FINAL TECHNICAL REPORT ON

CARBONIC ACID PRETREATMENT OF BIOMASS

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EXECUTIVE SUMMARY

This project sought to address six objectives, outlined below. The objectives were met through the completion of ten tasks.

1) Solidify the theoretical understanding of the binary CO_2/H_2O system at reaction temperatures and pressures. The thermodynamics of pH prediction have been improved to include a more rigorous treatment of non-ideal gas phases. However it was found that experimental attempts to confirm theoretical pH predictions were still off by a factor of about 1.8 pH units. Arrhenius experiments were carried out and the activation energy for carbonic acid appears to be substantially similar to sulfuric acid. Titration experiments have not yet confirmed or quantified the buffering or acid suppression effects of carbonic acid on biomass.

2) Modify the carbonic acid pretreatment severity function to include the effect of endogenous acid formation and carbonate buffering, if necessary. It was found that the existing severity functions serve adequately to account for endogenous acid production and carbonate effects.

3) Quantify the production of soluble carbohydrates at different reaction conditions and severity. Results show that carbonic acid has little effect on increasing soluble carbohydrate concentrations for pretreated aspen wood, compared to pretreatment with water alone. This appears to be connected to the release of endogenous acids by the substrate. A less acidic substrate such as corn stover would derive benefit from the use of carbonic acid.

4) Quantify the production of microbial inhibitors at selected reaction conditions and severity. It was found that the release of inhibitors was correlated to reaction severity and that carbonic acid did not appear to increase or decrease inhibition compared to pretreatment with water alone.

5) Assess the reactivity to enzymatic hydrolysis of material pretreated at selected reaction conditions and severity. Enzymatic hydrolysis rates increased with severity, but no advantage was detected for the use of carbonic acid compared to water alone.

6) Determine optimal conditions for carbonic acid pretreatment of aspen wood. Optimal severities appeared to be in the mid range tested. ASPEN-Plus modeling and economic analysis of the process indicate that the process could be cost competitive with sulfuric acid if the concentration of solids in the pretreatment is maintained very high (~50%). Lower solids concentrations result in larger reactors that become expensive to construct for high pressure applications.

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PROJECT SUMMARY

Task 1a: Xylan and Xylose were hydrolysed in 1% H_2SO_4 at 121 °C for varying reaction times. Samples were analyzed with high performance anion exchange (HPAE) and an ultra-violet spectrophotometer. Peak areas for xylan oligomers were integrated by completing a mass balance on samples of varying degrees of hydrolysis. This yielded an appropriate calibration for peaks representing oligomer concentrations, and confirmed theoretical expectations that the area of oligomer peaks was proportional to the molar concentration of the oligomer species. The effect of pressure on hydrolysis was tested by superpressurizing reactors with nitrogen—no pressure effect on hydrolysis was detected. Experiments were conducted to confirm pH predictions of CO_2 by comparing results to dilute H_2SO_4. Hydrolysis was done by subjecting a 1g/L xylan solution to H_2SO_4 and/or CO_2 or N_2 at 190˚C or 200˚C for 16 minutes.

Task 1b Experiments were conducted to develop an Arrhenius rate constant for sulfuric acid and CO_2. These experiments consisted of hydrolyzing xylan at varying temperatures and initial pressures of CO_2, while maintaining a constant pH of 3.4. Comparative results were generated using H_2SO_4. To characterize the species in the hydrolysate, samples were analyzed by pH, HPAE and UV absorbency in a spectrophotometer.

Task 1c: Repeat experiments of aspen samples were hydrolyzed and titrated against a standardized NaOH solution to determine molar concentration of acid species in the hydrolysate. In some experiments it appeared that the acid concentration of hydrolysate of reactions with CO_2 was significantly lower than the hydrolysate of reactions of wood and water alone. This confirms earlier work that consistently measured a higher pH in carbonic acid pretreated hydrolysates, compared to water-only pretreatment. However, in other titration experiments, the opposite result was observed, with the carbonic acid system having more accumulated acids present.

Task 2: A data set of pretreatment results on aspen using carbonic acid had been completed prior to the start of this project. Experiments continued to increase the reliability of the results and to achieve uniform performance between laboratory personnel. These objectives were achieved and reduced the scatter and uncertainty in the results.

Experiments were also carried out to determine whether the rate of mass transfer of CO_2 into or out of solution exerts an effect on the rate of hydrolysis. It was found that extra CO_2 could be dissolved into solution by allowing more time for the dissolution to occur, but that when the reactor was heated up to reaction temperatures there was no apparent effect on hydrolysis rates or extent.

Task 3: It was found that the severity function developed by Overend and Chornet adequately described the action of time and temperature on the pretreatment of aspen wood but not pure xylan. For aspen wood, no significant difference was detected between carbonic acid and water systems, thus the effect of the carbonic acid was negligible and did not need to be incorporated into the severity function. On xylan, however, it was necessary to take into account the action of the carbonic acid, and it was found that the severity function proposed by van Walsum did so adequately. Published literature results for the dilute acid pretreatment of softwood were used for comparing the fit of the severity function and the combined severity function for lower pH systems. It was found that the literature data had considerable scatter, but that the combined severity function did appear to offer a more predictive capability than the regular severity function, which does not take into account the pH of the system.

Task 4: Preliminary experiments using a 150 mL reactor were conducted using water and a range of CO_2 pressures and reaction temperatures to evaluate the reactor performance and to determine when the pressure (and hence temperature) inside the reactor reaches steady-state. This revealed optimal reaction conditions and will minimize variation between experimental results generated from small (15 mL) and larger (150 mL) reactors. The data also provided an accurate determination of the time required for the reactor to reach reaction temperature.

Task 5: The newly constructed 150 mL reactor was used in reactions that replicated the conditions of the 15 mL reactor. 1.0 grams of aspen wood, 80 ml of de-ionized water were reacted with and without CO_2 at 800 psig. Reaction temperatures were 180^{o}C, 200^{o}C, and 220^{o}C with reactions times of 8, 16, and 32 minutes. The reactor was preheated in a sand bath set to a temperature 40^{o}C above reaction temperature for two minutes. This allowed the reactor to quickly reach reaction temperature, as determined and reported in the previous progress report. The 150ml reactor successfully delivered the expected 10-fold increase in hydrolysate compared to the 15ml reactor. Results of the pH and UV analysis of the hydrolysate were consistent with those yielded by the 15ml reactor.

Task 6: Inhibition tests measured the rate of sugar consumption by *Saccharomyces cerevisiae* growing in batch culture of hydrolysate. It was found that inhibition of the yeast culture increased with severity of pretreatment above a mid level severity. Below this severity, little to no inhibition was observed. No difference was observed between the inhibition of hydrolysates prepared either with or without the presence of CO_2. To conduct the experiments, serum vials were charged with 21g/L of sterile growth medium containing 20ml of pretreatment hydrolysate. The vials were inoculated with 0.2ml of freshly grown cell broth and incubated. Glucose concentrations over time were determined via glucose assay (Infinity Glucose Reagent) and the HPAE when available.

Task 7: Enzyme digestibility tests measured enzymatic hydrolysis rates of pretreated solids by cellulase enzymes (Novozyme 188 and Iogen cellulase). It was found that more severe pretreatments enhanced enzymatic digestibility. The addition of pressurized CO_2 to the pretreatment system did not significantly increase enzymatic hydrolysis rates compared to water-alone pretreatment. To conduct the experiments, s erum vials were charged with a pH 5.0 buffer, preservative, enzyme and pretreated solid sample estimated to have 2g/L cellulose (calculated from dry weight of the solid residue) and incubated in a 40^{0}C shaker bath. Glucose concentrations over time were determined via glucose assay and the HPAE when available.

Tasks 8+ 9: Pretreatment costs for carbonic acid pretreatment are driven by the high cost of pretreatment reactors capable of containing the pressures used. This makes the cost of the carbonic acid system highly sensitive to reactor volume and thus the concentration of solids in the reactor. The cost of the reactor can be reduced by raising the concentration of the solids in the pretreatment reactor, which in turn reduces the size of the pretreatment reactor and thus diminishes the cost differential between dilute acid and carbonic acid equipment costs. If the solids concentration is put very high, and equal to that used in the NREL model, equipment costs become comparable to those for dilute sulfuric acid pretreatment. Cost of compressing CO_2 is relatively low compared to the equipment cost for the high pressure reactor vessel. About 50% of the total operating cost is due to the heat demand of the process. This is unavoidable due to difficulties in process heat recovery.

Unless the concentration of solids in the pretreatment reactor can be increased, use of carbonic acid for pretreatment proved to be more expensive than using sulfuric acid. This is mainly due to the use of high pressures such as 2000 psi in the pretreatment reactor.

Task 10: Work from this project has been presented at four international meetings: the ACS annual meeting in Orlando Florida, April 7 – 11, 2002, the 24th Symposium on Biotechnology for Production of Fuels and Chemicals in Gatlinburg, TN, April 28 - May 1, 2002, the 12th European Conference and Technology Exhibition on Biomass for Energy, Industry and Climate Protection in Amsterdam, NL, 17-21 June 2002, and the 25th Symposium on Biotechnology for Production of Fuels and Chemicals in Breckenridge, CO, May 3- 7, 2003. A brief paper was published in the proceedings of the Amsterdam conference and papers were submitted for peer-reviewed publication in the conference proceedings of the 25th symposium.

TASK 1a) Determine accuracy of theoretical pH predictions

Vanessa Castleberry, G. Peter van Walsum

Summary:

Xylan and Xylose were hydrolysed in 1% H_2SO_4 at 121 °C for varying reaction times. Samples were analyzed with high performance anion exchange (HPAE) and an ultra-violet spectrophotometer. Peak areas for xylan oligomers were integrated by completing a mass balance on samples of varying degrees of hydrolysis. This yielded an appropriate calibration for peaks representing oligomer concentrations, and confirmed theoretical expectations that the area of oligomer peaks was proportional to the molar concentration of the oligomer species. The effect of pressure on hydrolysis was tested by superpressurizing reactors with nitrogen—no pressure effect on hydrolysis was detected. Experiments were conducted to confirm pH predictions of CO_2 by comparing results to dilute H_2SO_4. Hydrolysis was done by subjecting a 1g/L xylan solution to H_2SO_4 and/or CO_2 or N_2 at 190˚C or 200˚C for 16 minutes.

Materials and Methods

Apparatus and Materials: DX500 Chromatography System (consists of Dionex GP50 gradient pump, ED40 electrochemical detector, AS3500 autosampler), Xylan (sigma), xylose (Sigma), 15 mL 316 stainless steel reactor vessels, glass serum vials, Techne SBL-2D fluidized aluminum oxide sand baths, Pharmco sulfuric acid at 95.0 to 98.0%, 18 ohm high resistance water, deionized water, spigot-attached vacuum apparatus with tubing, ultra pure helium gas, Eppendorf micro pipettes (various sizes), DU Series 500 Beckman Spectrophotometer, Eppendorf centrifuge 5417C, and centrifuge tubes. Fisher Scientific AR15 pH meter by Accumet Research; Ohaus Explorer digital scale Item #12140 (d=0.1mg); Pyrex glass beakers, graduated cylinders, burettes and flasks;

Procedures:

Solution preparation: A 1-g/L xylan solution was prepared with 0.25-g of dried xylan into 250 mL of DI water. For hydrolysis in 1% H_2SO_4, 18 M acid was added to the xylan solution to give a 1% solution. The acidified xylan was placed on an active stir plate with a magnetic stir bar in solution. For hydrolysis with varying pH conditions, 18 M H_2SO_4 was added to a 1 g/L xylan solution in varying amounts. The xylan solution was placed on an active stir plate with a magnetic stir bar in solution. It was then pipetted into the steel reactor vessels in 10-mL aliquots. The acid solution, in a range of pHs, would next be added to the reactor vessel in 1.1mL aliquots.

Hydrolysis: For experiments at 121C, the acidified xylan solution was pipetted into the glass reactor vessels in 10-mL aliquots. The vial was sealed with a rubber stopper and an aluminum crimping ring. Each vial was placed in the sand bath for the desired time. For reactions at higher temperature, 316 stainless steel reactors were used, pressurized with

CO_2 if necessary, and placed in a sand bath (190˚C or 200˚C) for 16 minutes. The samples were frozen for a later time to be analyzed by HPAE. Samples were run in duplicate.

Analysis: The samples were analyzed for polymer distribution using the HPAE. .The HPAE consists of a pump, a separation column, and a detector. Each prepared sample was pumped through the column to distinguish the amount of hydrolysis occurring at each time interval. To prepare for the run, a 500mmol NaOH solution was made and degassed for use as an eluent. Each of the samples was diluted by 1in 20 with deionized water. A set of standards was prepared using a 1g/L xylose solution. The stock xylose solution was diluted by 1:20, 1:25, 1:33, 1:50 and 1:100 to obtain standards at 50 mg/L, 40mg/L, 30 mg/L, 20 mg/L and 10 mg/L, respectively. The acidified xylose samples were further analyzed using a DU Series 500 Spectrophotometer to observe absorbency in the ultra-violet light range.

pH prediction: Most of the reactions carried out for testing the pH of carbonic acid at elevated temperature were done at 190 C for 16 minutes. This gives a log(severity) value of 3.85, where the severity function in its log form is calculated as per equation (1), first defined by Overend and Chornet (1987):

$$\log(R_O) = \log_{10}\Big(t \times \exp\big((T\text{-}100) / 14.75 \big) \Big) \qquad (1)$$

Where R_O is the severity, t is the reaction time expressed in minutes and T is the temperature expressed in degrees Celcius. The combined severity values for the experimental conditions, with the CO2 at an initial, room temperature pressure of 800 psi is 0.27. The combined severity function was defined by Chum et al. (1990) as:

$$CS = \log(R_O) - \text{pH} \qquad (2)$$

Where CS signifies the combined severity. In applying the combined severity factor to the carbonic acid system, van Walsum (2001) suggested the following equation, which estimates the value of the solution pH from the temperature and the partial pressure of the CO_2:

$$CS_{Pco_2} = \log(R_O) \text{ -}8.00 \times T^2 + 0.00209 \times T - 0.216 \times \ln(Pco_2) + 3.92 \qquad (3)$$

Where CS_{Pco_2} is the combined severity determined from the partial pressure of CO_2 and Pco_2 is the partial pressure of CO_2 in atmospheres.

The use of the term "partial pressure" under these conditions is somewhat misleading, since the temperature and pressure range of the pretreatment put CO_2 into its supercritical phase, while the water vapor is still present in the gas phase. Thus, equation (3) pertains more precisely to the fugacity of the CO_2 in the non-liquid phase. For a component in solution, fugacity and partial pressure are related by

$$\text{Ø}_i = f_i / y_i P \qquad (4)$$

Where Ø_i is the fugacity coefficient of component i , f_i is the fugacity, y_i is the molar fraction and P the overall pressure of the system (Smith and Van Ness, 1975). For a binary i,j gas mixture, the multicomponent Virial equation simplifies to:

$$\ln \varnothing_i = \frac{P}{RT}(B_{ii} + y_j^2\delta_{ij}) \qquad \text{with } \delta_{ij} = 2B_{ij} - B_{ii} - B_{jj} \tag{5}$$

Where R is the universal gas constant, T is the temperature in degrees Kelvin, y_j is the molar fraction of the solvent in the gas phase and B_{ij} is the second virial coefficient, which can be expressed by equation (6) (Smith and Van Ness, 1975)

$$B_{ij} = \frac{RT_{Cij}}{P_{cij}}(B^0 + \omega_{ij}B^1) \tag{6}$$

In which ω is the acentric factor. The parameters ω_{ij}, T_{cij}, and P_{cij} were calculated with mixing rules presented in Smith and Van Ness (1975), as follows:

$$\omega_{ij} = (\omega_i + \omega_j) / 2 \tag{7}$$

$$T_{cij} = (T_{ci}\, T_{cj})^{1/2} \tag{8}$$

$$P_{cij} = (Z_{cij}\, R\, T_{cij}) / V_{cij} \tag{9}$$

In which Z is the compressibility. Z_{cij} and V_{cij} are determined by:

$$Z_{cij} = (Z_{ci} + Z_{cj}) / 2 \tag{10}$$

$$V_{cij} = \left((V_{ci}^{1/3} + V_{cj}^{1/3}) / 2 \right)^3 \tag{11}$$

B^0 and B^1 were calculated as proposed in Smith and Van Ness (1975), as follows:

$$B^0 = 0.083 - 0.422 / T_r^{1.6} \tag{12}$$

$$B^1 = 0.139 - 0.172 / T_r^{4.2} \tag{13}$$

These equations can be solved iteratively starting with the known temperature, total pressure and assumed values for molar fractions. Initial estimates for molar fractions were obtained by assuming ideal gas behavior, which yielded convergent results. Results from these calculations for several reaction conditions are summarized in table 1a.1.

Table 1a.1. Results of thermodynamic determination of carbonic acid pH at elevated temperatures and pressures.

Temperature C	Total pressure (psia)	y_{co_2}	Z	Ø	f_{co_2} (psia)	pH
180	1941	.925	.850	.861	1606	3.54
190					1686	3.58
200	2165	.896	.854	.864	1766	3.62
210					1845	3.67
220	2416	.86	.872	.880	1924	3.71

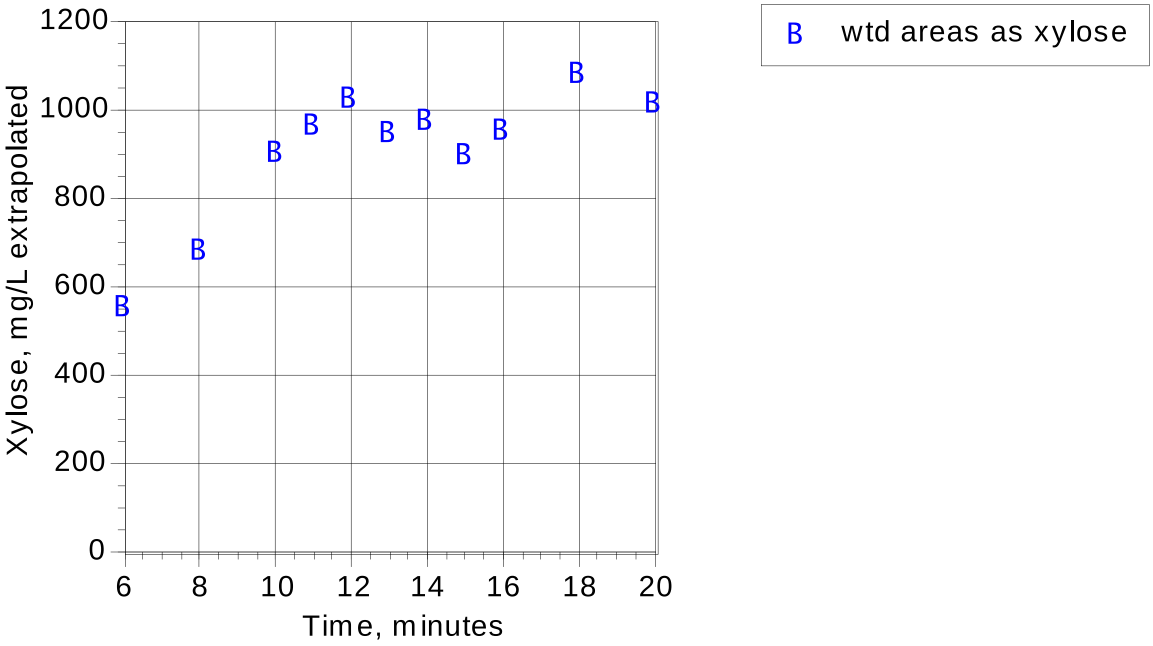
Previously reported [van Walsum, 2001] calculations for the pH of high temperature carbonic acid, which did not incorporate the fugacity coefficient calculations shown above, had predicted a pH of 3.56 at 190 C. This result is in very near agreement with the more thermodynamically correct value calculated in table 1a.1, and indicates that simplified assumptions about the gas phase behavior of CO2 may still be viable under these conditions.

Results

Oligomer quantification

Figure 1a.1 shows results from xylan hydrolysis at 121 C in 1% sulfuric acid. It can be seen that at about time 10 minutes, all of the 1 g/L xylan originally present has been hydrolyzed to oligomers small enough to be quantified by the HPAE. The relative stability of total oligomer concentration at times above 10 minutes demonstrates that the quantification of oligomers is relatively robust, since for all these cases the mass balance comes close to closure. At times less than 10 minutes, insufficient hydrolysis had occurred and large DP oligomers were not being detected.

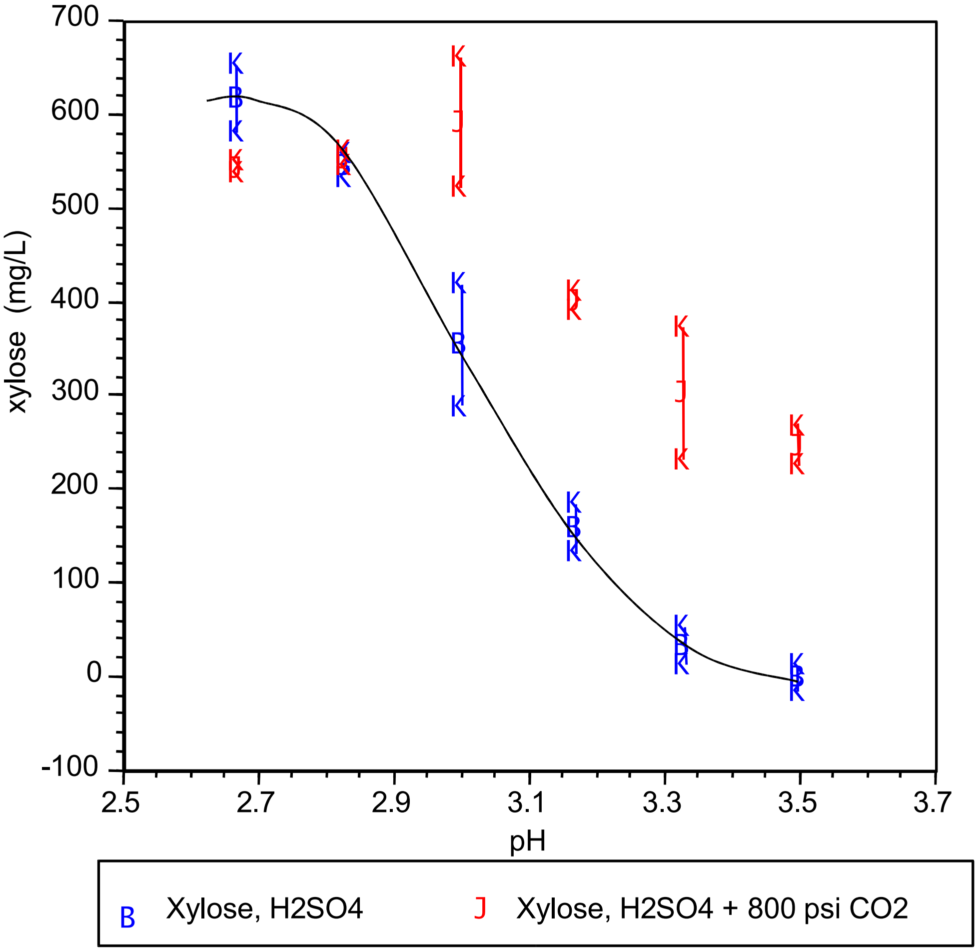
Figure 1a.1 Total xylose and xylan oligomers released in 1% H_2SO_4 hydrolysis of 1 g/L xylan at 121 C.



pH confirmation

Figure 1a.2 shows results of dilute sulfuric acid and carbonic acid hydrolysis of 1 g/L xylan at 190 C. The figure shows the effect of combining carbonic and sulfuric acids for hydrolysis of xylan. In this experiment it can be seen that down to a pH of about 3.2, carbonic acid is able to supplement the hydrolysis of the sulfuric acid. Below pH 3.2 the carbonic acid can no longer contribute to enhancing hydrolysis. It appears that carbonic acid on its own has an effective pH of about 3.4 Compared to the theoretical value of 3.58, this appears to show minor deviation from theoretically expected results. Van Walsum [2001] found similar deviation results, where the observed hydrolysis was equivalent to sulfuric acid at 3.37 and the predicted pH, using idealized gas calculations, had been 3.56 .

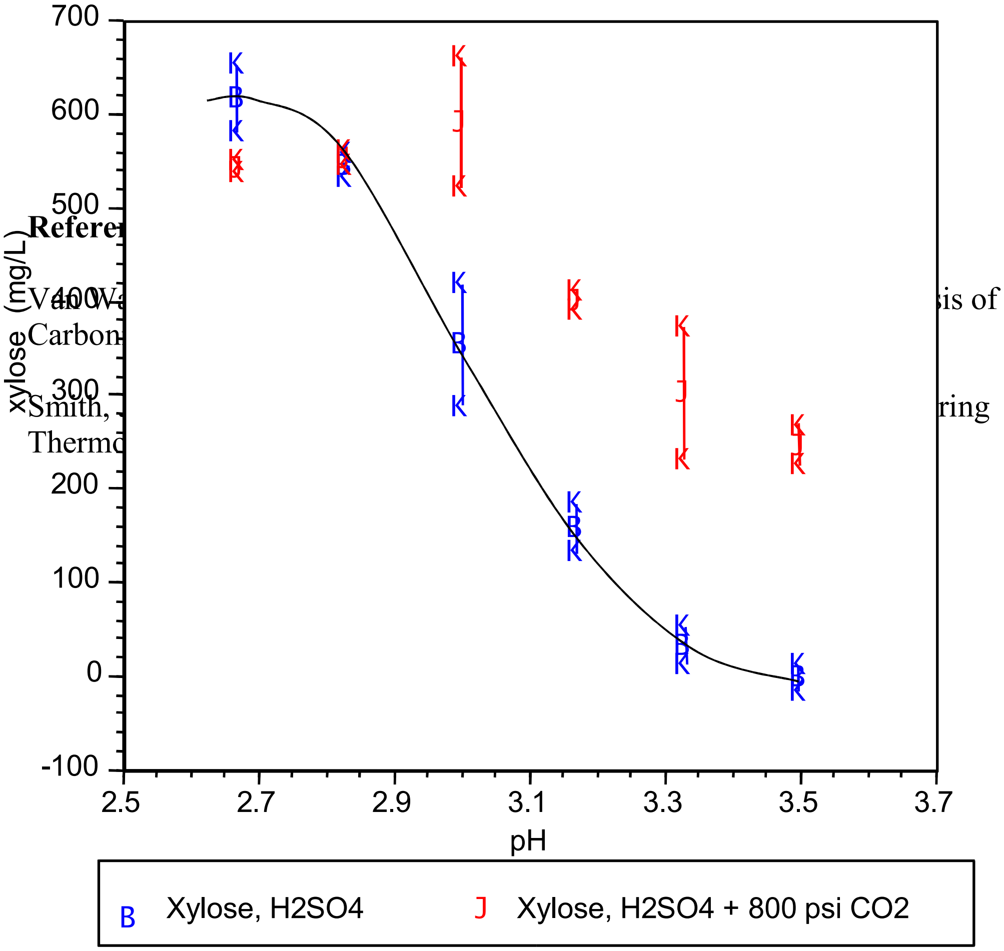
Figure 1a.2: Xylose accumulation in varying pH of H_2SO_4 and CO_2 with varying pH of H_2SO_4



Discussion & Conclusions

It was found that oligomer quantification based on a correlation of peak area to molar concentration of oligomers served to close a mass balance on partially hydrolyzed xylan solutions. This enabled quantification of xylan solubilization in cases of incomplete hydrolysis.

In the varying pH experiments, CO_2 at 800 psig most closely approximated H_2SO_4 at a pH of 3.4 and appeared to be able to assert some hydrolytic activity in the presence of other acids down to a pH of 3.2. Predictions of the pH of carbonic acid appear to have over estimated the effective pH by approximately 0.18 pH units. These results are in agreement with previously published findings.



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TASK 1b) Arrhenius equation for H_2CO_3

Vanessa Castleberry, G. Peter van Walsum

Summary:

Experiments were conducted to develop an Arrhenius rate constant for sulfuric acid and CO_2. These experiments consisted of hydrolyzing xylan at varying temperatures and initial pressures of CO_2, while maintaining a constant pH of 3.4. Comparative results were generated using H_2SO_4. To characterize the species in the hydrolysate, samples were analyzed by pH, HPAE and UV absorbency in a spectrophotometer.

Materials and Methods:

Apparatus and Materials: DX500 Chromatography System (consists of Dionex GP50 gradient pump, ED40 electrochemical detector, AS3500 autosampler), Xylan (sigma), xylose (Sigma), 15 mL 316 stainless steel reactor vessels, glass serum vials, Techne SBL-2D fluidized aluminum oxide sand baths, Pharmco sulfuric acid at 95.0 to 98.0%, 18 ohm high resistance water, deionized water, spigot-attached vacuum apparatus with tubing, ultra pure helium gas, Eppendorf micro pipettes (various sizes), DU Series 500 Beckman Spectrophotometer, Eppendorf centrifuge 5417C, and centrifuge tubes. Fisher Scientific AR15 pH meter by Accumet Research; Ohaus Explorer digital scale Item #12140 (d=0.1mg); Pyrex glass beakers, graduated cylinders, burettes and flasks;

Procedures:
Solution preparation: A new 1-g/L xylan solution was prepared with 2g of dried xylan into 2 L of DI water. For hydrolysis in 1% H_2SO_4, 18 M acid was added to the xylan solution in varying amounts to observe responses to a range of pHs side by side with CO_2. The xylan was placed on an active stir plate with a magnetic stir bar in solution.

Hydrolysis: The solution was pipetted into 150 mL, 316 stainless steel reactor vessels in 10-mL aliquots. To maintain a constant pH for the acids over the temperature range, the temperature dependence of the acid dissociations was taken into account when preparing solutions for use at different temperatures. This resulted in the lower temperature solutions being reacted at lower pressures of CO2 and lower concentrations of sulfuric acid. The reactor was closed, and if dictated, pressurized, and placed in a sand bath (170˚C (210˚C preheat), 180˚C (220˚C preheat), or 190˚C (230˚C preheat) for 14.5 minutes. Samples were preheated for 3 minutes in the 150 mL reactor. The samples were refrigerated until HPAE analysis was run. Samples were run in duplicate.

Analysis: The samples were analyzed using the HPAE. To prepare for the run, a 500mmol NaOH solution was made and degassed for use as an eluent. Each of the samples was diluted by 1in 20 with deionized water. A set of standards was prepared using a 100mg/L-xylose solution. The xylose solution was diluted by 1:2, 1:2.5, 1:3.3, 1:5 and 1:10 to obtain standards at 50 mg/L, 40mg/L, 30 mg/L, 20 mg/L and 10 mg/L. Each prepared sample was pumped through the column to distinguish the amount of hydrolysis occurring at each time interval.

The acidified xylose samples were further analyzed using a DU Series 500 Spectrophotometer to observe absorbency at 275 nm.

Results:

Results from the hydrolysis experiments are presented in figures 1b.1 and 1b.2, which show results for the carbonic and sulfuric acid systems, respectively. The slopes of the plots indicate activation energies of 11.9 kJ/mol for the sulfuric acid system and 12.6 kJ/mol for the carbonic acid system. It is not clear whether this difference is significant in terms of identifying a difference in the effective hydrolysis activity of the two acids. The R^2 values for the slopes of these plots are .988 and .976 respectively, which demonstrates a relatively good fit to the data in each case, but does not instill confidence in the significance of the effect.

