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Response of Thermochemical and Biochemical Conversion Processes to Lignin Concentration in Alfalfa Stems†

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The technologies currently in place to convert lignocellulosic biomass to energy are either biochemical or thermochemical, the efficiencies of which may vary depending on the composition of the feedstock. One variable that conversion technologists have wrestled with, particularly in the simultaneous saccharification and fermentation process, is biomass lignin content. While lignin is considered a recalcitrant to biochemical conversion, it can be a good source of combustion fuel, but the true effect of composition on thermochemical conversion has not been well quantified. In this study we examined the effect of lignin content of alfalfa stems on two biofuel conversion methodologies: (i) biochemical conversion using in-vitro ruminal fermentation as a surrogate for fermentability to ethanol and (ii) thermochemical conversion using pyrolysis. Lignin was found to account for little of the variation in pyrolysis product yield compared to biochemical conversion. Linear regression of lignin concentration on pyrolysis product yields resulted in few significant relationships whereas in-vitro gas production exhibited a strong negative response to lignin content. For alfalfa stems, lignin had a much larger effect on biological conversion potential than it did on thermochemical conversion potential. The results suggest that genetic modification or agronomic management of lignocellulosic biomass for bioenergy feedstock composition should be based on the intended energy conversion platform.

Introduction

The main technological question confronting the biorefinery investor is the choice of the conversion technology. Often the decision is based on the availability of the biomass, the logistics of handling it, and its processing characteristics. The technologies that are currently available for lignocellulosic conversion include both biochemical- or thermochemical-based platforms.[1] Biochemical conversion, often called the sugar platform, involves depolymerization of polysaccharides and fermentation of the resulting sugars. This is the technology of choice for the conversion of starch and simple sugars to fuel ethanol and related alcohols. Thermochemical conversion involves medium or high temperature degradation of biomass in an oxidized or reduced atmosphere to release the inherent energy (combustion) or to produce fuel intermediates (energy carriers) such as synthesis gas (syngas) and pyrolysis liquids. Both technologies can result in the production of transportation fuel from cellulosic biomass at varying economic penalties.[2]

Through plant breeding and advances in genomics, plants could be genetically engineered to tailor their composition to the desired conversion technology. Yields, conversion efficiencies, and ultimately the economics of biochemical-based biofuel production are all greatly impacted by feedstock composition.[3,4] For example, enzymatic hydrolysis of lignocellulose to free sugars is hindered by the presence of lignin because the latter acts as a physical barrier to hydrolytic enzymes, and because enzymes reversibly bind to lignin, resulting in inefficient use of the polysaccharide-degrading enzymes. When poplar (*Populus*) wood was subjected to acid hydrolysis, Chang and Holtzapple[5] found a strong negative correlation between lignin content and degradability of the biomass. Dien et al.[6] found that lignin content negatively influenced total glucose yield from dilute-acid pretreatment and enzymatic saccharification for several perennial herbaceous species including alfalfa (*Medicago sativa*), reed canarygrass (*Phalaris arundinacea*), and switchgrass (*Panicum virgatum*).

While there appears to be a negative impact of lignin on biochemical conversion, such may not be the case for thermo-

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chemical conversion. On the thermochemical conversion front, although the elemental composition (C:H:N:O) of most lignocellulosic biomass is similar, studies have shown that devolatilization behavior during pyrolysis differs among biomass feedstocks. Worasuwannarak et al.[7] showed that the concentration of cellulose, hemicellulose, and lignin affected the pyrolysis behavior of rice (*Oryza sativa*) straw, rice husk, and corncobs (*Zea mays*). Boateng et al.[8] found that compositional changes associated with maturity stage of switchgrass at harvest correlated with different concentrations of pyrolysis products at different reaction temperatures. Wood-extracted lignin, a byproduct in the pulp and paper industry, has long been used as boiler fuel. Lignin's energy content is comparable to those of certain bituminous coals and is greater than that of carbohydrates.[9]

These observations suggest that high lignin content is desirable for biomass combustion while low lignin content is desirable for enzymatic hydrolysis. It is well-known that the lignin content of most vegetative plant tissues increases during the course of physiological maturation.[10] Genetic variation in cell wall lignification has been demonstrated and exploited by plant breeders to improve forage digestibility by livestock.[11] Plant growth environment interacts with cell wall development to introduce even more variation in lignin concentration.[12] These factors combine to cause great natural variability in biomass lignification which may be of value to bioenergy production if conversion processes are responsive to these changes in lignin concentration.

