Identification and Quantitation of Organic Degradation Products in Dilute-Acid-Catalyzed Corn Stover Pretreatment Hydrolysates

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A variety of degradation products are produced upon pretreatment of lignocellulosic biomass with dilute acid. To date, the complexity of these samples has significantly limited the scope of efforts to perform summative analyses of degradation products. Qualitative and quantitative interrogation of hydrolysates is also paramount to identifying potential correlations between pretreatment chemistry and microbial inhibition in downstream bioconversion processes.

Chromatographic techniques have been used in combination with mass spectrometry (*i.e.*, HPLC-MS/MS and GC/MS) to qualitatively identify dozens of organic degradation products (*e.g.*, organic acids, phenols, aldehydes, etc.) in corn stover pretreatment hydrolysates. Additionally, a developing suite of analytical methodologies based on chromatographic separation of analytes with ultraviolet and MS detection modes has been applied to perform quantitative assessments of a variety of hydrolysate components as a function of pretreatment time, temperature, and pH. Correlations of product concentrations to the pretreatment severity function indicate differing responses of various compounds to the kinetic influences of temperature and reaction time.

Keywords: corn stover, severity function, organic acids, aliphatic acids, aromatic acids, aldehydes, phenols, biomass hydrolysates, pretreatment degradation products, HPLC analysis

1. Introduction

With the projected depletion of the world's petroleum reserves, there is escalating pressure to develop alternative, non-petroleum-based sources of energy [1-2]. Among energy alternatives, biomass-derived ethanol represents one of the more promising commodities for long-term sustainability of transportation fuels [3-6]. Currently, the most well-studied and near-commercial technology for conversion of biomass to ethanol involves dilute acid-catalyzed pretreatment of lignocellulosic feedstocks, followed by enzymatic hydrolysis of cellulose and fermentation of monomeric sugars to produce ethanol [7-10]. However, the pretreatment product mixture, commonly referred to as hydrolysate, contains not only cellulose and fermentable sugars, but also a wide variety of degradation products such as aliphatic and aromatic acids, phenols, and aromatic aldehydes. Many of these degradation products exert an inhibitory effect on downstream microbial processes [11-14], thus reducing the overall efficiency for bioconversion of lignocellulosics to ethanol. As a result, there is increasing impetus to develop reliable quantitative analyses for individual degradation products in order to advance a more fundamental understanding of lignocellulose pretreatment as well as subsequent microbial inhibition processes.

Generous effort has been extended towards analysis of degradation products in biomass hydrolysates, with varying degrees of success. Although gas chromatography coupled with flame ionization or mass spectrometry detection has been quite successful in identifying a variety of organic degradation products in lignocellulosic biomass [15-25], implementation of GC methodologies for quantitative work have suffered from inherent complexitites of derivatizing samples of unknown composition. Liquid-chromatography (LC) methods, employing post-column UV or refractive index detection, have historically suffered from incomplete resolution of analytes. As a result, LC analyses of degradation products in hydrolysate samples have typically employed multiple chromatographic modes and detection strategies, the choice of which depends on analyte class. For example, aliphatic acids have been determined using high performance anion-exchange chromatography with UV [24-25] or conductivity detection [25-27], ion-exclusion chromatography with UV detection [28], or electrophoretic methods [27, 29]. In contrast, LC analyses of aromatic acids, furans, phenolic compounds, and aldehydes have typically been accomplished using reversed-phase chromatography with refractive index [16, 30], UV [20, 23, 25, 26, 31] or mass spectrometry [26] detection.

In a recent paper, the authors reported the first example where aliphatic acid, aromatic acid, aldehyde and phenolic degradation products are determined simultaneously in a biomass pretreatment hydrolysate using liquid chromatography[43]. The relatively simple analytical procedure used requires an initial precipitation-filtration step, followed by liquid-liquid extraction and subsequent reversed-phase HPLC analysis with UV detection at 210 nm. Independent of sample type, the HPLC-UV method reported represents one of very few examples where 32 compounds: aliphatic acids, multifunctional-group aromatic acids, and phenolic compounds, are simultaneously separated and quantitated in a single chromatographic run.

There have been several reports seeking to correlate pretreatment severity or combined severity (CS) to fermentability of pretreated hydrolysates. Tengborg et al. [46] found that sulfuric acid pretreatment of sprucewood gave optimal sugars near CS 3.0 but that fermentability declined at this combined severity. Larsson et al. [44] conducted an extensive study of dilute acid hydrolysis of sprucewood at 76 different conditions, over a combined

severity range of 1.4 to 5.4. Their study looked at concentrations of glucose, mannose, xylose, furfural, 5-HMF, and acetic, formic and levulinic acids and the fermentability of the hydrolysates by *Saccharomyces cerevisiae.* Their results showed optimal sugar accumulations between combined severities (CS) of 2.0 and 3.4, maximum concentrations of furans in the vicinity of CS 3.2 to 3.6 and increasing acid concentrations with increasing CS. Fermentability, as measured by ethanol yield and productivity, decreased with increasing CS, with the greatest decreases occurring at approximately CS 3.

Bouchard et al. [45] presented an analysis that characterized the general chemical properties of the pretreatment products, without identifying the individual compounds. Results were presented characterizing qualities such as molecular weight distribution, abundance of O-acetyl groups, or the relative distribution of chemical bond types as determined by FTIR. Decomposition kinetics of xylose, galactose, mannose, glucose, 2-furfural, and 5-hydoxymethyl-2-furfural have been investigated over varying severities toward the end of enhancing methane fermentation [47,48]. Degrees of deacetylation of lignocellulose has been shown to correlate well to the severity factor [49].

The object of this study is to apply newly developed analytical techniques to the identification and quantification of a wide variety of biomass hydrolysis byproducts and to imcrease understanding of the kinetic factors contributing to the accumulation of these degradation products.

2. Materials and Methods

2.1 Chemicals and reagents

The solvents and reagents acetonitrile = MeCN (HPLC far UV grade, Acros, Fair Lawn, NJ), methyl *tertiary*-butyl ether = MTBE (EM Science, Gibbstown, NJ), methanol (Sigma-Aldrich, St. Louis, MO), methylene chloride (Sigma-Aldrich, St. Louis, MO), sulfuric acid (J. T. Baker, Philipsburg, NJ), phosphoric acid (J. T. Baker, Philipsburg, NJ), and ammonium bicarbonate (EM Science, Gibbstown, NJ) were reagent grade or better and used as received. The internal standard, *para*-*tert*-butylphenoxyacetic acid (Alfa Aesar, Ward Hill, MA) and 32 reference standards (Sigma-Aldrich St. Louis, MO): formic acid, malic acid, lactic acid, acetic acid, maleic acid, succinic acid, methylmalonic acid, fumaric acid, propanoic acid, levulinic acid, itaconic acid, gallic acid, 5-hydroxy-methylfurfural, 2-furoic acid, furfural, adipic acid, 3,4-dihydroxybenzoic acid, 3,5-dihydroxybenzoic acid, 3,4-dihydroxybenzaldehyde, 4-hydroxybenzoic acid, phenol, 4-hydroxybenzaldehyde, vanillic acid, syringic acid, vanillin, benzoic acid, syringaldehyde, 4-hydroxy-3-methoxycinnamic acid, 3-hydroxy-4-methoxycinnamic acid, 4-hydroxycoumarin, *ortho*-toluic acid and *para*-toluic acid were purchased in the highest available purity and used as received. Corn stover and its compositional analysis (see table 1) was kindly supplied by Mark Ruth at the National Renewable Energy Laboratory in Golden, CO. Distilled water was purified and deionized to 18 MΩ with a Barnstead Nanopure Diamond UV water purification system.

