Effect of Dissolved Carbon Dioxide on Accumulation of Organic Acids in Liquid Hot Water Pretreated Biomass Hydrolyzates

G. Peter Van Walsum,*,[1] Maurilio Garcia-Gil,[1] Shou-Feng Chen,[2] and Kevin Chambliss[2]

[1]Department of Environmental Studies; and [2]Department of Chemistry and Biochemistry, One Bear Place No. 97266, Baylor University, Waco, Texas, 76798-7266, E-mail: GPeter_Van_Walsum@Baylor.edu

Abstract

Liquid hot water pretreatment has been proposed as a possible means of improving rates of enzymatic hydrolysis of biomass while maintaining low levels of inhibitory compounds. Supplementation of liquid hot water pretreatment with dissolved carbon dioxide, yielding carbonic acid, has been shown to improve hydrolysis of some biomass substrates compared with the use of water alone. Previous studies on the application of carbonic acid to biomass pretreatment have noted a higher pH of hydrolyzates treated with carbonic acid as compared with the samples prepared with water alone. This study has applied recently developed analytical methods to quantify the concentration of organic acids in liquid hot water pretreated hydrolyzates, prepared with and without the addition of carbonic acid. It was observed that the addition of carbon dioxide to liquid hot water pretreatment significantly changed the accumulated concentrations of most measured compounds. However, the measured differences in product concentrations resulting from addition of carbonic acid did not account for the measured differences in hydrolyzate pH.

Index Entries: Analysis; aspen wood; carbonic acid; corn stover; pretreatment; organic acids.

Introduction

Conversion of lignocellulosic material to ethanol requires hydrolysis of carbohydrate polymers to their constituent sugars. Enzymatic hydrolysis is a common approach to hydrolysis and offers the benefits of mild reaction conditions and selective hydrolysis. To achieve useful rates of enzymatic hydrolysis, the lignocellulose must first be pretreated to reduce the recalcitrance of the substance to hydrolysis. Pretreatment accomplishes many alterations of the biomass. Depending on the technology

---

*Author to whom all correspondence and reprint requests should be addressed.

chosen, these effects typically include, to varying degrees: hydrolysis of the hemicellulose, solubilization of lignin and carbohydrate monomers and oligomers, and increased accessibility of the cellulose to cellulase enzymes *(1)*. Several pretreatment methods have been explored. Among the more commonly reported technologies are water-based methods such as steam explosion or liquid hot water, and dilute-acid pretreatments, in which mineral acids such as sulfur dioxide or sulfuric acid are used at low concentrations (on the order of 1%) and at temperatures usually below 200°C *(2)*.

Water-based and dilute acid pretreatments have undergone research and development for many years. Much of this research has been devoted to fuel production from biomass. Dilute-acid pretreatment offers good performance in terms of recovering hemicellulosic sugars, but the drawback is its use of sulfuric acid. Sulfuric acid is highly corrosive and its neutralization results in copious production of solid wastes, which can be costly to dispose of. The calcium sulfate resulting from neutralization has problematic solubility characteristics in that it becomes less soluble at higher temperatures, such as those encountered in a reboiler *(3)*.

One process that offers some benefits of acid catalysis without the drawbacks of sulfuric acid is the use of carbonic acid. The pH of carbonic acid is determined by the partial pressure of carbon dioxide in contact with water, and thus it can be neutralized by releasing the reactor pressure. Carbonic acid is relatively mild and hence does not offer the same hydrolytic capability of sulfuric acid. However, van Walsum *(3)* has demonstrated that at temperatures on the order of 200°C, carbonic acid does exhibit a catalytic effect on hydrolysis of xylan. Van Walsum observed enhanced release of xylose and low degree of polymerization xylan oligomers compared with pretreatment using hot water alone. On corn stover, Shi and van Walsum *(4)* found enhanced hydrolysis resulting from addition of carbon dioxide.

A curious phenomenon reported in several previous studies *(4–6)* is that the pH of the final hydrolyzate, when measured at ambient temperature and pressure, is higher when carbonic acid is used in the pretreatment, and lower when water alone is used. It has been hypothesized that the two pretreatment methods resulted in differential production and release of organic acids, resulting in the difference in final pH (6.0). This study seeks to test this hypothesis by quantifying the organic acids and other degradation products produced by liquid hot water and carbonic acid-catalyzed pretreatment. These concentration data can then be used to test whether or not the observed pH differences in these hydrolyzates can be explained by the quantity of acid species present. Feedstocks in this study are corn stover and aspen wood. Metrics of pretreatment effects include final pH and chemical concentrations.