The objective of the current study was to evaluate responsiveness of biochemical and thermochemical conversion processes to the range in lignification available in a candidate herbaceous biomass crop. Alfalfa was chosen because this species has been proposed as a feedstock for biochemical and thermochemical conversion.[13,14] It is envisioned that alfalfa hay would be fractionated into leaf and stem portions, with the leaves being used as a protein feed for livestock while the stems would be converted to energy.[15] Alfalfa stem samples were selected to provide the natural range in lignin concentration associated with genetic and environmental sources of biological variation for this species. In-vitro ruminal digestion was used as a surrogate for the biochemical conversion to ethanol, the extent of which was measured by gas production.[4] For thermochemical conversion pyrolysis was used. It was carried out at temperatures ranging from 500 to1100 °C. The reason for selecting pyrolysis is that it is the common thermal degradation process underlying combustion, gasification and fast pyrolysis oil (bio-oil) production technologies, and provides the fundamental gas evolution pathway for thermochemical conversion technologies.

Materials and Methods

Biomass Material. The alfalfa stem samples were selected from an ongoing breeding program targeted at the improvement of alfalfa as an animal feed. Alfalfa samples for the current study were selected from a set of 6937 individual samples collected as part of a breeding project focused on stem in-vitro fiber digestibility by rumen microbes. In that study, individual plants were harvested at the flowering stage of maturity during the primary spring growth and first summer regrowth cycles in 2002 and 2003 from a nursery planted at Becker, MN. Each harvested plant was manually separated into leaf and stem fractions after drying at 60 °C in a forced air oven. These alfalfa stem samples represent the variation observed in a multiyear breeding experiment due to a combination of genotype and growth environment effects, with only a small contribution from maturity differences. Twenty samples were chosen to represent the biological range in lignin concentration available in alfalfa. The selection protocol was to rank all 6937 alfalfa stem samples for Klason lignin concentration estimated by near-infrared reflectance spectroscopy calibrations[16] and then select 20 samples that covered the observed range in a nearly equidistant manner. The selected samples were analyzed for carbohydrate, lignin, and ash components by direct measurements as described below.

Dried samples were ground in a cyclone-type mill to pass a 1-mm screen and were subjected to chemical analysis for soluble carbohydrates, starch, cell wall polysaccharides, and Klason lignin. Soluble carbohydrates (glucose, fructose, and sucrose) were extracted with 80% (vol/vol) ethanol and analyzed by high performance liquid chromatography, HPLC.[6] The ethanol-insoluble residue was subjected to starch hydrolysis using a heat-stable α-amylase, followed by amyloglucosidase treatment and extraction with 80% ethanol.[6] Glucose from starch (in the supernatant) was analyzed by HPLC. Cell wall polysaccharides remaining in the insoluble residue were subjected to a two-stage sulfuric acid hydrolysis, and the neutral sugar residues were derivatized as alditol acetates and analyzed by gas chromatography.[16,17] Total uronic acids in the first-stage acid hydrolyzate were analyzed colorimetrically using galacturonic as the calibration standard.[18] On the basis of previous knowledge of alfalfa cell wall polysaccharide composition,[19] cellulose was estimated as cell wall glucose content, hemicellulose was considered to be the sum of xylose, mannose, and fucose residues, and pectin was the sum of arabinose, galactose, rhamnose, and uronic acids. Klason lignin was determined as the ash-free residue remaining after acid hydrolysis.[18] Ash content was determined by combustion in a muffle furnace at 450 °C overnight. All analyses were carried out in duplicate.