Table 1 Compositional analysis of Corn Stover used in this work.

Component	Mass fraction
Cellulose	0.371
Xylan	0.192
Arabinan	0.025
Galactan	0.016
Mannan	0.013
Lignin	0.207
Ash	0.052
Protein	0.038
Extractives	0.026
Acetate	0.024
Unknown	0.036

2.2 High performance liquid chromatography analysis

All HPLC analyses were carried out using a Dionex® DX-600 series liquid chromatograph (Dionex Corp., Sunnyvale, CA) as reported by Chen et al. [ref] The HPLC system consisted of an AS50 autoinjector, DG2410 degassing module, GS50 gradient pump, LC30 chromatography oven and UVD170U multiwavelength ultraviolet detector. Chromatographic separation was achieved using a 150 mm x 4.6 mm YMC™ Carotenoid S-3 column (Waters Corporation, Milford, MA). This is a C30 reversed-phase column, withstanding mobile phase compositions up to 100% water. An RP 18 Opti-Guard® column (Alltech Associates, Deerfield, IL) was employed to protect the analytical column. Gradient separations were carried out using aqueous 0.05% (v/v) phosphoric acid (pH 2.2-2.3) and water-acetonitrile (10:90) as the A and B solvents, respectively. Additional parameters employed in HPLC analyses were as follows: injection volume, 25 µl; column temperature, 30 ºC; flow rate, 1 ml/min.

Quantitation of target analytes was accomplished using a multipoint internal standard calibration curve. Response factors at 210 nm were determined for each analyte by dividing the peak area of the analyte by the peak area of the internal standard, and calibration curves were constructed by plotting a linear regression of the average response factor (n = 5) versus the analyte concentration for all calibration standards analyzed. Calibration curves were then used to determine analyte concentrations in all reference and hydrolysate samples. Acceptability criteria for identification of individual components using the HPLC method required that the retention time for a given analyte be within ± 2% of the average retention time for each respective standard used to construct the calibration curve for that analyte.

Identification of degradation products in hydrolysate samples was accomplished by comparing UV absorbance and retention time data with reference standards. Although 210 nm was used exclusively as the detection wavelength for quantitative purposes, UV absorbance was monitored at four wavelengths (210 nm, 254 nm, 275 nm, and 300 nm) during each chromatographic run. Absorbance ratios (e.g., A_{254}/A_{210}, A_{275}/A_{210}, etc.) were calculated as a function of retention time for both reference and hydrolysate samples and employed as spectroscopic metrics for analyte identification. Confirmation of component identity was achieved by spiking hydrolysate samples with a sufficient amount of a reference mixture to exactly double analyte concentration in a subsequent HPLC analysis. Figures 1 A and B illustrate chromatograms representing a prepared standard and hydrolysate samples, repectively.

2.3 Preparation of standards

Forty reference compounds reflecting a wide range of potential analytes were selected based on previous reports of hydrolysate composition [11, 15-31]. Reference standards and calibrators were prepared from the group of purchased reference standards using water as the diluent. All solutions were prepared in sufficient quantity to provide replicate analyses for each individual study and stored at 4 °C. The internal standard solution of *para-tert*-butylphenoxyacetic acid was prepared at a concentration of 2.5 mM in methanol.