Figure 1b.1 Plot of 1/T versus ln(K) for carbonic acid hydrolysis of xylan

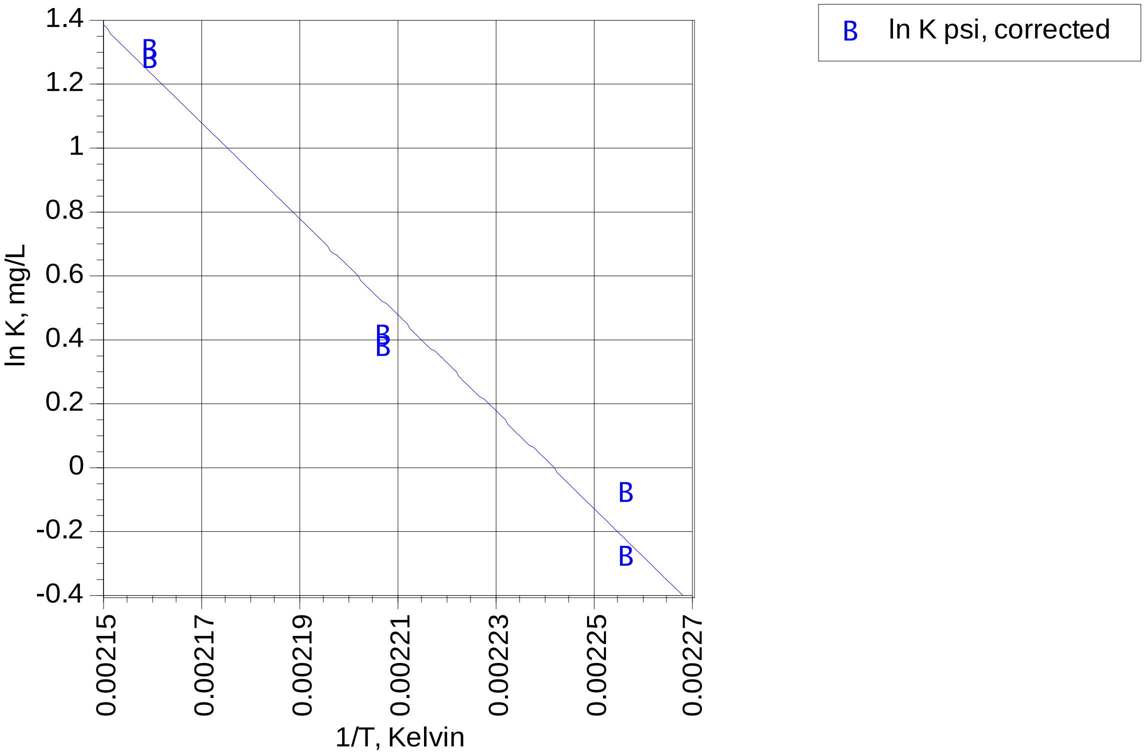
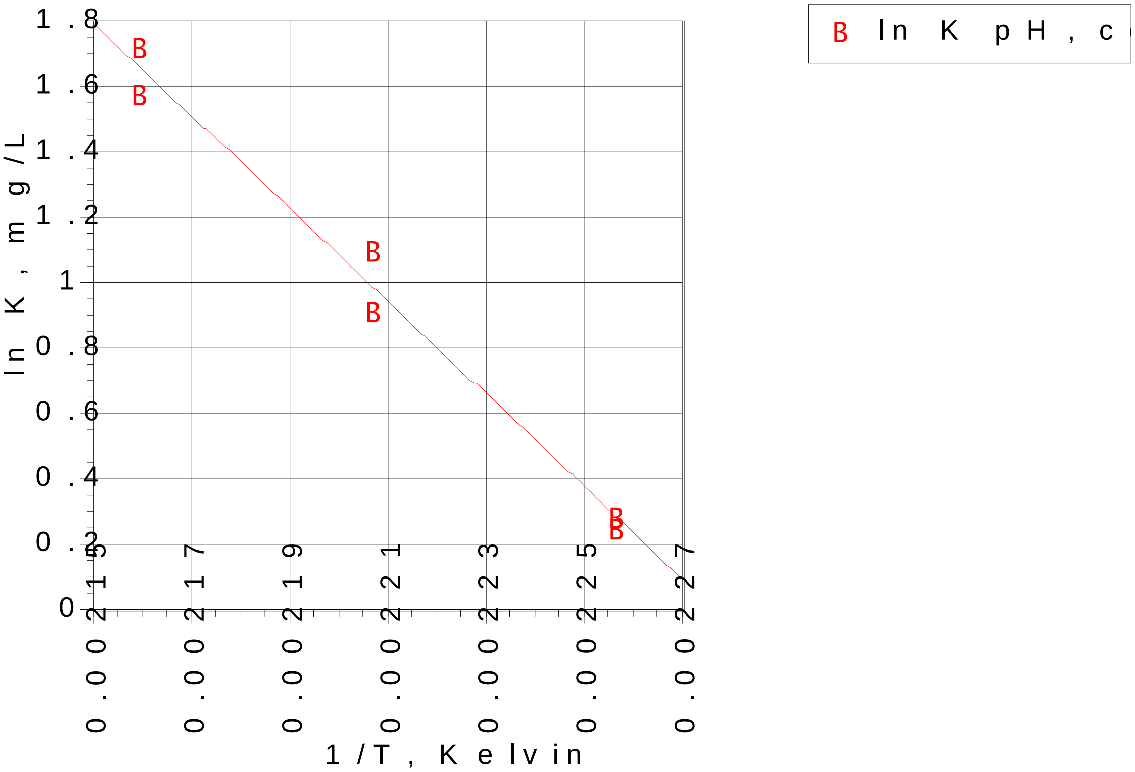


Figure 1b.2 Plot of 1/T versus ln(K) for sulfuric acid hydrolysis of xylan



Discussion and Conclusions

It appeared relatively straight forward to calculate the activation energy for both sulfuric and carbonic acids. There does not appear to be any significant effect on hydrolysis that could be attributed to the type of acid used. Temperature effects on the acid dissociations were taken into account when preparing the samples to achieve a constant pH at each temperature.

TASK 1c) Buffering Capacity of H_2CO_3

Vanessa Castleberry, G. Peter van Walsum

Summary:

Repeat experiments of aspen samples were hydrolyzed and titrated against a standardized NaOH solution to determine molar concentration of acid species in the hydrolysate. In some experiments it appeared that the acid concentration of hydrolysate of reactions with CO_2 was significantly lower than the hydrolysate of reactions of wood and water alone. This confirms earlier work that consistently measured a higher pH in carbonic acid pretreated hydrolysates, compared to water-only pretreatment. However, in other titration experiments, the opposite result was observed, with the carbonic acid system having more accumulated acids present.

Introduction

During previous experimentation using aspen wood, the hydrolysate pH of reactions with CO_2 was higher than reactions without. Since acetic acid may likely be the major contributor of H+ ions into solution,it is hypothesized that the presence of CO_2/carbonic acid may somehow reduce the release of acetyl groups, or in some other way reduce the activity of acid species in solution. This experiment attempted to close the “acid balance” by titrating hydrolysates and comparing titration results to analytical determination of acetic and formic acids in solution.

Materials + Methods

Apparatus and Materials: DX500 Chromatography System (consists of Dionex GP50 gradient pump, ED40 electrochemical detector, AS3500 autosampler), xylose (Sigma) , 15 mL 316 stainless steel reactor vessels, Techne SBL-2D fluidized aluminum oxide sand baths, J. T. Baker Glacial Acetic acid, Pharmco sulfuric acid at 95.0 to 98.0%, Mallinckrodt AR® sodium hydroxide solution at 50% w/w, Tetrabutylammonium hydroxide solution, 40% w/w; Heptafluorobutyric acid solution, 99%; 18 ohm high resistance water, deionized water, Fisher heated stirring plate, magnetic stir bars, glass beakers, Erlenmeyer flasks, Kjeldahl flasks, spigot-attached vacuum apparatus with tubing, ultra pure helium gas, Eppendorf micro pipettes (various sizes), DU Series 500 Beckman Spectrophotometer, Eppendorf centrifuge 5417C, and centrifuge tubes. Fisher Scientific AR15 pH meter by Accumet Research; Swagelock 150ml stainless steel reactor; Ohaus Explorer digital scale Item #12140 (d=0.1mg); Pyrex glass beakers, graduated cylinders, burettes and flasks; Aspen wood; Aspen hydrolysates; Aspen solids.

Procedures:

Hydrolysis: A sand bath was heated to 190 ˚C, another 230 ˚C. Aspen was ground and sifted, using the particles of 1 mm. 1.0 g of aspen was weighed and placed in 150-mL stainless steel reactor, along with 80 mL of water. Using 3 reactors, simultaneously, all samples at each parameter were generated at once. Depending on the sample, the reactor was charged either with water and wood or with water, wood and 800 psig of CO_2. The filled reactors were placed in the 230°C sand bath for a 3-minute preheat. Upon completion of preheat time, the reactors were transferred to 190°C sand bath for 15 minutes of reaction time. Reactions were quenched in ice bath after 15 minutes at target temperature.

Titrations: ~2 L of ultra pure water were brought to boil for 20-30 minutes. Remove from heat. The water was stoppered to cool and transferred to polyethylene bottles that were tightly closed. A titrant of ~0.01 M NaOH was mixed up, using newly bottled water. The titrant was stored in a tightly closed polyethylene container. KHP (Potassium Hydrogen Phthalate) was weighed and dried in a 100˚C oven overnight. The container was transferred to a dessicator and allowed it to cool. The # of moles in 20 mL of the newly made NaOH solution was calculated and, in the same number of moles of KHP the weight was calculated. That amount was weighed into a 125 mL flask. Enough degassed water was added to dissolve it (~20 mL). Fresh NaOH was poured into a 25 mL burette. The pH probe was placed into the KHP solution and titrated against the NaOH solution. The mV (potential) and pH were recorded after each mL of NaOH was added. Using the derivative of the end point, the exact concentration of NaOH solution was calculated. The exact molarity of the NaOH solution was $9.91954 * 10^{-3}$. Each hydrolysate titration was repeated 4 times and the average was used to derive an end point. For each tirtration, 25ml of hydrolysate was placed in a 150ml beaker which was then placed on a stir plate. The pH probe was placed in the beaker as well. The 0.1M NaOH was added incrementally (between 0.1ml and 1.0)ml and the pH was measured and recorded at each increment. The data will be plotted and evaluated to determine the number and concentrations of acids present in the hydrolysate.

Results:

Figures 1c.1 to 1c.7 show results from different experiments doing titrations on aspen wood hydrolysates. Figures 1c.1, 1c.4, 1c.6 show results of water pretreated aspen wood, figures 1c.2, 1c.5, 1c.7 carbonic acid pretreated aspen wood. Figure 1c.3 shows the calibration titration using KHP. It can be seen that in the experiment that generated figures 1c.1 and 1c.2, the wood hydrolysate has less overall acid than the carbonic acid hydrolysate, indicated by the lower volume of NaOH solution needed to reach the inflection point in the titration curve. Figures 1c.4 and 1c.5 show little difference between the two titrations, while 1c.6 and 1c.7 show both qualitative and quantitative differences: the CO_2 system has a much more gradually buffered-looking titration curve, indicating a variety of acid species.

Figure 1c.1 Titration of aspen hydrolysate without CO_2

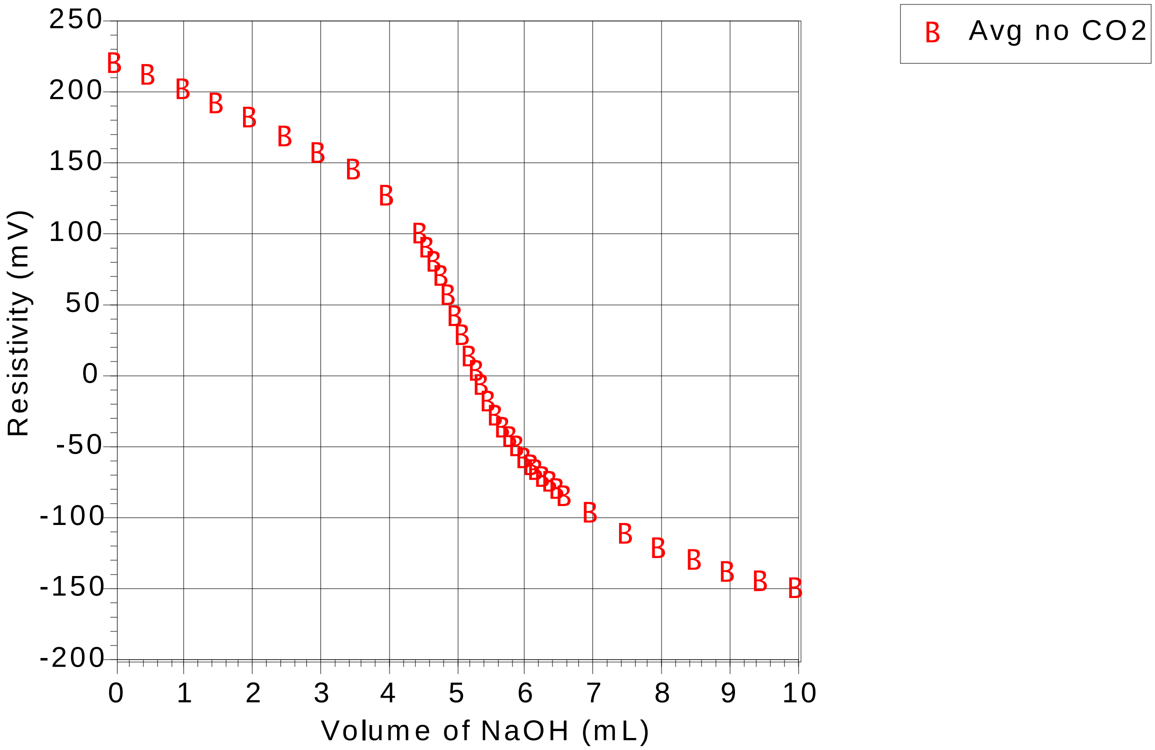


Figure 1c.2 Titration of aspen hydrolysate with CO_2

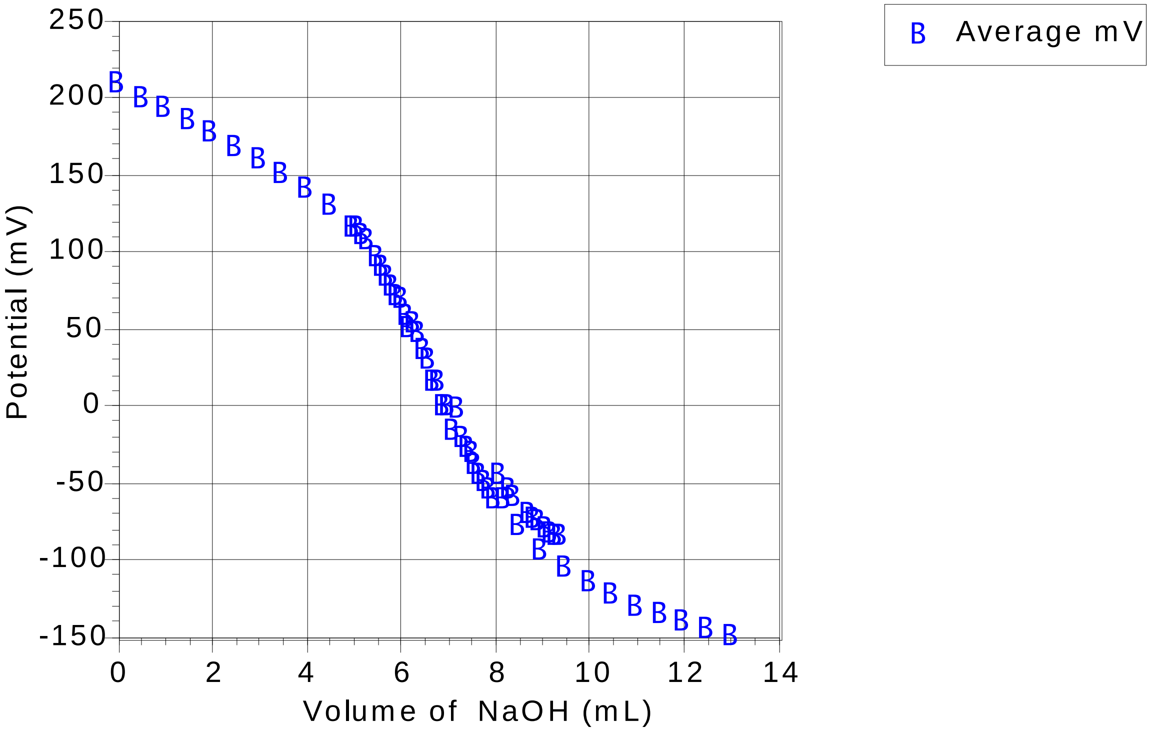


Figure 1c.3: KHP Standard Titration curve

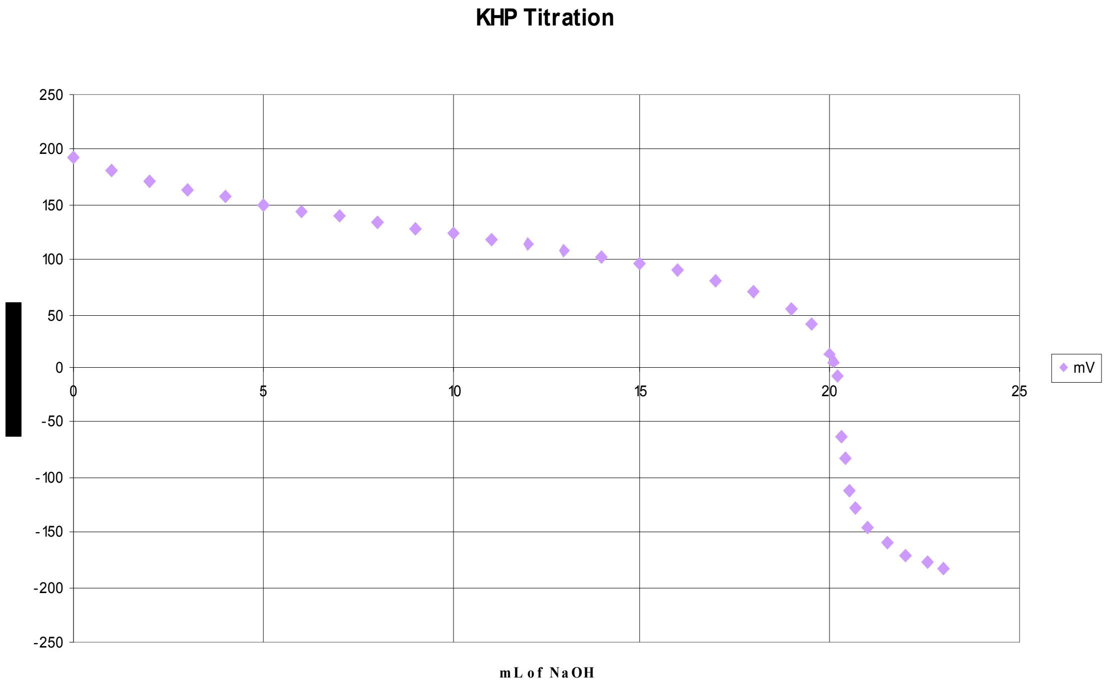


Figure 1c.4: Titration Curve of Water Pretreated Aspen

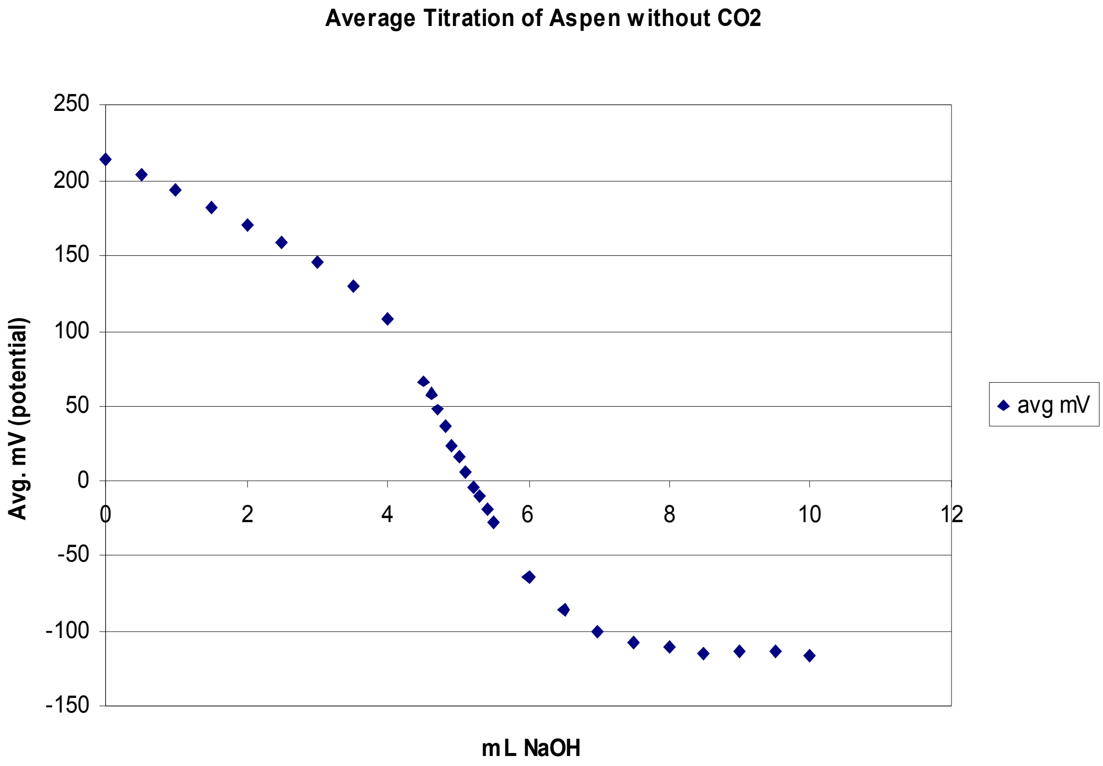


Figure 1c.5 Titration of Carbonic Acid Pretreated Aspenwood

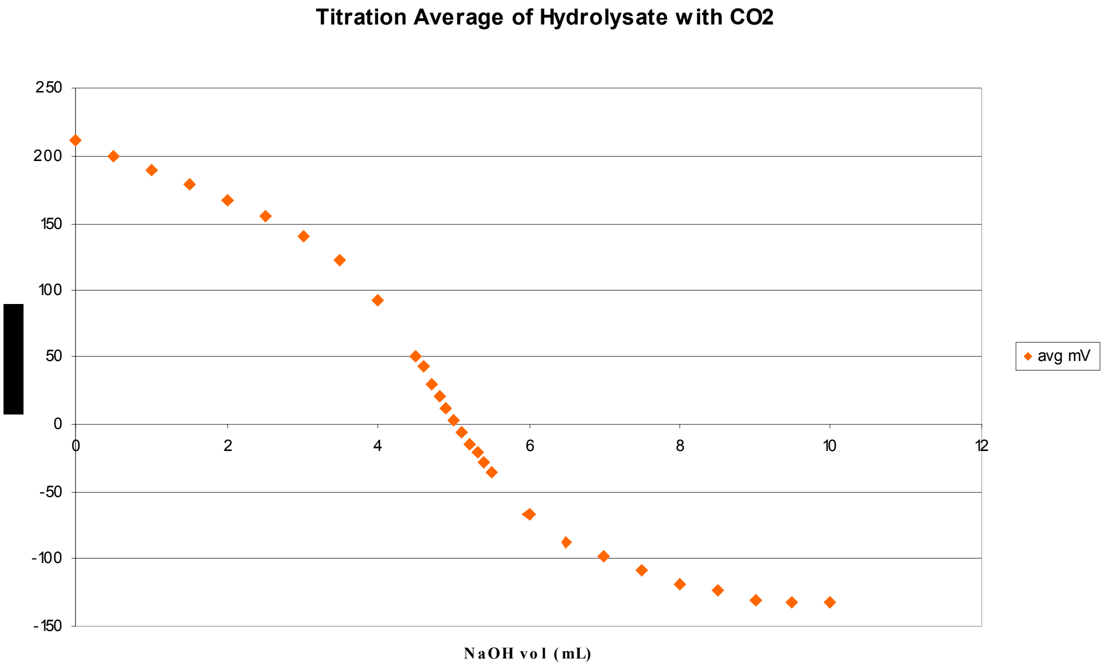


Figure 2c.6
pH Titration Results for Neutralization of Hydrolysate from Liquid Hot Water Pretreatment

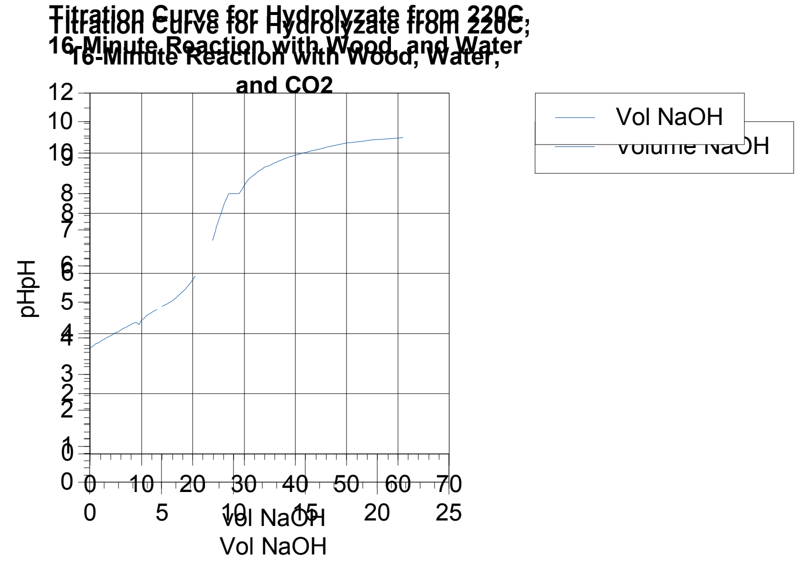
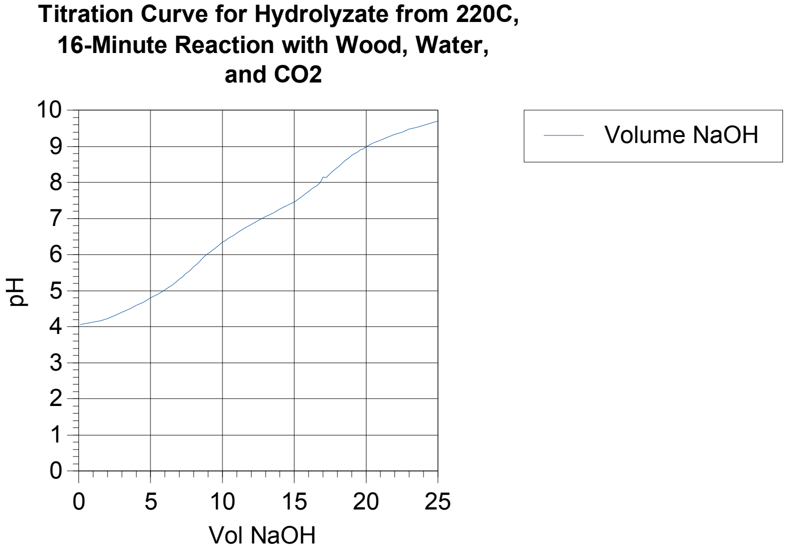


Figure 2c.7
pH Titration Results for Neutralization of Hydrolysate from Carbonic Acid Pretreatment



Discussion and Conclusions

The curious, and previously reported [1] result of carbonic acid having a net effect of increasing the final aspen wood hydrolysate pH has been confirmed for most experiments, but this result is not consistent with allof the titration studies undertaken. At this point there are inconsistent results in the titration results and so no conclusions can be drawn. Further work is being done to increase the reproducibility of these results.

TASK 2 Perform laboratory experiments on the pretreatment of raw biomass

John Lam, Robert McWilliams, Connaly Miller, G. Peter van Walsum

Summary

A data set of pretreatment results on aspen using carbonic acid had been completed prior to the start of this project [1]. Experiments continued to increase the reliability of the results and to achieve uniform performance between laboratory personnel. These objectives were achieved and reduced the scatter and uncertainty in the results.

Experiments were also carried out to determine whether the rate of mass transfer of CO_2 into or out of solution exerts an effect on the rate of hydrolysis. It was found that extra CO_2 could be dissolved into solution by allowing more time for the dissolution to occur, but that when the reactor was heated up to reaction temperatures there was no apparent effect on hydrolysis rates or extent.

Introduction

Previously published results showed a large degree of scatter in the data for Carbonic acid pretreatment of aspen wood. Further experiments were carried out to improve the data set and increase confidence in the results. With the increased number of data, a Q test at 99% certainty was used to eliminate outlier points and thus tighten up the results.

Through our studies of H_2CO_3 pretreatment of biomass, it has become apparent that there may be kinetic limitations affecting the consistency of hydrolysis results. A set of experiments was carried out to determine if the kinetics of solubilization of the CO_2 was affecting hydrolysis. A reactor was charged with a xylan solution and CO_2 at 800 psi. The pressurized reactor was then allowed to sit for a set period of time to allow mass transfer of the CO_2 into solution prior to heating and initiation of the reaction.

Materials and Methods

Apparatus and materials: A 15 mL reactor, 150 mL reactor, sand bath, a coffee grinder, raw aspen wood, a sieve, a balance, deionized water, pH meter, a tank of carbon dioxide (lab grade), beakers, graduated cylinders, glass vials, and a spectrophotometer with quartz cuvettes.

Procedures:

Feedstock preparation: Raw pieces of aspen wood are placed into a coffee grinder. After the coffee grinder turned the wood into fine chips, the chips are placed into a sieve and shaken. The desired size of the wood was 1 mm, and was collected for storage.

Aspen wood Pretreatment: The wood was taken to a balance and 0.1 grams is weighed. The 0.1 grams of wood was placed into a 15 mL reactor along with 8 mL of deionized water. For reactions involving carbon dioxide, 800 psi of carbon dioxide was added through a 1/8 inch stainless steel tube. The reactors are closed and placed in a sandbath.

Some reactors were reacted at 180 Celsius and some at 200 Celsius. The amount of time that each reactor was placed in the sandbath was 8 minutes, 16 minutes, and 32 minutes. For repetition of older results, no pre-heat time was used to shorten temperature transients. This was because some of the earlier reported results were generated without pre-heat times.

Post reaction pH: After the reaction, the reactor was taken out and the hydrolysate emptied into a container such as a beaker. The pH was quickly measured. A magnetic stirrer bar was placed into the beaker and the hydrolysate was stirred in order to accelerate de-gassing. The pH was measured every 5 minutes until constant pH was attained.

UV-absorption: 30 microliters of the hydrolysate was placed into a micro-centrifuge tube and centrifuged for 3 minutes at 12,000 rpm. The hydrolysate was then pipetted into a quartz cuvette with 2970 microliters of water. The cuvette was placed into a spectrophotometer and the ultraviolet absorbance at 270 nm was measured.

Carbohydrates: Were measured by HPAE-PAD using a Dionex GP50 gradient pump, an ED40 amperometric detector and a Carbopac column running an eluent of dilute NaOH.

Mass transfer experiments: Reactors were charged with aspen wood or xylan and water. The reactors were then pressurized with CO_2 and then treated in one of three methods: 1) the reactors were sealed and immediately immersed in the sand bath to initiate reaction; 2)the reactors were sealed and allowed to sit idle at room temperature for a specific period of time: 15 minutes to 24 hours prior to reaction--this allowed the solution to become saturated with CO_2 before reaction; and 3) the reactors were kept pressurized with CO_2 for an hour before sealing the reactor, which allowed even more CO_2 to go into solution, after which the reaction was then reacted. Conditions 1 and 2 were intended to compare the effects of saturation prior to reaction, while condition 3 allowed an even higher degree of saturation with CO_2. Hydrolysates were analyzed for pH, absorbance and xylose concentration.

Results

Tables 2.1 and 2.2 lists previous (McWilliams and van Walsum[1]) and newly generated (this study) results for aspen wood pretreatment experiments. As can be seen from the table, in most cases mean values from previous experiments have been confirmed and the error about the mean reduced. These same data are also plotted in figures 2.1 – 2.14. On most of these figures it can be seen that the more recent results show less variation. Figure 2.15 shows the combined, averaged results from McWilliams and van Walsum[1] and the additional points generated in this study.

Results from experiments investigating the effect of dissolving time on xylan hydrolysis are found in tables 2.3 and 2.4. Figures 2.16 and 2.17 show the same data plotted versus dissolving time. Figure 2.18 shows xylose concentrations measured in response to three different conditions for dissolving time: no extra time for dissolution, three hours for dissolving, and 800 psi of CO_2 pressure maintained during one hour prior to sealing the reactor. These three conditions represent progressively stronger conditions for dissolving

CO_2 prior to reaction. Although there appears to be a downward trend in the xylose concentration as solubilization intensity increases, the scatter in the repeats of each condition suggest that this may be arbitrary and not related to the degree of pre-reaction solubilization of CO_2. If the trend is valid, then it appears to function in the opposite, and less easily explained, direction than that indicated by the apparent trend in UV absorption.

Figure 2.19 shows results from increasing CO_2 pressure applied to the reactor when hydrolyzing xylan. It can be seen that up to an initial pressure of 200 psi the additional CO_2 offers advantages for hydrolysis. Above 200 psi the benefits of extra CO_2 diminish.

Table 2.1. Comparison of Previous (McWilliams) and Recent (Lam) pH and UV Absorbance of Aspen Wood Hydrolysate Pretreated with Hot Carbonic Acid.

