Kinetics of Xylose Oligomers Decomposition

An Undergraduate Honors College Thesis

In the

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By

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This thesis is approved.

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Project Description

Abstract

Hemicellose-derived oligomers are key components in hemicellulose depolymerization into the five-carbon carbohydrate xylose, a key operation in the production of cellulosic biofuels. Unfortunately, hemicellulose oligomers do not only depolymerize directly into xylose, but also into degradation compounds that reduce the overall production of cellulosic biofuels. Because commercially available oligomers are prohibitively expensive, this study was carried out using relatively inexpensive commercial grade xylose. Understanding the degradation of xylose is important for its impact on the economic viability of the monomeric sugars-to-ethanol conversion. From experimental data and ensuing statistical analysis, initial xylose concentrations did not result in different xylose degradation rates, confirming that, indeed, the reaction was first order. The average degradation rate of xylose at 120°C water, 120°C 1% acid, 200°C water and 200°C 1% acid were 0.002, 0.001, 0.024 and 0.409 min^{-1}, respectively. The significance of this study showed that subsequent kinetic studies to be conducted with precious xylose oligomers could be performed at 1 g per l, and the degradation rate will remain first order up to the concentration of 5 g per l.

Introduction

To convert feedstock into ethanol, cellulose and hemicellulose, must be released and hydrolyzed into monomeric sugars that can be utilized by

fermentation microorganisms. The release of monomeric sugars from the plant cell wall, dilute acid or hot water (autohydrolysis) pretreatment is followed by the enzymatic hydrolysis of hemicellulose and cellulose portions. The sugar-rich hydrolysate from these procedures is then fermented into desired products by microorganisms. In addition of being used for biofuel production, xylose oligomers can be used in fortified foods, anti-obesity diets, animal feeds, agricultural ripening agents, and yield enhancers because of their lower sweetness and high stability over a wide pH range [1]. Xylose oligomers have also been used in pharmaceuticals because of their prebiotic effect on humans and by inhibiting the growth of foreign pathogens [7].

Recent studies of bioethanol production by enzymatic processes clearly show that hemicellulose hydrolysis, such as pretreatment, is the single most expensive element for the process, representing about one-third of the overall processing cost [8]. However, the total costs for converting the cellulose fraction into fermentable sugars are close behind at about 25–30% of the overall processing costs [8]. Because radical improvements in hemicellulose and cellulose hydrolysis technologies could clearly have a significant impact on the cost of bioethanol production, these opportunities merit particular attention to make bioethanol competitive on the open market [8]. Hemicellulose is a polymer made-up of xylose, a five-carbon sugar. Unfortunately, hemicellulose does not depolymerize directly into xylose, but into a series of oligomers of various lengths. And, in turn, these oligomers can form products, such as formic acid, acetic acid and furfural that

inhibit enzymatic hydrolysis and fermentation [2,3, 5, 9]. Therefore it is critical to understand how these oligomers depolymerize. When available, these xylose oligomers are sold at a price of 200 euros per 10 mg. Moreover, not all oligomers are commercially available, and must be produced through a lengthy in-house purification process. Since these in-house produced oligomers are so valuable, it is important to understand the minimum concentrations at which they must be prepared, such that useful data can be generated from the experiments. The use of inexpensive xylose is useful for such experiments. Therefore, pretreating xylose in dilute acid, followed by monitoring its degradation using high-performance liquid chromatography with a refractive index (HPLC-RI) can generate useful data. By studying the rate of degradation of xylose, the kinetics of xylose depolymerization could be established. A kinetic model was developed based on experiments with 1 mg/mL, 2 mg/mL and 5 mg/mL xylose pretreated in dilute acid at three temperatures: 120, 160 and 200°C. The results of these experiments set the stage for oligomer depolymerization studies by determining the minimum concentration of xylose solutions that can be used in 1% dilute acid pretreatments. Knowing the minimum concentrations that can be used was critical to Chuan Lau's PhD experiments because precious in house-purified oligomers could be judiciously used.