In-Vitro Ruminal (IVR) Fermentation. In-vitro fermentations and measurements of gas production after 24 and 96 h of incubation were conducted as described by Weimer et al.[4] Ruminal inoculum

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was collected and composited from two rumen-fistulated Holstein cows (*Bos taurus*) 4 h after feeding a total mixed ration consisting of alfalfa silage, corn silage, corn grain, soybean (*Glyxine max*) meal, whole cottonseed (*Gossypium hirsutum*), and supplemental vitamins and minerals. Two in-vitro fermentation experiments were conducted, with samples run in duplicate within each fermentation experiment. Gas production data between runs were normalized using a standard alfalfa fiber sample run in triplicate within each run. The IVR gas accumulation at both fermentation times has been shown to correlate well with ethanol production in a 7-d yeast simultaneous saccharification and fermentation system, although which incubation time had the better correlation with ethanol production differed among forage types.[4]

Analytical Pyrolysis. Pyrolysis of the alfalfa stem samples was carried out in a Chemical Data System (CDS) Analytical (Oxford, PA) flash pyrolyzer (Pyroprobe). A more detailed experimental procedure is reported in Boateng et al.;[20] hence, only a brief description is provided herein. The pyroprobe consisted of a 1-cm quartz tube heated by a platinum filament of 2−3 mm diameter. The pyroprobe is capable of maintaining up to 1200 °C temperature with a nominal heating rate of approximately 20 °C ms^{-1} and uses helium as both carrier and purge gas. Pyrolysis was carried out at 500, 700, 900, and 1100 °C with a retention time of 20 s, the time when devolatilization was observed to be complete.[21] The pyrolysis products were comprised of charcoal and evolved gas with the latter consisting of a condensable gas fraction (CG) and a noncondensable gas fraction (NCG). The NCG was analyzed by gas chromatography (SRI, Torrance, CA) using a Shincarbon ST 80/100, 2 m × 2.0 mm packed column (Restek, Bellefonte, PA) with a predetermined temperature program.[21] Hydrogen detection was accomplished by a 4-filament Wheatstone bridge thermal conductivity detector (SRI Instruments, Torrance, CA). The yields of the major NCG produced by primary and secondary pyrolysis reactions were quantified by calibration with a standard gas mixture consisting of CO, CO_2, CH_4, C_2H_4, C_2H_6, C_3H_8, and C_4H_{10} in helium (custom-mixed by Scott Specialty Gases, Plumsteadville, PA). Char yield was determined gravimetrically by weighing the quartz tube before and after pyrolysis on a Mettler balance of a 10^{-1} mg accuracy. Condensable gas was determined as 100% minus the sum of the NCG and the product char.

Statistical Analysis. Each sample test for the pyrolysis experiment was done in triplicate resulting in 240 observations (20 samples × 4 temperatures × 3 replicates). For each triplicate data set, outliers (identified by Dixon's Q-test) were removed.[22] Of the 240 observations, 36 individual replicate analyses were removed for alfalfa stems at $P < 0.05$. The average pyrolysis product yields were calculated based on remaining replicates of each sample at each temperature. This resulted in a data set with 20 samples for each pyrolysis temperature. A one-way analysis of variance was done to compare pyrolysis product yields at the four temperatures.[23] Means separation tests were conducted using the *F*-protected least-significant difference procedure. Linear regressions of pyrolysis product yields as a function of lignin concentration, for individual pyrolysis temperatures, were determined, as were linear regressions of net normalized fermentative gas production as a function of lignin concentration. All statistical procedures were done using SAS for Windows, ver. 9.1 (SAS Institute Inc., Cary, NC).

Results and Discussion

Compositional Analysis. Table 1 shows the variation in lignin, cellulose, hemicellulose, pectin, and ash concentrations of the 20 alfalfa stem samples selected to represent the available biological range in lignin concentration of this potential bioenergy feedstock. The alfalfa stem sample with the highest lignin concentration had 48% more lignin than did the lowest concentration sample. Concentration variation for the major cell wall polysaccharide sugar components (cellulose, hemicellulose, and pectin) were in a similar range (22, 38, and 48%, respectively). While the relative ranges for noncell wall carbohydrates were much larger (several fold), absolute amounts of these carbohydrates were low. Cellulose concentration was moderately positively correlated ($r = 0.56$, $P < 0.01$) with lignin concentration among the alfalfa stem samples. Pectin and noncell wall carbohydrates declined ($r = -0.79$ and $r = -0.84$, $P < 0.001$, respectively) as lignin concentration of the samples increased. Changes in lignin concentration had no impact on either hemicellulose or ash concentrations.

