presumes that the lignin remaining in the pretreated sample is chemically unaltered. In a previous work *(5)*, the SSF performance of 15 hardwood and softwood samples collected from sawmills in the Appalachian region was investigated. These sawdust samples were subjected to an exhaustive compositional analysis, pretreated with dilute sulfuric acid at 160°C, reanalyzed for composition, and used in *Saccharomyces cerevisiae*-based SSF. In the present study, hardwood compositional and ethanol production data generated in this previous work were examined for statistically defendable trends.

Table 2
Statistical Analysis SSF Data

Data sets compared	*t*-Test of regression		Pearson-Product moment	
	p-value[a]	Trend	Coefficient	Trend
%EtOH vs %Lgn Native	<0.001	strong	−0.772	strong/inverse relationship
%EtOH vs %Gln Native	0.838	none	0.058	no significant relationship
%EtOH vs %Xyn Native	0.741	none	0.097	no significant relationship
%EtOH vs %Ash Native	0.524	none	−0.179	no significant relationship
%EtOH vs %LgnPretreated	0.032	weak	−0.555	possible/inverse relationship
%EtOH vs %GlnPretreated	0.038	weak	0.540	possible/direct relationship
%EtOH vs %XynPretreated	0.811	none	−0.067	no significant relationship
%EtOH vs %XynRemoved	0.296	none	0.289	no significant relationship

[a]Significant relationships are assumed to be those with p values less than 0.050 *(8)*.

TREND ANALYSIS

The data base shown in Table 1 was used for the analysis of trends *(5)*. The Kolmogorov–Smirnov test for normality was applied to each data set (columns) in Table 1 to ensure compliance with this prerequisite for application of the *t*-test. All data sets except the native xylan-content set passed the normality test. Graphical examination of these data confirmed that the pine value was an outlier that affected the normal distribution of the data set. When the pine value was removed, the entire data set conformed to the Kolmogorov-Smirnov criterion for normality.

Two tests for disclosing trends, the *t*-tests of regression and the Pearson Product Moment correlation (R), were applied to the data in Table 1. The results of these analyses, shown in Table 2, reflect a strikingly strong, inverse relationship between the ethanol production, shown as percent of theoretical, and the Klason lignin content before pretreatment (*see* Fig. 1). Moderate relationships were shown between ethanol yields and both the Klason lignin and glucan contents following pretreatment. No significant relationships were observed between ethanol yield and the glucan, xylan, or ash contents before pretreatment, or to the xylan or ash (not shown) contents following pretreatment. As an example of a data set

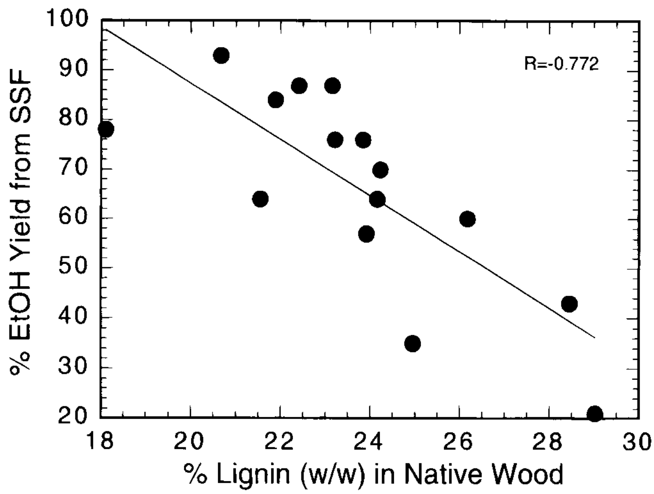

Fig. 1. Scatter plot of the percent theoretical yield of ethanol produced during SSF and the wt% Klason lignin determined in the untreated wood. These data produced a strong inverse correlation ($p < 0.001$). The least squares linear regression line was $y = 200.8 - 5.67x$.