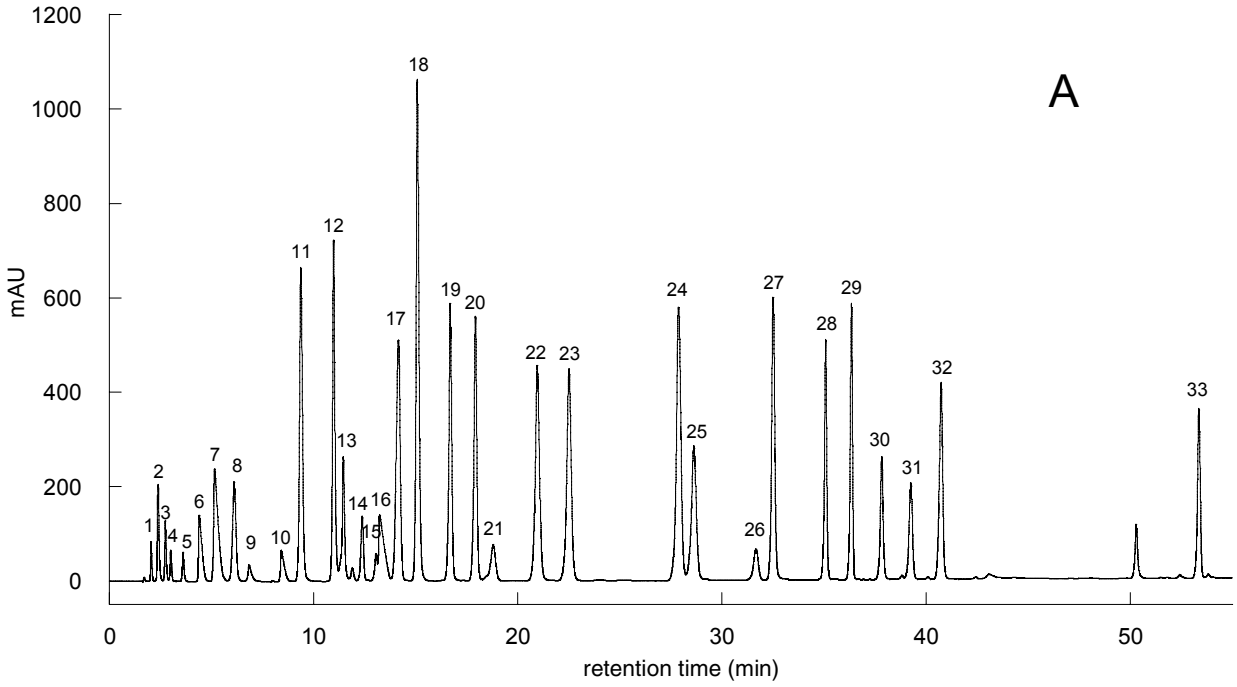
2.4 Hydrolysate samples

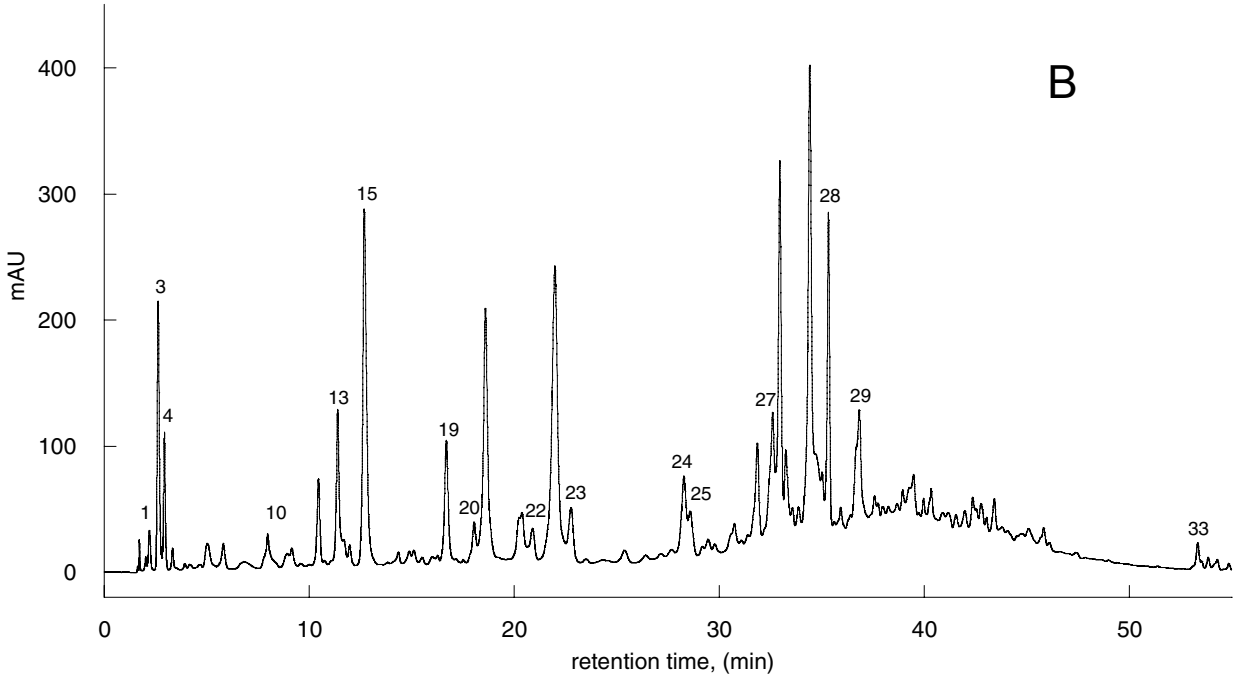
The corn stover hydrolysate analyzed in this work was generated using a previously reported procedure [12]. Briefly, corn stover was inserted into a 150 mL 316 stainless steel pressure vessel (Swagelok) with either water or dilute sulfuric acid Temperature control was achieved by pre-heating the reactor for 3 minutes in a sand bath at a temperature 40ºC higher than the intended reaction temperature. The reactor was then immediately transferred to a separate sand bath at the intended reaction temperature for the desired duration of reaction. Quenching was accomplished by immersing the reactor in an ice bath. Hydrolysis was carried out at three levels of reaction severity, as defined by Overend and Chornet [50]:

$$Severity = Ro = te^{\left(\frac{T-100}{14.75}\right)}$$

where t expresses the reaction duration in minutes and T the temperature in degrees celcius. Table 2 lists the experimental conditions tested in this study and the resulting

Figures 1A, 1B. Chromatograms generated by HPLC analysis of a prepared standard and hydrolysate samples, respectively.





values of log(Ro). Figure 2 illustrates the experimental space as a function of time and temperature.

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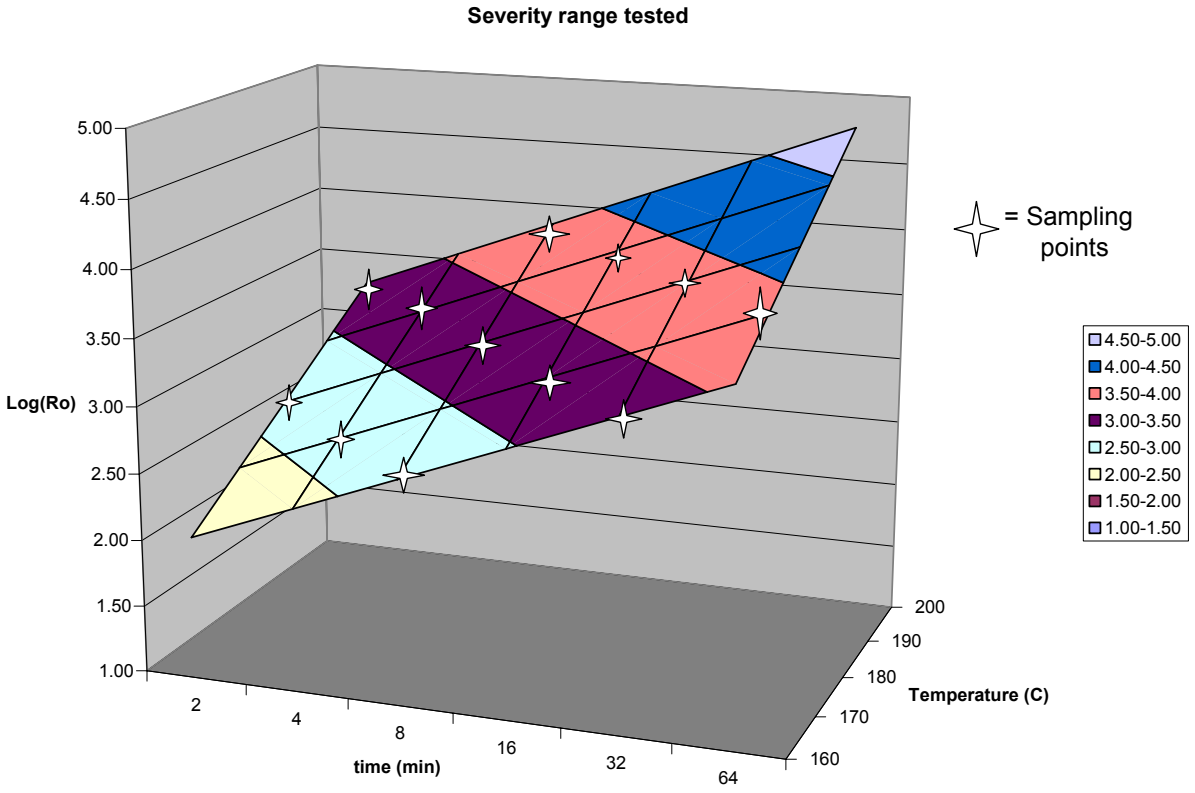
Table 2: Experimental Conditions and Calculated log(Ro) for Hydrolysis Experiments

Temperature / Time log(Ro)	160°C	170°C	180°C	190°C	200°C
2 minutes			**2.66**		**3.25**
4 minutes		**2.66**		**3.25**	
8 minutes	**2.67**		**3.26**		**3.85**
16 minutes		**3.27**		**3.85**	
32 minutes	**3.27**		**3.86**		
64 minutes		**3.87**			

Figure 2: Experimental space of log(Ro) conditions tested



Most reactions were carried out in the presence of 0.7% (w/v) sulfuric acid with initial corn stover solids at 10 g/L. At the center point of the severity experimental design (180°C, 8 minutes) additional experiments were carried out investigating higher and lower solids concentrations (1 and 100 g/L) and higher and lower acid concentrations (0% and 1.84% (w/v) sulfuric acid). After reaction, particulates were removed by filtration using Whatman glass-microfiber membrane filters (90 mm diameter; 0.45-•m pore size; VWR Scientific, Suwanee, GA), and samples were stored at 4 °C until processed for HPLC analysis.