Table 1. Variation in Lignin, Carbohydrate, and Ash Concentrations of 20 Alfalfa Stem Samples[a]

	% DM			
component	mean	standard error	minimum	maximum
Klason lignin	16.2	0.4	13.2	19.6
cellulose[b]	29.3	0.4	26.4	32.3
hemicellulose[c]	14.0	0.3	12.0	16.5
pectin[d]	13.7	0.4	10.8	16.0
noncell wall carbohydrates[e]	3.4	0.3	1.3	6.0
ash	5.8	0.2	4.3	7.6

[a] Samples were selected to represent the available biological range in lignin concentration of this potential bioenergy feedstock. [b] Cellulose defined as cell wall glucose content. [c] Hemicellulose defined as the sum of xylose, mannose, and fucose cell wall residues. [d] Pectin defined as the sum of arabinose, galactose, rhamnose, and uronic acids. [e] Noncell wall carbohydrates are the sum of soluble glucose, fructose, sucrose, and starch.

Table 2. Summary of Net Normalized Gas Accumulation (NNG), after 24 or 96 h of in-vitro Fermentation (Biological Conversion) and Percent Pyrolysis Product Yields (Thermochemical Conversion)[a]

product	mean	standard error	minimum	maximum
	fermentation			
NNG24, mL (g DM) $^{-1}$	167	3	113	205
NNG96, mLg DM) $^{-1}$	192	3	139	235
	pyrolysis			
CO_2, %	8.38	0.12	6.56	11.65
CO, %	1.72	0.10	0.144	6.74
CH_4, %	1.39	0.08	0.22	3.31
C_2H_6, %	0.54	0.05	0.12	3.21
C_3H_8, %	0.26	0.02	0.03	0.62
H_2, %	0.0313	0.0035	0.0003	0.1327
NCG[b], %	12.34	0.24	9.12	23.31
CG, %	77.21	0.54	61.54	86.16
char, %	10.69	0.59	2.06	28.04

[a] Pyrolysis yields were averaged across four temperatures (500, 700, 900, and 1100 °C) of 20 alfalfa stem samples selected to represent the available biological range in lignin concentration of this potential bioenergy feedstock. [b] NCG = CO_2 + CO + CH_4 + C_2H_6 + C_3H_8 + H_2.

In-vitro Fermentation. In-vitro gas production by mixed ruminal microbes has previously been shown to correlate with ethanol production in an enzymatic saccharification/yeast fermentation method developed by Dien et al.[6] The extent of in-vitro gas evolution serves as an indication of the potential extent of fermentation to ethanol in a biochemical conversion process. Table 2 summarizes in-vitro net normalized gas yields after 24- and 96-h fermentations. As is shown, ranges in gas production were large for alfalfa stem samples at both incubation times (81 and 69% for 24 and 96 h, respectively).

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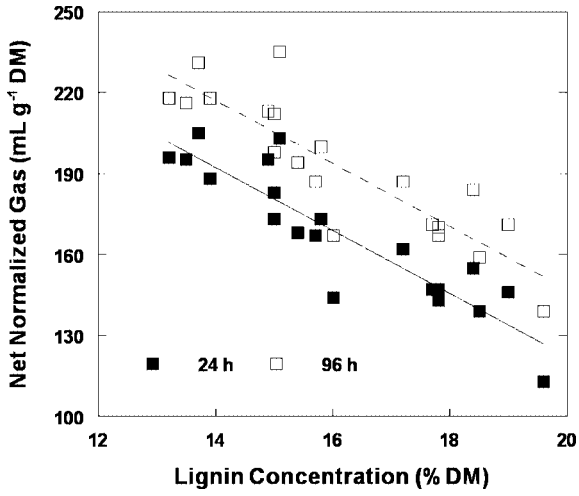

Figure 1. In-vitro rumen fermentative net normalized gas production from alfalfa stems at 24- and 96-h incubations as related to lignin concentration.