Materials and Methodologies

The commercial grade xylose was hydrolyzed in the stainless steel reactors (Figure 1) by 0 and 1% (v/v) sulfuric acid by submerging the reactors in a fluidized sand bath at 0 – 60 min and 120 and 200˚C in order to determine the reaction

kinetics of the xylose degradation rate. Table 1 shows a summary of the runs. After 1-min cooling via rinsing, the hydrolysates were analyzed using HPLC-RI. Xylose was quantified by using the peak height generated from the individual calibration curves [4].

Figure 1: Stainless Steel Reactors for Hydrolysis

The identity of the xylose was confirmed by co-chromatography with the available standard. The kinetic parameters describing the degradation of the xylose was established.

Table 1: Description of Experimental Plan

Compounds	Concentration (mg/mL)	Temperature (°C)	Acid Concentration (v/v%)	Replicates
Xylose	1 2 5	120 200	0 1	2

For each run, the appropriate concentration of xylose and solvent was prepared in a 40 mL beaker. The solution was thoroughly mixed by a stir bar on a stir plate while to insure uniform concentration. A 5 mL sample was added to each reactor. The reactors were tightened with a wrench to avoid any loss of the product during the hydrolysis. The reactors were then submerged into the sand bath at intervals of 10 minutes for 60 minutes. After the sand bath hydrolysis, the reactors were rinsed with cold water for 1 minute. The contents of each reactor were recovered. One mL of the hydrolyzate was transferred to a 15 ml glass tube and was neutralized by calcium carbonate. Fifty mg of calcium carbonate was added to water hydrolyzates, while 150 mg of calcium carbonate was added to 1% acid hydrolyzates. The glass tubes were centrifuge at 2000 *g* for 10 seconds and allowed to settle for an hour.

The samples were then filtered using a 0.2 μm filter and loaded into the HPLC-RI for analysis as in [4].

Analysis

An analysis of the results is listed below of different concentrations of xylose at the different temperatures and time. By showing this Statistical Analysis we should be able to prove that our model developed for the degradation for xylose can be used not only for 1 gpl (mg/mL), but 2 gpl and 5 gpl as well. Xylose was quantified by using the peak height generated from the individual calibration curves as shown in the chromatogram in Figure 1. The concentration in moles (C) was extrapolated for xylose from a calibration curve and was then generated for each time point of each concentration for the four conditions. In order to get a desirable degradation rate, k, for the degradation model, the plotted the natural log of the calculated concentration divided by the initial concentration was used.

$$\ln\left(\frac{C}{Co}\right) = -kt$$

(t) is the hydrolysis time. C is the measured concentration at different intervals of time in mmol/L, Co is the initial concentration measured, and k is the slope of degradation in (mmol/L)/minute. A Statistical Analysis of Regression was conducted for each of the experiments (concentrations of 1, 2, and 5 gpl at different temperatures and acid concentrations) with a Reduced and Full model to see if we could validate that our average k value between the three concentrations was valid. In order to see if the average k values were correct

and could be used, the null hypothesis was applied to the data. The null hypothesis, H_0, represents a theory that has been put forward, either because it is believed to be true or because it is to be used as a basis for argument, but has not been proved. In order to validate the null hypothesis, it must be proved that $H_0=k_1=k_2=k_3=0$ ($\delta_0=\delta_1=0$) for the three concentrations. In other words, Ho meant that there was no difference between the slopes [6].

A rejection of the null hypothesis meant that there was not sufficient evidence against H_0 in favor of H_1. Rejecting the null hypothesis then, suggested that the alternative hypothesis may be true and different degradation models for the different concentrations of xylose would need to be tested.