Materials and Methods

Feedstock

Corn stover was kindly supplied by the National Renewable Energy Laboratory in Golden, CO. Aspen wood chips were kindly supplied by the United States Department of Agriculture (USDA) Forest Products Laboratory in Madison, WI. Before pretreatment, the aspen wood was ground in a domestic brand coffee grinder and sifted to a particle size of between 0.5 and 1 mm. Corn stover was used as delivered. Dry weight of the feedstock was determined by oven drying. Carbon dioxide was standard laboratory grade, and H_2O was laboratory deionized quality.

Pretreatment

Two 1 g samples of biomass were weighed out and placed in separate 150-mL, 316 stainless steel reactors, along with 80 mL of deionized water each. One reactor was pressurized with 800 psi of CO_2 at room temperature; the other was left with air in the headspace at atmospheric pressure. CO_2 was added through a stainless steel tubing connection equipped with a valve and pressure gage *(5)*. Two sand baths equipped with digital temperature controllers were used for temperature control, one heated to the desired reaction temperature, and the second to a temperature 40°C more than the desired reaction temperature. The higher-temperature sand bath was used for preheating the reaction vessels for 3 min to quickly attain the desired reaction temperature. After preheating, the reactors were transferred to the reaction-temperature sand bath for the desired reaction duration. The reaction was quenched in an ice bath immediately after the reaction was complete. Previous research by McWilliams and van Walsum *(6)* had determined that a temperature range of 180–220°C was optimal for xylan solubilization. After pretreatment, suspended particles were removed by filtration using Whatman glass-microfiber membrane filters (90 mm diameter; 0.45-µm pore size; VWR Scientific, Suwanee, GA), and samples were stored at 4°C until processed for high-performance liquid chromatography (HPLC) analysis. Pretreatment conditions investigated in this study are summarized in Table 1. Further description of the pretreatment methods have been published previously *(5,6)*.

Analysis

Quantitative HPLC-ultraviolet (UV) analysis was performed on each sample in triplicate. The HPLC method used has been reported in detail previously *(7)* and is summarized below. For this work, several additional analytes were monitored that were not assessed in our previous work. All analyses were performed using a Dionex® DX-600 BioLC™ system (Dionex

Table 1
Experimental Conditions Applied in This Study

Experimental conditions				
Substrate	Corn stover		Aspen wood	
Cooking temperature (°C)	180		180	
Cooking time (min)	16		16	
CO_2 presence	800 psi CO_2	Without CO_2	800 psi CO_2	Without CO_2
Resultant (pH)	6.90	4.81	3.68	3.05
Predicted reaction pH in H_2CO_3 reactor	Approx 3.4 pH	–	Approx 3.4 pH	–

Resultant pH values were measured on room temperature, degassed hydrolyzate. pH prediction in the carbonic acid pretreatment reactor is after van Walsum *(3,4)*.

Corp., Sunnyvale, CA), equipped with a Model AS50 autosampler, Model DG2410 degassing module, and Model UVD170U ultraviolet detector. After extraction with MTBE, analytes were separated using a 25 cm × 4.6 mm, 5 mm, YMC™ Carotenoid S-3 column (Waters Corporation, Milford, MA) with an RP 18 Opti-Guard® column (Alltech Associates, Deerfield, IL) used to protect the analytical column. Nonlinear 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 used in HPLC analyses were as follows: injection volume, 25 μL; column temperature, 30°C; flow rate, 1 mL/min.

Identification of degradation products in hydrolyzate samples was accomplished by comparing UV absorbance and retention time data with reference standards. Absorbance was monitored at four different wavelengths in each analysis of reference and hydrolyzate samples. Quantitation of target analytes was accomplished using a multipoint internal standard calibration curve, with *p-tert*-butylphenoxyacetic acid as the internal standard. Acceptability criteria for identification of individual components in validation studies using high-purity reference samples 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. Since the publication of this analytical method *(7)*, and the conducting of this experimental study, it has been determined that there is a yet unidentified compound coeluting with the peak identified as lactic acid.

pH Calculations

Estimation of the predicted pH of the hydrolyzate was done through iterative calculation of the hydrogen ion concentration at equilibrium conditions, incorporating measured quantities of acid species and their respective p*Ka* values. Compounds for which p*Ka* values were not obtainable were

excluded from the calculation. Prediction of the pH within the carbonic acid pretreatment reactor was determined as per van Walsum and Shi *(3,4)*.

