0273-2289/94/4546—0611$04.20

SSF Comparison of Selected Woods from Southern Sawmills

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ABSTRACT

Simultaneous saccharification and fermentation (SSF) is recognized as an efficient approach to the cost-effective conversion of biomass to fuel ethanol. This methodology takes advantage of the relief in end-product inhibition realized by conducting cellulose hydrolysis and glucose fermentation in the same well-stirred vessel. In this study, 15 species of hardwoods and softwoods were collected from sawmills located in the Appalachian region of the southern United States. These wood samples were air-dried to 8–10% moisture, pretreated using a dilute sulfuric acid cooking scheme at 160°C, exhaustively washed, and applied to SSF with *Saccharomyces cerevisiae* D_5A. Although the glucan content of each wood was found to be relatively invariant throughout the samples tested, hemicellulosic sugar and lignin contents were unique to each wood. These and other differences in chemical composition were related to resulting ethanol yields from SSF.

Index Entries: Sawmills, simultaneous saccharification and fermentation, (SSF); cellulase; ethanol; Appalachia.

INTRODUCTION

In the continental United States, hardwoods represent the largest woody biomass resource. At 122 million green tons annually, hardwood

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production is twice that of softwood and one-half that of the largest biomass resource, com. (*1*). More than 11.5 million "as is" tons of hardwood sawdust were produced by sawmills in the United States in 1991 (*2*), much of it in West Virginia (*3*), Kentucky (*4*), and Ohio (*5*). This quantity of lignocellulosic waste could have been converted to more than 800 million gallons of ethanol (*6,7*) and, thus, represents a substantial underutilized resource (*8*). According to the forestry statistics of the United States, the net volume of hardwood growing stocks has increased 70% since 1952 (*2*). Currently, the United States is growing almost twice as much hardwood saw timber as is being used. Recent regulations set forth by the US Environmental Protection Agency have restricted the growth of the Appalachian sawmill industry (*9*). Sawdust, by regulation, is considered a solid waste that cannot be allowed to accumulate on site because of rainwater runoff into streams. Sawdust in many other areas is either used as kiln-boiler fuel or as animal bedding. However, the livestock situation and capital costs for mill retrofits do not make those options feasible in Appalachia. The choice for many mills is to pay to haul the waste to a landfill or to go out of business. Therefore, ethanol production in this region could resolve a numer of economic and environmental problems in Appalachia.

Today, Appalachian logging of stumpage (standing trees) continues at a fairly regular pace all year long. Much of the timber is often cut in adjacent counties and delivered to larger centralized sawmills. However, in the region, there are a fairly substantial number of "father and son" low-impact, portable mills. These mills yield total board feet that is so low that the mixed sawdust can be given away locally. Typically, the larger mills separate timber into groups and cut runs of specific species. The better-quality timber, with a substantial amount of "clear wood" free of knots, is considered "sawlogs" with the scraps, poorer-quality, and low-value species becoming the "pulp wood" (*10*). It is this potential for species-specific harvesting, found in large logging operations (*see* Fig. 1), that makes an in-depth look at the fermentability of individual southern woods necessary (i.e., instead of mixed hardwoods).

Successful fermentation of pretreated wood pulp, even after washing, may be expected to be unpredictable. Tree taxonomic relationships will become particularly important if leaves and bark from chipped woods are used as fermentation feed. Indeed, folklore uses and herbal medicine have shown many of the noncarbohydrate components of wood fractions (i.e., primarily aromatic extractives) to have novel properties and often unknown impacts on microbial growth and metabolism (e.g., Witch Hazel and other astringents) (*11,12*). The following study provided a unique opportunity to compare, in one laboratory, 15 wood samples for performance in SSF. Parallel values for the composition of native wood and the washed wood pulp after dilute acid pretreatment were also obtained.