Results and Discussion

Figure 1 presents the HPLC-RI chromatograms of 5 mg/mL of xylose in 200°C water reacted for 0 to 60 min. The retention time of xylose was determined to be 55 min. Results from Figure 1 show that close to 75% of initial xylose was lost at the end of the experiment which was at time 60 min. The degradation profiles of xylose as a function of time are presented in Figures 2 to 5. The degradation profile of xylose at 120˚C using water, 120˚C using 1 v/v% acid, 200˚C using water, and 200˚C using 1 v/v% acid, are shown in Figures 2, 3, 4, and 5, respectively.

An F-Test under the null hypothesis was run between all models of different concentrations at all four conditions. An F-Test on the model of 1 g/L vs. 2 g/L would take the assumptions that 2 g/L would follow the model of $Y = \beta_0 + \beta_1 X$ and

the 1 g/L concentration would follow the model of $Y = \alpha_0 + \alpha_1 X$. The second terms ($\beta_1 X$ and $\alpha_1 X$) in each of these equations are the slopes of the lines and the first terms (β_0 and α_0) are the y values. The two separate models of each concentration were then combined to the model $Y = \delta_0 * Z + \beta_0 + \delta_1 * X * Z + \beta_1 * X$. δ_0 was the difference between the y values ($\alpha_0 - \beta_0$) and δ_1 was the difference between the slopes ($\alpha_1 - \beta_1$). The "dummy" variable of Z was 0 for 2 g/L data and 1 for 1 g/L data. Then, Z was multiplied by the original hydrolysis time to obtain the actual dummy variable value. The hypothesis tested that both δ_0 and δ_1 were equal to 0, meaning that both lines had similar slopes and intercepts. The Full Regression Model took all variables into account [β_0, β_1, δ_0, δ_1] while the Reduced Regression Model only considered the variables that were related to the 2 g/L [β_0, β_1] experiments. Once the Full and Reduced Models were tested, the F values were calculated from the Sum of Squares of Regression for both models and the Sum of Squares Residual from the Full Model. The residual sum of squares is a measure of the amount of error remaining between the regression function and the data set and the Sum of Squares of Regression is the distance between each data point and the line of best fit is squared and then a summation of all the squares is tallied. The F value is then calculated by the relationship:

$$F = ((SSReg, Full - SSReg, Red) - 2)/(SSRes, Full)/(n - 4)$$

Appendix 1 presents a screen shot of the entire regression done in Excel for the Reduced and Full Models and 1 vs. 5 gpl at 200°C with 1% H_2SO_4 as the solvent. The F critical values were found in excel by the formula

$$= F.INV.RT(probability\ level, degrees\ of\ freedom\ 1, degrees\ of\ freedom\ 2)$$

The probability level used in this study was 0.05 or 5%.

Table 2: Statistical Analysis of Concentration Effect on Xylose Degradation Rates

F calculated vs. F Critical	1 gpl vs. 2 gpl	1 gpl vs. 5 gpl	2 gpl vs. 5 gpl
120 water	2.94<3.39	2.39<3.39	1.02<3.39
120, 1% acid	2.41<3.39	0.61<3.39	0.17<3.39
200 water	0.04<3.39	0.16<3.44	1.32<3.44
200, 1% acid	1.53<3.40	1.42<3.42	1.43<3.40
Confidence Interval	**1 gpl vs. 2 gpl**	**1 gpl vs. 5 gpl**	**2 gpl vs. 5 gpl**
120 water	93%	89%	62%
120, 1% acid	89%	45%	16%
200 water	4%	15%	71%
200, 1% acid	76%	74%	74%

The data in Table 2 shows that all the F calculated values for each concentration and condition were less than the F critical values. The F critical value was considered to be the breaking point where the Null Hypothesis was no longer valid. Therefore, since these calculated values were less than the F critical values the Null Hypothesis was not rejected. Also, the confidence intervals that corresponded to each of the calculate F values are shown. Since our confidence interval was 95% and all the values was less than 95%, thus the Hypothesis Null was not rejected. Therefore, a mean degradation rate (k) can be drawn from the averages of the three concentrations as shown in Table 3. In other words, there is no statistical difference of the slopes between 1, 2, and 5 mg/mL in all four hydrolysis conditions.