	McWilliams	**Lam**		**McWilliams**	**Lam**
H2O, CO2, 180 C, 16 min	**pH**	**pH**		**UV abs**	**UV abs**
1	4.89	4.03	1	0.134	0.118
2	3.54	4.39	2	0.243	0.147
3	3.89	3.76	3	0.133	0.127
4	4.04	4.04	4	0.132	0.175
5		3.79	5		0.186
mean	4.090	4.002		0.1605	0.1506
std deviation	0.496	0.226		0.048	0.026
% std dev/mean	12.1	5.7		29.7	17.5
H2O, CO2, 180 C, 32 min					
1	4.34	3.7	1	0.197	0.197
2	3.75	3.97	2	0.325	0.213
3	3.9	4.17	3	0.144	0.152
4	4.42	3.68	4	0.122	0.216
5		3.93			
mean	4.103	3.890		0.1970	0.1945
std deviation	0.284	0.183		0.079	0.026
% std dev/mean	6.9	4.7		40.0	13.2
H2O, CO2, 200 C, 16 min					
1	5.13	3.74	1	0.24	0.316
2	3.78	3.69	2	0.451	0.221
3	4.01	3.79	3	0.304	0.237
4		3.75	4		0.228
mean	4.307	3.743		0.3317	0.2505
std deviation	0.590	0.036		0.088	0.038
% std dev/mean	13.7	1.0		26.6	15.3

Table 2.2. Comparison of Previous (McWilliams) and Recent (Lam) pH and UV Absorbance of Aspen Wood Hydrolysate Pretreated with Liquid Hot Water.

	McWilliams	**Lam**		**McWilliams**	**Lam**
H2O, 180 C, 8 min	**pH**	**pH**		**UV abs**	**UV abs**
1	4.29	3.76	1	0.087	0.122
2	3.29	4.04	2	0.135	0.13
3	4.25	3.79	3	0.076	0.085
4	4.02	3.98	4	0.169	0.12
mean	3.963	3.893		0.1168	0.1143
std deviation	0.402	0.120		0.037	0.017
% std dev/mean	10.1	3.1		32.1	15.1
H2O, 180 C, 16 min					
1	3.95	3.75	1	0.153	0.171
2	3.22	3.61	2	0.185	0.189
3	3.56	3.52	3	0.168	0.22
4	3.78	3.63	4	0.139	0.166
mean	3.628	3.628		0.1613	0.1865
std deviation	0.273	0.082		0.017	0.021
% std dev/mean	7.5	2.3		10.6	11.3
H2O, 180 C, 32 min					
1	3.85	3.34	1	0.193	0.197
2	3.34	3.35	2	0.443	0.213
3	3.56	3.67	3	0.19	0.152
4	3.65	3.68	4	0.179	0.216
mean	3.600	3.510		0.2513	0.1945
std deviation	0.183	0.165		0.111	0.026
% std dev/mean	5.1	4.7		44.1	13.2
H2O, CO2, 180 C, 8 min					
1	4.7	4.17	1	0.091	0.111
2	3.62	4.36	2	0.456	0.114
3	4.23	4.36	3	0.099	0.106
4	4.29	4.74	4	0.092	0.025
5		5.16	5		0.107
mean	4.047	4.655		0.2157	0.0880
std deviation	0.303	0.330		0.170	0.037
% std dev/mean	7.5	7.1		78.8	41.5

Figure 2.1

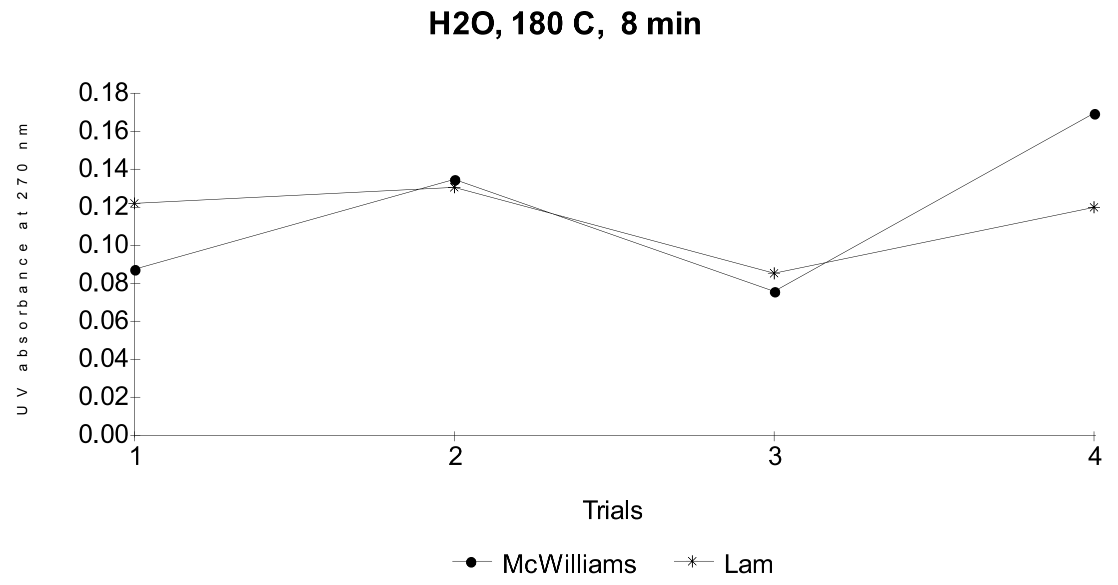


Figure 2.2

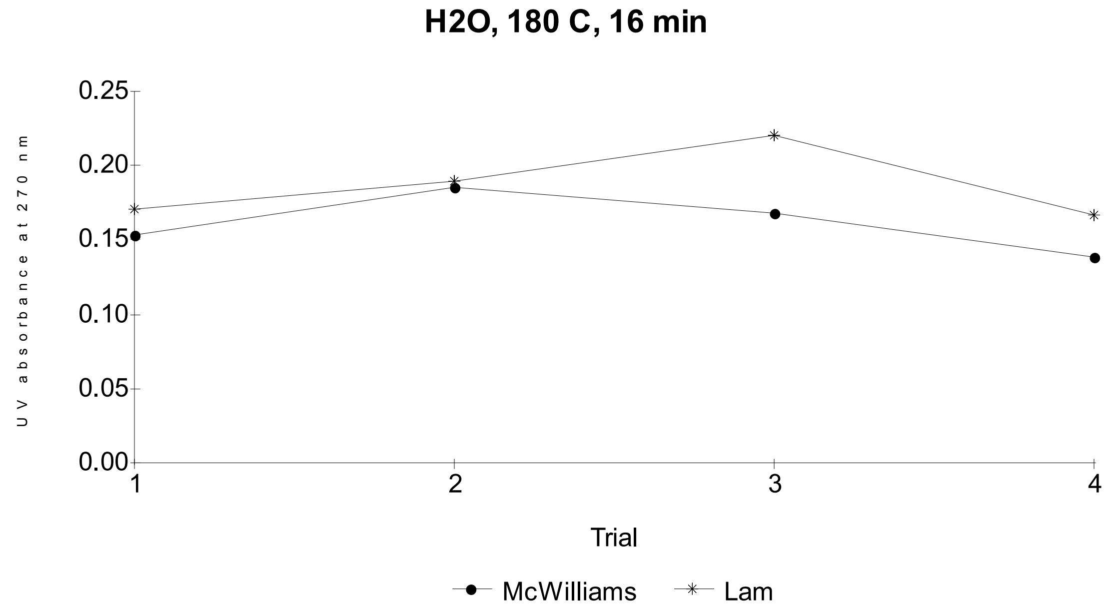


Figure 2.3

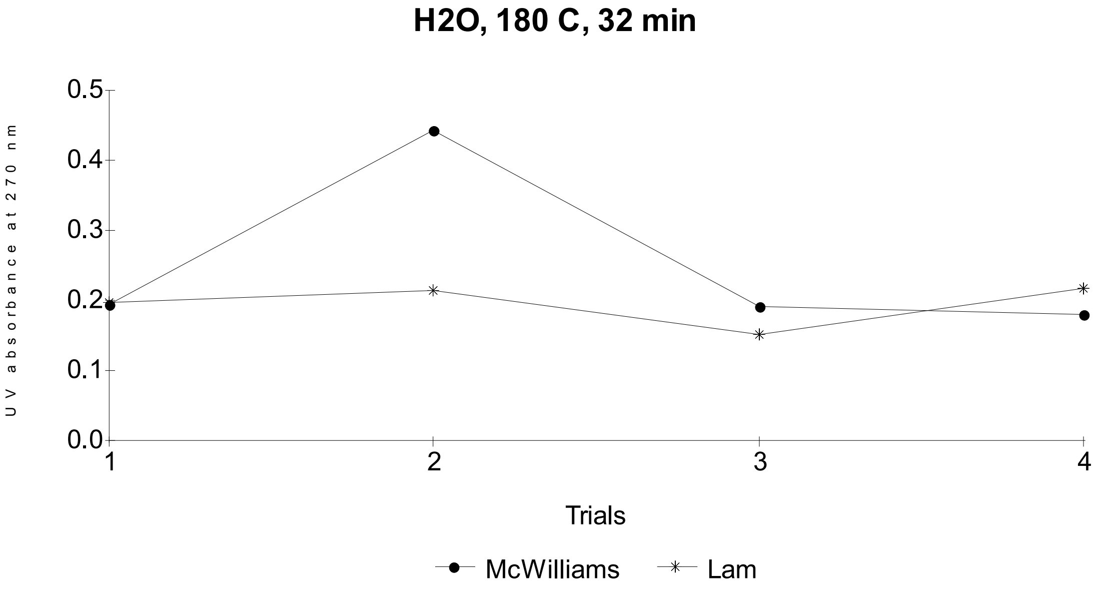


Figure 2.4

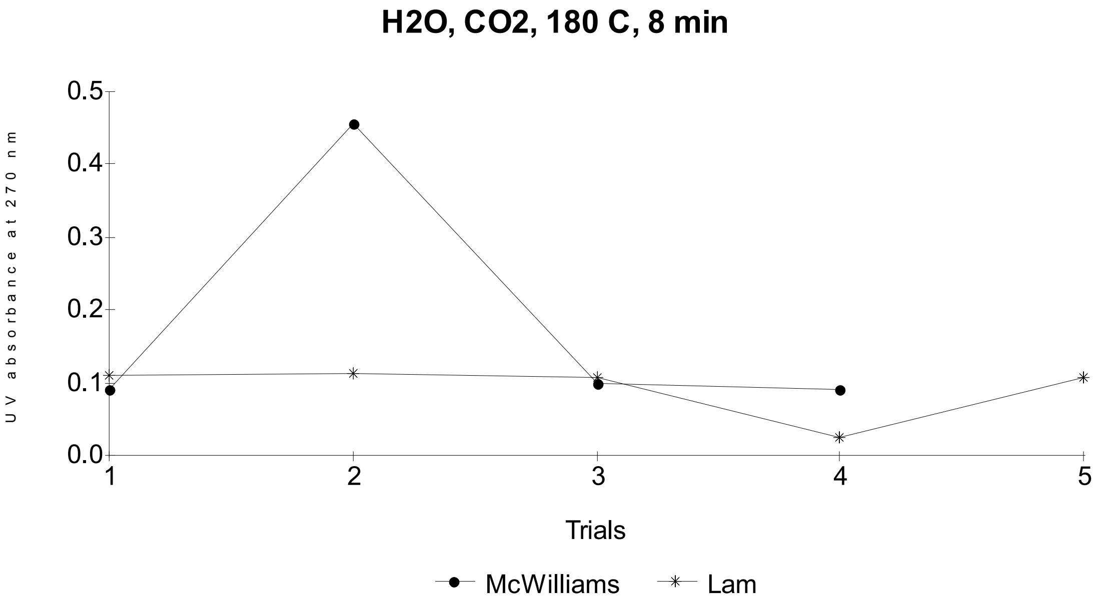


Figure 2.5

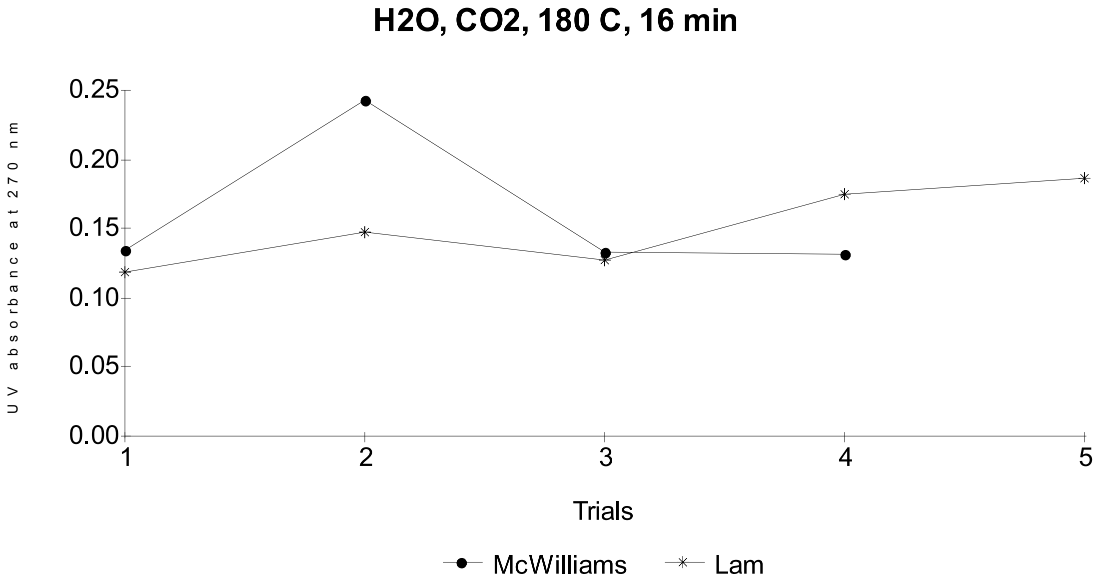


Figure 2.6

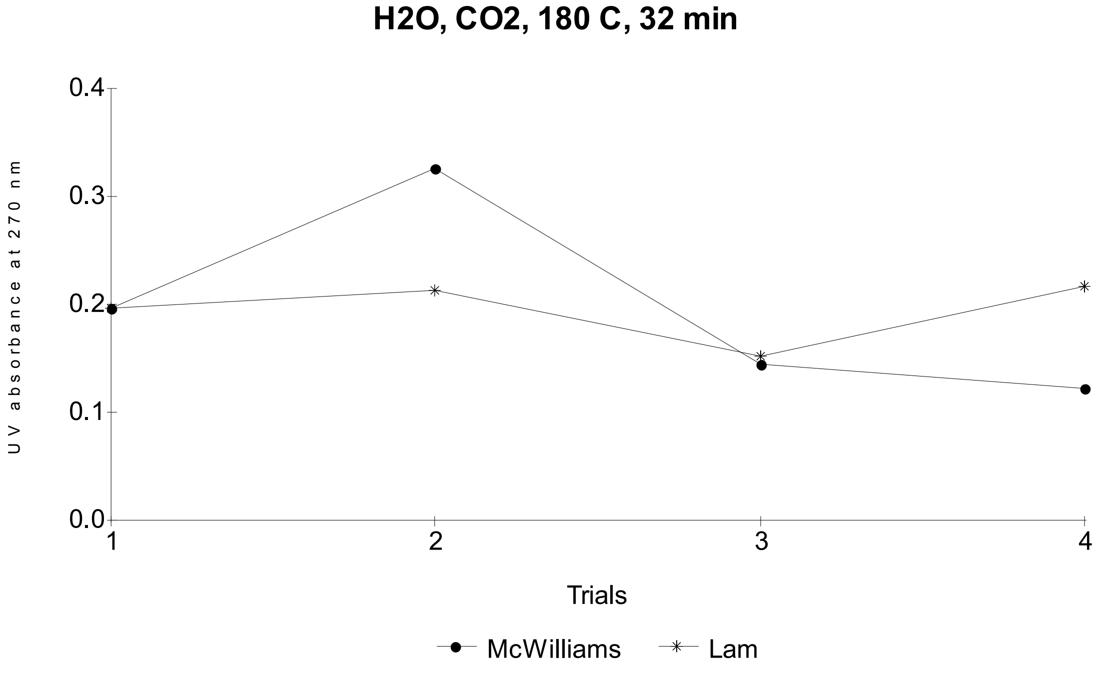


Figure 2.7

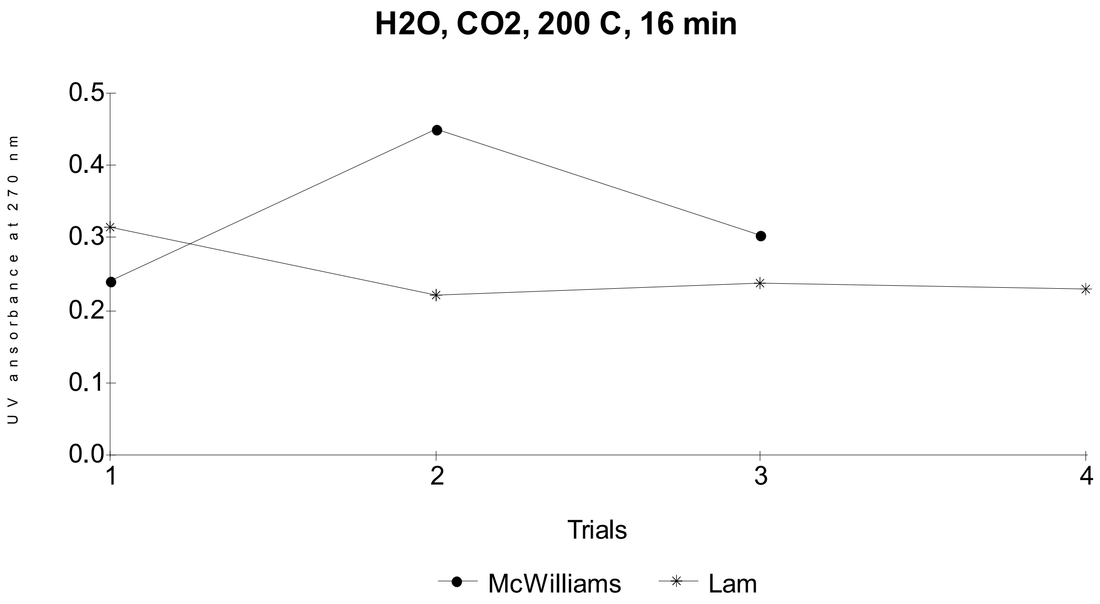
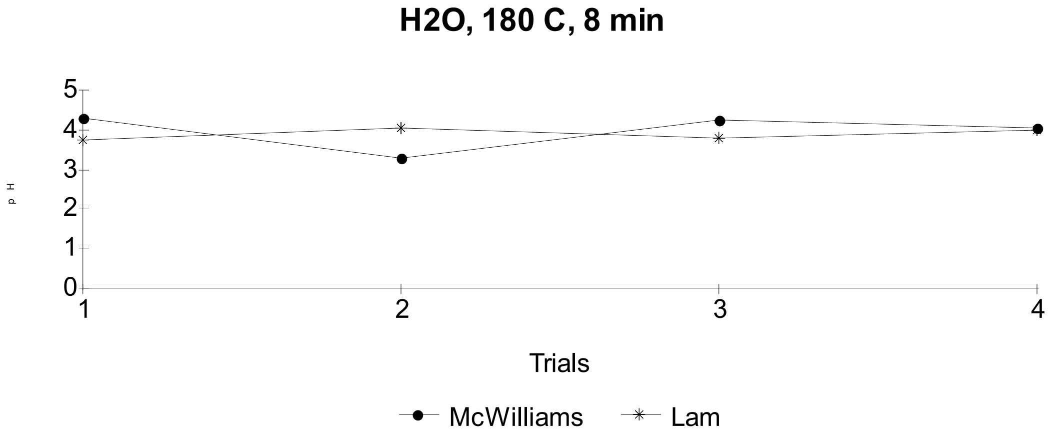

H2O, CO2, 200 C, 16 min
U V ansorbance at 270 nm
0.5
0.4
0.3
0.2
0.1
0.0
1
2
3
4
Trials
McWilliams
Lam

Figure 2.8


H2O, 180 C, 8 min
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1
2
3
4
Trials
McWilliams
Lam

Figure 2.9

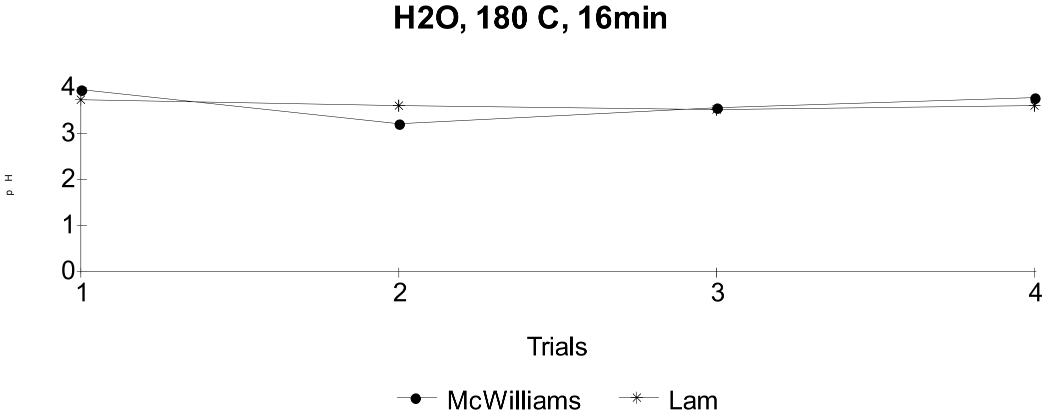


Figure 2.10

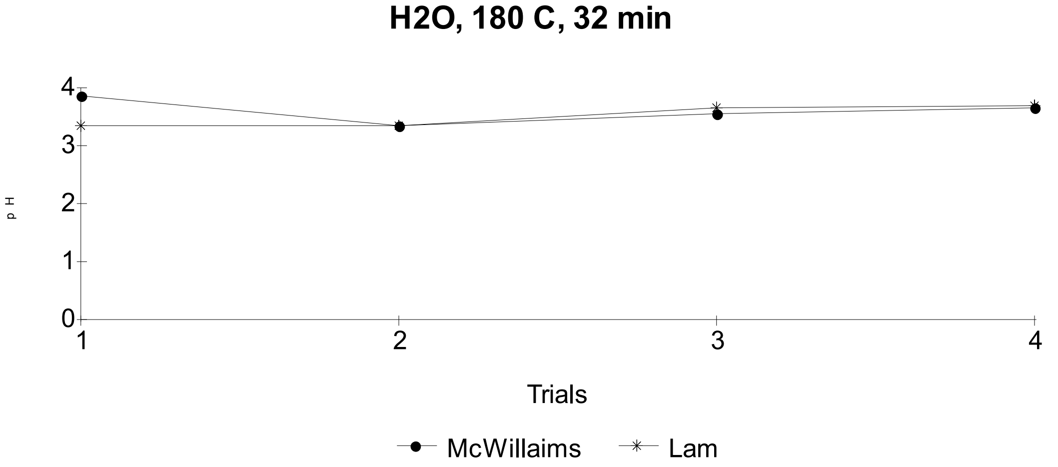


Figure 2.11

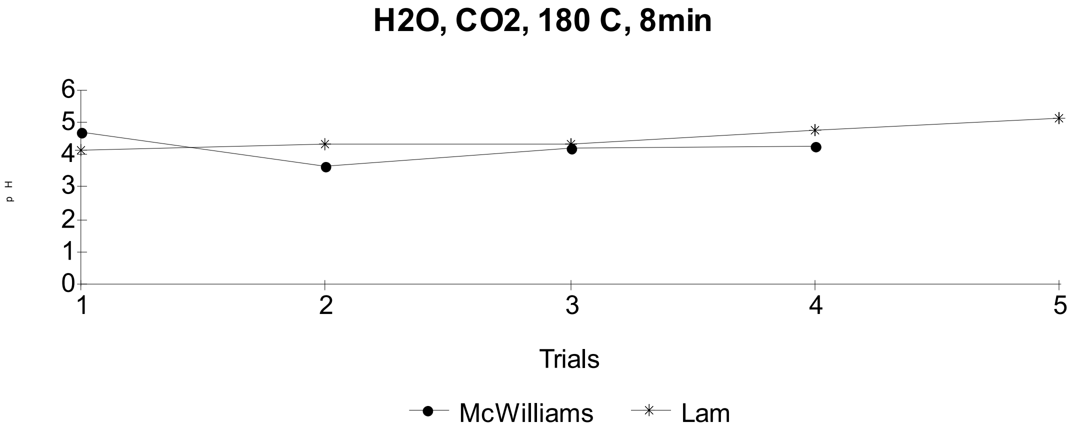


Figure 2.12

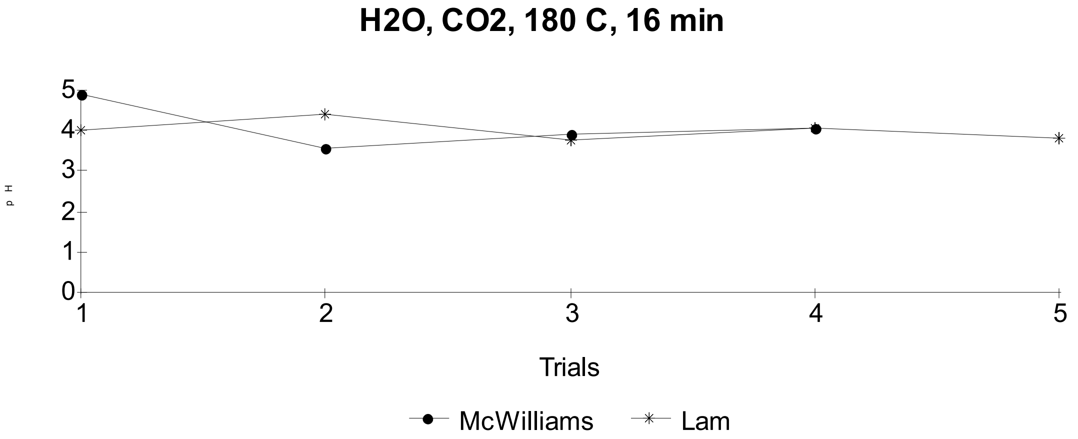


Figure 2.13

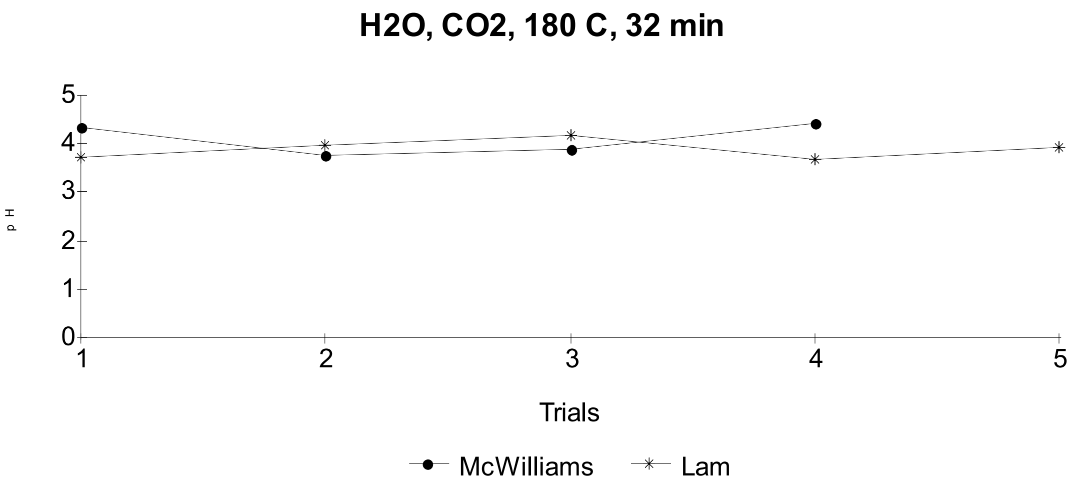


Figure 2.14

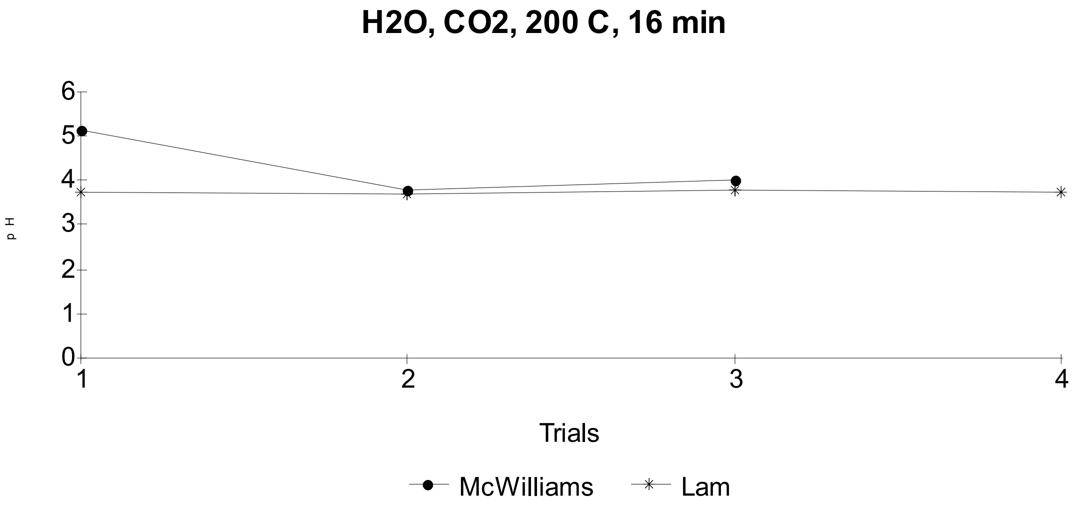


Figure 2.15 Response of UV absorbance, final pH and xylose concentrations versus reaction severity for Carbonic Acid Pretreatment of Aspen Wood: combined results from McWilliams and van Walsum [1] and this study

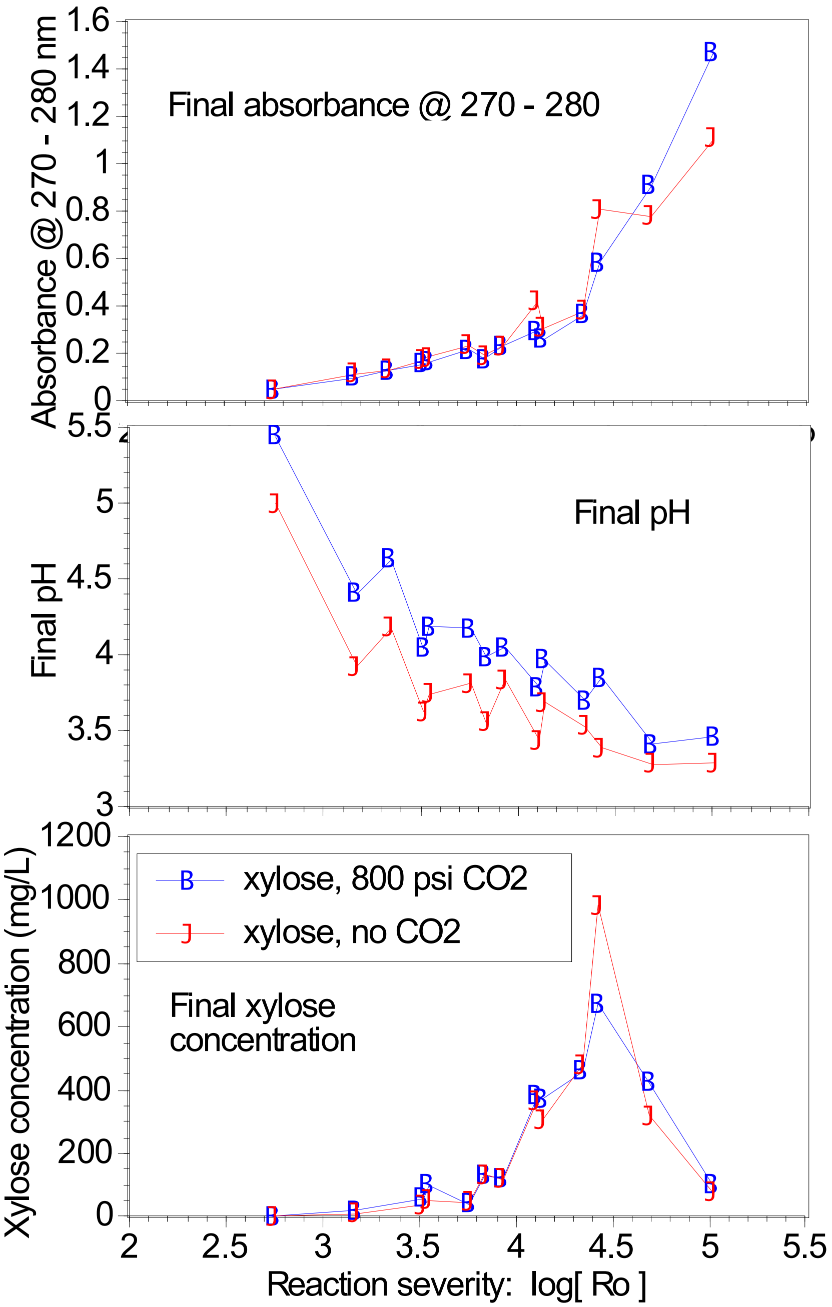


Table 2.3 pH and Absorbance Results for Varying Pre-reaction Solubilization Time

Sample	Time waited	Post reaction pH	pH	UV absorbance
1	0	4.86	5.94	0.069
2	0	5.21	6.42	0.166
3	0	4.91	6.08	0.093
4	0	4.76	5.71	0.1
5	0	5.54	6.46	0.085