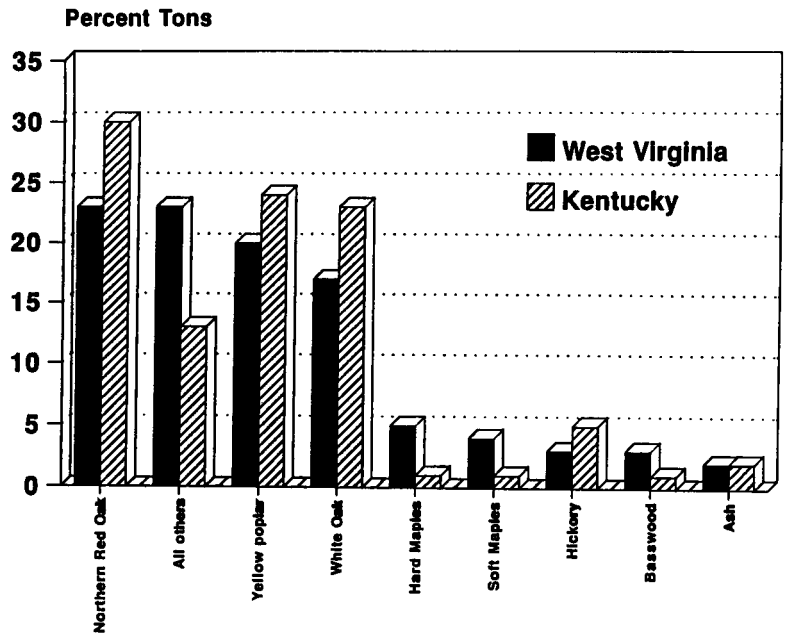

Fig. 1. Resources of hardwood sawdust by species for West Virginia (■, 1987) and Eastern Kentucky (▨, 1988) (3,4). Hard maples include sugar maple, black maple, and box elder; soft maples include red maple, silver maple, and white maple; white oak includes chestnut oak.

MATERIALS AND METHODS

Sources of Sawmill Wood

Samples of wood sawdust were obtained from green trees harvested on January 14, 1992. These samples, examined for particle size distribution only, were obtained from the following sawmills: Glandon Lumber, P.O. Box 306, McArther, OH 45651; Fouts Wood Products, Box 1162, Paintsville, KY 41240; Greentree Forest Products, Inc., Route 2, Box 199, Wallingford, KY 41093; Sawmiller, Incorporated, 17235 Haydenville Road, Haydenville, OH 43127; Blackwell Logging & Sawmill, P.O. Box 299, Elkview, WV 25071; Lannes Williamson Pallets, Inc., 2760 U.S. Route 35, Southside, WV 25187; and Crownover Lumber Company, McArthur, OH 45651. Wood samples used for SSF studies were identified (speciated) by L. Williamson.

Sample Handling

The samples of wood sawdust received from L. Williamson Pallets Inc. varied in moisture content from fairly dry (~8–12% moisture) to

almost dripping wet (~25–35% moisture). These samples were used for subsequent SSF studies. Wood samples were subjected to air-drying by spreading out the entire lot (20–25 kg) on plastic sheets in a temperature-controlled room for 10–20 d. During this drying procedure, great care was taken to ensure that wood samples were not mixed or mislabeled. Samples for the analytical workup were milled to 60 mesh using a Wiley analytical knife mill. Samples for pretreatment and SSF were reduced by milling through a 2-mm screen with a Wiley model #4 knife mill. The fraction finer than 100 mesh (~2% total) was discarded to ensure removal of trace dirt and bark.

Pretreatment

Dilute acid pretreatment was conducted on 500-g milled wood samples in a PARR 2-gal high-pressure stirred reactor. The reactor vessel used was constructed of Carpenter Cb20-3 stainless-steel alloy to ensure minimal contamination of the pretreated product. The condition for pretreatment of all wood samples was 30 min at 160°C in the presence of 0.73% w/w sulfuric acid following the method of Grohmann et al. (*13*). This acid concentration gave a prehydrolyze pH of 1.3–1.5. In all cases, the acid was added at the attainment of final reaction temperature (20 min from initiation of heating) using a Beckman model 110A high-performance liquid chromatography (HPLC) pump. When the pretreatment was complete, the reactor was removed from the heating mantle with an electric hoist and transported to a sink containing an ice-water bath. Cool-down usually required only 4–5 min. The treated wood pulp was separated from the liquor with a muslin screen and washed with approx 5 L of distilled water. Prior to preSSF compositional analysis, the samples were washed exhaustively with distilled water (i.e., against flowing water for 2 d).

Native and Pretreated Wood Analysis

The procedure used to analyze the native and pretreated samples for carbohydrates is based on the sequential hydrolysis of the samples with 72 and 4% sulfuric acid to ensure conversion of all polymeric sugars to monomers (*14*). After neutralization of the acidic digestion mixture with calcium carbonate and filtration, the samples were analyzed for neutral sugars by ion-moderated partition (IMP) chromatography using Aminex HPX-87P and HPX-87C columns with distilled water as the eluant and refractive index detection. Acid-insoluble lignin was determined by the Klason method (*14,15*). The filtrate generated from this method was measured spectrophotometrically at A_{205} for acid-soluble lignin (*16*). Total solids were determined by oven-drying at 105°C, and ash contents were determined after ignition at 575°C (*17*). All analytical testing was done in duplicate.