2.5 Sample preparation and extraction procedures

All reference samples, hydrolysate samples and calibration standards were prepared and extracted using the following procedure. Approximately 30 mL of each sample was initially treated with 1.5-2.0 g solid ammonium bicarbonate to adjust the pH to 7-8, and the resulting solution was stored at 4 °C for 30 minutes. Samples were subsequently filtered using Pall IC Acrodisc® hydrophilic polyethersulfone Supor® membrane syringe filters (25 mm diameter; 0.45-•m pore size; VWR Scientific, Suwanee, GA). The pH of the filtrate was adjusted to 1-2 by dropwise addition of concentrated sulfuric acid. Five-milliliter aliquots of the filtrate were subsequently transferred to 50 mL centrifuge tubes, and 50 •L of the methanolic internal standard mixture (26 •g) was added prior to extraction. Samples were contacted two times with 45 mL portions of MTBE on a rotating wheel at 25.0 ± 0.1 °C for 15 minutes. Following each extraction, samples were centrifuged at 4500 rpm for 3 minutes to ensure complete phase disengagement. The volume of the combined MTBE extracts was reduced to 1-2 mL under a stream of N_2 at 55 °C, using a Zymark® Turbovap LC™ concentration workstation (Zymark Corp., Hopkinton, MA). At this point, 1.50 mL of 18 MΩ water was added to the MTBE mixture, and the remaining MTBE was evaporated under a stream of N_2 at 55 °C. The resulting aqueous mixture was quantitatively transferred to a volumetric flask and diluted to exactly 5 mL with water. Aliquots of each sample (1.5 mL) were then transferred to 2 mL autosampler vials prior to HPLC analysis.

2.5 Extraction efficiency

The efficiency of analyte extraction using MTBE was determined using a modified literature procedure [33]. Two groups of controls were prepared in purified water and extracted using the sample preparation procedure described above. The first group was spiked with a precisely known concentration of each analyte and internal standard prior to extraction, while the second group was spiked with the internal standard only. Following extraction, the same precisely known concentration of each analyte added to samples in group one was added to samples from the second control group. Both groups were analyzed by HPLC, and the ratio of the response factors obtained for samples from control groups one and two were used to calculate the recovery for each analyte:

$$\%\,\text{recovery} = \frac{A_{X1}/A_{IS1}}{A_{X2}/A_{IS2}} \times 100\%$$

where A_{X1}, A_{IS1}, A_{X2} and A_{IS2} represent the peak areas for the analyte (X) and internal standard (IS) in groups one and two, respectively. Reported recoveries for the 32 reference compounds represent the average of three replicate determinations and raged from 20 to 90%, with an average recovery of 72% for all of the analytes.[43]

To verify the shelf life of the analysed samples, a hydrolysate sample was analyzed repeatedly over a five-day period and found to have no statistical change in analyte concentrations. Thus, the samples are generally presumed to be stable over this timeframe when stored at 4 °C. It is also noteworthy that the precision observed for five replicate analyses of this hydrolysate was not statistically different than that observed for the analysis of reference standards constituted in water despite a significantly more complex background.

3. Results

3.1 Identification and quantitation of products

40 compounds were identified of which 38 were quantified with some degree of certainty. Of these 38 compounds, 20 were quantified with a high degree of confidence. The data presented in the following sections reports on these 20 compounds. Table 3 lists the compounds identified and confidently quantified.

3.2 Effect of reaction severity on product accumulation

Temperature and reaction time were varied to result in 12 different reaction conditions representing three levels of reaction severity log(Ro) = 2.66, 3.26 and 3.86. It was observed that for most of the compounds measured, the traditional severity function did not provide a monotonic correlation to the accumulated concentrations of reaction products. For example, figures 3 and 4 illustrate the accumulation of various products (at two different concentration ranges) at a constant reaction severity of log(Ro) = 3.26. Since the reaction severity in these plots is constant over all conditions, it would normally be expected that product concentrations would also be constant. Clearly this is not the case, as the concentration at different temperatures is increasing for most products and is stable for relatively few. The implication of the data presented in figures 3 and 4 is that the effect of temperature on the accumulation of pretreatment byproducts is inadequately accounted for in the classic severity function.

In order to develop a reaction coordinate combining temperature and reaction duration that can represent product accumulation in a monotonic fashion, the severity function was modified by varying the value of the denominator in the exponent. Figure 5 presents data on the accumulation of formic acid versus different calculations of the severity function. It can be seen that an exponent denominator of 14.75, which is the value commonly used for analysis of biomass pretreatment, offers virtually no discrimination between different reaction conditions. To increase the relative contribution of temperature to the reaction coordinate, the exponent denominator in the severity function was altered. In the case of formic acid, an exponent denominator on the order of 10 gives a satisfactory monotonic response of formic acid concentration to reaction severity. Figure 6 illustrates a contrary example where the conventional severity function overcompensates for the effect of temperature. In this case, increasing the exponent denominator from 14.75 to 25 yields a satisfactory monotonic relation between concentration and calculated severity. Parallel analyses with the other measured products resulted in identification of different exponent denominators suitable for most compounds. Most compounds gave improved correlation to severity with lower denominators. 4-coumaric acid and Furulic acid were the only components to improve correlations with higher denominators. A monotonic correlation was not achieved for lactic acid. Table 3 lists the exponent denominator values found to be most effective at providing monotonic response of concentration to reaction severity. Based on this analysis, it appears that the kinetics generating compounds with low denominator values are more influenced by temperature changes while compounds with higher values are more influenced by reaction duration. Further analysis of these results could lead to kinetic insights into the production and accumulation of these minor products.

Table 3: Identified and quantified compounds reported in this study.