Sample	Time waited	Post reaction pH	pH final	UV absorbance
1	15 minutes	5.57	5.78	0.065
2	15 minutes	5.11	5.81	0.12
3	15 minutes	5.02	6.71	0.132
4	15 minutes	5.5	6.12	0.166

Sample	Time waited	Post reaction pH	pH final	UV absorbance
1	30 minutes	4.86	6.26	0.13
2	30 minutes	4.91	6.77	0.102

Sample	Time waited	Post reaction pH	pH final	UV absorbance
1	5 hours	5.51	6.44	0.166

Sample	Time waited	Post reaction pH	pH final	UV absorbance
1	24 hours	5.24	6.35	0.141
2	24 hours	5.32	6.4	0.126
3	24 hours	5.14	6.11	0.109
4	24 hours	5.35	6.65	0.161

Figure 2.16 pH values versus Solubilization time

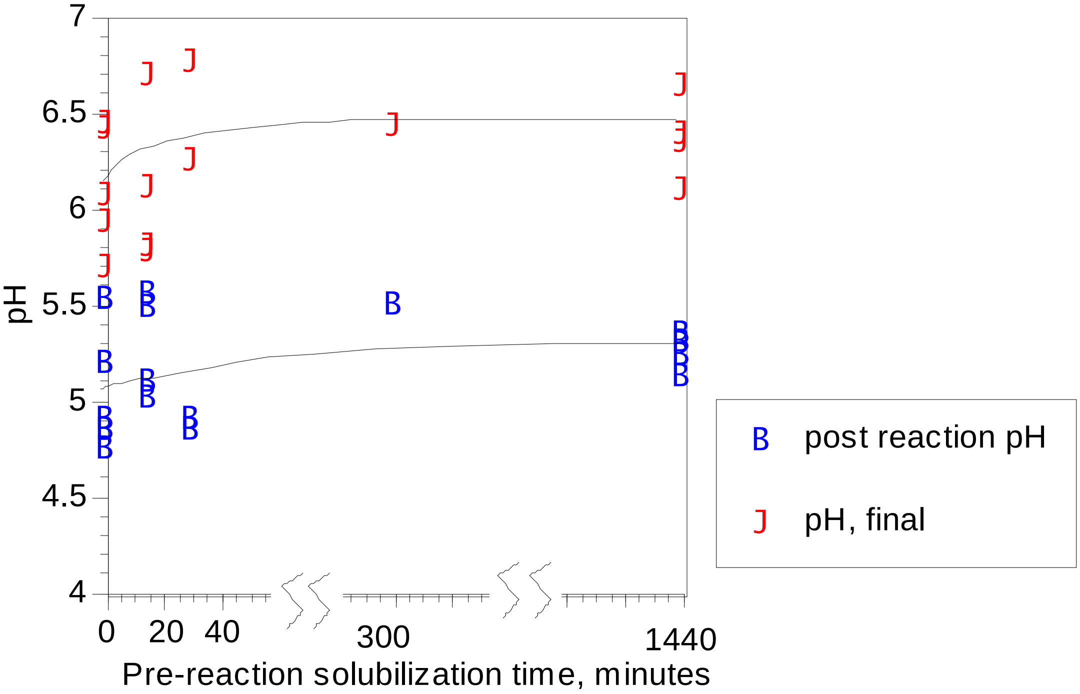


Figure 2.17 UV absorption vs Solubilization time

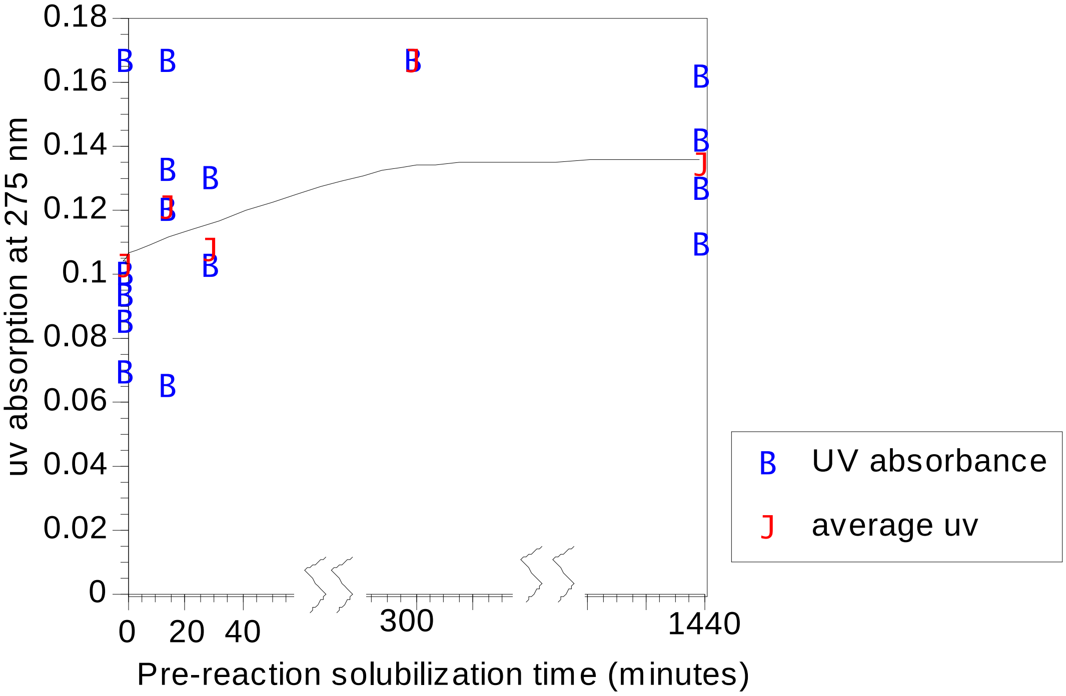


Figure 2.18 Effect on xylose concentration of increasing CO_2 solubilization prior to reaction.

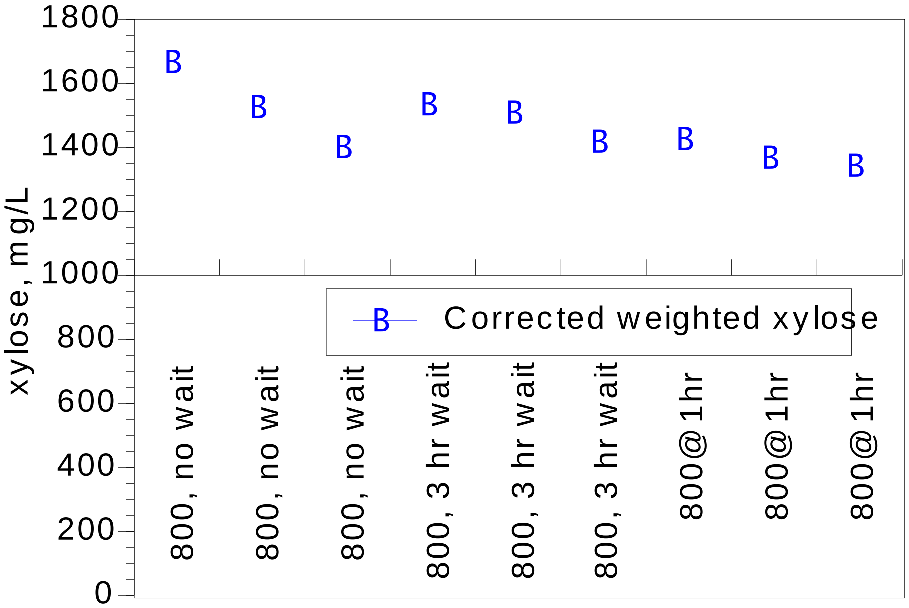
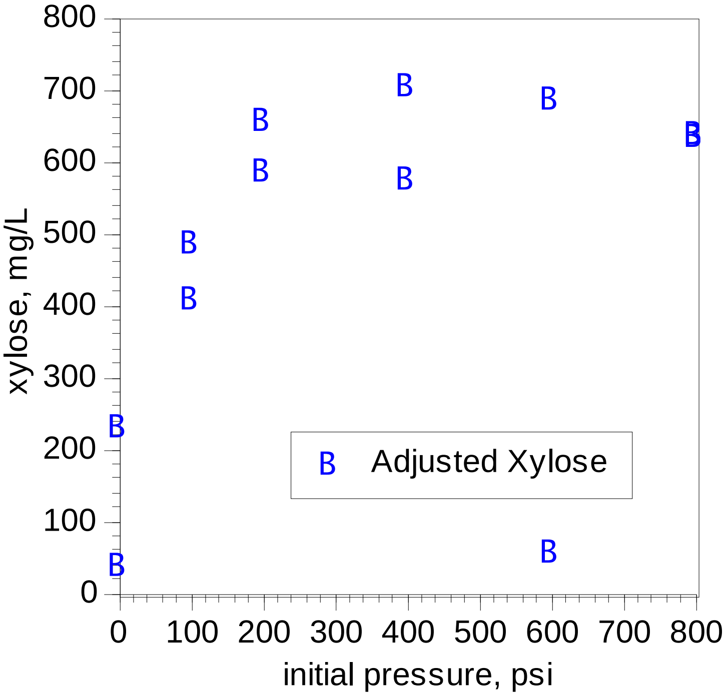


Figure 2.19: Response of xylose accumulation to increasing initial CO2 pressure.



Discussion

Tables 2.1 and 2.2 show how previous results have been confirmed and the accuracy of the experiment improved. This improvement is encouraging, since the final results include experiments carried out by three different researchers, over a period of more than one year—illustrating good reliability for the methods. Of the original data collection, the UV absorbance data were the most erratic. Repeat experiments have improved upon the pH data and have in some cases dramatically improved the UV absorption data. In most cases, the large error reported in the initial findings were due to extreme outlying points. Statistically. it is now possible to justify dropping these severe outlying points with a high degree of certainty.

For the mass transfer experiments, scatter in the data do prevent drawing any firm conclusions at this point. There does appear to be a mild trends associated with longer periods of time allowed for solubilization of the CO_2, but these trends appear to move in opposite directions for UV absorbance and xylose production, and thus are likely figments of the scatter in the data. Issues that still ought to be addressed with respect to this investigation are the amount of time needed to achieve equilibrium concentrations and whether or not super saturation of the solution is occurring in the reaction, since this could allow more acidic conditions than predicted by thermodynamic predictions of the equilibrium state of the system.

Results in figure 2.19, plotting xylose production versus CO_2 pressure are similar to those published by van Walsum in 2001[2], although van Walsum found a more continuous response to increased pressure throughout the range. It is possible that the quantity of xylose released in this more recent study was reaching a maximum due to completion of hydrolysis, as opposed to saturation with acid. However, if the observed result is more robust than this, it implies that carbonic acid pretreatment may be effective at lower pressures than currently used for most investigations. Because high pressures are expensive to implement in process conditions, this point deserves further exploration.

Conclusion

It appears that increased availability of CO_2 increases hydrolysis, but that saturation of the system prior to reaction has relatively little effect on hydrolysis. This is likely caused by the CO_2 coming out of solution as the reactor is heated up to reaction temperature. There may be a leveling off of hydrolysis enhancement at about 200 psi initial pressure of CO_2. This result should be confirmed because of its strong implications for reducing processing costs associated with high pressures.

References

[1] Robert C. McWilliams, G. Peter van Walsum, 2002. Comparison of Aspen Wood Hydrolysates Produced by Pretreatment with Liquid Hot Water and Carbonic Acid. Accepted for publication in *Applied Biochemistry and Biotechnology*

[2] Van Walsum, G. Peter, 2001. Severity Function Describing the Hydrolysis of Xylan Using Carbonic Acid. Applied Biuochemistry and Biotechnology, **91-93**: 317 - 329

TASK 3 Compare the results between hydrolysis of purified xylan and raw biomass.

John Lam, Robert McWilliams, G. Peter van Walsum

Summary

It was found that the severity function developed by Overend and Chornet [2] adequately described the action of time and temperature on the pretreatmentof aspen wood but not pure xylan. For aspen wood, no significant difference was detected between carbonic acid and water systems, thus the effect of the carbonic acid was negligible and did not need to be incorporated into the severity function. On xylan, however, it was necessary to take into account the action of the carbonic acid, and it was found that the severity function proposed by van Walsum[3] did so adequately. Published literature results for the dilute acid pretreatment of softwood [4,5] were used for comparing the fit of the severity function and the combined severity function for lower pH systems. It was found that the literature data had considerable scatter, but that the combined severity function did appear to offer a more predictive capability than the regular severity function, which does not take into account the pH of the system.

Results:

Figure 3.1(same as 2.15, above) shows how absorbance, final pH and xylose yield respond to varying reaction severity. In this case, the severity is calculated without taking into account the pH of the system. Because the results from the liquid hot water system mirror those of the carbonic acid system, it appears that the pH of the carbonic acid has no significant effect on the degree of hydrolysis. Thus, in the case of aspen wood, it appears that the simple severity function is adequate to characterize the extent of reaction.

The same result was not found with xylan. Figure 3.2 (same as 2.19 above) shows how the extent of hydrolysis for xylan is strongly influenced by the pressure of CO_2 in the reactor. In this case, the combined severity function proposed by van Walsum [3] was used to replot the data presented in figure3.2. In figure 3.3 it can be seen that the combined severity function does indeed offer a better characterization of the system than does the simple severity function, which for these data would have a single value.

Published results were used to asses the usefulness of the combined severity function at lower pH values. Reported values of dry matter remaining after hydrolysis were plotted versus the log of the severity function: log(Ro), and the combined severity function: CS = log(Ro) – pH. Results are shown in figures 3.4 and 3.5. It can be seen that the general linearity of fit is better with the combined severity function, especially at higher severities.

Figure 3.1 Response of UV absorbance, final pH and xylose concentrations versus reaction severity for Carbonic Acid Pretreatment of Aspen Wood: combined results from McWilliams and van Walsum [1] and task 2 of this study.

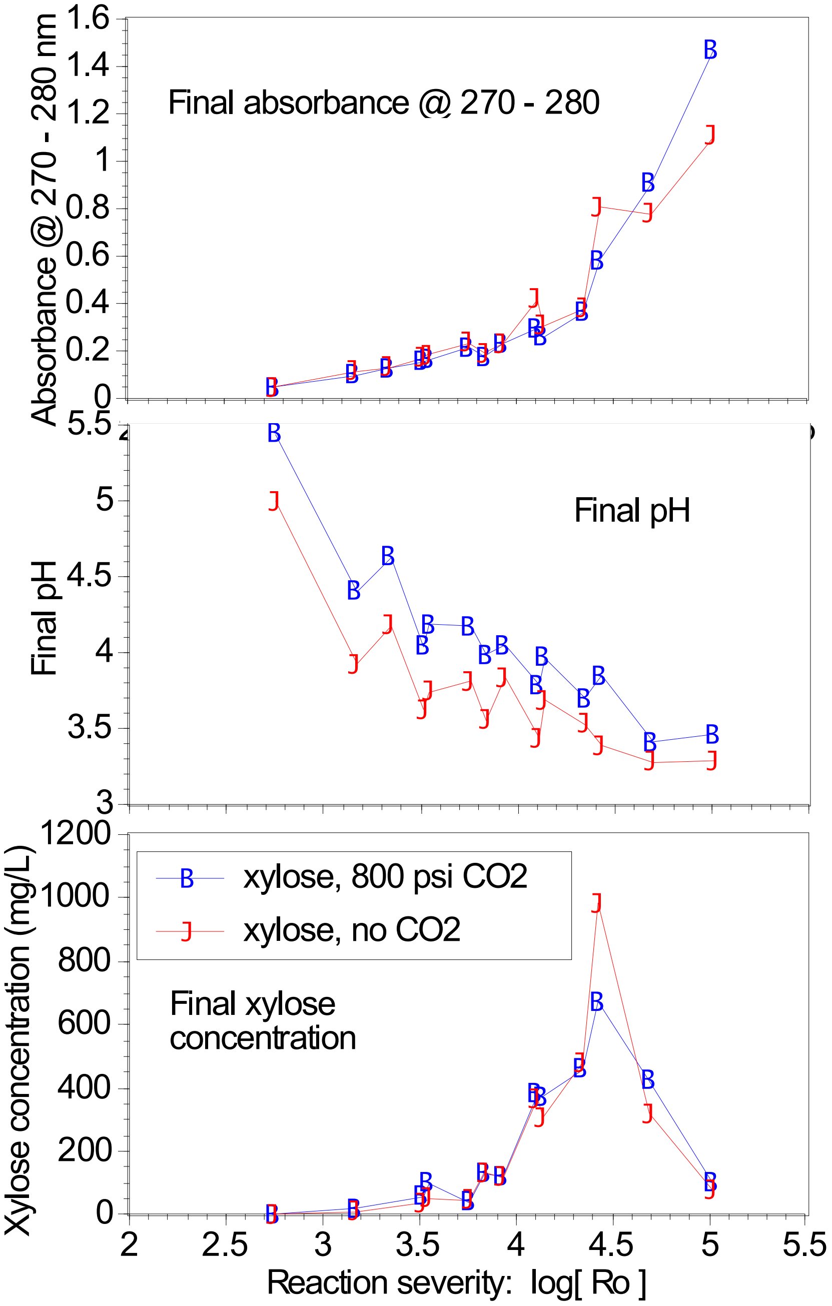


Figure3.2: Response of xylose accumulation to increasing initial CO_2 pressure.

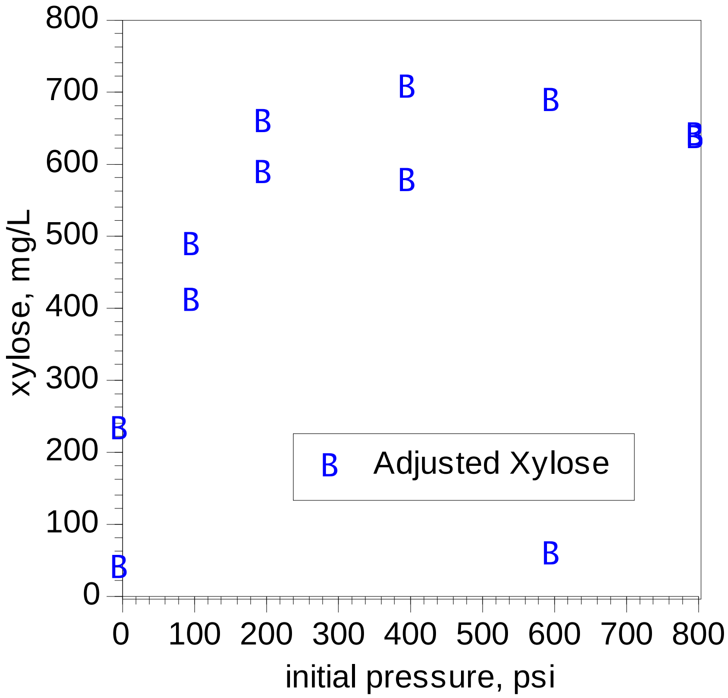


Figure 3.3 Xylose yield from hydrolysis of xylan at 190 C for 16 minutes, with combined severity reflecting varying pressures of CO_2.

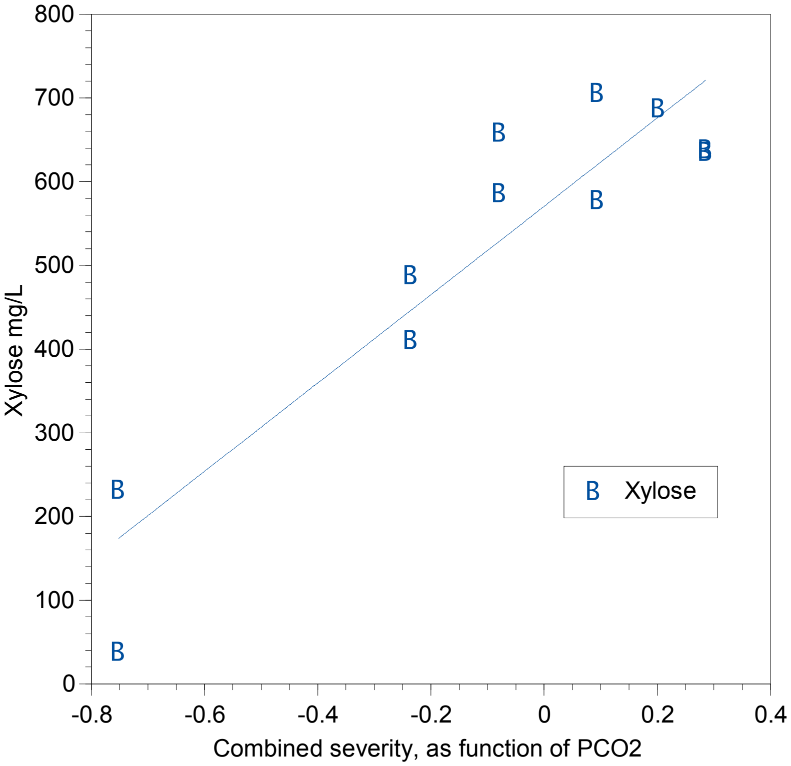


Figure 3.4 Residual dry matter versus combined severity for dilute sulfuric acid hydrolysis of pine wood [4]

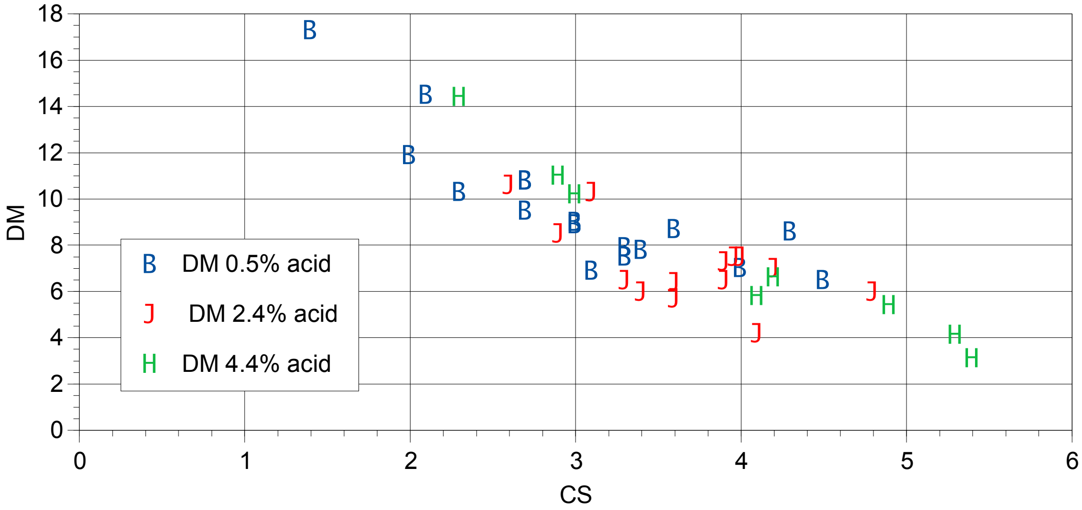
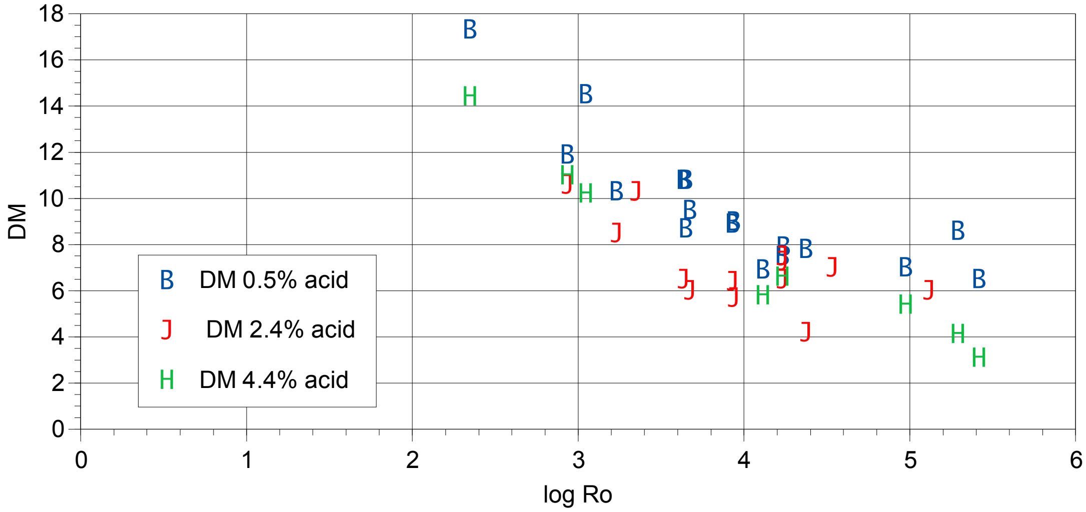


Figure 3.5 Residual dry matter versus log(Ro) for dilute sulfuric acid hydrolysis of pine wood [4]



Discussion + Conclusion

From this brief investigation of recent and previously published results, it appears that the combined severity function, which is often used to characterize acid-catalyzed pretreatments, does indeed offer a better fit to solubilization data than does the regular severity factor when pretreatment is carried out at a pH lower than that resulting from the endogenous production of acids through thermal degradation. When added acids, such as carbonic acid, result in a pH similar to that of the endogenous acid production, use of the combined severity function appears to offer no improvement over the standard severity function.

References:

[1] McWilliams, Robert C. , van Walsum, G. Peter. 2002. Comparison of Aspen Wood Hydrolysates Produced by Pretreatment with Liquid Hot Water and Carbonic Acid. Accepted for publication in *Applied Biochemistry and Biotechnology*

[2] Overend, R. P., Chornet, E. 1987. Fractionation of lignocellulosics by steam-aqueous pretreatments. Phil. Trans. R. Soc. Lond **A321,** 523-536

[3] van Walsum, G. Peter. 2001. Severity Function Describing the Hydrolysis of Xylan using Carbonic Acid. *Applied Biochemistry and Biotechnology*. **91-93:**317-329.

[4] Olsson, L. and Hahn-Hagerdal, B., Fermentation of lignocellulosic hydrolysates for ethanol production. *Enyme Microb. Technol.*, 1996. **18**: p. 312-331.

[5] Larsson, S., Palmqvist, E., Hahn-Hagerdal, B., Tengborg, C., et al., The generation of fermentation inhibitors during dilute acid hydrolysis of softwood. *Enz. Microb. Technol.*, 1998. **24**: p. 151-159.

TASK 4 – Test the performance of a larger capacity reactor

Robert C. McWilliams, G. Peter van Walsum

Summary

Preliminary experiments using a 150 mL reactor were conducted using water and a range of CO_2 pressures and reaction temperatures to evaluate the reactor performance and to determine when the pressure (and hence temperature) inside the reactor reaches steady-state. This revealed optimal reaction conditions and will minimize variation between experimental results generated from small (15 mL) and larger (150 mL) reactors. The data also provided an accurate determination of the time required for the reactor to reach reaction temperature.

Introduction

Up to this point, most experimental work with carbonic acid has been done on a small scale. This provided adequate volumes for studies of liquid hydrolysate composition, but does not provide sufficient volume of liquid or solid material to test hydrolysate inhibition or enzymatic hydrolysis rates of solids. Thus, this task served the purpose of testing the performance of a reactor to generate volume of samples for inhibition and enzymatic digestibility tests (task 5).

Materials + Methods

Apparatus and Materials:
150 mL stainless steel reactor (Swagelok Corporation), sand bath (Techne, Oxford UK, model SBL 2D) and temperature controller (Techne model TC-8D); laboratory quality de-ionized water; and carbon dioxide from a pressurized cylinder.

Experimental design: Using a 10-fold scale-up from the 15 mL reactor, 80 mL of de-ionized water was placed in the 150 mL reactor. The reactor was then placed in a sand bath at temperatures of 180°C, 200 °C, 220 °C, and 240 °C. At each temperature, initial CO_2 pressures were 0 psi, 200 psi, 400 psi, and 800 psi. While in the sand bath, internal reactor pressures were measured and recorded at the following time increments: 15 sec, 30 sec, 45 sec, 1 min, 2 min, 3 min, 5 min, 10 min, 20 min, 30 min, 45 min, and 1 hr. Pressures were evaluated to determine steady-state pressures/times at various temperatures and to correlate pressure/time intersections at different temperatures to yield a favorable pre-heat time to allow the larger reactor to be brought rapidly up to reaction temperature before placing the reactor in the sand bath at reaction temperatures.

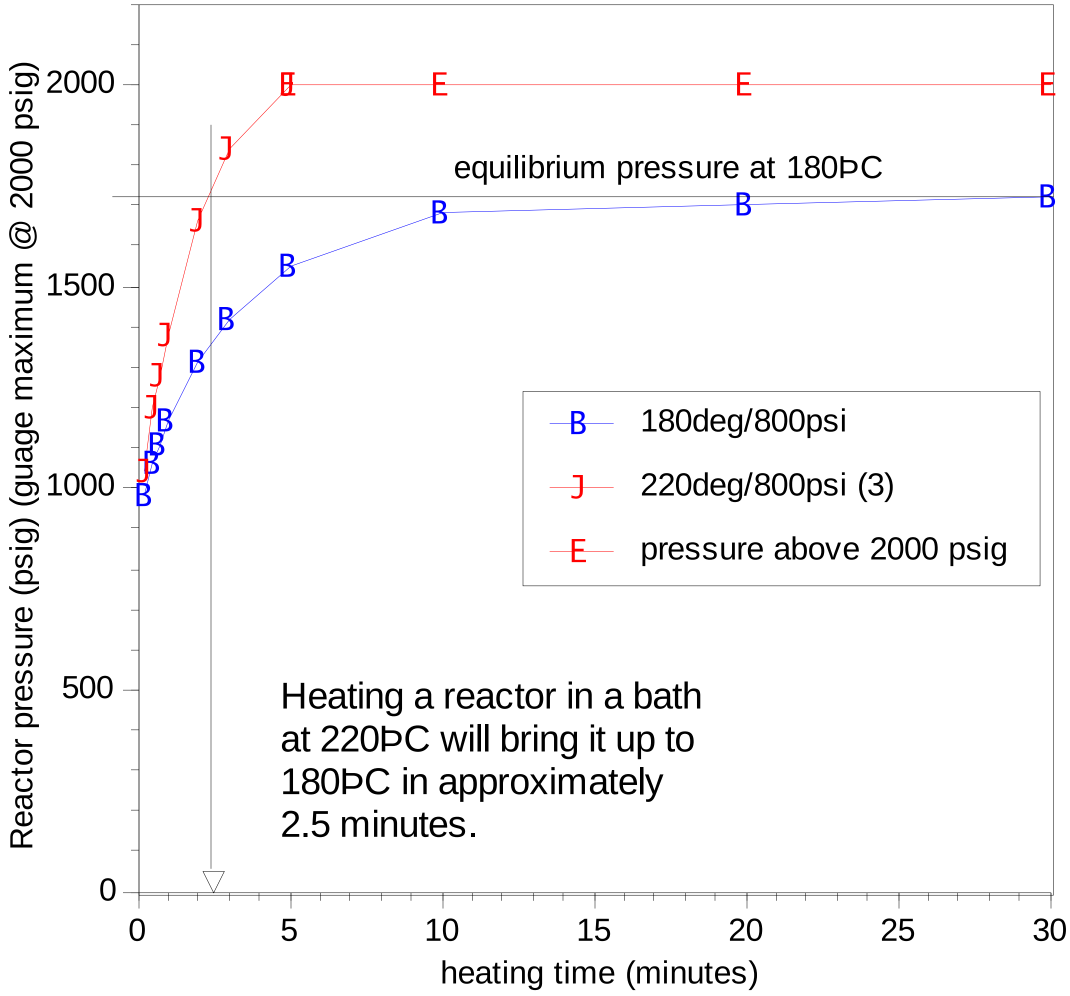
Results:

Pressures measured at various times are recorded in table 4.1. Arrows in the table indicate the time required to reach the equilibrium pressure at T – 40°C. Figure 4.1 shows pressure/time curves for the reactor at 180 and 220 °C, charged with 800 psi of CO_2.