Simultaneous Saccharification and Fermentation

SSF followed the method of Wyman et al. (*18*) and Spindler et al. (*19*), and was conducted on the well-washed wood samples in 250-mL calibrated shake flasks using cellulase from Genencor International (South San Francisco, CA) (Laminex; tested at 79 filter paper units [FPU]/mL, β-D-glucosidase (βG) from NOVO (Danbury, CT) (SP188; tested at 94 βGU/mL), and *Saccharomyces cerevisiae* D_5A. Laminex cellulase and SP188 were added to the fermentation vessel at titers at 25 FPU/g and 25 βGU/g substrate cellulose content, respectively. Shake flasks were inoculated with 10-mL aliquots of yeast cultures grown on 50 g/L glucose (dry cell mass of inoculum was 3–5 g/L). SSF experiments were conducted in 100-mL total final volume at 37°C in a shaking incubator controlled at 150 cycles/s. Solids loading was approx 10%, but was known precisely for each wood. Fermentations were performed in quadruplicate with Sigmacell-50 controls. Fermentations were monitored daily by removing aliquots with special cut-tipped pipets subsequent to ethanol and sugar analysis. Ethanol was determined using a Hewlett-Packard gas chromatograph, and was converted to percent theoretical yield following the procedure and assumptions given in Wyman et al. (*18*) and shown in Eq. 1. Fermentations were permitted to continue until ethanol accumulation no longer increased, a condition usually reached in as few as 3 d or as long as 7 d.

$$\text{Yield} = ([\text{EtOH}]_f - [\text{EtOH}]_o / 0.568\, f\, [\text{Biomass}])\, 100 \qquad (1)$$

where $[\text{EtOH}]_f$ = ethanol concentration at the end of the fermentation (g/L), $[\text{EtOH}]_o$ = ethanol concentration at the beginning of the fermentation (g/L), [Biomass] = dry biomass concentration at the beginning of the fermentation (g/L); and f = cellulose fraction of dry biomass (g/g).

RESULTS

The sawdust samples as received from the sawmills were approx 64% dry solids. After air-drying at 23°C, sieve analysis showed size distributions (data not shown) similar to the corn meal used by whole dry corn distillers (0.6–1.7 mm). However, some of the samples showed a small percentage of particles as large as 6–8 mm and even some splinters as large as 2 cm. Provisions to screen and regrind sawdust coming from varying sawmills will therefore be required. The size of the residue is highly dependent on the wood, moisture content, and particularly the sawmill equipment used. The 14 species of sawdust used in the sieve study came in ~0.45 kg lots from seven mills equally representing Ohio, Kentucky, and West Viriginia (*see* Materials and Methods).

The compositional analysis of the 15 wood samples native to the Appalachian region and supplied by one mill, Williamson Pallets, Inc., showed low levels of variance in glucan (cellulose) content ($\bar{x}$ = 46%; wood-to-wood standard deviation, *SD* = 3.3%). However, sycamore and buckeye were substantially above the average glucan content (i.e., 53 and 52.6% glucan, respectively) (Table 1). The hemicellulosic sugars proved to be more wood-specific, with xylan average content, $\bar{x}$ = 17.4% (SD = 2.9%), arabinan average content, $\bar{x}$ = 1.9% (SD = 0.4%), and mannan average content, $\bar{x}$ = 3.1% (*SD* = 2.6%). On the basis of glucan content alone, sycamore, buckeye, and poplar should provide the highest conversions to ethanol on a dry, native wood basis. The precision found for the duplicate wood values (not shown) was within 1 SD of the average SD for each compositional set (Table 1). The wood mass balances were closed to an average of 96.6% (i.e., without uronic acids which comprise ~1–2% of hardwoods).

The wood pulps were again subjected to compositional analysis following dilute sulfuric acid pretreatment (160°C, 30 min, 0.45% v/v H_2SO_4). After pretreatment, hickory, poplar, maple, and white oak showed the highest glucan content (Table 2). Experimental standard deviations found for the pretreated, washed wood pulps are similar to those found for the native wood compositional analysis.

The glucose concentration in each inoculum immediately prior to addition to the SSF shake flask was found to be < 2 mg/mL glucose (i.e., < 1% of the initial inoculum level) and, more importantly, < 0.1% ultimate ethanol potential considering the 1:20 dilution on inoculation. Thus, this glucose represents an inconsequential source of ethanol.