The biochemical conversion response via in-vitro fermentation to alfalfa stem lignification is graphically presented in Figure 1. The strong negative effect of lignin on fermentability of alfalfa stems is evident and is consistent with data on pretreated biomass. Kim et al.[24] reported that lignin concentration negatively affected ethanol production in ammonia-pretreated corn stover. Dien et al.[6] found total glucose release after cellulose hydrolysis of full-flower alfalfa stems to be lower than for bud-stage alfalfa which contained less lignin. The results are in accord with several studies that have shown the negative effect of lignin content on ruminal digestibility of forages.[10] In the present experiment, the alfalfa stem samples were not subjected to physical or chemical pretreatment beyond simple grinding, thus the effect of lignin content on fermentative gas production was direct and did not involve the generation of fermentation inhibitors in response to acid pretreatment. The effect of lignin concentration on the bioconversion of pretreated biomass materials is likely to be more complex and highly dependent upon feedstock type, pretreatment technology,[25,26] and bioconversion platform.[3,27]

Thermal Decomposition (Pyrolysis). The main products of high-temperature pyrolysis were NCG, char, and CG. We estimated CG by difference and this constitutes the fraction that would condense to pyrolysis liquids. Condensable gas was the largest pyrolysis product fraction and NCG was the smallest fraction. Dramatic differences in yields of individual pyrolysis products were observed among the alfalfa stem samples, averaged across pyrolysis temperatures (Table 2). Condensable gas yield was the least variable pyrolysis product with a 40% range between the lowest and highest lignin sample. In contrast, H_2 production increased by 442-fold over the same lignin range. Total noncondensable gas and char yields ranged over 2.5- and 13.6-fold, respectively, among the alfalfa stem samples.

Table 3 presents the yields of pyrolysis products as impacted by temperature, averaged across the 20 selected alfalfa stem lignin samples. Most product yields varied substantially with temperature, but temperature effects on yield were highly dependent on the redox state of the gaseous product. Hydrogen displayed the greatest relative increase in yield (187-fold increase) as pyrolysis temperature was increased from 500 to 1100 °C, while the hydrocarbon series CH_4, C_2H_6, and C_3H_8 increased 6.5-, 2.9-, and 0.6-fold, respectively, with increased temperature. Yields of the most oxidized products (CO_2 and CO) did not vary with temperature as expected. While some individual NCG showed large variation among pyrolysis temperatures, total NCG yield only increased 30% from 500 to 1100 °C (Table 3). Condensable gas yield showed an even smaller response to temperature (9% increase). However, char yields declined 2.6-fold with increased temperature.

Simple linear regression analyses were conducted between lignin concentration and all pyrolysis product yields at each of the four pyrolysis temperatures. No association was found between lignin concentration and total NCG, CG, char, and the individual noncondensable gases CO_2, CO, or C_2H_6 at any of the four pyrolysis temperatures. However, an association with lignin concentration was shown for the remaining three individual noncondensable gases (CH_4, C_3H_8 and H_2) at some but not all of the pyrolysis temperatures (Table 4). The data for the three significant regressions for C_3H_8 yield are shown in Figure 2 for illustrative purposes. Methane yield was positively associated with lignin concentration in the alfalfa stems at the lower pyrolysis temperatures (500 and 700 °C) and C_3H_8 yield was similarly positively associated with lignin at temperatures of 700, 900, and 1100 °C. In contrast, hydrogen gas yield was negatively associated with lignin concentration, but this regression was only significant at 1100 °C. It should be noted that these three component NCG made up less than 1.7 wt % of the feedstock in the primary pyrolysis reactions.

It was indicated earlier that pyrolysis is the common thermal decomposition process underlying combustion, gasification and bio-oil production. The implications of the results can be related to the effect of temperature and lignin concentration in light of these thermochemical processes. Syngas fuel quality might be characterized by cold gas efficiency, the amount of energy in the product gas compared with that of the parent biomass i.e.,

$$\eta = \frac{Q_{\text{Gas}}}{Q_{\text{Biomass}}} \times 100 \qquad (1)$$

where Q is the gross heating value, which will depend on the relative yields of the combustible gas components (paraffinic hydrocarbon and CO) and the noncombustible gas (CO_2). As a result, the combustion characteristics of the produced NCG would improve with increased pyrolysis temperature; however, there was no appreciable effect of lignin concentration in the narrow biological lignin range available. Moreover, the NCG produced represented only 12 wt % of the feedstock in the primary pyrolysis product.