Results

As noted in Table 1, all reactions were carried out under identical conditions of time and temperature, with only the presence of carbonic acid being varied. Table 1 also presents the measured pH of the hydrolyzates, which shows that the addition of carbonic acid caused the final (room temperature, degassed) hydrolyzate pH to be higher than when reacted with water alone, as has been observed previously *(4–6)*.

Corn Stover Pretreatment

Pretreated samples were analyzed in triplicate and the data shown represent average measurements. Analytical results showed excellent reproducibility, with the average analyte showing a standard deviation of less than 4% of the mean on the corn stover samples. Table 2 presents the mean value data for the samples. It can be seen that most compounds exhibit different concentration levels between the two pretreatment methods. For most measured compounds, the difference in concentrations between hot water and carbonic acid pretreated samples was far more than the standard deviation of the individual analyses. Applying a *t*-test to the data, all but five compounds (itconic, 3,4 dihydroxybenzoic acid, phenol, syringaldehyde, and 4-hydroxybenzaldehyde) showed more than 95% confidence in the difference between mean measurements. Of these five, only 4-hydroxybenzaldehyde showed less than 50% confidence in difference between the means. Thus for corn stover, the addition of carbonic acid appears to significantly change the accumulation of most degradation products. The nine most abundant products present in the two hydrolyzates are highlighted in Fig. 1. It can be seen that carbonic acid results in the increase of some compounds and the decrease in others. These relative differences are plotted in Fig. 2.

Aspen Wood Pretreatment

Parallel results for aspen wood are presented in Figs. 3 and 4 and Table 2. In general, it was found that the aspen wood samples demonstrated weaker analytic reproducibility, with the average standard deviation for the samples rising to about 7% of the mean. A *t*-test on the data shows that fewer compounds have a high level of confidence (95%) in the difference of their means. For aspen wood 13 of the 36 quantified compounds failed to show this level of confidence in the difference of means, compared with 5 of 33 for corn stover. Also for aspen wood, three compounds (fumaric, levulinic, and homovanillic acid) showed less than 50% certainty that the means were different, compared with only one compound for corn stover.

Table 2
Average Analyte Concentrations for Corn Stover and Aspen Wood Pretreated
With and Without Dissolved Carbon Dioxide

Compounds	pKa	Aspen wood		Corn stover	
		With CO_2	Without CO_2	With CO_2	Without CO_2
Formic acid	3.77	0.7642	0.8634	0.9863	0.8603
Malonic acid	2.83	0.0116	0.0105	0.0142	0.0244
Lactic acid[a]	3.86	1.3722	2.4773	1.5815	1.4145
Acetic acid	4.76	1.2853	1.3881	0.7581	1.4044
Maleic acid	1.93	0.0035	0.0034	0.0119	0.0073
Succinic acid	4.19	0.0265	0.0554	0.3093	0.5653
Fumaric acid	3.03	0.0034	0.0033	0.0193	0.0111
Propanoic acid	–	0.0000	0.0000	1.5315	1.2791
t-Aconitic acid	2.8	0.0000	0.0065	0.0128	0.0049
Levulenic acid	–	0.3879	0.3802	0.0467	0.1979
Glutaric acid	4.34	0.2474	0.3094	0.0981	0.3987
Itaconic acid	–	0.0008	0.0015	0.0056	0.0069
Gallic acid	4.41	0.0015	0.0023	0.0005	0.0002
5-Hydroxymethylfurfural	–	0.0296	0.0516	0.0599	0.0321
2-Furoic acid	–	0.0104	0.0119	0.0168	0.0084
Furfural	–	0.1650	0.3304	0.7044	0.4197
3,4 Dihydroxybenzoic acid	–	0.0009	0.0015	0.0019	0.0020
3,5 Dihydroxybenzoic acid	–	0.0001	0.0001	0	0
3,4 Dihydroxybenzaldehyde	–	0.0015	0.0023	0.0087	0.0038
Phenol	9.99	0.0090	0.0152	0.0081	0.0059
4-Hydroxybenzoic acid	–	0.0694	0.0837	0.0039	0.0024
2,5 Dihydroxybenzoic acid	–	0.0015	0.0022	0.0042	0.0011
4-Hydroxybenzaldehyde	–	0.0242	0.0263	0.0288	0.0324
Vanillic acid	–	0.0038	0.0051	0.0057	0.0041
Homovanillic acid	–	0.0007	0.0007	0.0003	0.0007
Caffeic acid	–	0.0029	0.0034	0.0035	0.0179
Syringic acid	–	0.0065	0.0086	0.0087	0
4-Hydroxy-acetophenone	–	0.0022	0.0032	0.0065	0.0020
Vanillin	–	0.0241	0.0335	0.0393	0.0368
4 HO-Coumaric acid	–	0.0094	0.0155	0.1921	0.2274
Syringaldehyde	–	0.0329	0.0407	0.0173	0.0176
Benzoic acid	4.2	0.0618	0.0665	0.0280	0.0323
4-Hydroxy 3-methoxy, ferulic acid	–	0.0033	0.0058	0.0342	0.0381
Sinapic acid	–	0.0182	0.0148	0.0026	0.0049
Salicyclic acid	2.97	0.0031	0.0034	0	0
4-Hydroxycoumarin	–	0.0094	0.0141	0.0037	0.0039
2-Toluic acid	–	0.0138	0.0213	0.0078	0
4-Toluic acid	4.36	0.0113	0.0140	0.0074	0
Calculated pH	–	4.69	4.61	4.61	4.63