Table 3: The Average Degradation Rate of Xylose at Variable Hydrolysis Conditions

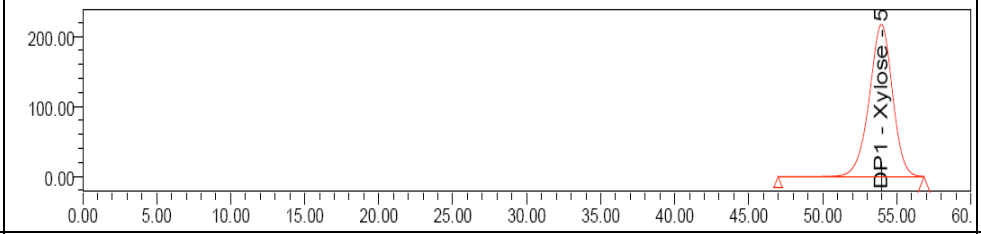
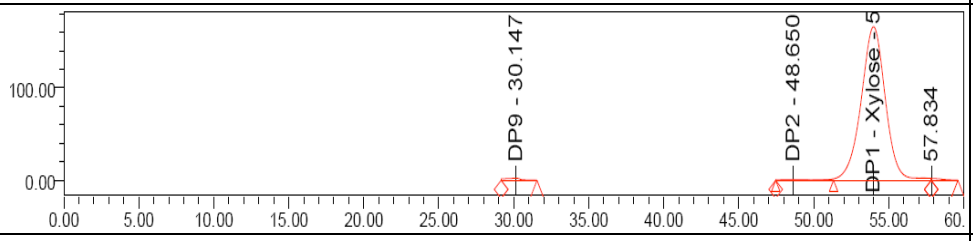
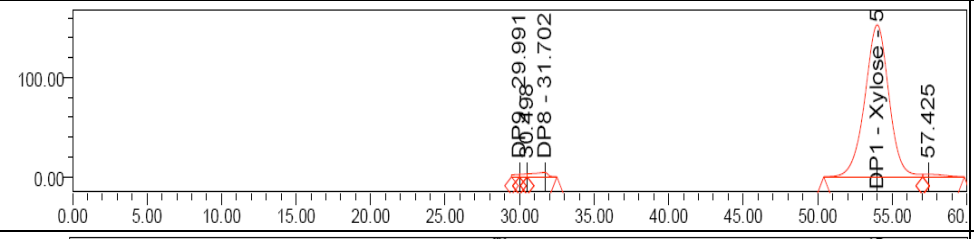
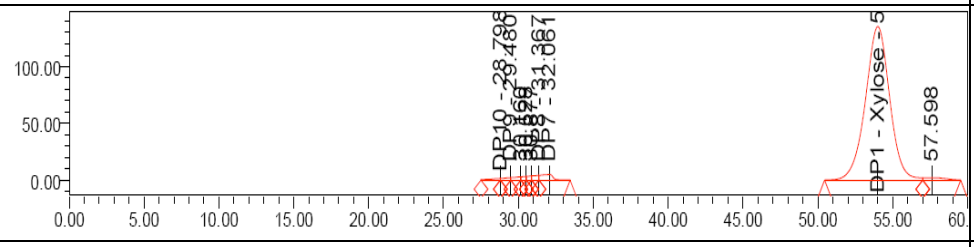
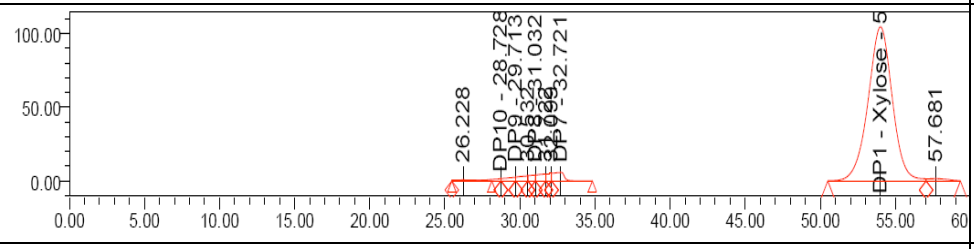
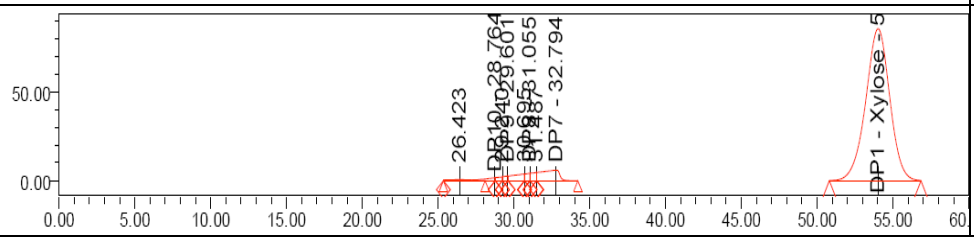
Mean k values	
120 water	0.002
120, 1%	0.001