Compound identified	*Compound well quantified*	*Average of measured concentrations (mmol)*	*Monotonic response exponent denominator*
1. Formic Acid	yes	*5.8*	*10*
2. Malonic acid		*na*	
3. Lactic Acid	Yes	*18.2*	*Na*
4. Acetic Acid	Yes	*25.2*	*10*
5. Maleic Acid	Yes	*0.41*	*5*
6. Succinic Acid		*0.94*	
7. Methylmalonic Acid		*na*	
8. Fumaric Acid	Yes	*2.1*	*10*
9. Propanoic Acid	yes	*11.6*	*6*
10. Levulinic Acid	yes	*2.1*	*11*
11. Galutaric acid		*0.48*	
12. Itconic Acid		*0.034*	
13. 2Hydroxy2Methybutyric acid		*0.20*	
14. Gallic Acid		*0.097*	
15. 5HO-methylfurfural	yes	*1.8*	*7*
16. 2-Furoic Acid		*0.049*	
17. Adipic Acid		*0.48*	
18. Furfural	yes	*16.9*	*7*
19. 3,4Dihydroxybenzoic Acid	yes	*0.0034*	*6*
20. 3,5Dihydroxybenzoic Acid		*0.00026*	
3,4dihydroxybenzaldehyde		*0.032*	
22. 4-Hydroxybenzoic Acid	yes	*0.0074*	*7*
23. 2,5Didydroxybenzoic acid		*0.013*	
24. Phenol		*0.045*	
25. 4-Hydroxybenzaldehyde	yes	*0.13*	*5*
26. Vanillic Acid	yes	*0.024*	*6*
27. Homovanillic acid		*0.0034*	
28. Caffeic acid	yes	*0.020*	*8*
29. Syringic Acid	yes	*0.012*	*6*
30. 4Hydroxylaceophenone		*0.023*	
31. Vanillin	yes	*0.046*	*6*
32. 4OH-Coumaric acid	yes	*0.068*	*20*
33. Syingaldehyde	yes	*0.033*	*6*
34. Benzoic acid		*0.055*	
35. Furulic acid	yes	*0.034*	*25*
36. Sinapic acid		*0.017*	
3HO,4Methoxycinnamic Acid	yes	*0.036*	*8*
38. Salicylic acid		*0.0058*	
39. 4HO-Coumarin		*0.0028*	
40. o-toluic acid		*0.034*	

Figure 3: Accumulation of high concentration reaction products vs temperature at constant log(Ro) = 3.26.

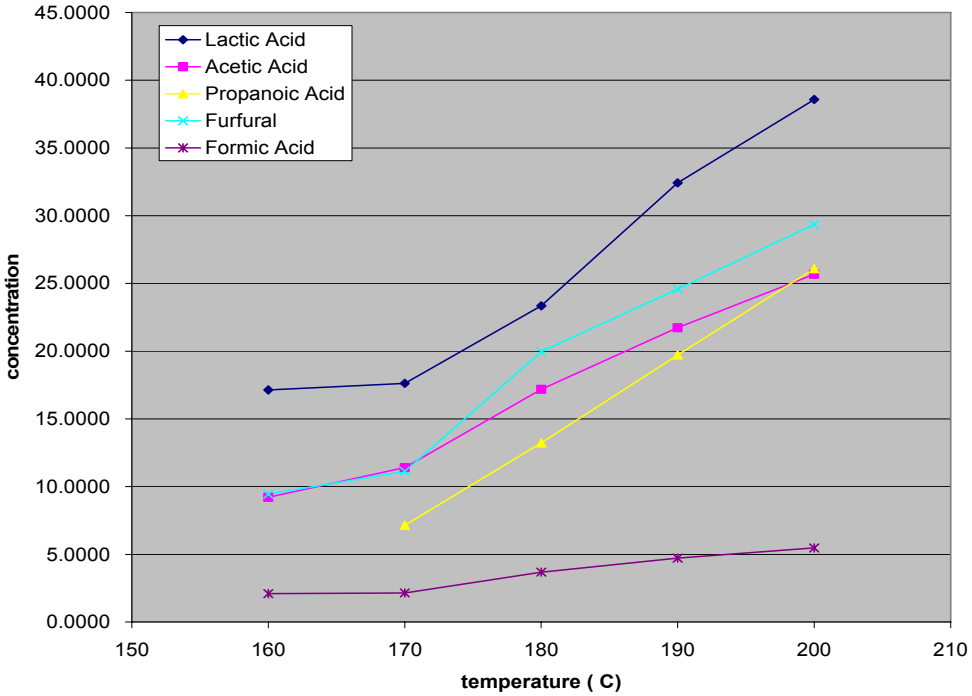


Figure 4: Accumulation of low concentration reaction products vs temperature at constant log(Ro) = 3.26.

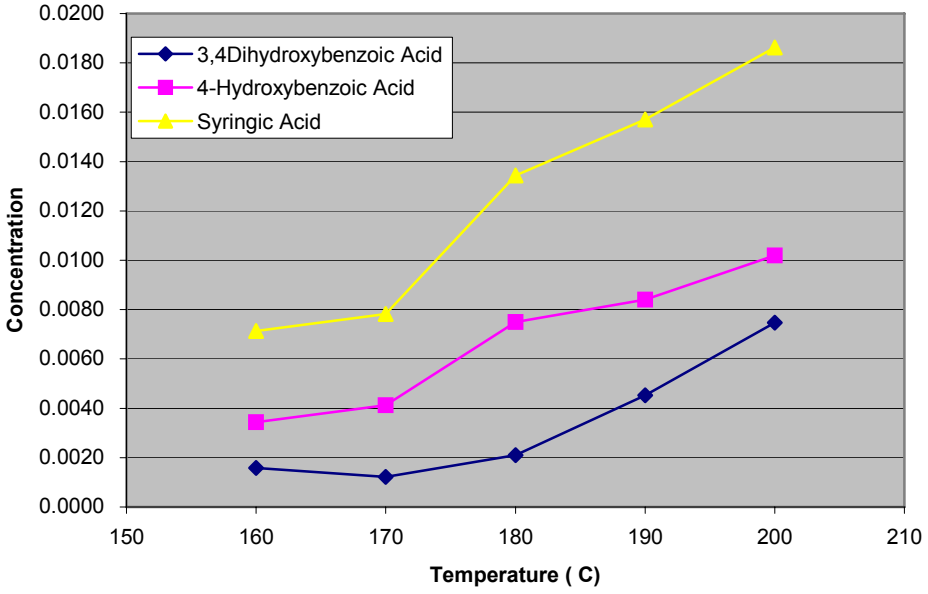


Figure 5: Concentrations of Formic acid vs Reaction Severity calculated with various exponent denominators