[a]Since the time these analyses were completed, it has been determined that the lactic acid peak detected with the reported method was coeluting with a yet unidentified compound.
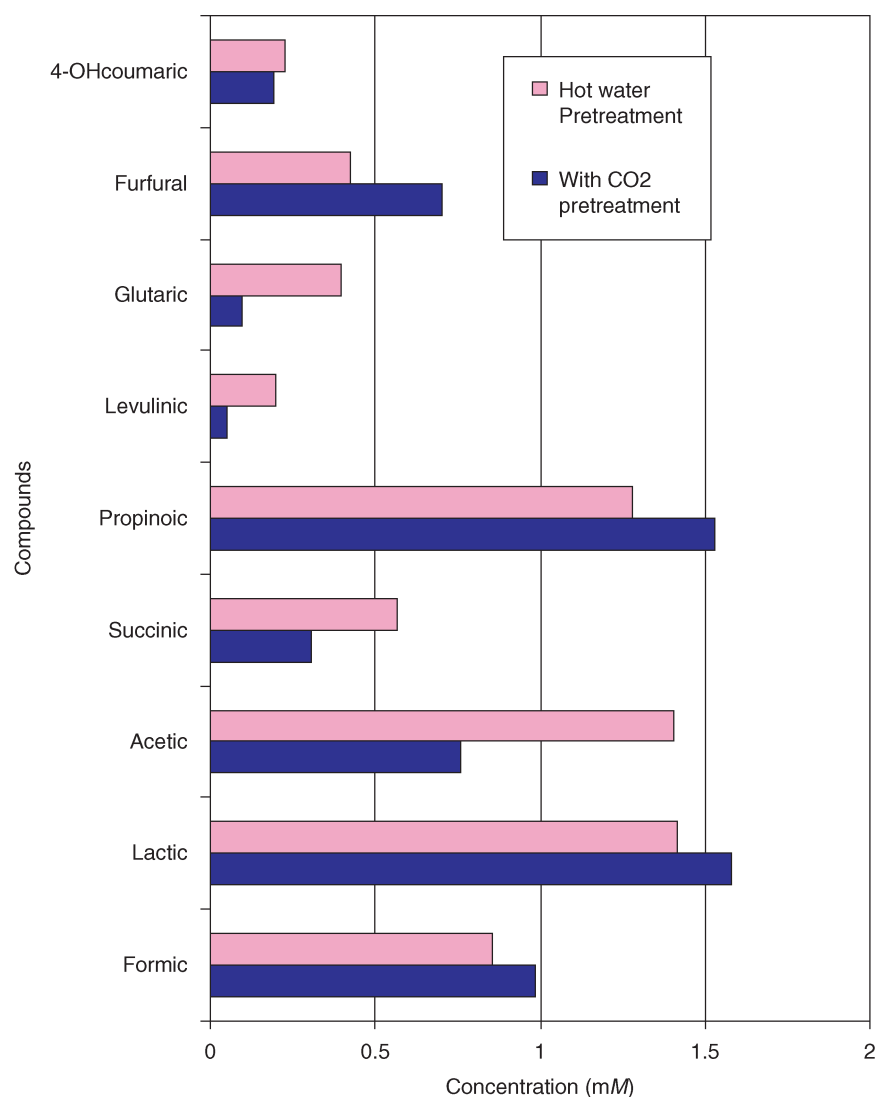
Fig. 1. Concentration of selected acid products in corn stover hydrolysate prepared in liquid hot water with and without dissolved carbon dioxide. (*Note*: lactic acid has been determined to coelute with a yet unidentified compound.)