Another important characteristic of the NCG is the H_2:CO ratio. Higher H_2:CO ratios and low CO_2 concentrations are desirable if one is targeting the produced gas for use as the feedstock for the production of liquid fuels via the Fischer−Tropsch process. The results indicate that CO yield was not correlated with lignin in alfalfa stems, although H_2 yield was related to lignin content at the highest temperature tested (1100 °C). As a result, regression analysis found no significant association between H_2:CO and lignin concentration. This indicates that one cannot rely on the biological range of lignin to substantially affect the production of syngas with Fischer−Tropsch synthesis quality based on the primary reactions alone. For high

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Table 3. Yields of Pyrolysis Products as Impacted by Temperature Across 20 Alfalfa Stem Samples[a]

	% DM										
temp	CO_2	CO	CH_4	C_2H_6	C_3H_8	H_2	NCG[f]	CG	char	H_2:CO ratio	HHV[g] (kcal kg^{-1})
500 °C	8.17	1.65	0.34[e]	0.24[c]	0.17[c]	0.0004[e]	10.21[d]	73.87[d]	15.93[b]	0.35[c]	1200[e]
700 °C	8.29	1.63	1.22[d]	0.56[b]	0.29[b]	0.0107[d]	11.63[c]	76.98[c]	11.39[c]	11.50[c]	2557[d]
900 °C	8.76	1.61	1.78[c]	0.61[b]	0.35[b]	0.0419[c]	12.97[b]	77.66[c]	9.41[c]	34.79[b]	3071[c]
1100 °C	8.27	2.00	2.22[b]	0.79[b]	0.27[ab]	0.0746[b]	13.23[b]	80.84[b]	6.05[d]	56.86[b]	3559[b]
SEM	0.24	0.20	0.06	0.10	0.04	0.0025	0.44	0.97	0.87	8.15	95

[a] Samples were selected to represent the available biological range in lignin concentration of this potential bioenergy feedstock. [b] Means in the same column not sharing a common superscript were different ($P < 0.05$). [c] Means in the same column not sharing a common superscript were different ($P < 0.05$). [d] Means in the same column not sharing a common superscript were different ($P < 0.05$). [e] Means in the same column not sharing a common superscript were different ($P < 0.05$). [f] NCG = $CO_2 + CO + CH_4 + C_2H_6 + C_3H_8 + H_2$. [g] HHV, higher heating value.

Table 4. Equation Statistics for Significant Linear Regressions of in-vitro Net Normalized Gas (NNG) Production and Pyrolysis Product (Thermochemical Conversion) Yields on Klason Lignin Concentration of Alfalfa Stem Samples[a]

product	h or °C	slope	intercept	R^2	fold change
		fermentation			
NNG	24	−1.175	356.96	0.82[d]	1.59↓
NNG	96	−1.177	381.89	0.78[d]	2.42↓
		pyrolysis			
CH_4	500	0.0223	−0.0234	0.49[b]	1.52↑
	700	0.0346	0.6659	0.22[b]	1.19↑
C_3H_8	700	0.0539	−0.5794	0.58[d]	3.69↑
	900	0.0450	−0.3763	0.33[c]	2.32↑
	1100	0.0529	−0.5834	0.55[d]	3.83↑
H_2	1100	−0.005	0.1557	0.23[b]	1.50↓

[a] Fold change refers to the magnitude of change in products predicted from the lowest to the highest Klason lignin concentration. [b,c,d] $P<0.05$, 0.01, 0.001, respectively.

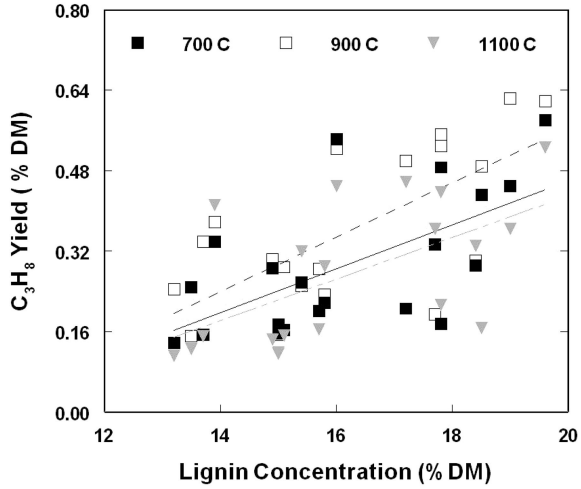

Figure 2. Relationships between alfalfa stem lignin concentration and yield of C_3H_8 by pyrolysis. Only data for pyrolysis temperatures that had significant regressions with lignin content (Table 4) are shown. Lines represent regressions.