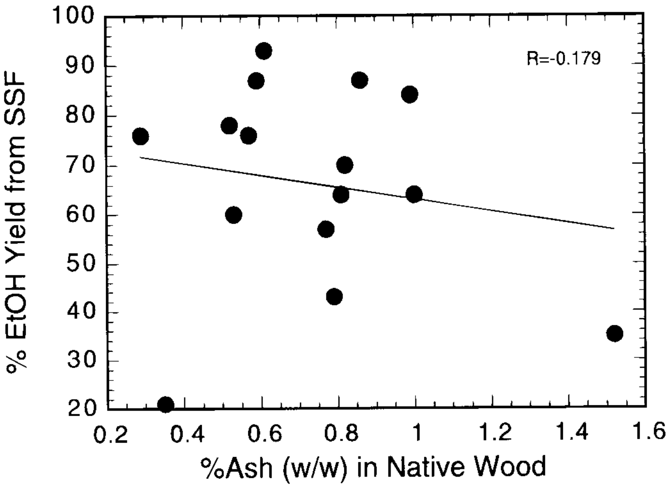

Fig. 2. Scatter plot of the percent theoretical yield of ethanol produced during SSF and the wt% ash determined in the untreated wood. These data produced no significant correlation ($p = 0.524$). The least squares linear regression line was $y = 75.3 - 12.2x$.

showing no significant correlation, the percent theoretical ethanol yield and ash contents are shown in Fig. 2.

DISCUSSION

We have taken advantage of a large data base of SSF data recently published to study possible trends relating ethanol production potential and feedstock compositional analysis The strong inverse trend found for ethanol yield and native wood Klason lignin content ($p < 0.001$) is unique and fully consistent with observations that biomass samples with lower lignin contents may excel in digestibility by providing fewer sites for enzyme adsorption, thus maximizing the apparent (available) concentration of active cellulases. This explanation is consistent with work by Chemoglazov et al. *(6)* and Ooshima et al. *(7)*, which showed that insoluble lignin hinders the accessibility of cellulases to cellulose, and irreversibly binds and inactivates cellulases. Because maximal ethanol yield was chosen for analysis, not extent of saccharification, we conclude that either lignin-mediated inactivation of cellulases or lignin-mediated inhibition of glucose fermentation resulted in a reduction of ethanol production by yeast.

Moderate relationships were found between the pretreated wood Klason lignin and glucan contents and ethanol yield. These data produced t-statistics that correlate to 99.978% and 99.972% likelihood, respectively, that Type 1 errors have not been made; i.e., that the null hypothesis has been correctly rejected and the slopes of the data are nonzero. Although not as compelling as the trend with native Klason lignin (99.999% confidence), these results are still important to understanding biomass conversion to ethanol. Because glucan is the polymer supplying glucose to the fermentation, this correlation is intuitively sensible; however, the glucan content of the native wood shows no such correlation. These results suggest that after pretreatment and subsequent washing steps, the remaining glucan (now in the absence of xylan, which was removed during pretreatment) becomes a direct predictor of ultimate convertability to ethanol. The Klason lignin content is largely unchanged by dilute acid preteatment when conducted at moderate temperatures (160°C), and continues to show an inverse relationship to ethanol yield.

The remaining data sets tested for correlation failed to reject the null-hypothesis for regression ($p > 0.050$); i.e., the probability that these sets are related is less than 99.95% and thus, uncertain *(8)*. No correlation was found for xylan contents and ethanol yield directly, or by comparing the xylan removed by pretreatment (*see* Table 2). To summarize, although we found no correlation between ethanol production potential and the native xylan or glucan contents, or to the extent of xylan removed during pretreatment, the moderately strong correlation between postpretreatment glucan content points to this component as a potentially reliable predictor of fermentability. The intuitive notion that the glucan content changed following pretreatment is supported by a Student's t-test performed on these two data sets (i.e., glucan content before and after dilute acid pretreatment), which verified that the change occurring after treatment was substantially greater than predicted by chance ($p < 0.001$). The strongest correlation found was inverse—between the Klason lignin content of the native woods and theoretical ethanol yield.

In reflection of the skepticism sometimes placed on statistical analyses of biological data, a certain degree of caution is always warranted; however, in the case of the strong trends observed for some of these data, the predictive nature of native Klason lignin and postpretreatment glucan contents regarding SSF effectiveness should be considered.

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