Comparing Figs. 2–4, it can be seen how the variation of the more abundant corn stover products appears to either increase or decrease in response to carbonic acid, whereas on aspen wood, the primary effect of the carbonic acid is to decrease product concentrations.

Calculation of pH From Organic Acid Concentrations

The theoretical pH of the hydrolyzates was calculated based on the concentration and p*Ka* of the various quantified organic acids. p*Ka* values were
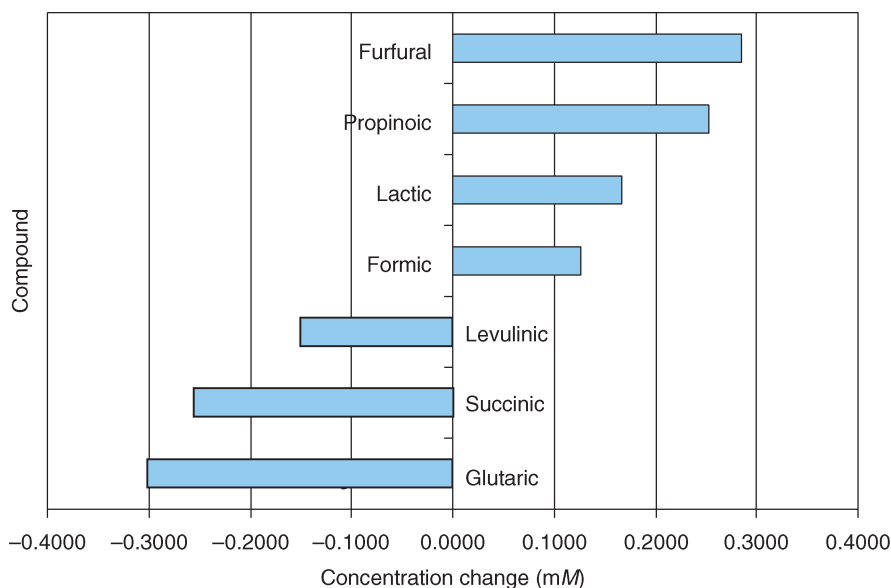
Fig. 2. Net difference in concentration of major acid products in corn stover hydrolysate resulting from addition of carbon dioxide. (*Note*: lactic acid has been determined to coelute with a yet unidentified compound.)

not available for some of the compounds present at lower concentration and these were excluded from the calculation. The bottom row of Table 2 lists the outcome of this calculation. It can be seen that the four hydrolyzates represent roughly similar pH ranges for their diverse mixtures of acids, coming in at approx pH 4.64. For aspen wood, the calculated pH values show a slightly higher level for the carbonic acid treated samples, but this difference is far smaller, and manifest at much higher pH, than the values measured experimentally (Table 1). For corn stover, the acid calculations show virtually the same pH in both hydrolyzates. In this case, the calculated pH values fall in the middle of the range measured experimentally.

Discussion

Corn stover samples were found to demonstrate more response to the presence of carbonic acid than aspen wood. This difference is owing in part to the greater scatter in the analytical results for aspen wood, reducing the confidence levels in the assessment of difference between means. In addition, this result may also reflect reduced influence of carbonic acid on the production of degradation products from aspen wood, in comparison with corn stover. This conclusion is in keeping with previously reported results where it was found that the influence of carbon dioxide on the release of xylose was more pronounced on corn stover than on aspen wood *(4)*.