SSF was based on *Saccharomyces cerevisiae* D_5A and Genencor International Laminex cellulase preparation. Figures 2–6 show the production of ethanol produced as a percentage of maximum theoretical conversion based on the total cellulose available in each wood and as function of fermentation time. The level of ethanol production from yellow poplar wood (i.e., 78% at 8 d with 1:1 FPU/βGU loading), for example, was comparable to that obtained by Spindler et al. (*19*) for aspen wood (*Populus tremuloides*) (i.e., 84% at 8 d with 1:2 FPU/βGU loading). Most fermentation profile curves are essentially hyperbolic, with several showing reductions in ethanol levels after 72 or 80 h. These differences in fermentation performance may reflect toxic effects on the yeast produced by minor components in some wood samples.

When comparing the results of multiple fermentation runs, as was done in this study, a challenging situation is caused by normal experimental variance. Sigmacell-50 was used as a control feedstock in all fermentation runs; however, the final ethanol conversions varied from 75 to 82% of theoretical yield (e.g., each fermentation set was conducted in quadruplicate and the values averaged). This situation was reconciled by

Table 1
Compositional Analysis of Southern Wood Feedstocks, Reported on a % Dry Wt Basis

Name	Glucan	Xylan	Arabinan	Galactan	Mannan	Klason lignin	Acid-soluble lignin	Total ash	Total %
White oak	43.61	17.95	2.43	0.38	2.93	23.21	4.05	0.57	95.13
Beechwood	42.89	20.76	1.47	nd[a]	0.94	26.17	1.53	0.53	94.29
Hickory	45.78	19.90	1.17	nd	0.61	24.16	1.56	1.00	94.18
White pine	46.43	8.79	2.43	nd	11.66	29.02	0.39	0.35	99.07
Sycamore	53.06	17.11	1.71	nd	2.68	23.15	3.58	0.59	101.88
White ash	48.31	17.36	1.66	nd	1.42	28.45	2.19	0.79	100.18
Chestnut oak	44.02	19.98	2.29	nd	3.47	22.41	4.92	0.86	97.95
Ohio buckeye	52.63	14.92	2.07	nd	2.84	23.92	1.70	0.77	98.85
Red oak	43.43	18.94	1.94	nd	2.65	25.76	4.28	0.44	97.44
Wild cherry	46.01	19.81	1.83	nd	2.08	23.84	2.02	0.29	95.88
Yellow poplar	49.91	17.40	1.84	1.21	4.70	18.10	3.69	0.52	97.37
Lynn basswood	46.69	17.45	2.07	1.07	2.75	21.54	2.94	0.81	95.32
White walnut	46.22	16.53	1.80	nd	2.59	21.88	3.98	0.99	93.99
Soft maple	44.88	17.34	2.76	nd	2.86	20.66	4.94	0.61	94.05
Hard maple	42.80	16.79	1.68	nd	2.90	24.95	3.34	1.52	93.98
Average standard deviation, $\overline{SD}$	0.81	0.57	0.12	0.12	0.20	0.11	0.12	0.02	Mean 96.6% $\overline{SD}$ = 2.5

[a]nd = not detected.

Table 2
Compositional Analysis of Southern Wood Feedstocks Following Dilute Acid Pretreatment Results Reported on a % Dry Wt Basis

Name	Glucan	Xylan	Arabinan	Galactan	Mannan	Klason lignin	Acid-soluble lignin	Total ash	Total %
White oak	62.01	1.98	nd[a]	nd	nd	32.95	3.94	0.095	100.98
Beechwood	60.35	2.46	nd	nd	nd	35.86	2.17	0.045	100.88
Hickory	63.72	1.94	nd	nd	nd	32.03	2.72	0.045	100.46
White pine	54.62	0.98	nd	nd	0.32	39.56	0.30	0.039	95.82
Sycamore	61.27	2.34	nd	nd	nd	31.06	1.60	0.042	96.31
White ash	59.00	2.50	nd	nd	nd	35.72	1.20	0.071	98.49
Chestnut oak	59.49	3.82	0.78	nd	1.13	32.93	1.81	0.040	100.00
Ohio buckeye	60.78	3.46	0.79	nd	0.86	31.16	0.93	0.083	98.06
Red oak	58.16	1.91	0.39	nd	0.53	35.27	1.82	0.041	98.12
Wild cherry	58.70	1.89	0.36	nd	1.20	37.44	1.02	0.071	100.68
Yellow poplar	62.98	3.70	0.71	nd	0.98	29.73	1.30	0.043	99.44
Lynn basswood	61.39	2.46	0.40	1.31	1.32	31.66	1.35	0.049	99.94
White walnut	57.08	2.12	0.40	nd	0.54	35.32	1.60	0.009	97.07
Soft maple	62.60	1.64	0.37	nd	0.56	30.47	2.36	0.036	98.04
Hard maple	56.40	3.81	nd	nd	1.77	35.67	1.51	0.37	99.53
Average standard deviation, $\overline{SD}$	0.44	0.21	0.04	0.15	0.04	0.16	0.06	0.01	Mean 98.9% $\overline{SD}$ = 1.66

[a]nd = not detected.