Pressure experiment results

Temp (C)	CO_2 pres room temp (psig)	P at 15sec	P at 30sec	P at 45sec	P at 60sec	P at 120 sec	P at 3min	P at 5min	P at 10min	P at 20min	P at 30min	P at 45min
180	0	0	0		0	0	0	60	80	100	**120**	120
180	200	200	220	235	250	260	280	305	360	400	410	420
180	400	460	480	500	520	580	620	670	690	690	690	690
180	800	980	1060	1110	1170	1315	1420	1550	1680	1710	1720	**1730**
200	0 (1)	80	120	135	150	200	240	300	350	380	380	380
200	0 (2)	0	0	0	0	60	90	140	200	210	210	210
200	0 (3)	0	0	0	0	60	90	150	200	210	210	210
200	200	220	240	265	280	310	340	400	445	460	460	460
200	400	460	500	520	550	620	665	715	760	780	780	780
200	800 (1)	1020	1120	1230	1300	1520	1685	1880	2000+	2000+	2000+	2000+
200	800 (2)	980	1050	1130	1200	1380	1500	1640	1820	1900	1920	**1930**
200	800 (3)	980	1120	1260		1540	1660	1800	1940	2000	2000	2000
220	0 (1)	0	0	0	0	80	**120**	200	280	300	300	300
220	0 (2)	0	0	0	0	70	115	190	280	310	320	320
220	200	240	260	280	300	350	400	500		600	600	600
220	400	500	550	585	610	700	770	880	980	1000	1000	1000
220	800 (1)	980	1060	1150	1220	1400	1500	1640	1800	1820	1820	1820
220	800 (2)	1040	1200	1300	1420	1680	1820	2000+	2000+	2000+	2000+	2000+
220	800 (3)	1040	1200	1280	1380	1660	**1840**	2000+	2000+	2000+	2000+	2000+
240	0	0	0	0	60	140	230	350	410	460	460	460
240	800 (1)	1080	1160	1260	1400	1600	1780	2000	2000+	2000+	2000+	2000+
240	800 (2)	1040	1200	1320	1420	1760	**1930**	2000+	2000+	2000+	2000+	2000+

Figure 4.1. Determination of reactor heat up time at reaction temperature + 40°C



Discussion: At 0 psi for all temperatures, the larger reactor had a slower initial heat-up time compared with experiments conducted under the same conditions with a 15ml stainless steel reactor of similar design. Given the larger size of the reactor, this was expected. Typically, at this initial pressure it took 2 minutes for the pressure to begin to increase gradually followed by a more pronounced pressure increase and finally a "leveling-off" as the reactor's internal pressure reached steady-state. At 200 psi, 400 psi, and 800 psi, initial pressures, there was a gradual initial increase, followed by a more rapid increase between 3 and 5 minutes, then returning to a gradual increase and leveling-off between 5 and 45 minutes. This experiment provided an excellent data set to determine pressure/time intersections of reactions at different temperatures. From this data set it was determined that a 40 degree temperature increase (keeping initial pressure the same) results in reaching reaction pressure of the target reaction temperature in considerably less time. For example, a reaction at 180°C and 800 psi initial CO_2 pressure took 30 minutes to reach a steady-state pressure of 1720 psi. In contrast, a reaction at 220°C and 800 psi took between 2 and 3 minutes to reach the same pressure. Therefore, when conducting reactions at 180°C, preheating the reactor (with the water or water/CO_2, and wood already inside) at 220°C would allow the reactor to reach steady state much faster and yield more consistent results.

Conclusion: The 150 mL reactor design is adequate in design to allow the scale up of previous experiments and to produce a larger volume of product for further evaluation. Results from experiments with water alone were comparable to published steam table data at each temperature [1] and consistent with results for the 15 mL reactor under the same circumstances. The determination of an accurate preheat time and temperature allows the reactor to begin the reaction at steady-state temperature which will provide more consistency and a truer picture of the reaction a the established reaction time.

References:

[1] Chemical Rubber Company, Handbook of Chemistry and Physics, 62nd Edition, CRC press, Boca Raton, FL, 1982-2, E-17, 18

TASK 5 – Generate larger samples at selected reaction conditions

Robert C. McWilliams, G. Peter van Walsum

Summary

The newly constructed 150 mL reactor was used in reactions that replicated the conditions of the 15 mL reactor. 1.0 grams of aspen wood, 80 ml of de-ionized water were reacted with and without CO_2 at 800 psig. Reaction temperatures were 180°C, 200°C, and 220°C with reactions times of 8, 16, and 32 minutes. The reactor was preheated in a sand bath set to a temperature 40°C above reaction temperature for two minutes. This allowed the reactor to quickly reach reaction temperature, as determined and reported in the previous progress report. The 150ml reactor successfully delivered the expected 10-fold increase in hydrolysate compared to the 15ml reactor. Results of the pH and UV analysis of the hydrolysate were consistent with those yielded by the 15ml reactor.

Introduction

Up to this point, most experimental work with carbonic acid has been done on a small scale. This provided adequate volumes for studies of liquid hydrolysate composition, but does not provide sufficient volume of liquid or solid material to test hydrolysate inhibition or enzymatic hydrolysis rates of solids. This task served the purpose of testing generating volume of samples for inhibition and enzymatic digestibility tests (tasks 6 and 7).

Materials + Methods

Apparatus and Materials: 150 mL stainless steel reactor (parts supplied by Swagelok Corporation); two sand baths (Techne, Oxford UK, model SBL 2D) and temperature controllers (Techne model TC-8D); laboratory quality de-ionized water; and carbon dioxide from a pressurized cylinder, ground Aspen wood using a domestic coffee grinder, stainless steel sieve (2 mm to 500 micron mesh), pH meter (Acumet AR-15, Fisher Scientific), spectrophotometer (Beckman, DU-600), centrifuge (Eppendorf) laboratory balance (Ohaus, Explorer)

Experimental: Aspen wood chips are ground using a domestic coffee grinder and sifted in the stainless steel sieve. The wood particles are allowed to separate and the particles between 0.5 mm and 1 mm are retained for experimental use. 1 gram samples of the 0.5 – 1 mm wood particles are weighed and placed in the reactor. In addition to the wood, 80 mL of water is added. The reaction temperatures (180, 200, 220°C) and times (8, 16, 32 minutes) selected are based on previous results using the smaller reactor. At each of these sets of conditions, reactions were run using wood and water alone (as previously described) and wood, water, and CO_2 at 800 psi. The reactor was placed in one sand bath set at a temperature 40 degrees Celsius higher than the desired reaction temperature for three minutes. This allowed the reactor to reach steady-state before immersion into the sand bath at reaction temperature. The pH of the hydrolysate was measured. Finally, spectrophotometric measurements of the hydrolysate (1% solutions) were made using the spec ength 190 nm and 350 nm. Absorption peak compar s with and without CO_2 to evaluate the c produc entially inhibitory to enzymatic dige

Resu

Tabl illustrate data generated to date using the larger volume react

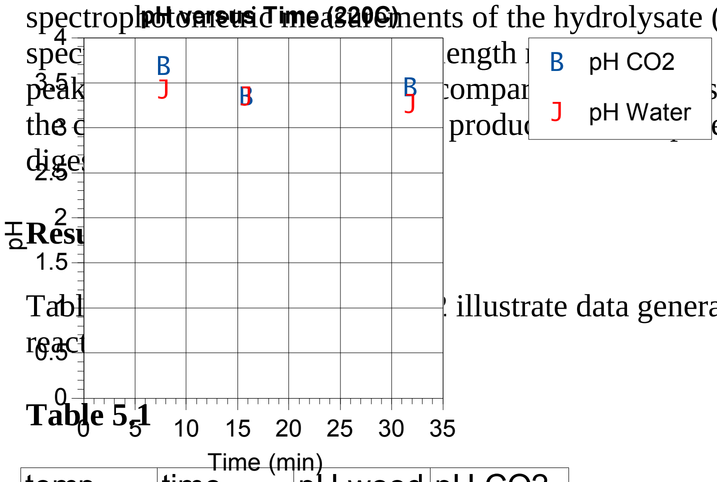


Table 5.1

temp	time	pH wood	pH CO2
200C	8	3.81	3.76
200C	16	3.51	3.96
200C	32	3.44	3.58
220C	8	3.68	3.42
220C	16	3.34	3.34
220C	32	3.44	3.26

Figure 5.1

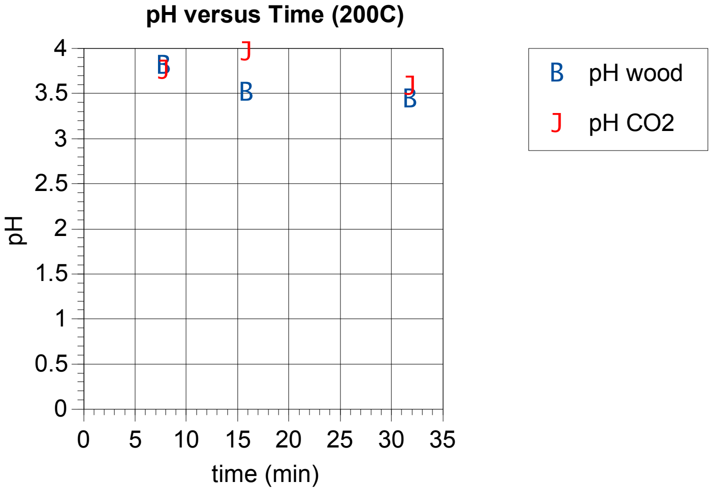


Figure 5.2

Discussion and Conclusion: The 150ml reactor met all performance expectations generating larger volumes of hydrolysate which was consistent with hydrolysate yielded by the smaller reactor. This larger volume of hydrolysate will enable faster analysis of toxicity and an applied understanding of the composition of the hydrolysate.

Task 6: In vitro determination of inhibition

Damon Yourchisin, G. Peter van Walsum

Summary

Inhibition tests measured the rate of sugar consumption by *Saccharomyces cerevisiae* growing in batch culture of hydrolysate. It was found that inhibition of the yeast culture increased with severity of pretreatment above a mid level severity. Below this severity, little to no inhibition was observed. No difference was observed between the inhibition of hydrolysates prepared either with or without the presence of CO_2. To conduct the experiments, serum vials were charged with 21g/L of sterile growth medium containing 20ml of pretreatment hydrolysate. The vials were inoculated with 0.2ml of freshly grown cell broth and incubated. Glucose concentrations over time were determined via glucose assay (Infinity Glucose Reagent) and the HPAE when available.

Introduction

The organisms used for the fermentation of ethanol are often inhibited by the degradation products produced during pretreatment. Enzymatic hydrolysis can also be impaired by inhibitors produced during pretreatment. Inhibitory compounds originate from: the hydrolysis of extractive components, organic and sugar acids (e.g. acetic, formic, glucuronic, galacturonic) esterified to hemicellulose; solubilized phenolic lignin derivatives; the degradation of solubilized compounds (e.g. furfural, hydroxymethyl furfural); and the release of corrosion products (e.g. metal ions)]. The production of inhibitors has been documented for dilute-acid, steam-explosion, acid-hydrolysis and liquid hot water pretreatments. To date no published studies have looked at inhibition resulting from carbonic acid pretreatment. Treatments to eliminate or reduce inhibitors, such as ion exchange and over-liming, add considerable expense to processing systems.

Materials + Methods

Apparatus and materials

Two Techne Fluidised Sand Baths, model SBL-2d, with temperature controllers.
Beckman DU 520 General Purpose UV/Vis Spectrophotometer (with single cell module)
Fisher Scientific AR15 pH meter by Accumet Research
150ml stainless steel immersible reactors
Eppendorf Reference series auto pipettes
Ohaus Expolorer digital scale Item #12140 (d=0.1mg)
Domestic brand coffee grinders
Fisher Scientific U.S.A. Standard Testing Sieve, ASTME-11 Spec #18 (1mm opening)
Standard laboratory de-ionized quality water
Standard laboratory grade carbon dioxide
Vacuum filter with water aspirator
Schleicher and Schuell Sharkskin 100Cir. 5.5cm Microfilter paper
Sigma Yeast Malt Agar @ 41g/L
Sigma Yeast Malt Broth @ 21g/L
Fleischmann's Active Dry Yeast

Fisher Scientific Marathon 21000R centrifuge
Market Forge Sterilmatic STM-E autoclave
Thermolyne MaximixII type 37600 mixer
SIGMA Diagnostics - Infinity™ Glucose Reagent (Procedure 17-UV) Kit
Glass serum vials (50mL)
Aspen wood chips (provided by USDA Forest Products Laboratory in Madison, WI)
Sigma D-Glucose Anhydrous

Experimental Methods

The experiments followed a general methodology regardless of reaction conditions. The general steps included: grinding the feedstock, pretreatment, preparation of hydrolysate, separating the solids from the liquids, microbial culturing, inhibition tests, analysis of the samples. These steps are detailed below and summarized in figure 6.1.

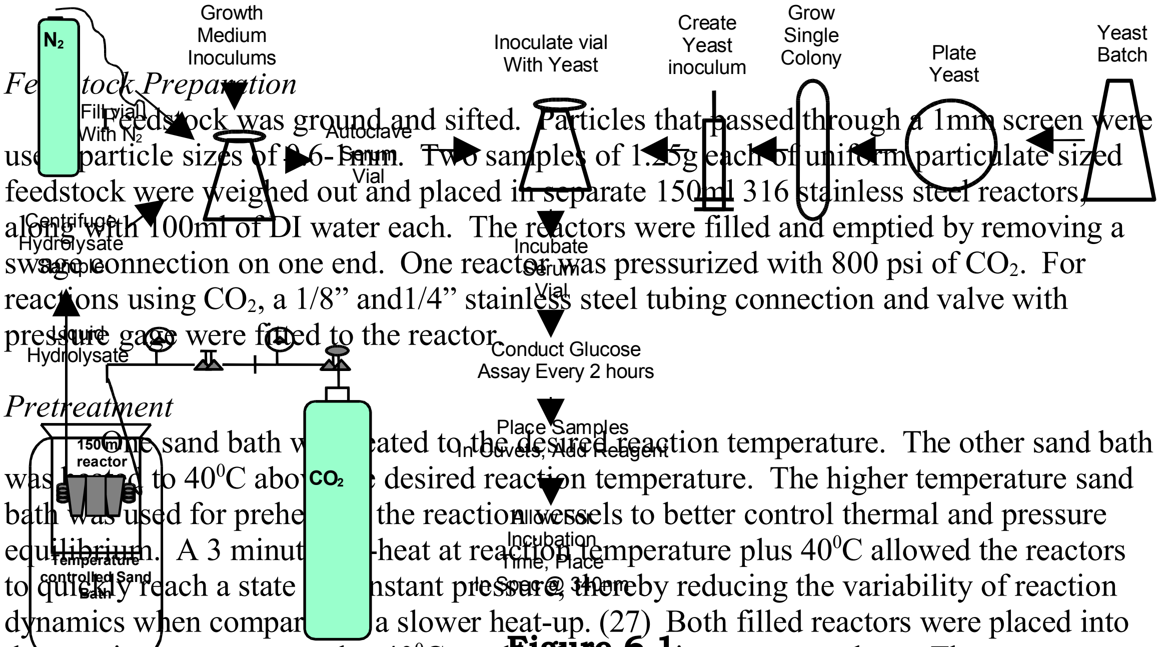


Figure 6.1

Feedstock Preparation

Feedstock was ground and sifted. Particles that passed through a 1mm screen were used, particle sizes of 0.6-1mm. Two samples of 1.25g each of uniform particulate sized feedstock were weighed out and placed in separate 150ml 316 stainless steel reactors, along with 100ml of DI water each. The reactors were filled and emptied by removing a swage connection on one end. One reactor was pressurized with 800 psi of CO_2. For reactions using CO_2, a 1/8” and1/4” stainless steel tubing connection and valve with pressure gage were fitted to the reactor.

Pretreatment

One sand bath was heated to the desired reaction temperature. The other sand bath was heated to 40^{0}C above the desired reaction temperature. The higher temperature sand bath was used for preheating the reaction vessels to better control thermal and pressure equilibrium. A 3 minute pre-heat at reaction temperature plus 40^{0}C allowed the reactors to quickly reach a state of constant pressure, thereby reducing the variability of reaction dynamics when compared to a slower heat-up. (27) Both filled reactors were placed into the reaction temperature plus 40^{0}C sand bath for 3 minutes as a preheat. They were immediately removed and placed in the reaction temperature sand bath for the desired reaction duration. The reaction was quenched in an ice bath immediately after the reaction duration time period was complete.

Reaction times consisted of short durations (4-8 minutes), a mid-range duration (16 minutes), and long-range durations (32-64 minutes). Pretreated samples were extracted from the reactors and placed in glass collection bottles for the next step.

Preparation of hydrolysates

Solids were filtered out from the hydrolysate samples generated using vacuum filter and microfilter paper. The solids remaining on the filter paper were washed with DI water 3 times (12ml DI water total). During the final rinse the vacuum filter was allowed to work for 3 minutes and then shut off to collect solids. The solids were placed in weighing tins in preparation for enzymatic hydrolysis experiments. The liquids were then centrifuged to remove all solids. The original liquid was placed into centrifuge bottles and centrifuged at 4000rpm for 15minutes at 15^{0}C. The centrifuge bottles were emptied using a pipette, to ensure no solids were present, into clean collection bottles. The liquid hydrolysate was now prepared for experimentation.

Microbial culturing

A new batch of yeast agar, yeast broth, and yeast were prepared for each experiment according the manufacturers directions. Yeast agar and broth were prepared in the following manner: 500ml of water were brought to a boil and 20.5grams of yeast agar or 10.5grams of yeast broth were placed into it. Once all solids were dissolved, the mixtures were autoclaved. After autoclaving, 20ml of the agar were aseptically transferred from the autoclaved flask to a Petri dish and 10ml of the broth were aseptically transferred from the autoclaved flask to a culture tube under the laminar flow hood. Three plates and three culture tubes were created each time to ensure growth and to act as a back-up during each step if contamination occurred. A new batch of bakers yeast was prepared according to manufacturers directions: 2 1/4 teaspoons of yeast was added to 1/4 cup of water at 1000F with 1 teaspoon of glucose. The mixture was let to stand for 10 minutes. Once growth of yeast was confirmed, they were plated for isolation under the laminar flow hood using the fresh yeast agar plates. After plating, the Petri dishes were placed in the incubator for 48 hours at 30^0C along with the culture tubes of broth (although nothing was in them yet). After 48 hours, the plates and broth culture tubes were removed and put under laminar flow hood in order to aseptically transfer a single yeast colony from the agar plate to the broth culture tube. An isolated colony from the plate was aseptically transferred into the 10ml of broth in the culture tube using a loop. The plates were properly disposed of and the culture tubes were placed into the incubator at 30^0C for 48 hours.

Preparation of test vials

Twenty milliliters of the previously generated liquid hydrolysate were placed into serum vials. 20ml was the maximum amount of liquid per vial. The vials contained from 0% to 100% hydrolysate. The remaining 20ml of the less than 100% hydrolysate was made up using DI water. Yeast broth at 21g/L (0.42g) was weighed and placed into each serum vial to serve as a known growth medium. Prior to autoclaving the serum vials N_2 gas was bubbled in them for 30 seconds. Each vial was capped immediately and sealed after removal from the N_2 injector. Once all vials were sealed, they were placed in the autoclave for 20 minutes at 121^0C. The pH for each of these samples was tested and found to be within the tolerance range of yeast, as shown in table 6.1 below for the most recently performed experiment.

Table 6.1 pH of hydrolysates prior to yeast cultivation

Exp 138:pH of hydrol+nutrient	
Liquid Samp	pH
T/t/P	
180/8/0	5.69
180/8/800	5.87
200/16/0	5.04
200/16/800	5.05

220/4/0	5.25
220/4/800	5.39
180/64/0	5.01
180/64/800	4.97
0% hydrol	6.31

Inhibition test

Once the vials were cooled they were placed in the incubator to bring them to the same temperature as the yeast broth that would be transferred to them. Once the yeast broth had been incubated for exactly 48 hours, the serum vials were each injected 0.2ml of the vortexed yeast inoculum from the culture tubes using a 1ml syringe. This was performed under the laminar flow hood and each serum vial top was flamed prior to needle insertion. This became time zero (T=0) for the experiment. Samples were taken for the next 12 hours every 2 hours, except for the first 4 hours, T+4 was the second measurement.

Glucose Assay

At each testing period, 0.2ml of sample was removed from the serum vials and place into a 1ml microcentrifuge tube. The sample was diluted 5:1, so 0.8ml of DI water was added to each centrifuge tube. This was done because the yeast broth contained 10g/l dextrose and the glucose assay was only good for up to 5g/l, but 2g/l was best. Next, 1500 microliters of glucose reagent was placed into a 2ml cuvette for each sample, minimizing the time the glucose reagent is out of the refrigerator as much as possible. Using the spectrophotometer, UV measurements of the reagent only were taken and recorded. This was done by placing each cuvette in the spectrophotometer set at 340nm absorbance and measuring the AUs. Then, 15 microliters of each sample were removed and placed in their respective 2ml cuvettes. The cuvettes were covered and incubated for 14 minutes (ambient room temperature should be between 20^{0}C and 22^{0}C). Serum vials were immediately placed in the incubator at 30^{0}C . After 14 minutes, each cuvette was again placed in the spectrophotometer set at 340nm absorbance and was measured and recorded. The recorded AUs of each cuvette with reagent only were subtracted from the recorded AUs of the cuvettes with sample and reacted reagent. A glucose standard of 2g/l was run at each sampling time in order to compare the samples to a known standard to determine glucose generated throughout the experiment. At T+24 the dilution was only 1:1 for the samples and at T+48 there was no dilution of sample prior to analysis with the assay. The glucose assay was found to be linear up to 3g/L of glucose.

This information was used when calculating theoretical glucose yield of samples prior to experimentation to ensure they would be within the range of the assay's testing limits. It was also used to determine the correct g/L of glucose standard to run as a standard for each experiment, which was chosen to be a constant 2g/L for each experiment. A control was also used for each yeast inhibition experiment by running a sample that contained 0% hydrolysate but all the other components of the nutrient broth.

Results

Data were analyzed using the DU 500 Spectrophotometer, pH meter and Infinity Glucose Reagent Assay kit. Two metrics were used, total recovered glucose and reaction speed. The raw numbers were averaged amongst duplicates within experiments and graphed (Absorbance Units versus time, transposed into Glucose yield versus time).

Figure 6.2 shows that there is no effective difference in the yeast growth rates in hydrolysates produced either with or without the presence of CO2. These experiments were carried out at the mid point severity of the study, at 200 C and 16 minutes reaction time.

Figure 6.2

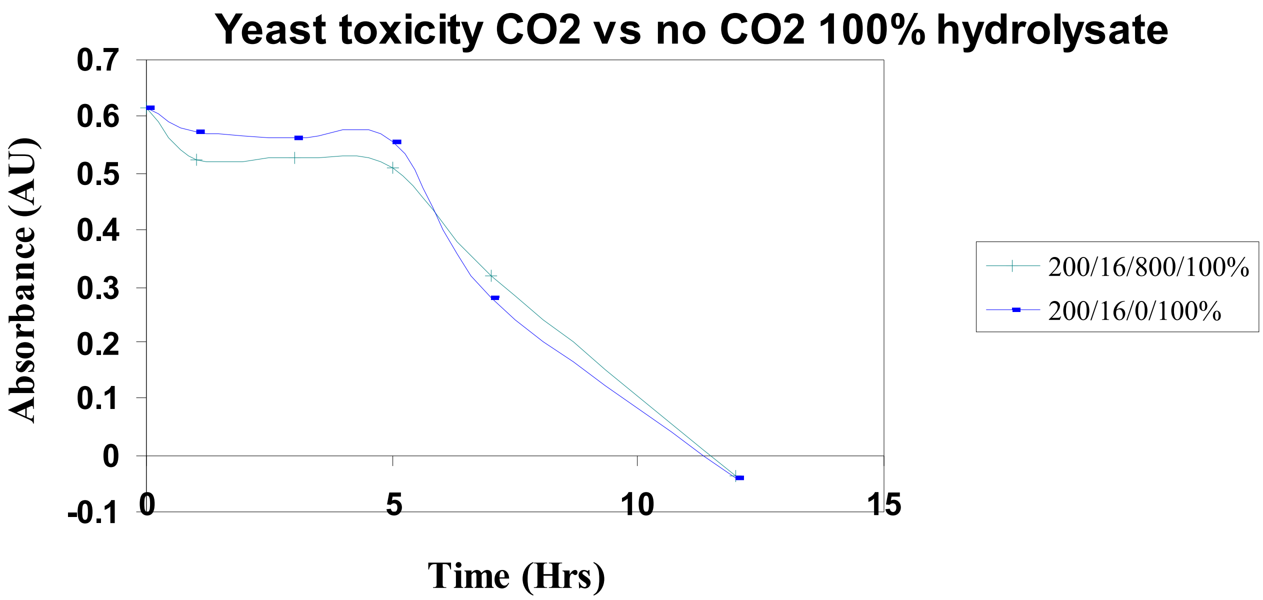


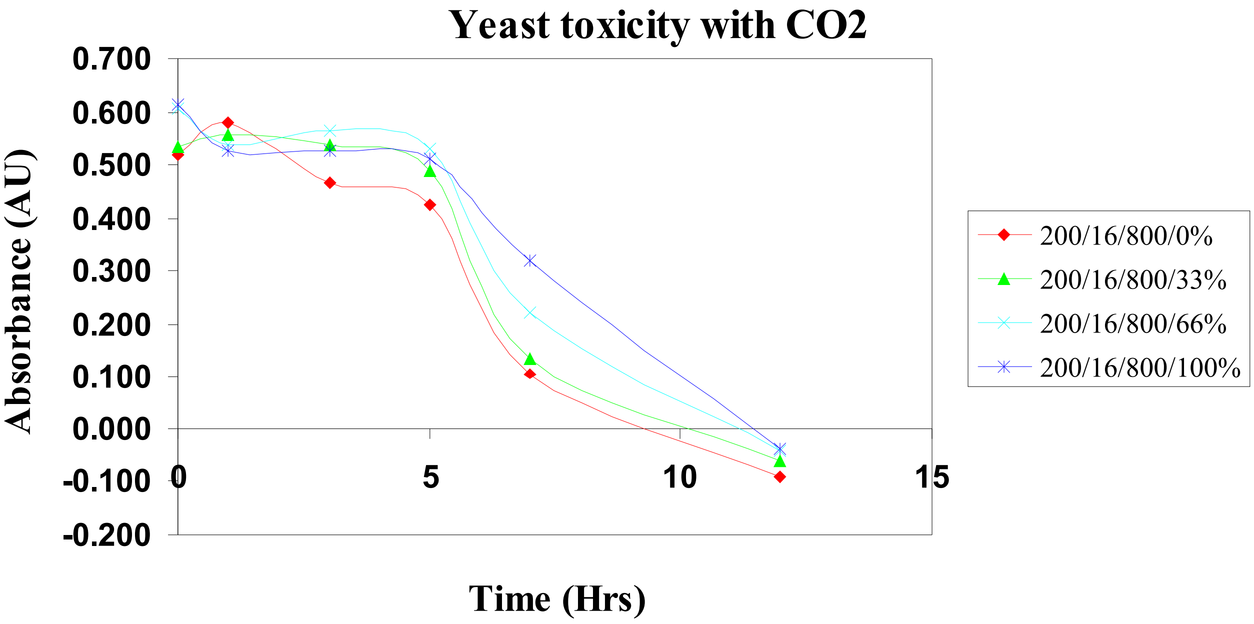
Figure 6.3 below, again shows that there is no difference between samples that contain CO_2 and samples without CO_2. In this case, a marked difference is evident in the inhibition of the yeast by hydrolysates produced at high (220 C, 32 minutes) and low (180 C, 8 minutes) severities.

Figure 6.3

At the lowest tested severity parameter of 180^0C for 8minutes, there was almost no inhibition when compared to the control that was run at each experiment. However, at the highest tested severity parameter of 220^0C for 32minutes, there was an almost 50% reduction in the speed of glucose consumption by the yeast. This division of inhibition as it correlates to severity is well repeated among the samples.

Figure 6.4 illustrates the effect of dilution of the inhibition by the hydrolysates. It can be seen that diluted hydrolysates are less inhibitory, thus there appears to be a relatively continuous dose-response to the inhibitors.

Figure 6.4



Discussion

The midpoint pretreatment condition of 200^{0}C for16minutes could be an optimal condition for reduced microbial inhibition. For severities around the midpoint and below, there was no improvement in the reduction of microbial inhibition. A possible explanation for this result is that at the midpoint and below conditions, there are not enough inhibitory compounds released to effect the hearty yeast organisms. However, the more severe reaction conditions, above the midpoint, are continuing to breakdown the biomass and releasing more and more inhibitory compounds into the hydrolysate, thereby inhibiting the yeast by making their environment more difficult to survive in, but not impossible and not killing them.

Conclusion

Pretreatment with carbonic acid showed no significant advantage when compared to pretreatment with liquid hot water. Differences between the samples could be attributed to testing error since in some cases the samples with carbonic acid showed reduced inhibition rates and yields and in other cases they showed an increase in inhibition rates and yields.

Task 7: Determine enzyme digestibility of pretreated solids

Damon M. Yourchisin, G. Peter van Walsum

Summary

Enzyme digestibility tests measured enzymatic hydrolysis rates of pretreated solids by cellulase enzymes (Novozyme 188 and Iogen cellulase). It was found that more severe pretreatments enhanced enzymatic digestibility. The addition of pressurized CO_2 to the pretreatment system did not significantly increase enzymatic hydrolysis rates compared to water-alone pretreatment. To conduct the experiments, s erum vials were charged with a pH 5.0 buffer, preservative, enzyme and pretreated solid sample estimated to have 2g/L cellulose (calculated from dry weight of the solid residue) and incubated in a 40^0C shaker bath. Glucose concentrations over time were determined via glucose assay and the HPAE when available.

Introduction

The main purpose of pretreatment is to enhance fiber reactivity to enzymatic hydrolysis. The rate of enzymatic digestion is important because higher rates increase productivity and allows for use of less enzyme. Increased fiber reactivity is accomplished through a variety of mechanisms: solubilization of hemicellulose , removal of lignin , reduction of particle size, alteration of the cellulose characteristics such as degree of polymerization, abundance of cellulose chain ends and crystallinity. Effective pretreatments in general approach or exceed 80 % of theoretical cellulose conversion upon subsequent hydrolysis of a representative hardwood feedstock (e.g. poplar) using moderate (e.g. 10 to 15 FPU/g cellulose) cellulase loadings. Such conversions are achieved in a period on the order of five days, although this is highly feedstock-dependent.

Materials + Methods

Apparatus and materials
Two Techne Fluidised Sand Baths SBL-2d with temperature controllers.
Beckman DU 520 General Purpose UV/Vis Spectrophotometer (with single cell module)
150ml stainless steel imersible reactor
Ohaus Expolorer digital scale Item #12140 (d=0.1mg)
Cole Parmer 2ml disposable plastic cuvettes
Domestic brand coffee grinders
Fisher Scientific U.S.A. Standard Testing Sieve, ASTME-11 Spec #18 (1mm opening)
Standard laboratory de-ionized quality water
Standard laboratory grade carbon dioxide
Vacuum filter with water aspirator
Schleicher and Schuell Sharkskin 100Cir. 5.5cm Microfilter paper
Blue M drying oven model OV-18A set at 100°C and 40^0C
New Brunswick Scientific Reciprocal Water Bath model R76
Thermolyne MaximixII type 37600 mixer
SIGMA Diagnostics - Infinity™ Glucose Reagent (Procedure 17-UV) Kit

Glass serum vials (50mL) and (100mL)
Novo Nordisk's cellobiase (Novozyme 188L cellulase enzyme)
Iogen cellobiohydrolase, endoglucanase, and B-glucosidase (Iogen cellulase enzyme)
Citric Acid, 99%
Benzoic Acid, 99%
Sulfuric Acid, 72%
Sodium Hydroxide Solution, 50/50 w/w
Aspen wood chips (provided by USDA Forest Products Laboratory in Madison, WI)
Aspen wood liquid hydrolysate
Avicel pure dried cellulose
Sigma D-Glucose Anhydrous

Experimental Design

Enzyme digestibility of pretreated solids at varying parameters around the midpoint of (200^{0}C/16min/with and without CO_2) for both aspen wood and corn stover was measured. An assessment of hydrolysis enhancement of pretreatment with carbonic acid (CO_2) was then made. The general steps included: preparation of the feedstock, pretreatment, separating the solids from the liquids, drying some of the solids and calculating dry weight; preparation of the testing vials, enzymatic hydrolysis tests, performing a glucose assay on the samples. The following procedure is summarized in figure 7.1, below.