From Figs. 2 and 4, it appears that the effect of carbon dioxide on aspen wood pretreatment is to reduce the concentration of degradation
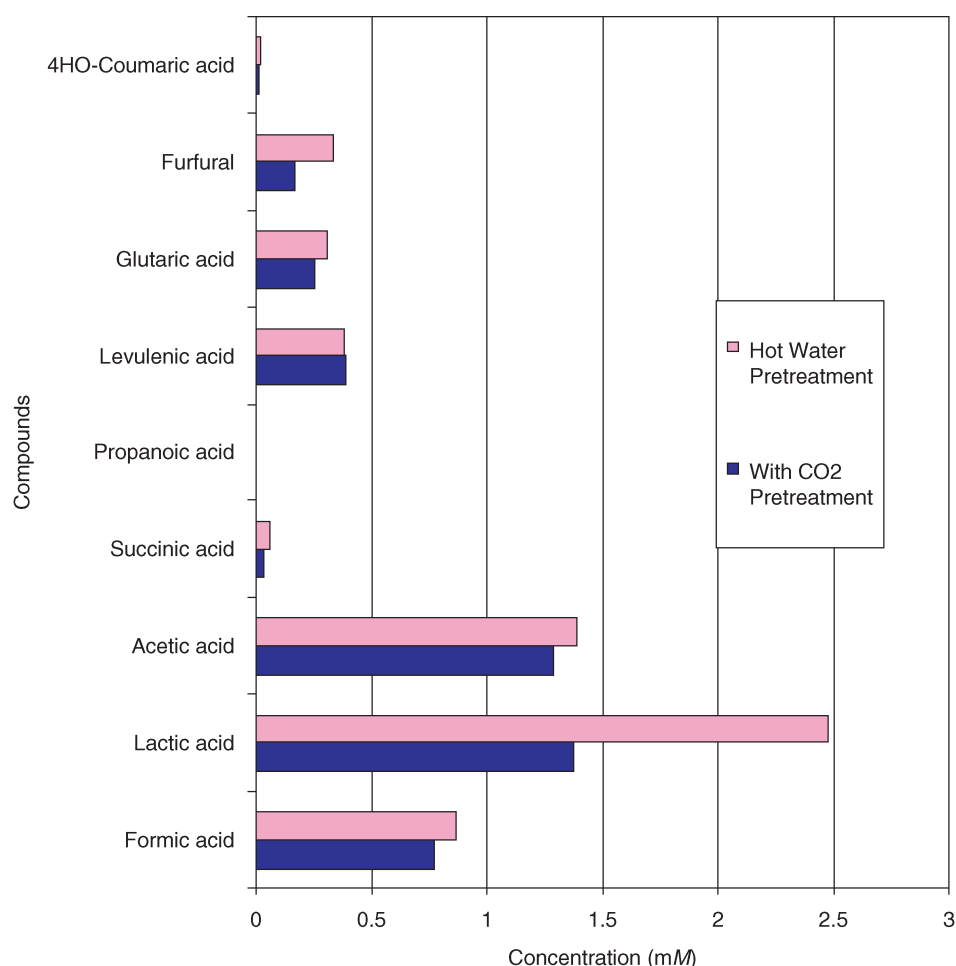
Fig. 3. Concentration of selected acid products in aspen wood hydrolysate prepared in liquid hot water with and without dissolved carbon dioxide. (*Note*: lactic acid has been determined to coelute with a yet unidentified compound.)

products, whereas on corn stover it appears to have differing effects for different compounds. The aspen results suggest a lessened degree of hydrolysis action on aspen wood with the addition of carbon dioxide to the reactor, suggesting that with aspen wood, the carbonic acid may possibly be working as a buffer rather than an acid. The predicted pH of the carbonic acid system under reaction conditions is approx 3.4, which is higher than the room temperature pKa of some of the stronger acids present. However, it is not known how the pKa values of the various organic acids present change in response to temperature, making it difficult to further assess this possibility.