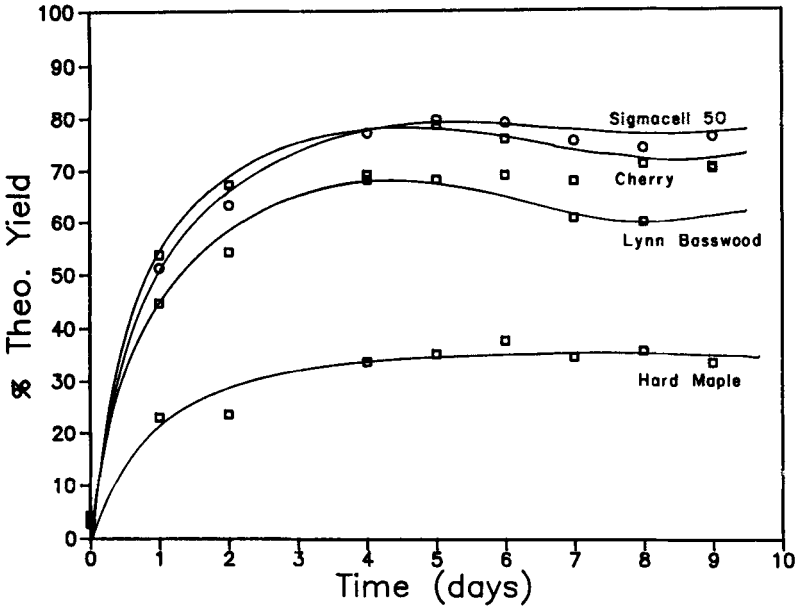

Fig. 2. SSF profile of sawdust from wild cherry, lynn basswood, hard maple, and Sigmacell-50. Ethanol production is shown as a percentage of maximum theoretical conversion based on the total cellulose available in each wood sample following dilute acid pretreatment and extensive water washing.

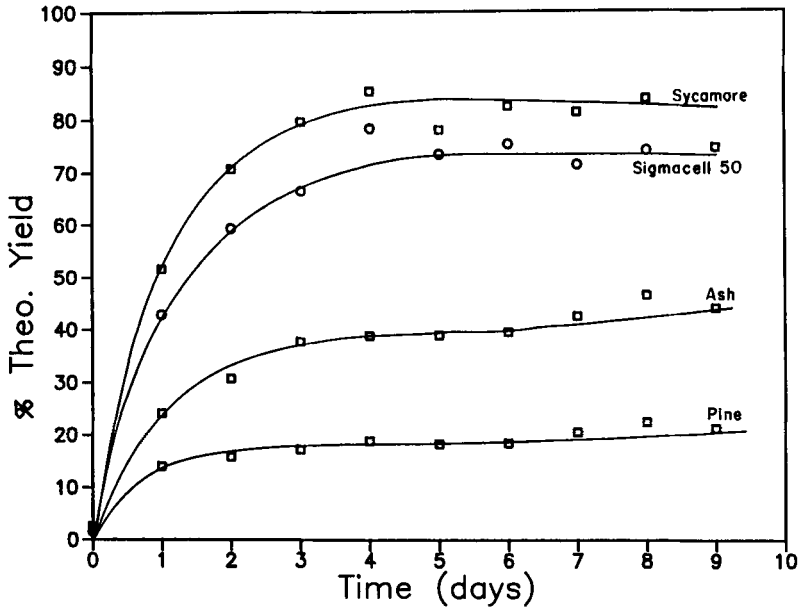

Fig. 3. SSF profile of sawdust from sycamore, white ash, white pine, and Sigmacell-50. Wood treated as in Fig. 2.