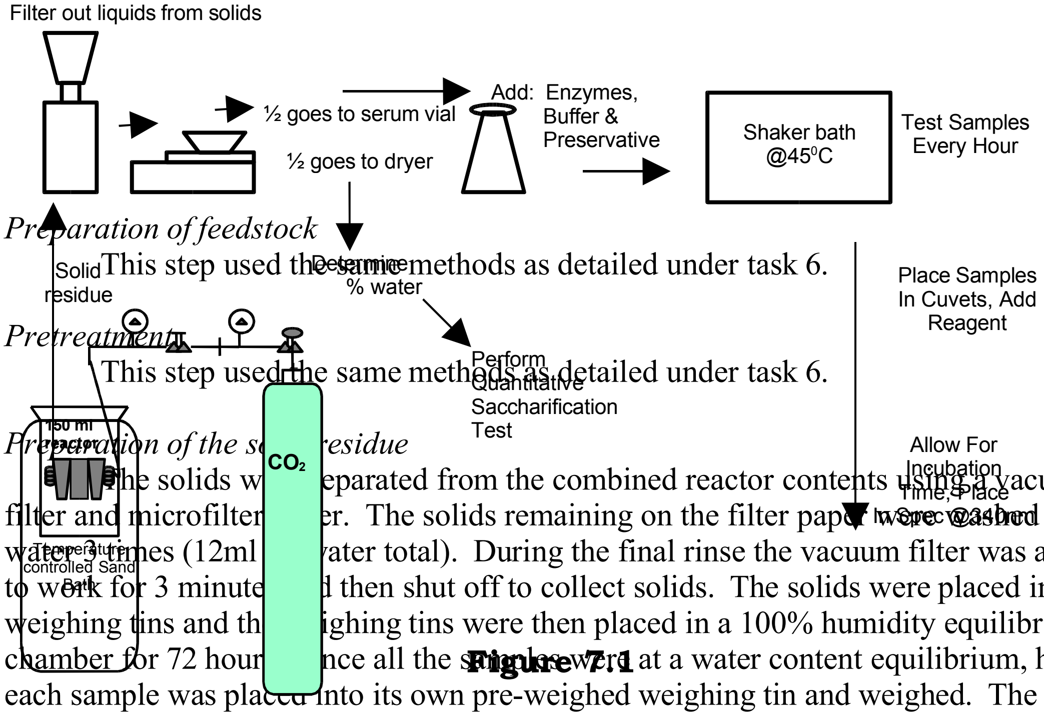


Figure 7.1

Preparation of feedstock

This step used the same methods as detailed under task 6.

Pretreatment

This step used the same methods as detailed under task 6.

Preparation of the s[illegible]residue

The solids w[illegible]eparated from the combined reactor contents using a vacuum filter and microfilter[illegible]er. The solids remaining on the filter paper were washed with DI water 3 times (12ml [illegible]ater total). During the final rinse the vacuum filter was allowed to work for 3 minute[illegible]d then shut off to collect solids. The solids were placed in weighing tins and th[illegible]ighing tins were then placed in a 100% humidity equilibrium chamber for 72 hour[illegible]nce all the samples were at a water content equilibrium, half of each sample was placed into its own pre-weighed weighing tin and weighed. The weight of the tin, and the wet sample were recorded for each sample. The original wet samples were placed back into the equilibrium chamber to ensure their water content would not change prior to experimentation. The weighed wet samples were placed into the dryer oven at 101^{0}C for 72 hours. Once the samples were dry, they were weighed again to determine water content of the original samples still in the equilibrium chamber.

Preparation of the testing vials

Each sample now had a known moisture content. Quantitative saccharification (se below) found the solids to be approximately 63% cellulose. This was used to then determine the amount of sample needed for each testing vial to achieve a final glucose level of less than 2g/L in the post hydrolysate

Next the pH 5.0 buffer solution with preservative was prepared. This was done by mixing citric acid with DI water and adding benzoic acid to the mixture at 0.49%. The mixture was then titrated with sodium hydroxide (NaOH) until the pH was raised to 5.0 and remained constant.

Finally, the amount of enzyme mixture was determined. Enzyme loadings were set to 20 cellulase units per gram of solid sample and 100 units of beta-glucosidase per gram of solid sample.

Vials were prepared so that each sample was done in duplicate. Once the buffer solution and solid samples were added to the vials, they were prepared to begin the test. (29,31,32,33,34)

Enzymatic hydrolysis tests

The addition of the enzymes was used as time zero (T=0). After a sample of each testing vial was taken at time zero the hydrolysis testing began. Immediately after taking the time zero samples the testing vials were placed in the shaker bath at 40^{0}C. The shaker bath oscillator was set to medium (1/2 way on the dial or number 5). The serum vials were then removed for testing every 2 hours for up to 12 hours. They were then tested at 24 hours, 48 hours and 120 hours.

Analysis of the sample

The enzymatic hydrolysis samples did not have to be diluted, so the sample taken from each serum vial was placed directly into its corresponding cuvette at each sampling hour. Also, the control that was also used for each enzymatic hydrolysis experiment was a sample that contained buffer, preservative and enzymes but no pretreatment sample.

Quantitative saccharification

This process was done in accordance with NREL LAP-002. Sulfuric acid (H_2SO_4) was prepared by adding 99% H_2SO_4 to water to achieve a 72% H_2SO_4 . The density of H_2SO_4 was close to double that of water so a balance was used to determine the amounts needed. The dried samples from the enzymatic hydrolysis tests were used. Samples of approximately 0.4 grams were dispensed into separate test tubes. Duplicates of each sample were again created for each experiment. All masses were immediately recorded once they were close to the 0.4 grams and before the effects of moisture in the air could affect the weights. A standard of pure cellulose (Avicel) was also measured in triplicate with each experiment. It was also dried in the dryer oven for 72 hours to ensure no moisture content when weighed. The tubes with sample in them were placed in a tube rack which was further placed on ice. The 72% H_2SO_4 mixture was added to each of the tubes creating an acid to sample ratio of 0.01ml/mg. Once all tubes had sufficient H_2SO_4, they were placed into the reciprocal water bath set at 30^0C and the shaker mechanism set to half way, or number 5 on the dial. The tubes were immersed in the shaker bath for 2 hours. Every 15 minutes they were stirred with glass stirring rods that stayed in each of the tubes to prevent sample loss or cross contamination. The stirring was key to breaking up clumps and ensuring uniform acid penetration to all the sample. While the tubes were in the bath, a set of serum vials corresponding to each test tube was prepared for the next step of the quantitative saccharification test. The 100mL serum vials were placed on ice and water was added to each serum vial. The total water added to each vial was 0.27mL/mg of sample minus 20ml. For example, if sample tube #3 has 400mg of sample, then 400 x 0.27 – 20 = 88ml of water were placed into serum vial #3. The 20ml of water subtracted from 0.27mL/mg ratio was used to rinse the contents of the test tubes and stirring rods into the serum vials to ensure complete mass transfer. At the end of the 2 hours, the content of each tube was transferred to their respective serum vials. Each tube and stirring rod was washed with 20ml of water and again transferred to its respective serum vial. Each serum vial was then capped and sealed. They were taken off the ice and placed in the autoclave for 1 hour. Once the autoclaving was complete and the serum vials were cool enough to be handled, they were analyzed.

Analysis of the Quantitative Saccharification Samples

The glucose analysis method used for the quantitative saccharification samples was the enzymatioc glucose assay, as was done with the enzymatic hydrolysis samples and yeast inhibition samples.

Results

Data were analyzed using the DU 500 Spectrophotometer and Infinity Glucose Reagent Assay kit. Two metrics were used, total recovered glucose and reaction speed, after the raw numbers were graphed (Absorbance Units versus time, transposed into Glucose yield versus time).

Figure 7.2, below, shows rapid hydrolysis of all samples pretreated at a similar severity. Note that there is no difference between samples with or without carbonic acid. Also, the graph shows that after 24 hours there was little difference in the yield, even when the testing continued for 120 hours (not shown, but the time it is thought to take for enzymes to achieve about 90% hydrolysis) there was very little difference in the yield.

Figure 7.3, below, shows that reaction severity played a major role in the rate and yield of enzymatic hydrolysis and that as the reaction severity increased so to did the rate and final yield of the enzymatic hydrolysis throughout the range of severities. Again, however, the presence or absence of carbonic acid appears to have played no distinguishing role in rates or extents of enzymatic hydrolysis.

Figure 7.2 Enzymatic Hydrolysis of Pretreated Aspen Wood

Figure 7.3: Enzymatic hydrolysis of Aspen wood pretreated at varying severity.

Discussion

The midpoint pretreatment condition of 200^{0}C for16minutes does not appear to be the optimum condition for enzymatic hydrolysis. It is clear that the optimal severity of 200^{0}C for 32minutes that produces the maximum xylose sugars as found by McWilliams (2002) from the pretreatment step is not the optimal severity for enzymatic hydrolysis. The rates and yields of enzymatic hydrolysis continued to increase as the reaction severity of the pretreatment increased. It is possible that the enzymatic hydrolysis rates and yields would continue to increase past the maximum severity that was tested of 220^{0}C for 32minutes. A possible explanation for this result is that the more sever reaction conditions are continuing to breakdown the hemicellulose and solubilize the lignin, allowing the enzymes more and easier access to the cellulose

Conclusion

Pretreatment with carbonic acid showed no significant advantage when compared to pretreatment with liquid hot water. Differences between the samples could be attributed to testing error since in some cases the samples with carbonic acid showed improved enzymatic hydrolysis rates and yields and in other cases they showed a decrease in enzymatic hydrolysis rates and yields.

The reaction severity did have a significant effect on the enzymatic hydrolysis yields of the samples. On average, for every 10 fold increase in severity, the enzymatic hydrolysis yield increased by about 30%. More testing would be needed to determine the increase in rate. Also, data points need to be tightened and a standard for slope determination amongst the samples would need to be determined.

References

McWilliams R. C., Van Walsum G. P., 2001, *Comparison of Aspen Wood Hydrolysates Produced by Pretreatment with Liquid Hot Water and Carbonic Acid*, 3-17

McWilliams, R.C. 2002 Master's Thesis, Comparison of Aspen Wood Hydrolysates Produced by Pretreatment with Liquid Hot Water and Carbonic Acid, p1-17

Tasks 8 + 9: Model Process Operating Parameters

Kemantha Jayawardhana, G. Peter van Walsum

Summary:

Pretreatment costs for carbonic acid pretreatment are driven by the high cost of pretreatment reactors capable of containing the pressures used. This makes the cost of the carbonic acid system highly sensitive to reactor volume and thus the concentration of solids in the reactor. The cost of the reactor can be reduced by raising the concentration of the solids in the pretreatment reactor, which in turn reduces the size of the pretreatment reactor and thus diminishes the cost differential between dilute acid and carbonic acid equipment costs. If the solids concentration is put very high, and equal to that used in the NREL model, equipment costs become comparable to those for dilute sulfuric acid pretreatment. Cost of compressing CO_2 is relatively low compared to the equipment cost for the high pressure reactor vessel. About 50% of the total operating cost is due to the heat demand of the process. This is unavoidable due to difficulties in process heat recovery.

Unless the concentration of solids in the pretreatment reactor can be increased, use of carbonic acid for pretreatment proved to be more expensive than using sulfuric acid. This is mainly due to the use of high pressures such as 2000 psi in the pretreatment reactor.

Introduction

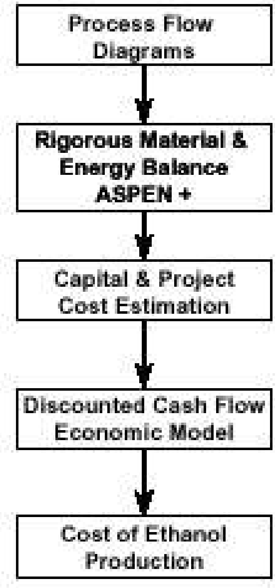
Computer aided design and simulation gives the process engineer the ability to evaluate more alternates in more detail than was possible by hand calculations. Simulation, as it will be used in this section, will refer to the creation of a mathematical model or representation of a chemical process. Aspen Plus^R (Advanced Systems for Process Engineering) is used to model the Carbonic Acid Pretreatment process at the research laboratory of environmental studies department. It is capable of solving steady-state material and energy balances, calculates phase and equilibria, and estimates physical properties of thousands of chemical compounds and capital costs of equipment. Originally developed for the Department of Energy (DOE) by Massachusetts Institute of Technology (MIT) in 1987, Aspen plus requires the user to write an input file containing process specifications. However, later versions of Aspen Plus incorporate a Graphical User Interface (GUI), making the simulation software more user friendly.

Aspen Plus utilizes three mechanisms to simulate chemical processes: unit operation blocks, Fortran blocks and design specifications (design-specs). Unit operation blocks represent processes taking place in an actual chemical plant (i.e. compressors, pumps, reactors, heat-exchangers, etc.,). A Fortran block is used for feed-forward control, allowing the user to enter code to control variables in an Aspen Plus flowsheet. A design-spec is used for feedback control, allowing the user to set values for any flowsheet variable. The user then chooses another flowsheet variable for Aspen Plus manipulation. The design-spec varies the manipulation flowsheet variable to achieve the specified set variable value.

The software is also able to handle recycle streams. When a stream is encountered in a simulation, which is calculated further ahead in the process (such as a recycle stream), Aspen Plus assumes an initial value for the stream. A stream of this nature is called a “tear-stream”. The program will solve the tear-stream iteratively until it obtains a solution. There are many other benefits associated with Aspen Plus. The program includes an extensive chemical-compound property database, with the ability to handle heterogeneous compounds such as coal or MSW (Municipal Solid Waste). Aspen Plus allows the user to solve the flowsheet in a specified sequence if the default sequence did not converge.

Biomass to ethanol process developed by the National Renewable Energy Laboratory (NREL) was used to design the carbonic acid pretreatment process. The Process design and economic modelling approach used by the NREL is given in figure 9.1. Same approach was used for the construction of the carbonic acid pretreatment process except that instead of discounted cash flow method, a straight line depreciation method was due to lack of economic data.

Figure 9.1 : NREL’s Approach to Process Design and Economic Modeling

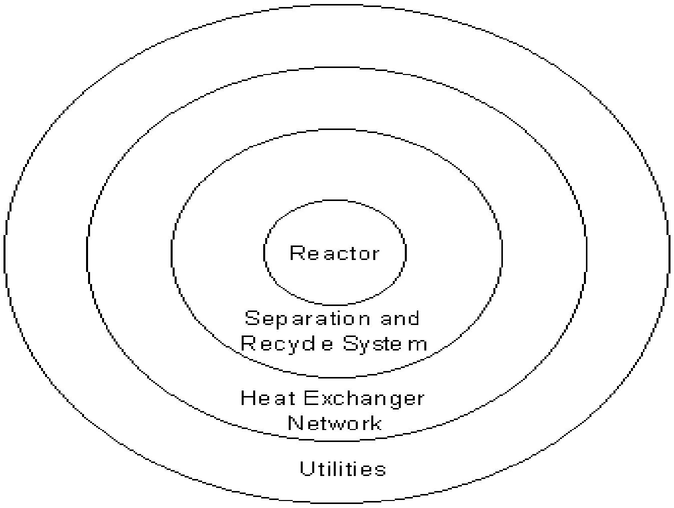


Methods

Process Design & Simulation

The first step was to develop the preliminary process flow diagram (PFD). Aspen Plus model library was used to select all the necessary unit operation blocks. The “onion model” (figure 9,2) approach for process design was used to construct the PFD.

Figure 9.2: Onion model approach to chemical process design



Once the process flow diagram was sketched out, aspen plus software was used to build the process model. Figure 9.3 below depicts the carbonic acid pretreatment process flowsheet generated by the aspen plus. The unit operation blocks used for the pretreatment process are given in the Table 9.1.

Reactor (B1) in this pretreatment process was modeled as a stoichiometric reactor (Rstoic), since the kinetic data for many pretreatment reactions were not available. However, a commercial unit will be screw type plug flow reactor with a structure similar to that given in Figure 9.4.

Rstoic models a reactor when reaction kinetics are unknown or unimportant and when stoichiometry and reaction extent or conversion are known. It can also perform product selectivity and heat of reaction calculations. Preliminary investigations carried out at the department of environmental studies, showed that carbon dioxide compression to 2000 psi is difficult and expensive to implement in commercial scale. Therefore, in consultation with van Walsum and others, the process was modified such a way that the carbon dioxide would be injected to biomass and water stream (stream no. 4) at a pressure of 800 psi. Hence, the compression duty was lowered by a factor of 2. To raise carbon dioxide pressure from 1 atm (14.7 psi) to 800 psi, a multi-stage compressor (B13) was used instead of a single-stage compressor mainly due to higher efficiency in the former. The stream 1, which contains biomass and water, was pressurized to 800 psi using pump B7. The mixing of carbon dioxide to stream 4 (biomass & water at 800 psi) was carried out using an in-line CO_2 mixer B8 (figure 9.5). The mixer model in Aspen Plus determines the combined outlet stream temperature and phase condition by performing an adiabatic phase equilibrium flash calculation on the composite feed streams.

A heat exchanger (B11) was employed to preheat incoming stream using outgoing product stream (stream no. 16). Then, the preheated stream 5 was pressurized to 2000psi using pump B12. Heater B9 was employed to heat stream 12 to 220 ^{0}C.

Hence at the inlet of the reactor B1, conditions of input stream were always maintained at 2000psi and 220 ^{0}C. Selection of these conditions was based on the laboratory experiments carried out by van Walsum and others at the department of environmental studies. The product stream from the reactor (B1) was flash cooled using flash drum (B3) in order to separate vapors from liquid phase. The flash models available in Aspen Plus model library determine the thermal and phase conditions of a mixture with one or more inlet streams. The separator (B6) was employed to separate carbon dioxide and steam and resulting recycle streams no. 10 and 9 were sent to B13 and B7 respectively. The liquid stream from flash drum was sent to Pneumapress filter (B10) where it was separated into filter cake (stream no. 13) and filtrate (stream no. 8). This separation was done basically to extract heat from the liquid stream. Therefore, stream 8 was pressurized to 800psi using pump B14 and sent to the heat exchanger (B11). The filter cake was sent to a mixing tank (B4) through a screw conveyor (B2) and at B4, filtrate and cake were thoroughly mixed in order to obtain slurry. This product slurry was further cooled using a cooler (B5) before being sent to the next level of the biomass process.

Figure 9.3: Aspen Plus Process Flow Diagram (PFD)

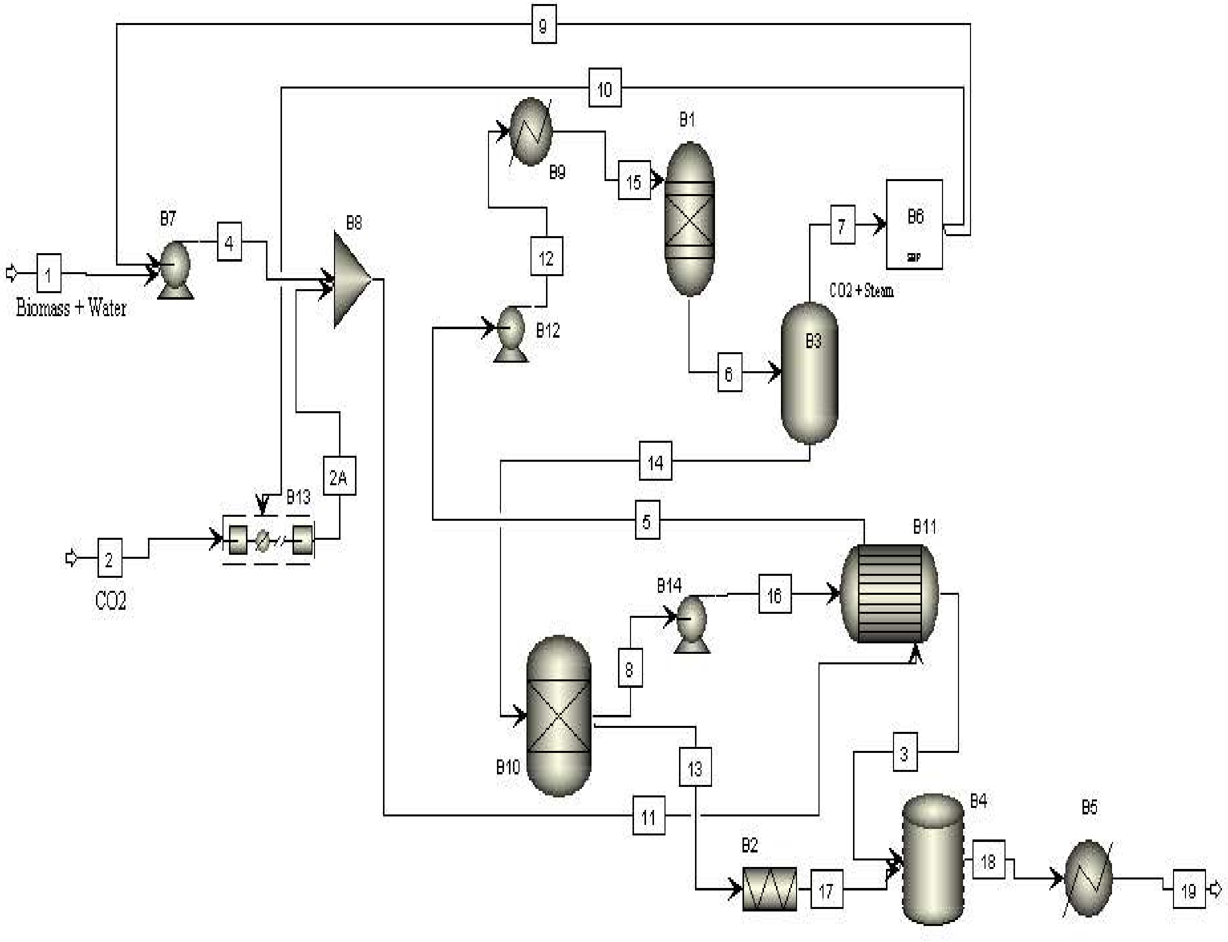
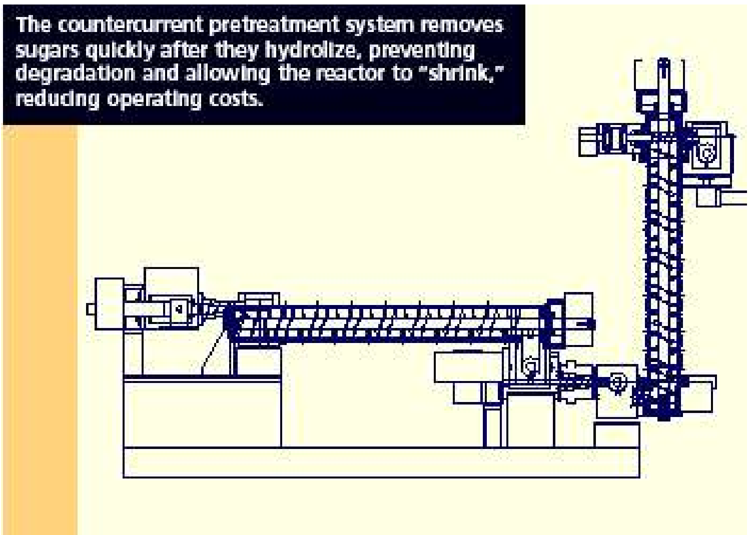

9
10
B1
B9
15
B7
4
B8
B6
SEP
7
1
Biomass + Water
12
CO2 + Steam
B12
B3
6
14
2A
B13
2
CO2
5
B11
B14
16
8
B10
13
3
11
B4
B5
B2
17
18
19

Table 9.1: Unit operation blocks

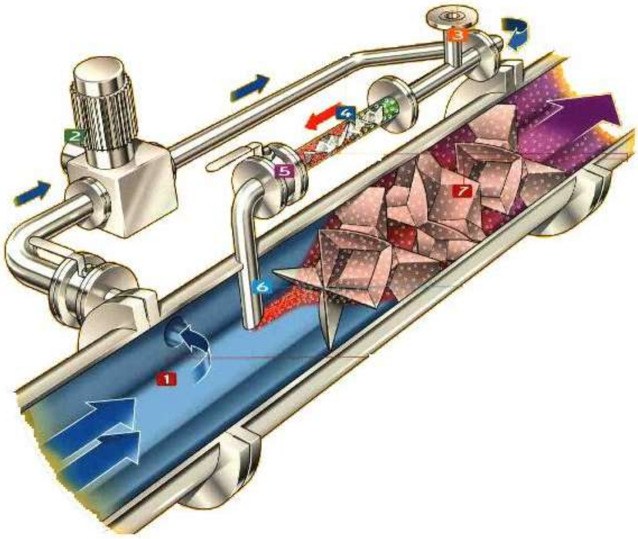
Block Number	Description
B1	Reactor (Rstoic)
B2	Screw mixer
B3	Blowdown tank & screw conveyor
B4	Slurrying tank & tank agitator
B5	Cooler
B6	Reflux drum & condenser
B7	Feed pump
B8	In-line CO_2 mixer
B9	Heater
B10	Pneumapress filter
B11	Heat exchanger
B12	Loading pump
B13	CO2 compressor
B14	Primary filtrate pump

Figure 9.4: Pretreatment Reactor


The countercurrent pretreatment system removes sugars quickly after they hydrolize, preventing degradation and allowing the reactor to "shrink," reducing operating costs.

Source: NREL, DOE/GO-102000-1114

Figure 9.5: In-line CO_2 mixer



Source: http://www.lenntech.com/ozone_mixing.htm

Component data and databases

Physical property data for many of the key components used in the simulation for the ethanol from lignocellulose process are not available in the standard Aspen Plus property databases. Indeed, many of the properties necessary to successfully simulate this process are not available anywhere. In addition, inputting the available properties into each simulation is awkward and tedious, and mistakes can easily be introduced into the simulation.

The Aspen simulator handles three classes of compounds:

1. Those (such as ethanol) that are involved in vapor liquid equilibrium;
2. Those (such as $CaSO_4$) that are solids only and are identifiable; and
3. Solids (such as coal) that are identifiable by attribute only.

For compounds involved in vapor liquid equilibrium, the simulator must have a complete set of properties to allow it to do flash calculations. For materials such as glucose and xylose, which are commonly solids, but will be used exclusively in aqueous solution in the process, will be treated as liquids. The second class, which includes cellulose, is assumed to comprise conventional solids whose property requirements are very minimal. A conventional solid can (unlike non-conventional solids that must be described by attributes) be defined by a chemical formula.

The minimum physical properties required by Aspen depend on the calculation routes selected for fundamental properties such as liquid, vapor and solid enthalpy and density. In general, because of the need to distill ethanol and to handle dissolved gases the standard NRTL (non-random two liquid or Renon) route is used. This route includes the NRTL liquid activity coefficient model, Henry's law for the dissolved gases, and RKS (Redlich-Kwong-Soave) equation of state for the vapor phase, is used to calculate properties for components in the liquid and vapor phases. It also uses the Ideal Gas (IG) at 25 ^{0}C as the standard reference state, thus requiring the heat of formation at these conditions (Table 9.2).

Table 9.2: Required Properties

Liquids/Gases	Conventional Solids
Critical Temperature	Heat of Formation
Critical Pressure	Heat Capacity
I.G. Heat of Formation	Density
Vapor Pressure	
I.G. Heat Capacity	
Heat of Vaporization	
Liquid Density	

An in-house physical property database developed by NREL reported all these properties for components listed below (Table 9.3) and was used for this carbonic acid pretreatment process.

Glucose, although generally considered a solid at the temperatures involved in the ethanol process, is exclusively in aqueous solution. It will therefore be modeled as a liquid, although it will never exist as a pure liquid in the process. Xylose, like glucose, is generally considered a solid at the temperatures involved in the ethanol process, but is exclusively in aqueous solution.

Table 9.3: Compounds included in the In-House Database developed by the NREL

Compound name	Formula	Database Name	Database Alias	Normal State
Glucose	$C_6H_{12}O_6$	GLUCOSE	C6H12O6	Liquid
Xylose	$C_5H_{10}O_5$	XYLOSE	C5H10O5	Liquid
Cellulose	$C_6H_{10}O_5$	CELLULOS	C6H10O5	Solid
Xylan	$C_5H_8O_4$	XYLAN	C5H8O4	Solid
Lignin	$C_{7.3}H_{13.9}O_{1.3}$	LIGNIN	CXHXOX	Solid
Solsld	$CH_{1.48}O_{0.19}S_{0.00013}$	SOLSLDS	CHXOXSX	Liquid

Hence, xylose too, will be modeled as a liquid although it will never exist as a pure liquid in the process. Cellulose and xylan are considered to be solids throughout the process and will never be in solution. Additionally, both cellulose and xylan are polymers, but their molecular weight formula will be taken as the repeat unit only. The other properties are determined on a weight basis and then converted to mole basis, using the molecular weight of a repeat unit. Lignin is considered to be solid throughout the process and will never be in solution. Soluble solids (Solslds) are the non-identifiable solids that will be dissolved in aqueous solutions throughout the simulation. Therefore, they will never exist as a pure liquid in the process.

Input & basis

After careful investigation of the NREL model, the basis for the carbonic acid model was selected as 2000 dry metric tonnes/day of biomass. This number represents the economically feasible collection distance for biomass [Wooley et al., 1999].

Biomass: 2000 dry tonnes/day
Total biomass flowrate: 2,000,000 kg/day (= 83,333.33 kg/hr)
Water flow with 52 % solids = 83333 (1-0.52)/0.52 = 76,923 kg/hr (4273 kmol/hr)

Yellow Poplar biomass composition was used as the feedstock composition.

Table 9.4: Biomass Composition

Component	% Dry Basis
Cellulose	42.85
Xylan	23.61
Arabinan	2.55
Mannan	0.18
Galactan	1.39
Lignin	20.15
Acetate	2.25
Ash	7.01

However, when used in the simulation biomass is considered as consist of only cellulose, xylan, lignin and soluble solids (solslds). Hence, arabinan, mannan, galactan, acetate and ash constituted the soluble solids. Then the composition used for simulation is as follows:

Table 9.5: Modified biomass composition used for simulation

Component	% Dry Basis
Cellulose	42.85
Xylan	23.61
Lignin	20.15
Solslds	13.39

For the successful convergence of carbonic acid model, the simulation required several inputs. These inputs include stream input (Table 9.6) and block input (Table 9.7).

Reactions:	Conversion (%)
CELLULOSE + WATER => GLUCOSE	6.5
XYLAN + WATER => XYLOSE	75.0
LIGNIN => LGNSOL	5.0

Table 9.6 Stream Inputs

Stream No.	Flowrate (kg/hr)	Temperature (^{0}C)	Pressure (psi.)
Biomass			
Cellulose	41225	20	14.7
Xylan	22716	20	14.7
Lignin	19392	20	14.7
Total	83333		
Water	76923	20	14.7
Carbon Dioxide	146	20	14.7

Table 9.7 Block Inputs

Block Number	Block Name	Temperature (0C)	Pressure (psi.)	Valid Phases	Split Faction
B1	Rstoic Reactor	220	2000		
B2	Screw Mixer		800	Solid only	
B3	Flash Drum	205	250		
B4	Mixing Tank		14.7	Liquid Only	
B5	Cooler	37	14.7		
B6	Separator		250		0.995 (St. 10, CO_2)
B7	Pump		800		
B8	In-line Mixer		800	Vapor-Liquid	
B9	Heater	220	2000		
B10	Press Filter		250		0.99 (St. 8, Water)
B11	Heat Exchanger	64 (Hot St. outlet)			
B12	Pump		2000		
B13	Compressor		800 (last stage)	Vapor Only	
B14	Pump		800		

Calculation of CO_2 flowrate

As a part of modeling, CO_2 & water mixing data for high temperatures were estimated using Aspen Plus property estimation method and compared with original data for accuracy. However, it is found that at high temperatures CO_2 solubility prediction using Aspen Plus to be unreliable. Therefore, Henry's law is used to theoretically predict the CO_2 solubility in water at different temperatures and pressures.

Henry's Law: $X_{CO2}= P_{CO2}* K_H$
Henry's constant for CO_2: K_H
K_H= -1.70367*10-2*X^2 + 6.155342*X + 78.22759 [van Walsum, 2001]

Aspen Plus Simulation Results

In this study aspen plus model was used to calculate the heat & energy demand of the process units. Then, equipment cost and energy cost were calculated in 2001 US dollars. Simulation results were generated for the following pretreatment conditions (Table 9.8).

Table 9.8 Pretreatment Conditions

Parameters	
Pressure (psi)	Temperature (^{0}C)
2000	220
2000	200
2000	180
1600	220
1600	200
1600	180
1200	220
1200	200
1200	180
1000	220
1000	200
1000	180
800	220
800	200
800	180

For each set of parameters, a corresponding material and energy balance sheet was generated by the Aspen Plus software.

Reactor Thickness Calculation

Use of carbonic acid for pretreatment resulted in very low acidity inside the pretreatment reactor. Due to this low acidic level, fabrication of pretreatment reactors on commercial scale can be carried out using less expensive metal alloys. In this study, for the purpose of reactor thickness calculations and the cost of reactors, it is assumed that pretreatment reactors are fabricated using stainless steel 316 L (SS 316).

Pretreatment reactor residence time was varied from 2 min. to 10 min. and the corresponding reactor thickness and size were calculated for all the pretreatment conditions (i.e. from 2000 psi 220 ^{0}C to 1200 psi 180 ^{0}C).