acid	
200 water	0.024
200, 1% acid	0.409

The average degradation rate of xylose at 120°C water, 120°C 1% acid, 200°C water and 200°C 1% acid were 0.002, 0.001, 0.024 and 0.409 min^{-1}, respectively. The significance of this study showed that subsequent kinetic studies to be conducted with precious xylose oligomers could be performed at 1 g per l, and the degradation rate will remain first order up to the concentration of 5 g per l. These results were very important for PhD candidate, Chuan Lau, because he could conduct his oligomer degradation experiments with 1 g p l, knowing that his results could be extrapolated to concentrations up to 5 g p l.

This research is important to meet the growing demands for an independent and sustainable source of fuel; a considerable amount of work has been done to make the production of biofuels feasible on an industrial scale. The overall reliance of petroleum as the main source of energy will drive us towards a global recession within the next fifty years [8]. Currently, corn is the most common bioethanol feedstock in the United States. However, cellulosic biofuels hold a promising future because they can be produced from the abundant but relatively inexpensive raw materials, which will not create competition to our food source [8]. Other reasons for understanding and evaluating biofuel systems include net lifecycle energy balance, net lifecycle greenhouse gas balance, and economics [9].

In addition to containing the degradation products, the hydrolysates can also be contaminated with bacteria that inhibit the fermentation process. This can significantly reduce the yield of ethanol and increase the amount of undesirable products, such as lactic or furic acid [5]. Some of the most common contaminants are lactic acid bacteria such as Streptococcus, Lactobacillus, Lactococcus, and Leuconostoc. To reduce the contamination problems, ethanol producers add large amounts of antimicrobial agents, such as virginiamycin, monensin, and penicillin, which later pollute wastewater effluents [5]. Overall, the use of antibiotics and the production of degradation compounds are intrinsic burdens to the production of biofuels because of the challenges made by the antimicrobial-resistant strains of bacteria and the inhibition caused by the degradation compounds, which lead to the increased production costs. A better understanding of the depolymerization kinetics of hemicellulose can lower production costs, and thus, creating a sustainable approach to produce biofuels to meet our energy demands

Reaction Time (min)	HPLC Chromatogram (Set 6)	Xylose Concentration (mol/mL)
0		33.60
10		25.70
20		23.63
30		20.90
40		16.15
50		13.22