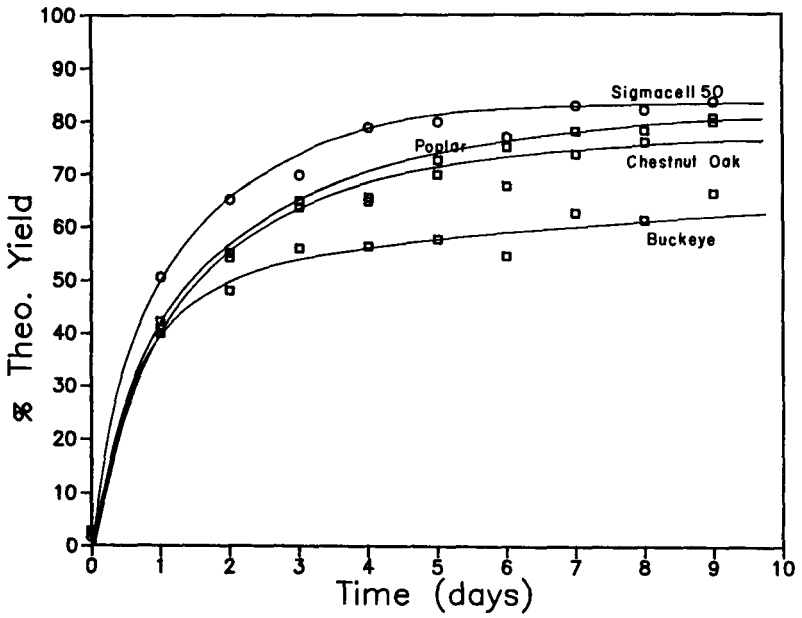

Fig. 4. SSF profile of sawdust from yellow poplar, chestnut oak, buckeye, and Sigmacell-50. Wood treated as in Fig. 2.

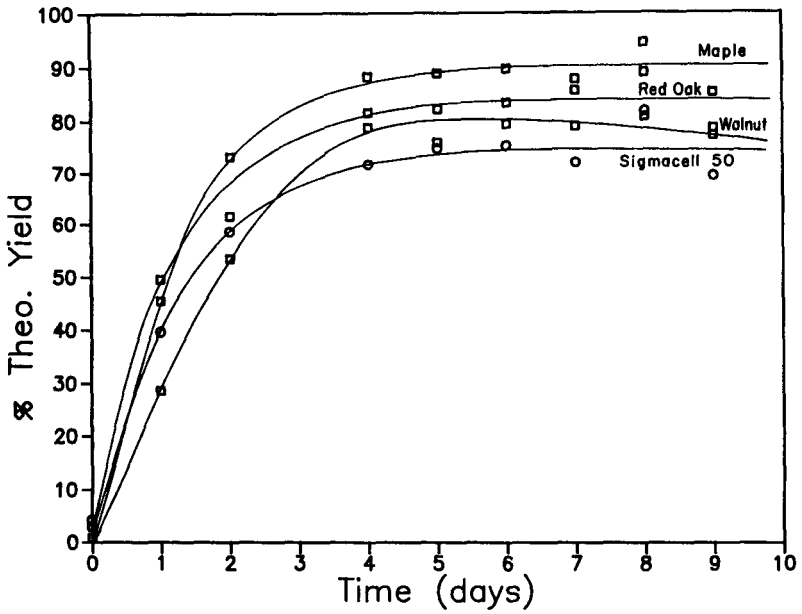

Fig. 5. SSF profile of sawdust from soft maple, red oak, white walnut, and Sigmacell-50. Wood treated as in Fig. 2.

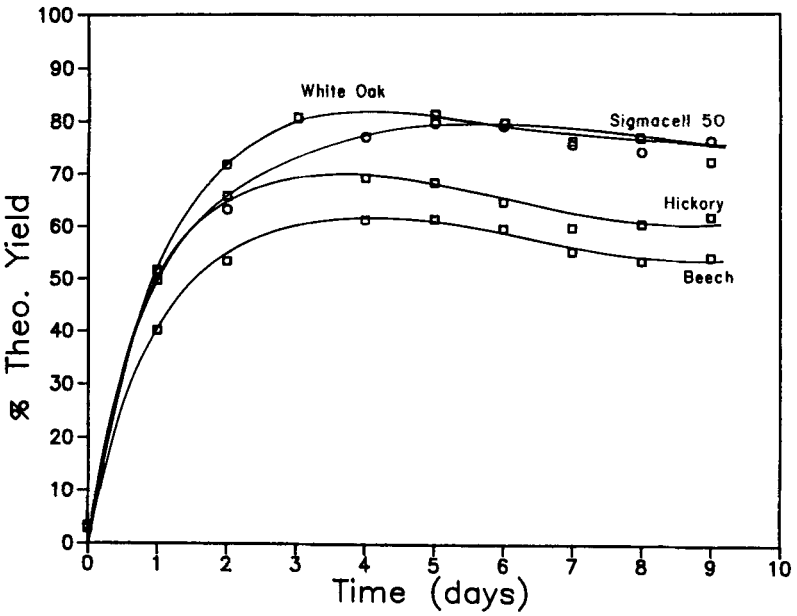

Fig. 6. SSF profile of sawdust from white oak, hickory, beech, and Sigmacell-50. Wood treated as in Fig. 2.