The thickness of the reactor can be calculated using the following equation:

$t = P*R/(S*E-0.6*P)$ (Perry and Green n.d.)

t- Reactor thickness (in.)
R-Radius of the reactor (in.)
P-Pressure of the reactor (psi.)
S-Maximum allowable stress (psi.)
E-0.85 (joint efficiency)

As it can be seen from the equation, increasing radius will increase the thickness of the reactor. In order to accommodate 83333 kg/hr, use of a single reactor would drastically increase the thickness of the reactor making it infeasible to build a reactor. Use of several smaller reactors in parallel can be a more economically feasible method, since it avoids the thickness issue. However, use of several reactors will require use of many auxiliary equipment and instrumentation for process control. Having analyzed costs of auxiliary equipment, the optimum number of reactors to be employed in pretreatment process was determined to be three.

Table 9.9 shows required reactor radii for a pretreatment residence time of 4 min. In the first column of the table, calculated reactor radius was given as R_1= 17.487 in. This corresponds to a tubular reactor, which is 80 % liquid by volume inside the reactor and 10 m in height. Similarly R_2 represents a reactor, which is 10m high, and filled with liquid up to 90%. R_3 and R_4 are radii for 12 m high reactors, which are filled with liquid up to 80% and 90% respectively.

Table 9.9 Determination of Reactor Radius

Base data				
Res. Time (min)	4			
flow rate (l/min)	3600.8			
Total Vol inside (l)	14403.2			
	80% filling	90% filling	80% filling	90% filling
Reactor Vol (l)	18004	16003.6	18004	16003.55556
No. of Reactors	3	3	3	3
Volume of one reactor (l)	6001.3	5334.5	6001.3	5334.5
Volume of one reactor (m3)	6.0	5.3	6.0	5.3
Volume of one reactor (gal)	1428.9	1270.1	1428.9	1270.1
h (m)	10	10	12	12
R (m)	0.43717	0.41215	0.39908	0.37626
R (in.)	17.4871	16.4870	15.9635	15.0505
	R1	R2	R3	R4
t=P*R/(S*E-0.6*P)				
E	0.85	0.85	0.85	0.85

Table 9.10 shows the variation of reactor thickness with variations in reactor pressure and temperature at 4 min. residence time. In this table, t_1 is the thickness corresponding to R_1 and t_2 corresponds to R_2 and so on. For the calculation of reactor cost, reactor is considered as 90% liquid by volume and of 12m in length. The corresponding radius is R_4 and the thickness is t_4 (Table 9.10).

NREL reported that when built using Hastelloy C, the pretreatment reactor would cost $ 2,505,084.00, which is 50% more expensive than to build with SS316. Hence, a same size SS316 reactor would cost only $ 1,670,056 (2000 $). For Table 9.10, reactor thickness was known for different pretreatment conditions at residence time of 4 min. Hence, the material volume for the fabrication of reactor could be calculated using following equation.

$$V= 2*\pi*r*h*t$$

With these data, reactor cost can be calculated for different pretreatment conditions as given in the Table 9.11.

Table 9.10 Variation of Reactor Thickness with Pressure and Temperature at 4 min. Residence Time

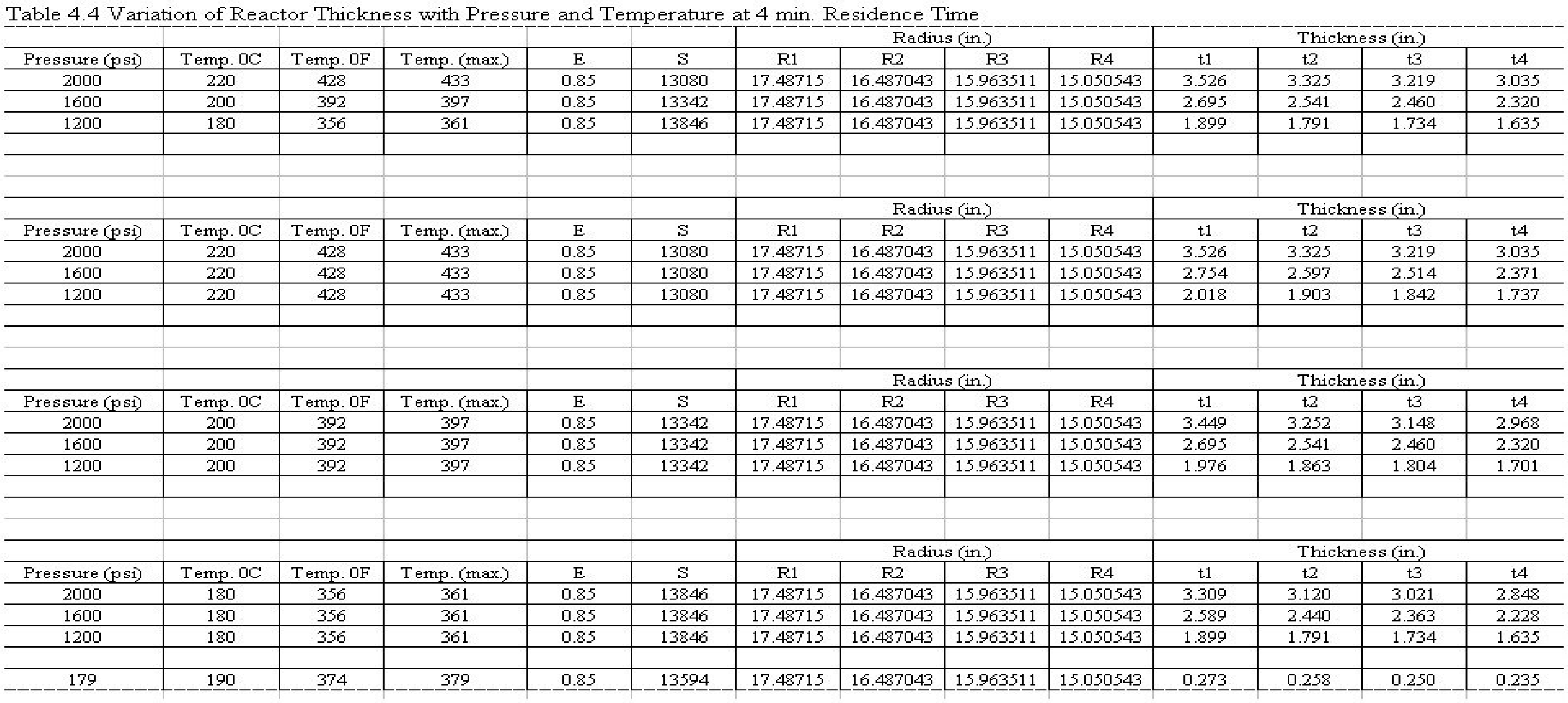


Table 4.4 Variation of Reactor Thickness with Pressure and Temperature at 4 min. Residence Time

						Radius (in.)				Thickness (in.)			
Pressure (psi)	Temp. 0C	Temp. 0F	Temp. (max.)	E	S	R1	R2	R3	R4	t1	t2	t3	t4
2000	220	428	433	0.85	13080	17.48715	16.487043	15.963511	15.050543	3.526	3.325	3.219	3.035
1600	200	392	397	0.85	13342	17.48715	16.487043	15.963511	15.050543	2.695	2.541	2.460	2.320
1200	180	356	361	0.85	13846	17.48715	16.487043	15.963511	15.050543	1.899	1.791	1.734	1.635

						Radius (in.)				Thickness (in.)			
Pressure (psi)	Temp. 0C	Temp. 0F	Temp. (max.)	E	S	R1	R2	R3	R4	t1	t2	t3	t4
2000	220	428	433	0.85	13080	17.48715	16.487043	15.963511	15.050543	3.526	3.325	3.219	3.035
1600	220	428	433	0.85	13080	17.48715	16.487043	15.963511	15.050543	2.754	2.597	2.514	2.371
1200	220	428	433	0.85	13080	17.48715	16.487043	15.963511	15.050543	2.018	1.903	1.842	1.737

						Radius (in.)				Thickness (in.)			
Pressure (psi)	Temp. 0C	Temp. 0F	Temp. (max.)	E	S	R1	R2	R3	R4	t1	t2	t3	t4
2000	200	392	397	0.85	13342	17.48715	16.487043	15.963511	15.050543	3.449	3.252	3.148	2.968
1600	200	392	397	0.85	13342	17.48715	16.487043	15.963511	15.050543	2.695	2.541	2.460	2.320
1200	200	392	397	0.85	13342	17.48715	16.487043	15.963511	15.050543	1.976	1.863	1.804	1.701

						Radius (in.)				Thickness (in.)			
Pressure (psi)	Temp. 0C	Temp. 0F	Temp. (max.)	E	S	R1	R2	R3	R4	t1	t2	t3	t4
2000	180	356	361	0.85	13846	17.48715	16.487043	15.963511	15.050543	3.309	3.120	3.021	2.848
1600	180	356	361	0.85	13846	17.48715	16.487043	15.963511	15.050543	2.589	2.440	2.363	2.228
1200	180	356	361	0.85	13846	17.48715	16.487043	15.963511	15.050543	1.899	1.791	1.734	1.635
179	190	374	379	0.85	13594	17.48715	16.487043	15.963511	15.050543	0.273	0.258	0.250	0.235

Equipment Cost Calculation

From the aspen plus model, the equipment requirements were determined. The number of each piece of equipment required was determined on the basis of mass flow rate through each equipment. From the thickness calculations, it was found that three reactors are required to accommodate mass flow rate of 83333 kg/hr. Also, it was found that given the solid concentration of the fluid, it is necessary to use more than one Pneumapress filter.

Two scenarios were considered to calculate equipment cost. Scenario 1 assumes a reactor installation cost factor of 1.2. Table 9.12 lists equipment costs for this scenario at 2000 psi and 220 ^{0}C at 4min. Scenario 2 assumes a reactor installation cost factor of 2.29 and the corresponding equipment costs are given in Table 9.13. Equipment costs were obtained from vendor quotations when possible, however majority of equipment costs were derived from NREL's economic model.

Table 9.11 Reactor Cost at Different Pretreatment Conditions (Residence Time 4 min.)

Parameters	t4 (in.)	t4 (m)	R4 (m)	H4 (m)	Material volume (m3)	Original Price (2000 $)
NREL Reactor	0.4962	0.0124	0.7933	12	0.742	1670056
CO2 Model						
2000 psi, 220 0C	3.0350	0.07587	0.376	12	2.153	4846878
2000 psi, 200 0C	2.9683	0.07421	0.376	12	2.105	4740436
2000 psi, 180 0C	2.8480	0.07120	0.376	12	2.020	4548291
1600 psi, 220 0C	2.3706	0.05927	0.376	12	1.681	3785890
1600 psi, 200 0C	2.3198	0.05799	0.376	12	1.645	3704670
1600 psi, 180 0C	2.2278	0.05570	0.376	12	1.580	3557842
1200 psi, 220 0C	1.7369	0.04342	0.376	12	1.232	2773880
1200 psi, 200 0C	1.7005	0.04251	0.376	12	1.206	2715716
1200 psi, 180 0C	1.6346	0.04086	0.376	12	1.159	2610421

If process changes are made and the equipment size changes, the equipment is not generally re-costed in detail. The following exponential scaling expression was used to determine the new cost of the scaled unit.

$$\text{New Cost} = \text{Original Cost} * \left[\frac{\text{New Size}}{\text{Original Size}}\right]^{\text{exp}} \text{ [Wooley et al. 1999b]}$$

Instead of size, a characteristic that is linearly related to size can be used in the above expression. Some other characteristics that can be used in the equation include heat duty for a heat exchanger when ΔT is constant. Generally these related characteristics are easier to calculate and give the same result as resizing the equipment each time. The scaling exponent was obtained from standard reference such as Garrett (Wooley et al. 1999b). The installation costs were taken from the NREL economic model in order to have consistency. Since original equipment costs were calculated in different cost years, the following cost indices were used to calculate their cost in 2001 US dollars (Table 9.13).

Table 9.13 Plant Cost Indices (Chemical Engineering Progress 2001)

Year	Index
1996	381.7
1997	386.5
1998	389.5
1999	390.6
2000	394.1
2001	396.5

Energy Cost Calculation

Energy cost of the pretreatment section was determined using energy demand values calculated by the Aspen Plus model for pretreatment equipment. The following table lists all the assumptions made in this energy cost evaluation (Table 9.14). For pumps and compressors such as B7, B12, B14 and B13, power requirements were determined by the Aspen Plus model. However, some internal components of major process units such as reactor screw, tank agitators were not modeled in this process and hence their power requirements were not known.

Table 9.12 Equipment Cost for Carbonic Acid Pretreatment Process (2000 psi, 220 0C, and 4 min.)
(Scenario 1: Reactor Installation Factor of 1.2)

Equip No	No. Rqd	No. Spares	Equip Name	Scaling Stream	Stream Flow (kg/hr)	New Stream Flow (kg/hr)	Size ratio	Original Equip. cost (per unit)	Base year	Total Original Equip. Cost (Reqd. & Spares)	Scaling Exponenet	Scaled Cost in base Year	Installation Factor	Installed Cost	Installed Cost in 2001 $
B8	1		In-line CO2 Mixer	11	53630	208280	3.88	1500	1997	1500	0.48	2877	1	2877	2951
B7	1	1	Feed Pump	4	358810	203879	0.57	61368	1998	122736	0.7	82628	2.8	231359	235516
B12	1	1	Loading pump	5	358810	208280	0.58	61368	1998	122736	0.7	83873	2.8	234843	239064
B3	1		Blowdown Tank	6	270300	208280	0.77	64100	1997	64100	0.93	50302	1.2	60362	61924
B3	1		Screw Conveyor	14	225140	160401	0.71	59400	1997	59400	0.78	45597	1.3	59276	60810
B10	3		Pneumapress Filter	SLD 14	50299	62647	1.25	1575000	2000	4725000	0.6	5390218	1.05	5659729.4	5694196
B14	1	1	Primary Filtrate Pump	8	136350	97012	0.71	32549	2000	65098	0.79	49749	3.56	177106	178184
B11	1	1	Heat Exchanger	Area/ft2	12532	6805	0.54	132800	1997	265600	0.68	175347	2.1	368228	377755
B13	1	1	CO2 Compressor	2A	98040	4401	0.04	278200	2000	556400	0.34	193695	1.3	251803	253336
B9	1	1	Heater	Duty (Cal/s)	7863670	3890560	0.49	158374	1996	316748	0.68	196289	2.1	412208	428191
B2	1		Screw Conveyor	17	225140	63390	0.28	59400	1997	59400	0.78	22103	1.3	28734	29477
B4	1		Tank Agitator	18	358810	160401	0.45	36000	1997	36000	0.51	23877	1.2	28652	29394
B4	1		Slurrying Tank	18	358810	160401	0.45	44800	1997	44800	0.71	25294	1.2	30353	31138
B5	1	1	Cooler	Duty (Cal/s)	277820	2185707	7.87	29544	1996	59088	0.68	240250	2.1	504524	524086
B1	3		Reactor	15			1.00	4846878	2000	14540634	1	14540634	1.2	17448761	17555021
B6	1		Separator (Condenser)	Duty (Cal/s)	277820	5556935	20.00	29544	1996	29544	0.68	226568	2.1	475792	494241
B6	1		Separator (Reflux drum)	Duty (Cal/s)	277820	5556935	20.00	11900	1997	11900	0.93	192994	2.1	405287	415773
			Total												26611058
			Additional Equipment											Cost (99 $)	
A205	1		Hydrolysate Mix Tank Agitator											34546	
C202	1		Hydrolysate Washed Solid Belt Conveyor											114375	
H200	1		Hydrolysate Cooler											125160	
P205	2	1	Pneumapress Feed Pump											151994	
P225	1	1	ISEP Elution Pump											42815	
P226	1	1	ISEP Reload Pump											73685	
S221	1		ISEP											2699169	
T205	1		Hydrolysate Mixing Tank											39220	
			Total											3280964	3330522
			Grand Total												29941580

Table 9.13 Equipment Cost for Carbonic Acid Pretreatment Process (2000 psi, 220 0C, and 4 min.) (Scenario 1: Reactor Installation Factor of 2.29)

Equip No	No. Rqd	No. Spares	Equip Name	Scaling Stream	Stream Flow (kg/hr)	New Stream Flow (kg/hr)	Size ratio	Original Equip. cost (per unit)	Base year	Total Original Equip. Cost (Reqd. & Spares)	Scaling Exponenet	Scaled Cost in base Year	Installation Factor	Installed Cost	Installed Cost in 2001 $
B8	1		In-line CO2 Mixer	11	53630	208280	3.88	1500	1997	1500	0.48	2877	1	2877	2951
B7	1	1	Feed Pump	4	358810	203879	0.57	61368	1998	122736	0.7	82628	2.8	231359	235516
B12	1	1	Loading pump	5	358810	208280	0.58	61368	1998	122736	0.7	83873	2.8	234843	239064
B3	1		Blowdown Tank	6	270300	208280	0.77	64100	1997	64100	0.93	50302	1.2	60362	61924
B3	1		Screw Conveyor	14	225140	160401	0.71	59400	1997	59400	0.78	45597	1.3	59276	60810
B10	3		Pneumapress Filter	SLD 14	50299	62647	1.25	1575000	2000	4725000	0.6	5390218	1.05	5659729.41	5694196
B14	1	1	Primary Filtrate Pump	8	136350	97012	0.71	32549	2000	65098	0.79	49749	3.56	177106	178184
B11	1	1	Heat Exchanger	Area/ft2	12532	6805	0.54	132800	1997	265600	0.68	175347	2.1	368228	377755
B13	1	1	CO2 Compressor	2A	98040	4401	0.04	278200	2000	556400	0.34	193695	1.3	251803	253336
B9	1	1	Heater	Duty (Cal/s)	7863670	3890560	0.49	158374	1996	316748	0.68	196289	2.1	412208	428191
B2	1		Screw Conveyor	17	225140	63390	0.28	59400	1997	59400	0.78	22103	1.3	28734	29477
B4	1		Tank Agitator	18	358810	160401	0.45	36000	1997	36000	0.51	23877	1.2	28652	29394
B4	1		Slurrying Tank	18	358810	160401	0.45	44800	1997	44800	0.71	25294	1.2	30353	31138
B5	1	1	Cooler	Duty (Cal/s)	277820	2185707	7.87	29544	1996	59088	0.68	240250	2.1	504524	524086
B1	3		Reactor	15			1.00	4846878	2000	14540634	1	14540634	2.29	33298051.9	33500831
B6	1		Separator (Condenser)	Duty (Cal/s)	277820	5556935	20.00	29544	1996	29544	0.68	226568	2.1	475792	494241
B6	1		Separator (Reflux drum)	Duty (Cal/s)	277820	5556935	20.00	11900	1997	11900	0.93	192994	2.1	405287	415773
			Total												42556868
			Additional Equipment											Cost (99 $)	
A205	1		Hydrolysate Mix Tank Agitator											34546	
C202	1		Hydrolysate Washed Solid Belt Conveyor											114375	
H200	1		Hydrolysate Cooler											125160	
P205	2	1	Pneumapress Feed Pump											151994	
P225	1	1	ISEP Elution Pump											42815	
P226	1	1	ISEP Reload Pump											73685	
S221	1		ISEP											2699169	
T205	1		Hydrolysate Mixing Tank											39220	
			Total											3280964	3330522
			Grand Total												45887390

However, these are typical instruments that must be employed in this sort of process for optimum product output. Hence, power requirements of such equipment were assumed to be same as those provided in the NREL economic model. For example, reactor screw (B1) power requirement was taken as same as WM202 given in the NREL model. Hence, the total electricity cost was calculated as given in the Table 9.15.

Table **9.14 Assumptions for Energy Cost Calculation**

Cost year for Analysis	2001
Life of Equipment	10
Ethanol Density (kg/l)	0.7894
Operating Days per Year	330
Operating Hours per Year	7920
Electricity ($/kWhr)	0.04
Cooling Water ($/1000 lb)	0.055
Cooling Water Temperature (^{0}C)	15
Steam (2000 psi) $/1000 lb	4.50

The cooling duty and the heating duty of different process units were determined by the Aspen plus model. These data were used to calculate the total cooling duty and the total heating duty for the carbonic acid pretreatment and finally the total energy cost of the pretreatment process. These results are summarized in tables 9.16, 9.17 and 9.18.

Table 9.15 Electricity Cost

Equipment Name	Number	Power (kW)	Cost ($/hr)	Cost ($/yr)
Feed Pump	B7	268	10.72	84902
Loading Pump	B12	451	18.04	142877
Primary Filtrate Pump	B14	162	6.48	51322
Compressor	B13	235	9.4	74448
Reactor Screw (3 Nos.)	B1 (WM 202)	863.08	34.5	273424
Screw Conveyor	B3 (WC 201)	36.96	1.5	11709
Screw Conveyor	B2 (WC 202)	44.59	1.8	14126
Tank Agitator	B4 (WT 232)	25.55	1.0	8094
Press Filter (3 Nos.)	B10 (WS 202)	133.8	5.4	42388
Total		2219.98		703290

Table 9.16 Cooling Duty/Cost

Equipment Name	Number	Duty (kW)	Temp. diff. 0C	Flow Rate (kg/s)	Flow Rate (kg/hr)	Cost ($/yr)
Cooler	B5	9151	17	128.2	461395	442164
Separator/condenser	B6	23266	70	79.1	284890	273016
Compressor Cooler	B13	689	10	16.4	59057	56596
Total				224	805342	771775

Table 9.17 Heating Duty/Cost

Equipment Name	Number	Duty (kW)	Latent Heat (kJ/lb)	Flow Rate (lb/hr)	Cost ($/hr)	Cost ($/yr)
Heater	B9	16289	1138	51529	231.9	1836506
Total						1836506

Table 9.18 Annual Total Energy Cost

Electricity	703290
Cooling Duty	771775
Heating Duty	1836506
Total Energy Cost (2001$)	3,311,571

Sensitivity Analysis

Introduction

Sensitivity analyses were conducted to answer the question of whether there is optimum pretreatment condition or conditions that could be used in commercial scale. Laboratory results from Yourchisin and others indicate that the best process conditions were in the vicinity of 2000 psi, 220 ^{0}C at 4 min. These conditions must further be analyzed in terms of costs to reach a conclusion. Hence, to answer these questions and to better understand the process conditions and their effect on the overall cost of carbonic acid pretreatment, several sensitivity analyses were carried out using Aspen Plus modeling results.

Variation of Total Cost with Temperature

As explained in previous sections, equipment cost and operating cost were calculated for different pretreatment conditions. However, reactor residence time remained constant at 4 min. throughout this calculation. NREL, in their economic model used the discounted cash flow method to calculate the yearly total equipment cost for different process sections. However, owing to time constraints and lack of economic data to carry out such a rigorous cash flow analysis, we used straight-line depreciation with 20% per year depreciation level for equipment. With this assumption, it was possible to calculate total cost of pretreatment per year for different pretreatment conditions as given in the Table 9.19. All values are in 2001 US dollars. Figure 9.6 depicts the variation of the total cost with temperature at different reaction pressures.

Table 9.19 Variation of Total Cost at Constant Pressure

Parameters		Total Equipment Cost ($)	Equipment Cost (per year) ($)	Operating Cost (per year) ($)	Total Cost (2001$/yr)
Pressure	Temperature				
2000	220	45887390	9177478	3311571	12489049
2000	200	45061023	9012205	2742546	11754751
2000	180	43736623	8747325	2208698	10956023
1600	220	38481612	7696322	3280963	10977285
1600	200	37921785	7584357	2712051	10296408
1600	180	36909742	7381948	2178089	9560037
1200	220	31506117	6301223	3250150	9551373
1200	200	31105209	6221042	2681126	8902168
1200	180	30379441	6075888	2147277	8223165
1000	220	28138493	5627699	3234846	8862545
1000	200	27808264	5561653	2665821	8227474
1000	180	27224859	5444972	2131973	7576945
800	220	24847209	4969442	3219542	8188984
800	200	24594033	4918807	2650517	7569324
800	180	24131714	4826343	2116669	6943012

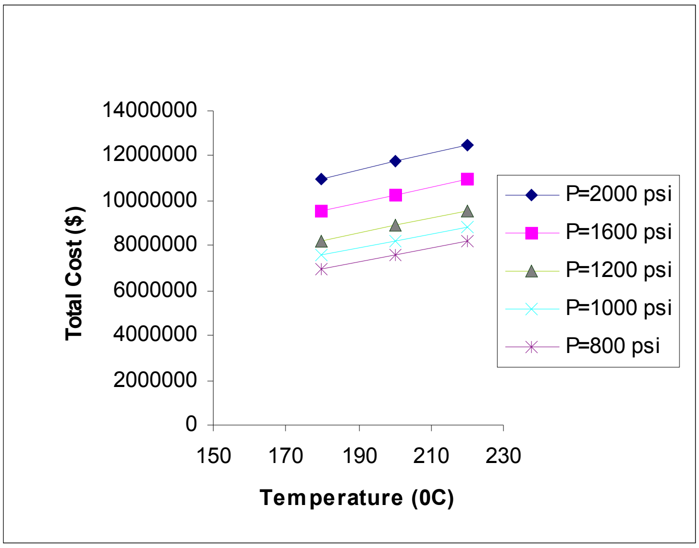


Figure 9.6 Variation of Total Cost with Temperature

Variation of Total Cost with Pressure

In this analysis, total cost variation with pressure is observed for a given temperature. As in the first analysis, for this analysis too, a 20% straight-line depreciation is assumed to calculate yearly equipment cost for pretreatment section (Table 9.20). Same information was presented in graphical form to better understand the trend (Figure 9.7).

Table 9.20 Variation of Total Cost at Constant Temperature

Parameters		Total Equipment Cost	Equipment Cost (per year)	Operating Cost (per year)	Total Cost (2001$/yr)
Pressure	Temperature				
2000	220	45887390	9177478	3311571	12489049
1600	220	38481612	7696322	3280963	10977285
1200	220	31506117	6301223	3250150	9551373
1000	220	28138493	5627699	3234846	8862545
800	220	24847209	4969442	3219542	8188984
2000	200	45061023	9012205	2742546	11754751
1600	200	37921785	7584357	2712051	10296408
1200	200	31105209	6221042	2681126	8902168
1000	200	27808264	5561653	2665821	8227474
800	200	24594033	4918807	2650517	7569324
2000	180	43736623	8747325	2208698	10956023
1600	180	36909742	7381948	2178089	9560037
1200	180	30379441	6075888	2147277	8223165
1000	180	27224859	5444972	2131973	7576945
800	180	24131714	4826343	2116669	6943012

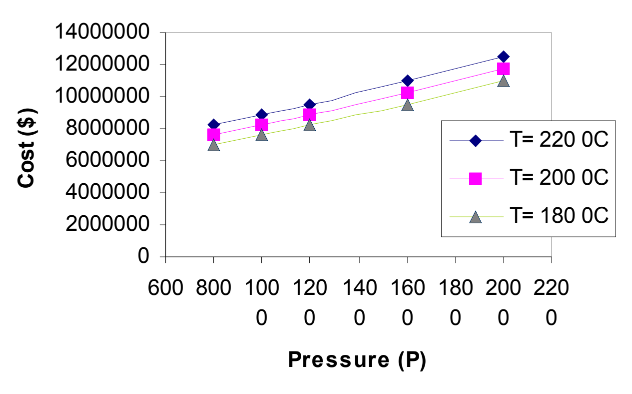


Figure 9.7 Total Cost Variation at Constant Temperature

Glucose and Xylose Yield at Different Severities

McWilliams (2002) has reported xylose yield for aspen wood at different severities. From that, average xylose yields were calculated for different pretreatment conditions such as temperature and residence time. In his analysis, McWilliams used only 1% solid solution. However, in the current study, the material stream flow to the reactor consists of 52% solids. Also, total flowrate to reactors is about 3600.8 l/min. Hence, xylose flow rate was calculated in mg/hr as given in Table 19. Then, xylose flow rate is converted to mol/yr using molecular weight of xylose monomers.

The effectiveness of biomass pretreatment is often correlated using a severity function, which combines the effects of time and pretreatment temperature into one function.

$R_0 = t * \exp[(T-100)/14.75]$ (Overend and Chornt)

Yourchisin (2002) has reported glucose yields from aspen wood at different severities. Since values reported are limited in number (only three data points), a trend line was constructed along the data points by plotting percentage of glucose yield against Log R_0 (logarithmic of reaction severity R_0) as given in the Figure 9.8.

Table 9.21 Xylose Yield at Different Severities

Residence Time (min.)	Temp. (0C)	Xylose (mg/l)	Xylose @ 52% solid	Xylose (mg/hr)	Xylose (mol/hr)	Xylose (mol/yr)
2.5	180	N/A				
6.5	180	19.13	271	58486828	390	3085389
14.5	180	57.56	816	175980231	1172	9283587
30.5	180	129.7	1839	396536413	2641	20918714
2.5	200	N/A				
4	200	100.2	1420	306345016	2041	16160795
6.5	200	46.15	654	141096033	940	7443320
14.5	200	379.5	5379	1160258817	7728	61207803
30.5	200	670.5	9504	2049943444	13654	108141849
2.5	220	120.3	1705	367797459	2450	19402632
4	220	371.1	5260	1134577199	7557	59853005
6.5	220	273.3	3874	835569788	5566	44079295
14.5	220	423.5	6003	1294781578	8624	68304360
30.5	220	101.2	1435	309402351	2061	16322081

(N/A: Not Available)

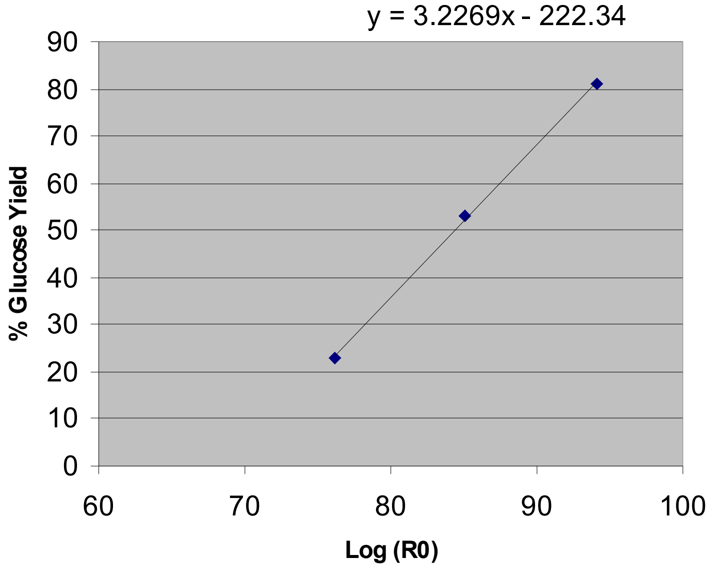


Figure 9.8 Percentage Glucose Yield Vs. Log R_0

The equation of the trend line was used to calculate the percentage of glucose yield at other severities that were not reported previously. The results are summarized in Table 9.22.

Table 9.22 Percentage Yield of Maximum Glucose

Temperature (^{0}C)	Time (min.)	R_0	Log R_0	% Glucose yield
180	8	1.35434E+76	76.13172736	23
200	16	1.31416E+85	85.11864699	53
220	32	1.27517E+94	94.10556663	81
180	4	6.77169E+75	75.83069737	22
180	16	2.70868E+76	76.43275736	24
180	32	5.41736E+76	76.73378735	25
180	64	1.08347E+77	77.03481735	26
200	4	3.28539E+84	84.516587	50
200	8	6.57078E+84	84.817617	51
200	32	2.62831E+85	85.41967699	53
220	4	1.59396E+93	93.20247664	78
220	8	3.18791E+93	93.50350664	79
220	16	6.37583E+93	93.80453663	80

Yourchisin in his calculations assumed that an average sample contained 60% cellulose by weight. However, it was found that on average, pretreated aspen wood solids have 64.2% cellulose by weight. Therefore, the reported glucose yield from cellulose (i.e. 2 g/l) was lower than the actual value. Hence, the actual 100% (or maximum) glucose yield is calculated as follows:

$2 \text{ g/l} * 64.2/60 = 2.14 \text{ g/l}$

Using this value, glucose yields at different severities are found in g/l. Finally, using glucose molecular weight, total glucose flowrate in mol/yr was calculated for each pretreatment condition. The data are reported in Table 9.23.

Calculation of Ethanol Revenue

In this study, it was assumed that ethanol is produced from fermentation of glucose and xylose. According to the NREL model, 85% of xylose is converted to ethanol while 92% of glucose is converted to ethanol. Since extent of fermentation has not been evaluated in the laboratory, the same conversions are used for the current analysis too.