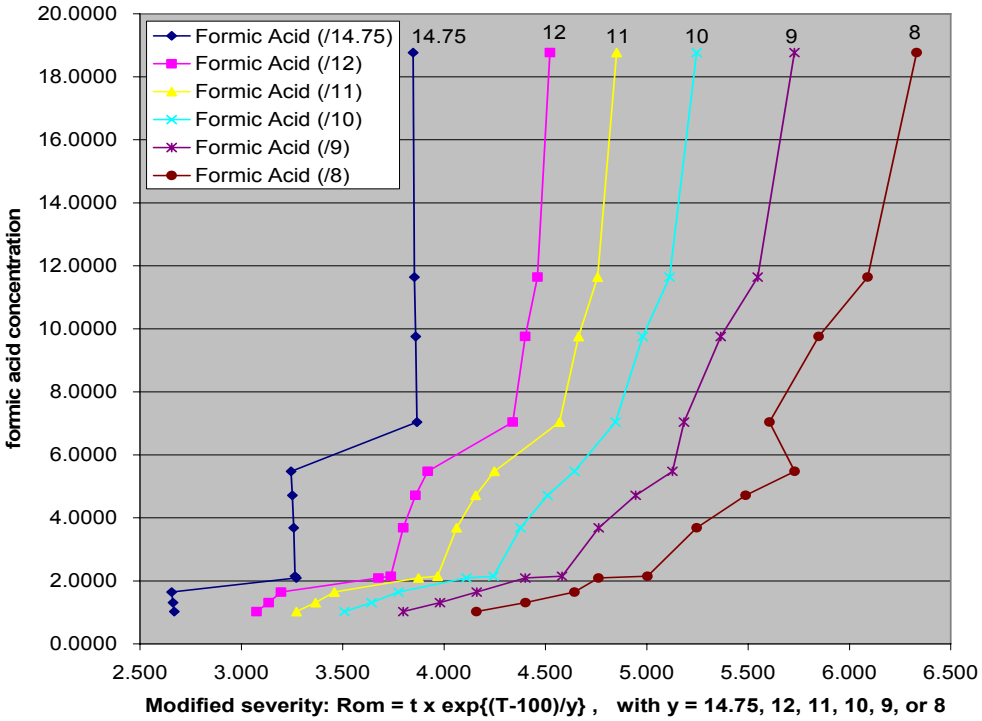
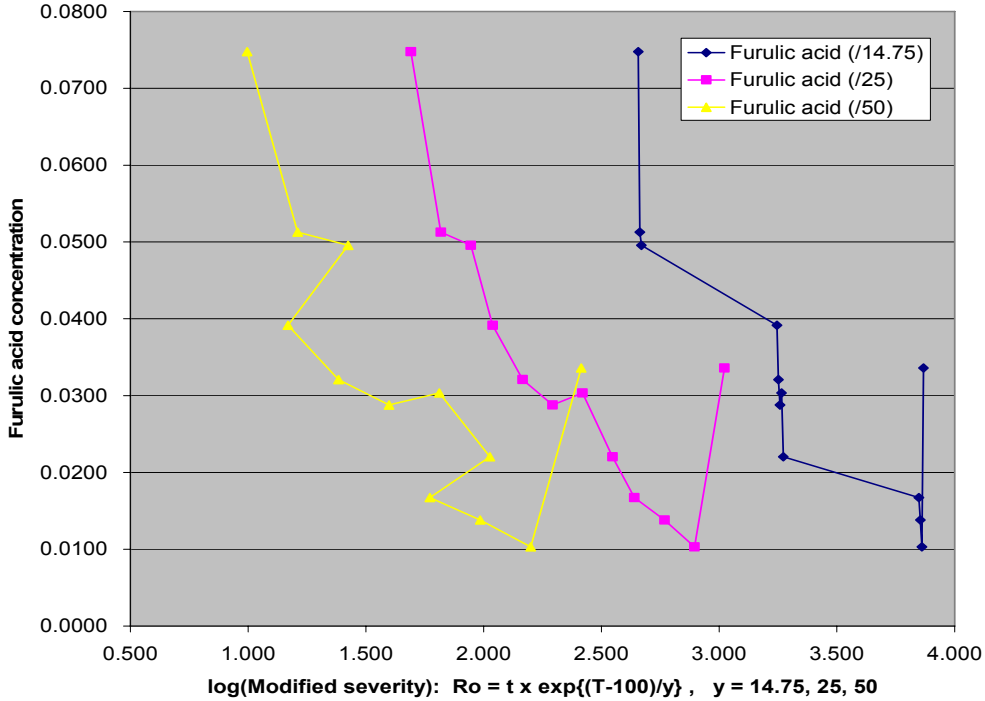


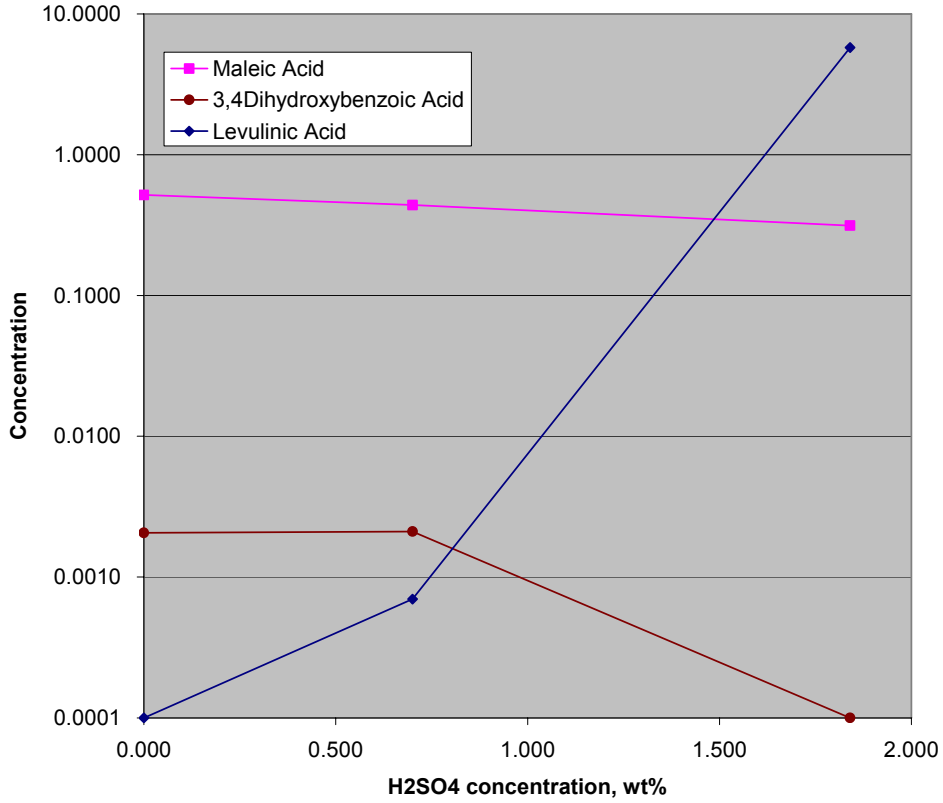
Figure 6: Concentrations of Furulic acid vs Reaction Severity calculated with various exponent denominators.