averaging the four Sigmacell-50 values (from four run series) and normalizing the wood fermentation values to this averaged value. The conversion to ethanol shown in Table 3 reflects the ranking of wood feedstocks based on this treatment. The average biomass conversion efficiency value for Sigmacell-50 from five fermentation runs was 78 ± 3%. Maple, sycamore, and red oak produced the highest theoretical yields of ethanol (93, 87, and 87%, respectively) in 4 d. Walnut, poplar, cherry, and white oak were also efficiently converted. Pine, hard maple, and ash produced low levels of ethanol.

DISCUSSION

In the execution of the present study, an experimental approach was chosen to reduce the high-element experimental array possible with an SSF examination of 15 wood samples. First, we had to choose a viable protocol for chemically pretreating all the woods. Although it is undoubtedly true that some enhancement in the saccharification of some woods is possible following alternative pretreatments to dilute acid at 160°C for 30 min, we chose this method because of the good general success reported by others with similar woods, especially poplars (*20–22*). The next experimental step, SSF, utilizes a cellulase preparation introduced to the pretreated wood pulp in the presence of an ethanologen. In this study, we

Table 3
SSF Comparison of Selected Woods from Southern Sawmills

Wood Common Name	% Theoretical yield	Fermentation time
Soft maple	93; SD = 2.9	4 D[a]
American sycamore	87; SD = 6.0	4 D
Red oak	87; SD = 2.8	4 D
White walnut	84; SD = 3.4	5 D
Sigmacell-50	78; SD = 3.0	5 D
Poplar	78; SD = 1.6	8 D
Wild cherry	76; SD = 5.1	4 D
White oak	76; SD = 3.6	4 D
Chestnut oak	70; SD = 1.8	8 D
Hickory	64; SD = 5.6	4 D
Lynn basswood	64; SD = 0.6	6 D
Beechwood	60; SD = 2.8	4 D
Ohio buckeye	57; SD = 3.0	7 D
White ash	43; SD = 7.9	8 D
Hard maple	35; SD = 2.2	6 D
White pine	21; SD = 4.8	8 D

[a]Calculated from quadruplicate data at the maximum ethanol yield value. SD is standard deviation. D = day.

chose Laminex cellulase and the yeast *Saccharomyces cerevisiae* D_5A, again based on published experience, primarily with aspen conversion (*18*). In summary, no effort was made to optimize either pretreatment conditions, saccharification, or yeast used for fermentation. This eclectic approach yielded results varying only for wood type, thus defining the primary objective of the study.

The results from SSF show the effects of multiple inputs to overall efficiency, including the possible existence of yeast toxins or enzyme inhibitors in the wood pulps, and/or the differences in availability of cellulose too celulases in the wood (i.e., the structure of woody tissue, especially after pretreatment). Red oak, for example, is notorious for maintaining an open-pore structure, whereas white oak fills in these channels when the wood matures. A more general view of wood structure and permeability is possible as well, since hardwoods tend to produce vessels or pores that run longitudinally along the direction of growth and are of substantial size (i.e., 50–300 μm diameter for early wood) (23). Softwoods (i.e., pine, in this study) do not have vessels, but longitudinal tracheids that result in a more filled-in structure. The ethanol conversion data shown in Table 3 support the correlation between an open-pore structure and higher cellulase accessibility.

However, successful conversion of woods to ethanol via fermentation also requires a stable ethanol-producing microorganism. Evidence for toxicity may be apparent for lynn basswood, hickory, and beech in

Figs. 2 and 6 (i.e., an abnormality to the hyperbolic ethanol production curve). Also, toxicity effects may be more subtle and not readily detectable beyond ethanol production profiles.

The taxonomic outline of relative woods (Table 4) shows that some orders contain families and genera that produce compounds not compatible with fermentation (*24–26*). It is also possible that some of these compounds, especially those that are aromatic or heterocyclic, may be adsorbed to cellulose fiber of lignins during pretreatment, thus preventing this step from effectively "cleaning" the wood pulp of all extractives (*27*). For example, higher wood theoretical yields and faster fermentation time tended to follow order groupings: Sapindales > Roseales > Fagoles > Juglandales > Ranales > Malvales > Contortae. Differences within orders, such as within Rosales, might be explained by their further breakdown into families (sycamore and rose, or maple and horse chestnut). Correlations within those families (i.e., rose and hickory) often show genus members known to contain important secondary metabolites and reactive tannins, alkaloids, and antibiotics (*11*). This might explain why sycamore ferments better than rose (wild cherry, choke cherry) and maple better than horse chestnut (Ohio buckeye), and so forth. However, the high conversion of walnut (known to contain jugalones) and the moderate conversion of poplar (free of obvious toxins), argue for a complex explanation. Further work will be required to determine which, if any, of these compounds is synergistic or antagonistic to the SSF process. Still, wood-use folklore suggests that different methods of pretreatment may be preferable for extractive cleaning of different woods.