Table 9.23 Glucose Yield at Different Severities

Residence time (min.)	Temp. (0C)	% Glucose yield	Glucose (g/l)	Glucose (g/g)	Glucose (g/hr)	Glucose (mol/hr)	Glucose (mol/yr)
4	180	22.36	0.479	0.134	11169516	62053	491458725
8	180	23	0.492	0.138	11489216	63829	505525522
16	180	24.3	0.520	0.146	12138607	67437	534098704
32	180	25.27	0.541	0.151	12623152	70129	555418693
64	180	26.24	0.562	0.157	13107697	72821	576738683
4	200	50.39	1.078	0.302	25171375	139841	1107540481
8	200	51.36	1.099	0.308	25655920	142533	1128860471
16	200	53	1.134	0.318	26475151	147084	1164906638
32	200	53.3	1.141	0.320	26625010	147917	1171500449
4	220	78.42	1.678	0.470	39173233	217629	1723622237
8	220	79.39	1.699	0.476	39657778	220321	1744942227
16	220	80.36	1.720	0.482	40142323	223013	1766262216
32	220	81	1.733	0.486	40462023	224789	1780329013

Using xylose and glucose mole flowrates, the total ethanol flow can be determined for different pretreatment conditions. Finally, by using ethanol selling price of $ 1.57 (99 $) as calculated by NREL, the ethanol revenue from glucose and xylose can be obtained as given in Tables 9.24 and 9.25.

Table 9.24 Ethanol Revenue from Glucose

Residence time (min.)	Temp. (0C)	Glucose (mol/yr)	EtOH (mol/yr)	EtOH (gal/yr)	EtOH ($/yr) (1999$)
4	180	491458725	904284054	12536789	19682759
8	180	505525522	930166961	12895624	20246130
16	180	534098704	982741615	13624507	21390476
32	180	555418693	1021970396	14168366	22244335
64	180	576738683	1061199176	14712225	23098193
4	200	1107540481	2037874485	28252630	44356629
8	200	1128860471	2077103266	28796489	45210488
16	200	1164906638	2143428214	29716003	46654125
32	200	1171500449	2155560827	29884207	46918205
4	220	1723622237	3171464916	43968471	69030499
8	220	1744942227	3210693697	44512330	69884358
16	220	1766262216	3249922477	45056189	70738216
32	220	1780329013	3275805384	45415023	71301587

Table 9.25 Ethanol Revenue from Xylose

Residence Time (min.)	Temp. (0C)	Xylose (mol/yr)	EtOH (mol/yr)	EtOH (gal/yr)	EtOH (1999 $/yr)
2.5	180	N/A			
6.5	180	3085389	4379710	60719	95329
14.5	180	9283587	13178051	182698	286835
30.5	180	20918714	29694115	411672	646326
2.5	200				
4	200	16160795	22940249	318038	499320
6.5	200	7443320	10565793	146482	229976
14.5	200	61207803	86884476	1204547	1891138
30.5	200	108141849	153507355	2128191	3341260
2.5	220	19402632	27542035	381837	599483
4	220	59853005	84961341	1177885	1849279
6.5	220	44079295	62570559	867464	1361919
14.5	220	68304360	96958038	1344204	2110401
30.5	220	16322081	23169194	321212	504304

Variation of Capital Cost with Pretreatment Residence Time

As seen before, capital cost or equipment cost is a function of reactor cost since it is the most expensive single piece of equipment. However, reactor cost is a function of reactor size or more precisely residence time. Hence, capital cost can be expressed as a function of reactor residence time. In the Table 9.26, capital cost is calculated for different pretreatment residence times at 2000 psi and 220 ^{0}C.

Table 9.26 Capital Cost Variation with Residence Time

Residence Time (min.)	Pressure (psi.)	Temperature (0C)	Reactor Cost (2001 $)	Capital Cost (2001 $)
2	2000	220	15539720	27926279
4	2000	220	33500831	45887390
6	2000	220	46619159	59005718
8	2000	220	62237690	74624249
10	2000	220	77797111	90183670

Currently not all the xylose and glucose are converted to ethanol. Therefore, there were for all pretreatment conditions lost ethanol revenue, which, could be calculated as follows;

Lost ethanol revenue from xylose = 100% xylose yield – actual xylose yield

Lost ethanol revenue from glucose = 100% glucose yield – actual glucose yield

The results are summarized in Table 9.27.

Table 9.27 Lost Ethanol Revenue from Xylose and Glucose

Lost Ethanol Revenue from Xylose			Lost Ethanol Revenue from Glucose		
Residence Time (min.)	Temp. (0C)	Lost EtOH ($/yr)	Residence time (min.)	Temp. (0C)	Lost EtOH ($/yr)
2.5	180	N/A	4	180	N/A
6.5	180	31625695	8	180	83625318
14.5	180	31434189	16	180	N/A
30.5	180	31074698	32	180	70873794
			64	180	53186196
2.5	200	31721024			
4	200	31221704	4	200	N/A
6.5	200	31491048	8	200	N/A
14.5	200	29829886	16	200	44383531
30.5	200	28379764	32	200	49401872
2.5	220	31121541	4	220	46687032
4	220	29871745	8	220	62400200
6.5	220	30359105	16	220	62400200
14.5	220	29610623	32	220	43807655
30.5	220	31216720			

(N/A: Data not available)

Then, all this information can be plotted in one graph to find the effect of residence time on capital cost and ethanol revenue as shown in Figure 9.9. It can be seen from this graph that low residence times are critical to reducing costs associated with carbonic acid pretreatment.

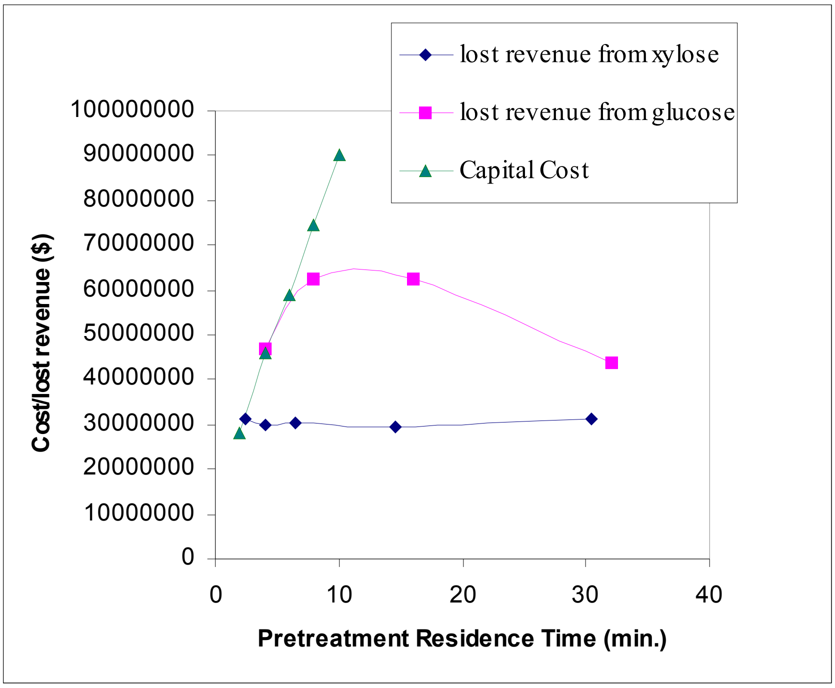


Figure 9.9 Cost Vs. Pretreatment Residence Time (P=2000 psi, T= 220 ^{0}C)

Comparison of Carbonic Acid Pretreatment and Sulfuric Acid Pretreatment

The central hypothesis of this research is that there will be negative incremental cost for carbonic acid pretreatment process compared sulfuric acid process. In other words, cost of carbonic acid pretreatment would be less than that of sulfuric acid process.

From sensitivity analysis, it is seen that the optimum pretreatment conditions are (Figure 9.9):

P= 2000 psi, T= 220 ^{0}C, Residence time= 4 min.

At these conditions,

Total Equipment cost (2001 \$, Scenario 1) = \$ 45,887,390
Energy cost (2001 \$) = \$ 3,311,571
Ethanol revenue from glucose (1999 \$) = \$ 69,030,499
Ethanol revenue from xylose (1999 \$)= \$ 1,849,279
Total revenue (1999 \$)= \$ 70,879,778
Total revenue (2001 \$)= \$ 71,950,414

Comparison of sulfuric acid pretreatment process and carbonic acid pretreatment process show that in the sulfuric acid process, there are some additional operating costs due to the use of reagents and the waste disposal as compiled from NREL report (Table 9.28).

Table 9.28 Reagents Cost and Waste Disposal Costs (Wooley et al. 1999b)

Component	Stream No	kg/hr	ton/yr	Price (\$/ton)	Year	Total Cost (\$/yr)
Sulfuric Acid	STRM0710	2128	16854	25	2001	421,344
Lime	STRM0745	913	7231	70	2001	506,167
Gypsum disposal		2433	19269	22		423,926
Total						1,351,437

This cost is added to the energy cost since these costs are due to the sulfuric acid pretreatment. Hence, the total variable operating cost for sulfuric acid system is given as 7 million US dollars approximately, in Table 9.29.

Table 9.29 Comparison of Costs of Carbonic Acid and Sulfuric Acid Processes

Pretreatment Method	Capital Cost (2001 \$) @ 2000 tonnes/day	Variable Operating Cost (2001 \$) @ 2000 tonnes/day
Carbonic Acid (Scenario 1)	45,887,390	3,311,571
Carbonic Acid (Scenario 2)	29,941,580	3,311,571
Sulfuric Acid	29,235,023	7 MM (approx.)

(Cost indices: 1999: 390.6, 2001: 396.5)

To test the hypothesis, the results from carbonic acid process have compared with cost figures from sulfuric acid process in the Table 27 above. Also, ethanol revenue from both systems are compared in the Table 9.30.

Table 9.30 Comparison of Ethanol Revenue of Carbonic Acid and Sulfuric Acid Processes

Pretreatment Method	Ethanol Revenue (2001 $) @ 2000 tonnes/day
Carbonic Acid	71,950,414
Sulfuric Acid	81,757,569

(Cost indices: 1999: 390.6, 2001: 396.5)

Conclusion

Although at the beginning of the deign process it was assumed that reactor cost could be lowered due to the use of stainless steel instead of Hastelloy C, high pressures associated with pretreatment (such as 2000 psi) made it extremely expensive to build reactors out of SS316 L. One option that can be used to minimize this cost is to clad the reactor with polymeric material such as Teflon and to reinforce it with stainless steel. Hence, Pretreatment reactor turned out to be the most expensive piece of equipment. It is about 2 times the cost of NREL Hastelloy reactor. About 50% of the total operating cost is due to the heat demand of the process. This is unavoidable due to difficulties in process heat recovery.

Overall, laboratory results have shown that pretreatment effectiveness is a function of time and temperature, and that high CO_2 pressure offers only limited benefits on selected substrates. Thus, lower pressure values are likely to offer similar performance at reduced cost.

Acknowledgements

Contributions made by following departments and organizations are greatly appreciated; Department of Environmental Studies, Baylor University; National Renewable Energy Laboratory (NREL); and the U.S. Department of Energy. Special thanks are due to Mark Ruth, National Renewable Energy Laboratory (NREL); Susan Power Bratton, Environmental Studies Department Chair; Larry Lehr, Department of Environmental Studies; Shauck Maxwell and Monty Suffern, both of Baylor Aviation Science Department.

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Yourchisin, D.M. 2002 Master's Thesis, Comparison of the Microbial Inhibition and Enzymatic Hydrolysis Rates of Liquid and Solid Hydrolysates Produced from Pretreatment of Biomass with Carbonic Acid and Liquid Hot Water, p21-36

Task 10) – Documentation and publication of results

Work from this project has been presented at four international meetings: the ACS annual meeting in Orlando Florida, April 7 – 11, 2002, the 24th Symposium on Biotechnology for Production of Fuels and Chemicals in Gatlinburg, TN, April 28 - May 1, 2002, the 12th European Conference and Technology Exhibition on Biomass for Energy, Industry and Climate Protection in Amsterdam, NL, 17-21 June 2002, and the 25th Symposium on Biotechnology for Production of Fuels and Chemicals in Brekenridge, CO, May 3- 7, 2003. A brief paper was published in the proceedings of the Amsterdam conference and papers were submitted for peer-reviewed publication in the conference proceedings of the 25th symposium.

The list of presentations and papers includes:

Oral presentation, van Walsum et al., ACS annual meeting in Orlando Florida, April 7 – 11, 2002, 34 slides

Oral presentation, van Walsum et al., 24th Symposium on Biotechnology for Production of Fuels and Chemicals in Gatlinburg, TN, April 28 - May 1, 2002, 35 slides.

Poster presentation, Jayawardhana and van Walsum, 24th Symposium on Biotechnology for Production of Fuels and Chemicals in Gatlinburg, TN, April 28 - May 1, 2002, 14 slides.

Poster presentation, Yourchisin and van Walsum, 24th Symposium on Biotechnology for Production of Fuels and Chemicals in Gatlinburg, TN, April 28 - May 1, 2002, 11 slides.

Poster presentation, Castleberry and van Walsum, 24th Symposium on Biotechnology for Production of Fuels and Chemicals in Gatlinburg, TN, April 28 - May 1, 2002, 18 slides.

Poster Presentation, van Walsum et al. 12th European Conference and Technology Exhibition on Biomass for Energy, Industry and Climate Protection in Amsterdam, NL, 17-21 June 2002, 15 slides.

Conference proceeding paper, van Walsum et al.12th European Conference and Technology Exhibition on Biomass for Energy, Industry and Climate Protection in Amsterdam, NL, 17-21 June 2002, 4 pages. (See appendix 1)

Yourchisin and van Walsum, paper submitted to Applied Biochemistry and Biotechnology. (See appendix 1)

Jayawardhana and van Walsum, paper submitted to Applied Biochemistry and Biotechnology. (See appendix 1)

Appendix 1: Publications

Comparison of the Microbial Inhibition and Enzymatic Hydrolysis Rates of Liquid and Solid Hydrolysates Produced from Pretreatment of Biomass with Carbonic Acid and Liquid Hot Water

Damon Yourchisin
G. Peter van Walsum*

ABSTRACT (draft in progress)

Achieving production of renewable ethanol from lignocellulosic biomass would be promoted if hydrolysates could be produced that allow rapid and high yield conversion of cellulose to fermentable sugars and have a low inhibition to fermenting organisms such as *Saccharomyces cerevisiae*. This research quantified the enzymatic digestibility of the solid component and the microbial inhibition of the liquid component of pretreated aspenwood hydrolysates. Products of liquid hot water and carbonic acid pretreatment were compared. Pretreatment temperatures tested ranged from 180^{0}C to 220^{0}C, reaction times were varied between 4 and 64 minutes.

The research was conducted in 2 parts. Part one was In-vitro inhibition of yeast in liquid hydrolysate, done by measuring the rate of glucose uptake by an anaerobic culture of *Saccharomyces cerevisiae* growing in hydrolysates produced at different reaction severities. The degree of inhibition was correlated to the severity of pretreatment and to the presence or absence of carbonic acid. Part 2 focused on enzymatic hydrolysis of pretreated solids by measuring rates and yield of glucose accumulation through enzyme digestion of pretreated solids. Hydrolysis rates were correlated to severity of pretreatment and to the presence or absence of carbonic acid.

Both microbial inhibition rates and enzymatic hydrolysis rates showed no difference between pretreatments containing carbonic acid and pretreatments containing no carbonic acid. When microbial inhibition rates were examined with increasing reaction severity, the inhibition increased as the reaction severity increased, but only above a midpoint severity parameter of 200^{0}C for 16 minutes. Below this midpoint severity parameter there was little to no inhibition of the yeast. When enzymatic hydrolysis rates and yields were examined with increasing reaction severity, both the rates and yields displayed an increase from the lowest tested reaction severity to the highest tested reaction.

* Damon Yourchisin, graduate student, presenter
G. Peter van Walsum, assistant professor

Modeling of Carbonic Acid Pretreatment Process Using ASPEN-Plus

Kemantha Jayawardhana*
G. Peter van Walsum

ABSTRACT (Draft in progress)

Use of carbonic acid instead of sulfuric acid for the pretreatment of biomass would offer environmental benefits for the production of renewable fuels and chemicals. The viability of this substitution depends on the economics of the process. Laboratory work in the Department of Environmental Studies at Baylor University has assessed the process parameters of the carbonic acid system. Economic costs and benefits of the process is determined using Aspen Tech's ASPEN PLUS process modeling software.

Aspen Tech's ASPEN PLUS process modeling software is being used to model carbonic acid pretreatment of biomass process. ASPEN PLUS is used because of the thorough treatment of thermodynamic interactions and its status as a widely accepted process simulator. The physical property data for many of the key components used in the simulation for the pretreatment process are derived from the In-house database (INHSPCD) developed by National Renewable Energy Laboratory (NREL). Because of the need to distil ethanol and to handle dissolved gases the standard NRTL (Non-Random Two Liquid or Renon) route is used as the main property method. The pretreatment reactor is modeled as a "black box" reactor due to unavailability of reaction kinetics. Stoichiometric data are used to define reactions. The Aspen-Plus model developed is used to calculate energy costs of carbon dioxide compression with energy recovery options for pretreatment process. Laboratory data is used to calculate ethanol revenue from carbonic acid pretreatment for different reaction severities. Model results indicating economic advantages and disadvantages of the carbonic acid system, compared to water-only and sulfuric acid based systems are discussed.

ANNALYSIS OF CARBONIC ACID PRETREATMENT HYDROLYSATES DERIVED FROM ASPEN WOOD AND CORN STOVER

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ABSTRACT: The purpose of this work was to determine the effectiveness of carbonic acid (H_2CO_3) as a biomass pretreatment for the production of fuels and chemicals through enzymatic hydrolysis and fermentation. Acids commonly used for pretreatment, such as sulfuric acid or sulfur dioxide, present corrosion and pollution problems, and the waste generation associated with the use of sulfuric acid can result in prohibitive waste disposal costs. Pretreatments using only water as a reagent, such as steam explosion and liquid hot water pretreatment, have no independent means of controlling pH, limiting their flexibility. The use of H_2CO_3 allows control of reaction pH by manipulating CO_2 pressure in the reactor. The source of the CO_2 could be the fermentation processes downstream of the pretreatment system.

The approach used was to conduct batch pretreatment tests over a variety of temperature, carbon dioxide pressure and reaction duration conditions. The product was analyzed for pH, UV absorption, soluble carbohydrate concentrations, toxicity to yeast and enzymatic hydrolysis rates. Previous results have shown that H_2CO_3 is an effective hydrolysis catalyst for purified compounds. This study shows that the effectiveness at increasing hydrolysis product concentrations from raw biomass is variable and depends on the nature of the substrate. Compared to water-only pretreatments, the use of H_2CO_3 results in a higher final pH of the hydrolyzate and may therefore reduce the concentration of organic acids present in the hydrolysate.

Keywords: Carbonic acid, pretreatment, aspen wood, corn stover

1 INTRODUCTION

The use of biomass-derived ethanol as an alternative to fossil fuels offers many benefits: reduced greenhouse gas emissions, reduced exhaust emissions, decreased reliance on imported fuel sources, an improved agricultural economy, reduction of trade deficit and increased national security [1]. In order to produce ethanol in sufficient quantity to displace a substantial portion of petroleum-based fuels, lignocellulosic materials must become a significant source of feedstock [2]. In order to achieve useful rates of enzymatic hydrolysis, the lignocellulose must first be pretreated to reduce the recalcitrance of the substrate to hydrolysis. Pretreatment accomplishes many alterations of the biomass, including: hydrolysis of the hemicellulose, solubilization of lignin, and increased accessibility of the cellulose to cellulase enzymes [3].

Several pretreatment methods have been explored to varying degrees. The most commonly reported technologies include dilute-acid pretreatment, in which sulfuric acid is used in low concentrations (on the order of 1%) and at temperatures usually less than 200°C [4,5]; and steam explosion, which exposes the substrate to steam at elevated temperature and then explosive decompression to physically break apart the plant fibers [6]. Often, steam explosion is coupled with acid catalysis by impregnating the substrate with sulfur dioxide prior to steam treatment [7]. Other techniques include ammonia fiber explosion (AFEX) and treatment with liquid hot water [8,9]. Some methods that have been examined less thoroughly include treatment with supercritical fluids [10] and carbonic acid [11,12,13].

Steam-explosion and dilute-acid pretreatment have undergone research and development for many years. Dilute acid pretreatment offers good performance in terms of recovering hemicellulose sugars, but suffers from its use of sulfuric acid. Sulfuric acid is highly corrosive, and its neutralization results in copious production of calcium sulfate, which has a problematic solubility characteristic in that it becomes less soluble at higher temperatures, such as those encountered in a reboiler [11]. Typically, steam explosion suffers from low hemicellulose yields and an inhibitory hydrolysate.

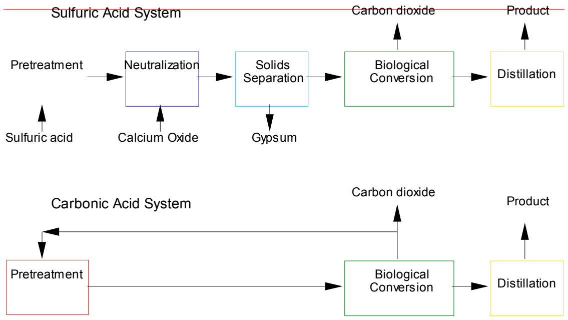

Figure 1: The Carbonic Acid process configuration offers simplification compared to Sulfuric Acid systems.

One process that may offer 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 fugacity of carbon dioxide in contact with water, thus it can be neutralized by releasing the reactor pressure. Carbonic acid is relatively mild and does not offer the same hydrolytic capability of sulfuric acid. However, van Walsum has demonstrated that at temperatures on the order of 200°C, carbonic acid does exhibit a catalytic effect on hydrolysis of xylan [11]. Puri and Mamers compared steam explosion of biomass with and without carbon dioxide pressurization and reported enhanced enzymatic degradation with the carbonic acid enhanced steam explosion [12]. However, McWilliams and van Walsum found that aspen wood hydrolysates produced by carbonic acid pretreatment showed no evidence of an increase in reaction severity resulting from the addition of the carbonic acid [13]. Carbonic acid also offers an elegant simplification of the pretreatment process configuration, in that it eliminates the need for several unit operations (see figure 1). This study seeks to further investigate the action of carbonic acid pretreatment on biomass.

2 MATERIALS AND METHODS

2.1 Materials

Aspen wood chips were kindly supplied by the USDA Forest Products Laboratory in Madison WI. Corn stover was kindly supplied by the National Renewable Energy Laboratory in Golden CO. Prior to pretreatment, the biomass was ground in a domestic brand coffee grinder and sifted to a particle size of between 0.5 and 1 mm. Dry weight of the biomass was determined by oven drying. Carbon dioxide was standard laboratory grade, the H_2O was of de-ionized quality. NaOH was purchased as an analytical grade, 50% aqueous solution.

2.2 Methods

Reactions were performed in one of two reactors: either a 15 mL or a 150ml 316 stainless steel vessel. The reactors were filled and emptied by removing a swage connection on one end. For reactions using CO_2, a stainless steel tubing connection and valve were fitted to the reactor to allow introduction of CO_2 from a gas cylinder. Pressure of the CO_2 was regulated using a high-pressure regulator on the CO_2 cylinder. The reaction temperature was controlled by quickly immersing the reactor in a fluidized sand bath (Techne, Oxford UK, model SBL 2D) with temperature controller (Techne model TC-8D) that maintained temperatures in the bath to +/- 1°C.

Reaction temperatures ranged from 180 °C to 220 °C. Reactions were carried out for durations ranging from 2 to 32 minutes before removing the reactor from the sand bath and quenching the reaction in a cold-water bath. Extra time was allowed for heating up the reactor. For some experiments, the heating up of the reactors was done in a separate bath maintained at the reaction temperature plus 40 °C. This allowed for the transient heating time to be reduced. Further descriptions of the reactors and hydrolysis procedure have been reported previously [11].

In each of the reactions, ground biomass and water were put into the reactor in a 1:80 mass ratio. In the samples reacted with carbonic acid, the reactors were also charged with 800 psi of CO_2 at room temperature. At most conditions, triplicate experiments were conducted, though some points had fewer experimental data while others were repeated more than three times. Outliers in the data were rejected at the 99% confidence level using a Q test.

Hydrolysate absorbance was analyzed using an UV-visual spectrophotometer (Beckman Corporation, Fullerton CA, model DU 500). Absorbance at 270 to 280 nm correlates to furan concentration, which in turn has been correlated to microbial toxicity [14]. Analysis of pH used a pH meter (Fisher Scientific, Acumet research model AR15) and was done with the samples depressurized and at room temperature. Soluble carbohydrate concentrations were measured using high performance anion exchange chromatography with pulsed amperometric detection (HPAE-PAD) and a Dionex Carbopac PAX-100 column.

3 RESULTS

Results are presented for pretreatment of aspen wood and corn stover. Absorbance, pH and xylose yield results for the aspen wood hydrolysate represent an expansion and improvement upon data presented in an earlier publication [13].

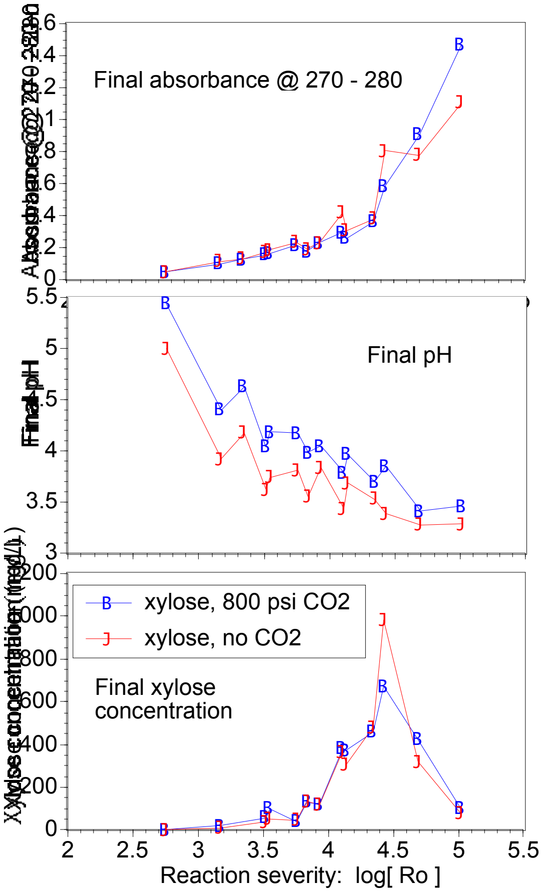

Figure 2: Response of absorbance at 275 nm, final pH and xylose concentration versus reaction severity for aspen wood pretreated in either water or carbonic acid.

1. Absorbance

McWilliams and van Walsum [13] found that hydrolysates from aspen wood pretreatment showed no influence of the carbonic acid on the absorbance at 275nm, compared to hot water pretreatment. Repeats of these experiments confirmed this earlier conclusion with increased certainty. Averaged results from the improved data set are presented in figure 2. Absorbance is seen to be a strong function of reaction severity.

Pretreatment tests on corn stover showed that here too the absorbance of the hydrolysate at 275 nm showed no influence from the presence of carbonic acid during pretreatment. Figure 3 shows a bar graph depicting the differences in hydrolysate measurements between carbonic acid and liquid hot water pretreatment. Results shown are averages taken from triplicate experiments. It can be seen that the distribution of differences in absorbance at 275nm is random and in many cases there is little difference between the water and carbonic acid.

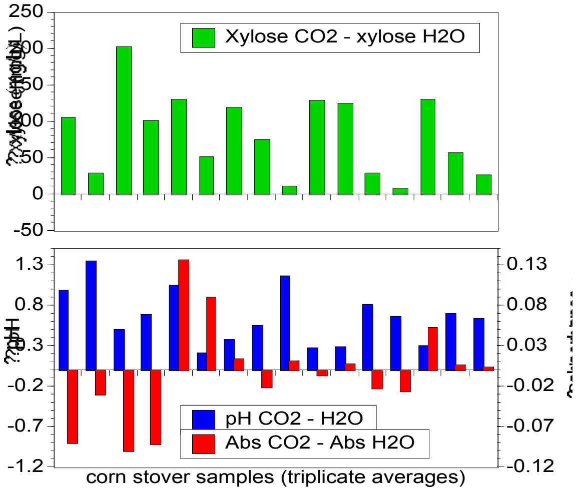

Figure 3: Differences in average xylose, pH and absorbance values for hydrolysates generated from carbonic acid and water pretreatment of corn stover.

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3.2Xylose yield

McWilliams and van Walsum [13] found that hydrolysates from aspen wood pretreatment showed no influence of the carbonic acid on the concentration of xylose, compared to hot water pretreatment. Repeats of these experiments confirmed this earlier conclusion with increased certainty. Averaged results from the improved data set are presented in figure 2. It can be seen that very high severity conditions result in a diminished xylose yield.

Contrary to the results on aspen wood, corn stover did show an influence of carbonic acid on the concentration of xylose released. At every severity tested (see figure 3), corn stover showed more xylose was released when pretreated with carbonic acid than when pretreated with water alone.

3.3Residual pH

McWilliams and van Walsum [13] found that the final pH of hydrolysates from aspen wood pretreatment were consistently higher than the final pH of hydrolysates produced using water alone. Repeats of these experiments confirmed this earlier conclusion with increased certainty. Averaged results from the improved data set are presented in figure 2. It can be seen that higher severity results in lower final pH but that the difference in pH between the two pretreatment systems is more or less constant.

Similar pH results have been recorded with corn stover. Figure 3 shows the differences in pH measured on corn stover at different reaction conditions and severity. Figure 4 shows pH results for aspen wood and corn stover at selected reaction severities. It can be seen that for both substrates the residual pH is higher when pretreatment was done with carbonic acid, and that the residual pH of corn stover is considerably higher than aspen wood.

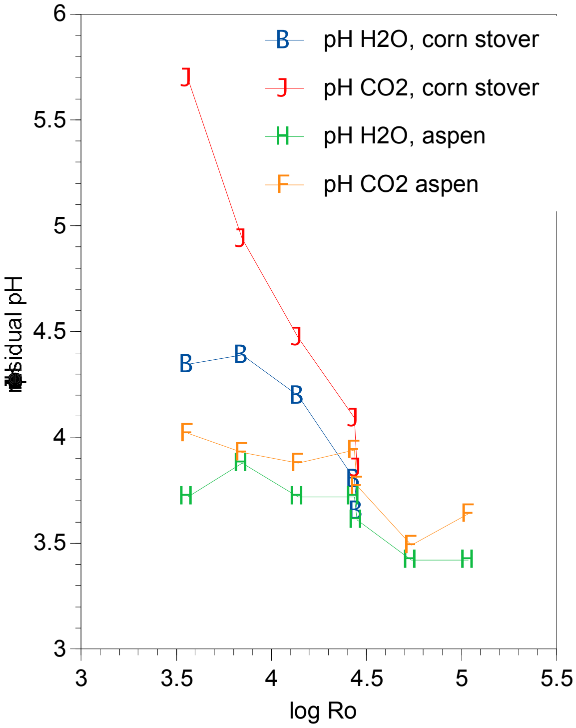

Figure 4: Residual pH of aspen wood and corn stover samples pretreated with either water or carbonic acid at varying severities.

4 DISCUSSION

McWilliams and van Walsum reported [13] that hydrolysates from pretreated aspen wood appeared to be little affected by the presence or absence of carbonic acid. This was contrary to earlier findings by van Walsum [11] that showed that carbonic acid was active against pure xylan. This difference was attributed to the acetyl groups present in aspen wood that effectively lowered the pH low enough to render the acid contribution of carbonic acid irrelevant. It is curious, therefore, that the one difference that did appear to be caused by the use of carbonic acid was a decrease in the acid concentration of the final hydrolysate. Repeat experiments done for this work have reaffirmed these earlier conclusions. An explanation for this phenomenon of increased pH has not yet been formulated.

Corn stover differs from aspen wood in several respects, the most apparent of which appears to be its reduced ability to autohydrolyse. Corn stover has on the order of one third the concentration of acetyl groups as does aspen wood, and so it is not surprising the note that the final pH of the corn stover hydrolysate is higher than that of the aspen wood hydrolysate. From the xylose concentrations, we see that unlike aspen wood, hydrolysis of corn stover does improve with the addition of carbonic acid. This additional hydrolysis must be caused by the carbonic acid, since the higher residual pH of the hydrolysate suggests that the organic acid concentration is lower for the carbonic acid pretreated material. With more severe pretreatment conditions, one would anticipate a higher absorbance for the carbonic acid pretreated corn stover, but this does not appear to be the case. Although this is difficult to explain, it is a positive result, in that it demonstrates that compared to liquid hot water pretreatment, carbonic acid pretreated corn stover may achieve a higher xylose yield without increasing sugar degradation.

5 CONCLUSIONS

Results have shown that the value of carbonic acid as an enhancement to hydrolysis in pretreatment is substrate-dependent. This study confirmed that carbonic acid has little apparent affect on the severity conditions of pretreatment of aspen wood, but that it does have a positive effect on pretreatment of corn stover. Results have also confirmed that the residual pH of carbonic acid pretreated biomass is higher than liquid hot water pretreated biomass—for both aspen wood and corn stover. An explanation for this phenomenon is still lacking.

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