3.3 Effect of acid concentration on product accumulation

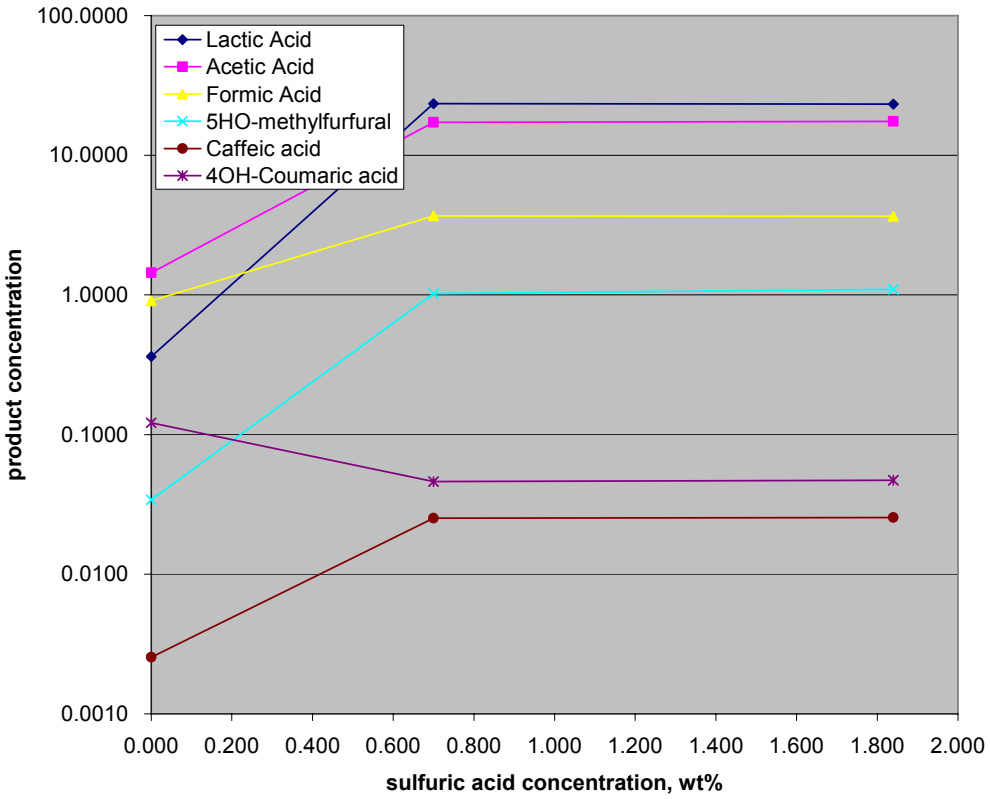
Most experiments carried out in this study were done with 0.7 wt% sulfuric acid. In order to test the affects of different acid concentrations, additional experiments with either 0% or 1.84% sulfuric acid were carried out at the central severity condition of 180°C and 8 minute reaction time. Responses of product concentrations have been grouped into three types. The first type is products that are either positively or negatively affected by the presence of acid. These responses appear to be acid limited, i.e. more acid appears to accelerate the trend. Figure 7 illustrates the decreasing response of maleic acid, and 3,4Dihydroxybenzoic acid to increasing acid concentration, as well as a strong positive response of levulinic acid to increasing sulfuric acid.

Figure 7 Response of product concentrations to varying sulfuric acid concentration: 180°C, 8 minutes, 10 g/L solids. Increasing or decreasing product concentrations



The second type of response shows an increase in concentration in response to acid, but no further increase with additional acid. Thus, this appears to represent reactions in which the presence of acid initiates an accumulation response but this response is not limited by the acid concentration—i.e. additional acid has no additional affect. Figure 8 illustrates increasing trends for lactic, acetic, formic and caffeic acids and 5hydroxy-methylfurfural as well as a decreasing tend for 4-coumaric acid.

Figure 8 Response of product concentrations to varying sulfuric acid concentration: 180°C, 8 minutes, 10g/L solids. Flattening response to acid concentrations



The third type of response consists of initial accumulation of product with decreasing concentration at higher acid levels. This response appear to indicate both productive and destructive action of acids on the products measured. Figure 9 shows this trend applies for the majority of the compounds quantified in this study.

3.4 Effect of solids concentration on product accumulation

Most experiments carried out in this study were done with 10 g/L corn stover in 0.7% sulfuric acid. In order to test the affects of different solids concentrations, additional experiments with either 1g/L or 100 g/L corn stover carried out at the central severity condition of 180°C and 8 minute reaction time. Responses of product concentrations have been grouped into two types. The first type of response was product accumulations that appear to have increased linearly with initial solids feed concentration. The seven products that appear to have this linear response include: fumaric acid, 4-hydroxybezaldehyde, syringaldehyde, 3,4 dihydroxybenzoic acid, 4-hydroxybenzoic acid, levulinic acid and vanillin. Figure 10 illustrates the accumulation trend for these compounds.

Figure 9: Response of product concentrations to varying sulfuric acid concentration: 180°C, 8 minutes, 10g/L solids. Productive and destructive response to acid

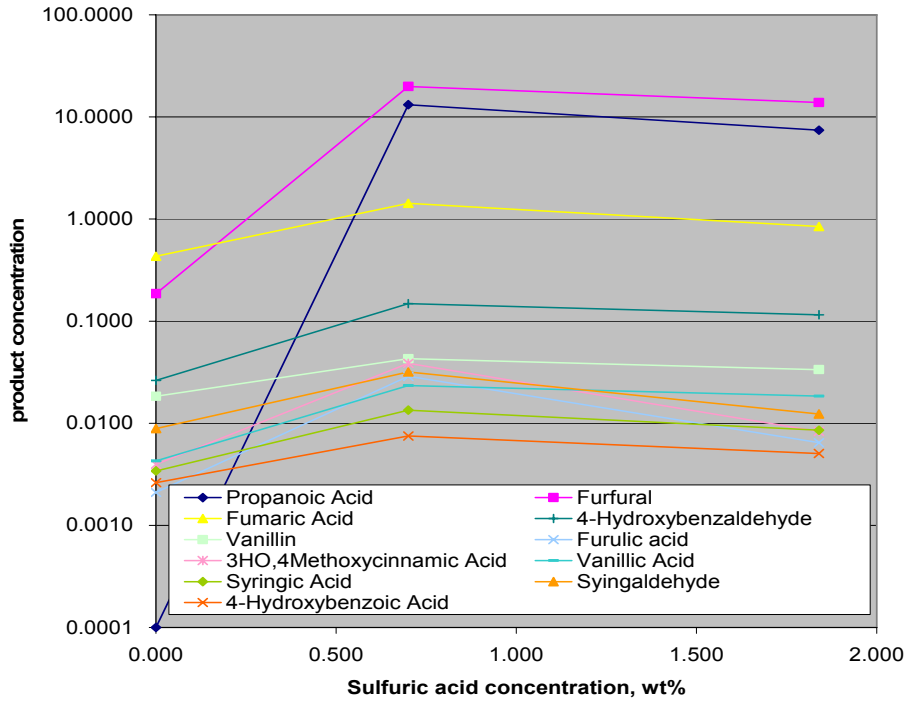
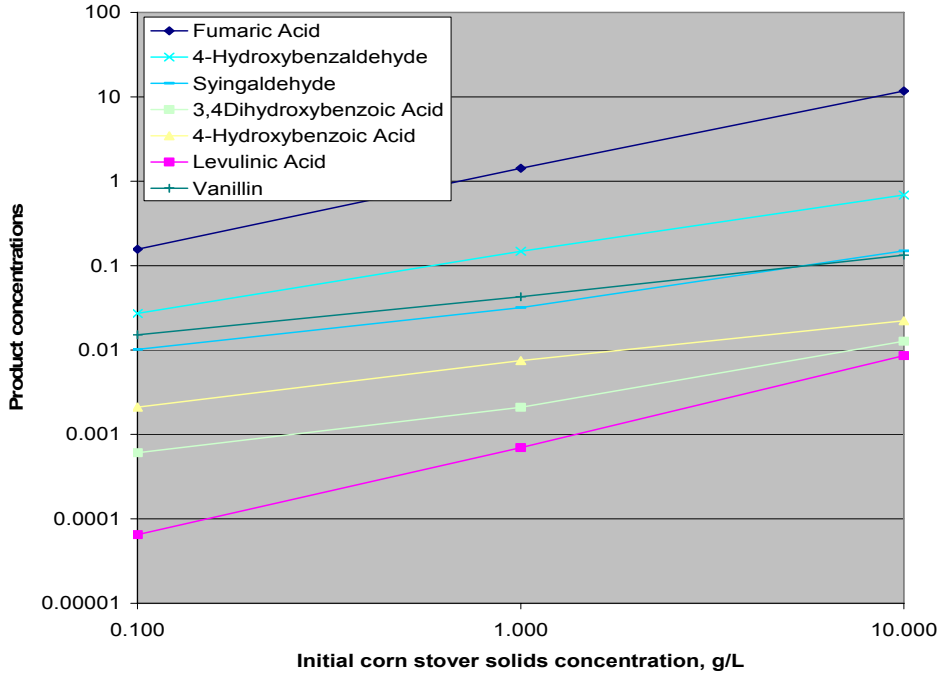