Hard maples (sugar maple) contain 2% sugars, which, during syrup harvest, must be prevented from "turning" (i.e., acid production from bacterial spoilage) by special treatment in the field. However, the dilute-acid pretreatment should effectively remove all such carbohydrates, rendering all maples similar. The fact that there is a large disparity in fermentation results among the maples tends to support a structural difference in woody tissue as the cause. Taxonomically, all three maple species should represent premium fermentation candidates. One must conclude, therefore, that the potential for native wood toxicity does not predict poor ethanol production.

Future work with these southern sawmill woods should investigate the cause of wood-specific ethanol production by monitoring transient (steady-state) and final glucose levels, and periodic assay of yeast cell viability. A dramatic increase in the former and decrease in the latter could signal the timed release of a yeast toxin. An early and dramatic decrease in available glucose may reflect substrate resistance to cellulase action; however, determination of expected steady-state glucose concentrations is not immediately apparent. It is also clear that optimizing pretreatment conditions and cellulase loadings could substantially enhance the yield from some woods.

Table 4
Taxonomy and Folklore of Woods from Southern Sawmills Used in This Study

#	Common name	Order	Family	Genus	Species	Comments
1	White ash	Contortae	Oleaceae	*Fraxinus*	*americana*	Members of other families produce poisons
2	Lynn basswood	Malvales	Tiliaceae	*Tilia*	*americana*	Members of other families produce caffeine
3	White pine	Conniferales	Pinaceae	*Pinus*	*strolus*	Known to produce many organic extractives
4	White cherry	Rosales	Rosaceae	*Prunus*	*serotina*	Some species produce hydrocyanic glucosides; sap known to be sedative, antitussive, and astringent
5	American sycamore	Rosales	Platanacae	*Platanus*	*occidentals*	No strong toxins; members of other families known to produce toxins
6	Ohio buckeye	Sapindales	Hippocastanaceae	*Asculus*	*hippocastrum*	Some species produce a sapogenin glycoside used by native Americans to stun fish; members of other families produce poisons
7	Hard (sugar) maple	Sapindales	Aceraceae	*Acer*	*saccharum*	Sap has high sugar content; some species produce diuretic volatile oils
8	Soft maple	Sapindales	Aceraceae	*Acer*	*sacchrinum*	Sap has high sugar content
9	Chestnut oak	Fagales	Fagacea	*Quercus*	*prinus*	All species contain large amounts of tannins that precipitate metals and enzymes
10	Red oak	Fagales	Fagacea	*Quercus*	*rubra*	Same as above
11	White oak	Fagales	Fagacea	*Quercus*	*alba*	Same as above
12	Beechwood	Fagales	Fagacea	*Fagus*	*grandifolia*	Same as above
13	Yellow poplar	Ranales	Magnoliaceae	*Liriodendron*	*tulipfera*	No strongly toxic products
14	Hickory	Juglandales	Juglandaceae	*Carya*	*ovata*	Contains jugalone, which is inhibitory to fungal mycelial growth
15	White walnut	Juglandales	Juglandaceae	*Juglans*	*cinerea*	Used to treat clothing in 19th century

Mixtures of local hardwoods, not pure species, may ultimately be the actual feedstock for ethanol production in this Appalachian region because sawmills cannot routinely segregate sawdust, even though it is typical for an individual mill to cut a few days' run of a particular wood. The techniques of feeds microscopy, now used at South Point Ethanol, suggest that these different woods can be quantitatively differentiated in a mixture. A distillery, using feeds microscopy, could determine and blend a particular mash bill containing similar contents of different woods. This study has shown the advantage of such capability.

ACKNOWLEDGMENTS

The 15 different species of wood sawdust used in this study were supplied by Lannes Williamson Pallets, Inc., Southside, WV. This company was anxious to assist in the study and suspended normal operations for one full day in order to select, saw, collect from each individual log, and ship 25-kg lots to NREL. This work was funded by the Ethanol from Biomass Program of the Biofuels Systems Division of the US Department of Energy.

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