The pH values calculated from the concentrations of the organic acids present in the hydrolyzates (Table 2) are in the range to be expected from a mixture containing many diluted organic acids. However, the calculated
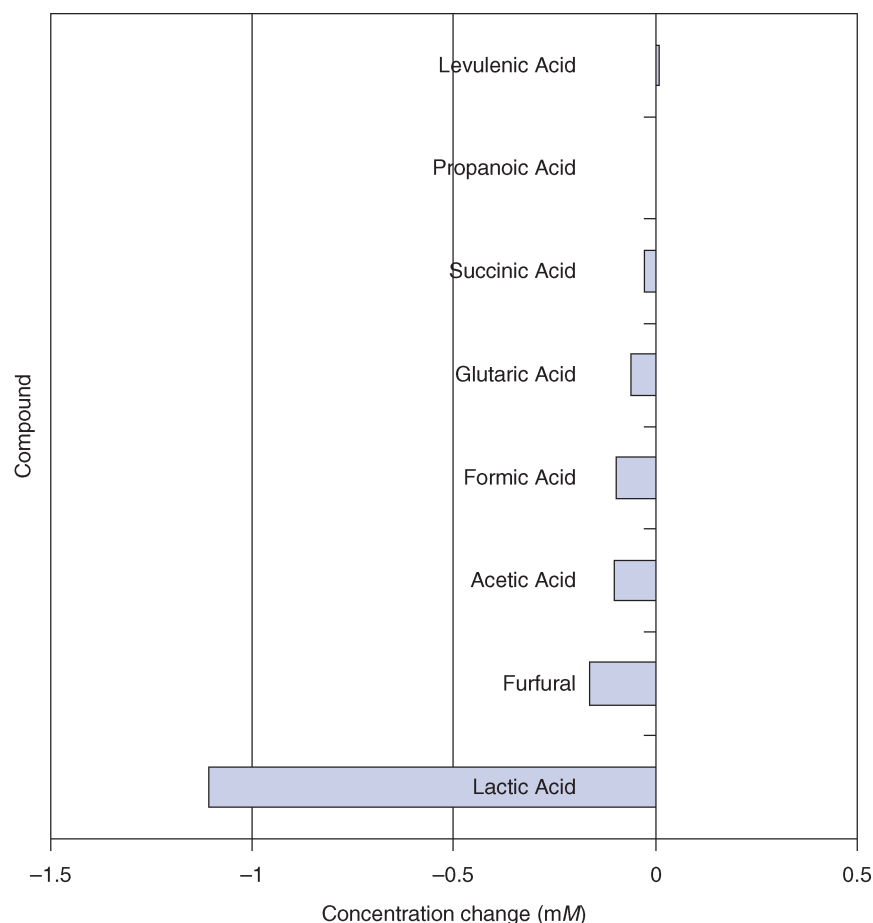
Fig. 4. Net difference in concentration of major acid products in aspen wood hydrolysate resulting from addition of carbon dioxide. (*Note*: lactic acid has been determined to coelute with a yet unidentified compound.)

values were not in accord with the measured pH values. For aspen wood, the measured pH values were substantially lower than the calculated values. This indicates that there may remain acid species in the sample that were not quantified in this study. Also, the measured increase in pH resulting from the addition of carbon dioxide, which was observed in this study and has also been consistently reported in previous studies, was not adequately accounted for by the compounds measured in this study. This too suggests an incomplete analysis of the compounds present.

Conclusions

A newly developed analytical procedure was applied to determine the concentrations of organic acids and other degradation products present in pretreatment hydrolyzates of aspen wood and corn stover. It was observed

that the addition of carbon dioxide to liquid hot water pretreatment significantly changed the accumulated concentrations of most measured compounds. However, these different concentration values failed to explain the consistently observed higher pH levels associated with carbonic acid pretreatment.

Acknowledgments

References

1. McMillan, J. D. (1994), *ACS Symp. Ser.* **566,** 292–324.
2. Quang, A. N., Tucker, M. P., Keller, F. A., and Eddy, F. P. (2000), *Appl. Biochem. Biotechnol.* **84–86,** 561–576.
3. van Walsum, G. P. (2001), *Appl. Biochem. Biotechnol.* **91–93,** 317–329.
4. van Walsum, G. P. and Shi, H. (2004), *Biores. Technol.* **93,** 217–216.
5. Yourchisin, D. M. and van Walsum, G. P. (2004), *Appl. Biochem. Biotechnol.* **113–116,** 1073–1086.
6. McWilliams, R. and van Walsum, G. P. (2002), *Appl. Biochem. Biotechnol.* **98–100,** 109–121.
7. Chen, S.-F., Mowery, R., Castleberry, V. A., van Walsum, G. P., and Chambliss, C. N. (2006), *J. Chrom. A.* **1104,** 54–61.