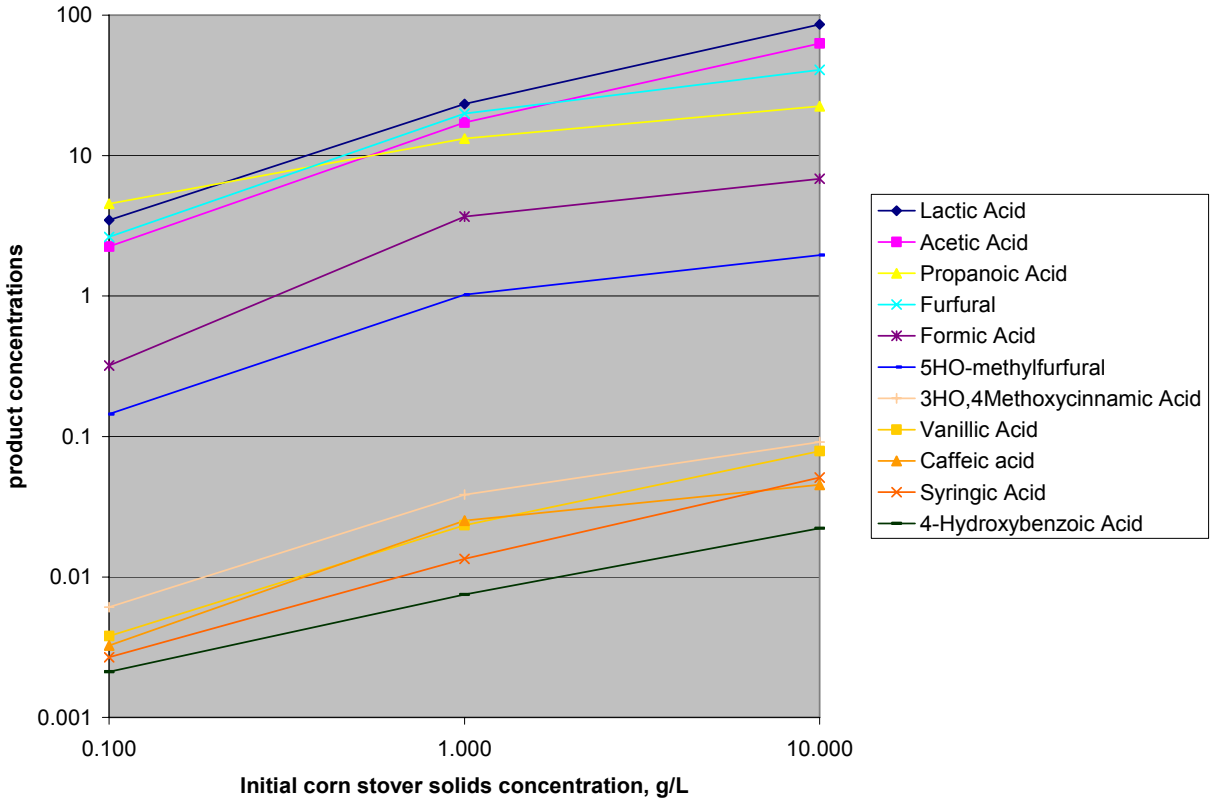
Figure 10: Response of product concentrations to varying initial corn stover solids concentration: 180°C, 8 minutes, , 0.7wt% H_2SO_4. Linear or increasing response to solids



The second type of response was product accumulations that increase, but not linearly, with initial solids feed concentration. The remainder of the measured compounds fall into this category. This result is most likely the outcome of mass transfer limitations at the higher solids

loading. Why some compounds are affected by such limitations and others are not is an interesting starting point for more detailed investigation. Figure 11 illustrates the accumulation trend for these compounds.

Figure 11: Response of product concentrations to varying initial corn stover solids concentration: 180°C, 8 minutes, 0.7wt% H_2SO_4. Diminishing response to increased solids.



4. Conclusions

A relatively simple analytical protocol for the determination of potentially inhibitory degradation products derived from lignocellulosic biomass has been applied to the study of acid pretreatment of cornstover. The use of liquid chromatography in combination with UV detection at 210 nm should render the method available to a wide variety of users and attract the attention of other researchers investigating biomass pretreatment and microbial inhibition processes. Improved and simplified analytical procedures such as the method reported here will enable more comprehensive analysis of other chemical conversions taking place during the thermochemical pretreatment processes.

The severity function as it is commonly described functioned poorly as a means of discriminating between different reaction conditions making use of variable combinations of temperature and reaction time. It was found that most of the products measured demonstrated kinetics that indicated a greater influence of temperature on their rate of accumulation than is

predicted by the widely used severity function. It was found that manipulation of the Temperature contribution to the severity function could result in a monotonic response of product accumulation to reaction severity, but that this required manipulation was different for different compounds. Thus, there appears to be no one severity function that can describe a universal effect on accumulation trends for these various products.

Acid concentration was found to affect the accumulation trends of different compounds in various ways, with some reactions appearing to be acid limited and others not. Solids concentration was seen to have a universally positive influence on product accumulations, with some products responding linearly to increased solids concentration, but most demonstrating reduced kinetics at higher concentrations, presumably due to mass transfer limitations.

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