H_2:CO ratios, one would have to design a process that includes a shift reaction between H_2O and CO, or by char−CO_2 reactions.

For thermochemical conversion with an emphasis on pyrolysis oil production, 500 °C is the desired process temperature.[28] The results showed no relationship of CG production with alfalfa stem lignin concentration at this temperature. However, we also observed no correlation between lignin content and CG production at the higher temperatures, which was unexpected, as these higher molecular weight compounds are thought to crack to form low molecular weight gases at high temperature. It should be noted that CG was estimated by difference in the study and, therefore, no generalized conclusion can be drawn on the impact of lignin concentration on potential bio-oil production.

Comparison between Conversion Technologies and Lignin Concentration. The predicted impact of lignin concentration on both conversion technologies was tested by regression analysis. For both incubation times in the in-vitro fermentation, significant negative linear regressions were found for in-vitro gas production with Klason lignin concentration (Table 4). With the exception of H_2 production at 1100 °C, the significant pyrolysis regressions (CH_4 at 500 and 700 °C, C_3H_8 at 700, 900, and 1100 °C) had positive relationships between product yield and Klason lignin concentration. Although the absolute slope coefficients for the pyrolysis regression equations were much smaller than for in-vitro gas production, the magnitude of the slope coefficients was driven by the very different actual amounts of these products because the measurement scales differed for the processes (milliliters gas per gram DM for the in-vitro assay vs pyrolysis product as a percent of DM). The predicted fold change in product yields based on these regression equations, using the lowest and highest lignin concentrations in the alfalfa sample set, were of similar relative magnitude for both in-vitro fermentative gas production and pyrolysis (1.59 to 2.42 and 1.19 to 3.83, respectively). However, it should be noted that the proportion of the variation in product yields accounted for by Klason lignin concentration was greater for in-vitro gas production ($R^2 = 0.82$ and 0.78 for 24 and 96 h, respectively) than observed for pyrolysis ($R^2 = 0.22$ to 0.58), suggesting that lignin content per se had less of an effect on thermochemical conversion than on biochemical conversion. Also, the fact that only six of 36 possible product × temperature combinations for the pyrolysis data showed significant regressions with alfalfa stem lignin concentration argues for thermochemical conversion being relatively insensitive to lignocellulosic biomass composition.

Conclusions

Alfalfa stem samples representing the biological range in lignin concentration obtained from a multiyear breeding experiment, encompassing genetic and environmental variation, were subjected to biochemical (in-vitro gas production by mixed rumen microbes) and thermochemical (high-temperature pyrolysis) conversion processes. Lignin concentration was negatively related to in-vitro gas production at both incubation times examined, and accounted for approximately 80% of the variation in in-vitro gas production. In contrast, yields of only three noncondensable gases (CH_4, C_3H_8, and H_2) were related to lignin concentration and these relationships were only observed for a limited set of pyrolysis temperatures. Also, the significant regressions only accounted from 22 to 58% of the variation in yield of these gases. In total these three gases accounted for

(28) Boateng, A. A.; Mullen, C. A.; Goldberg, N.; Hicks, K. B.; Jung, H. G.; Lamb, J. F. S. Production of bio-oil from alfalfa stems by fluidized-bed fast pyrolysis. *J. Ind. Eng. Chem. Res.* **2008**, *47*, 4115.

less than 1.7 wt % of the alfalfa stem feedstock in the primary pyrolysis reactions. Within the available biological range in lignin concentration, biochemical conversion was strongly and negatively affected by lignin content of alfalfa stems but lignin had limited impact on thermochemical conversion. Ultimately the utility of controlling lignin content of alfalfa stems, and presumably other biomass feedstocks, for thermochemical conversion will be extremely dependent on the process and the desired products. The yield and efficiency of thermochemical processes may thus be relatively independent of lignin concentration. This is a desirable feature for commercial biorefineries geared to handle a variety of different biomass feedstocks. Moreover, the relative lack of effect of lignin concentration on pyrolysis yields may eliminate the need for genetic modification of feedstock crops or management of biomass production for composition, which can be in conflict with maximizing biomass yield.

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