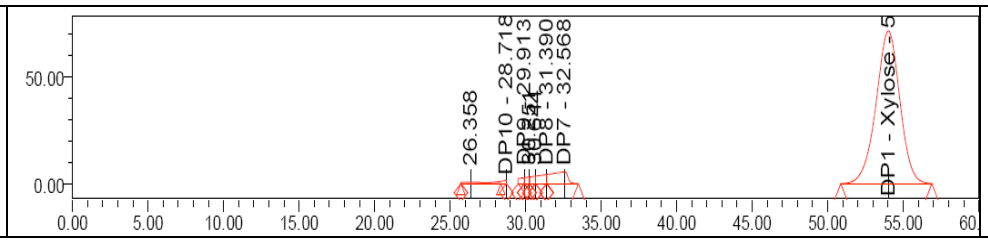
60		11.06

Figure 1: Chromatogram Sets for 200°C in water for 5 (mg/mL)

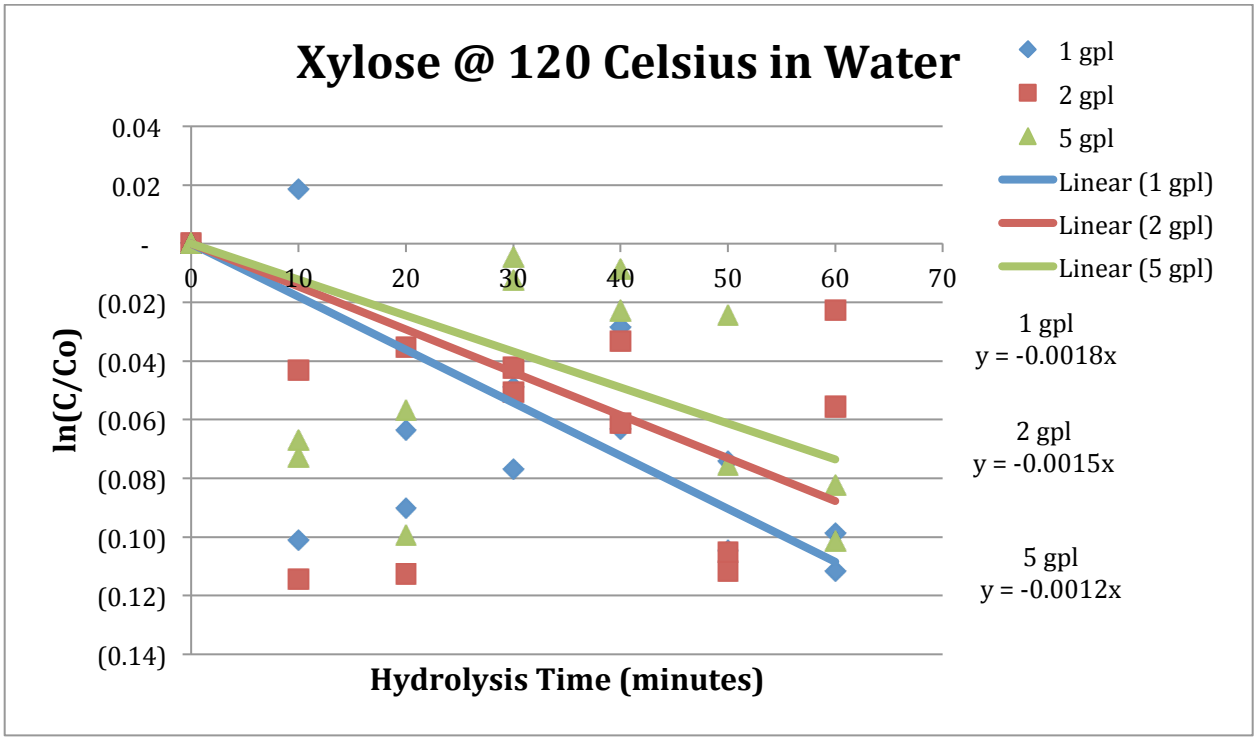


Figure 2: Degradation Profile of Xylose at 120°C in Water

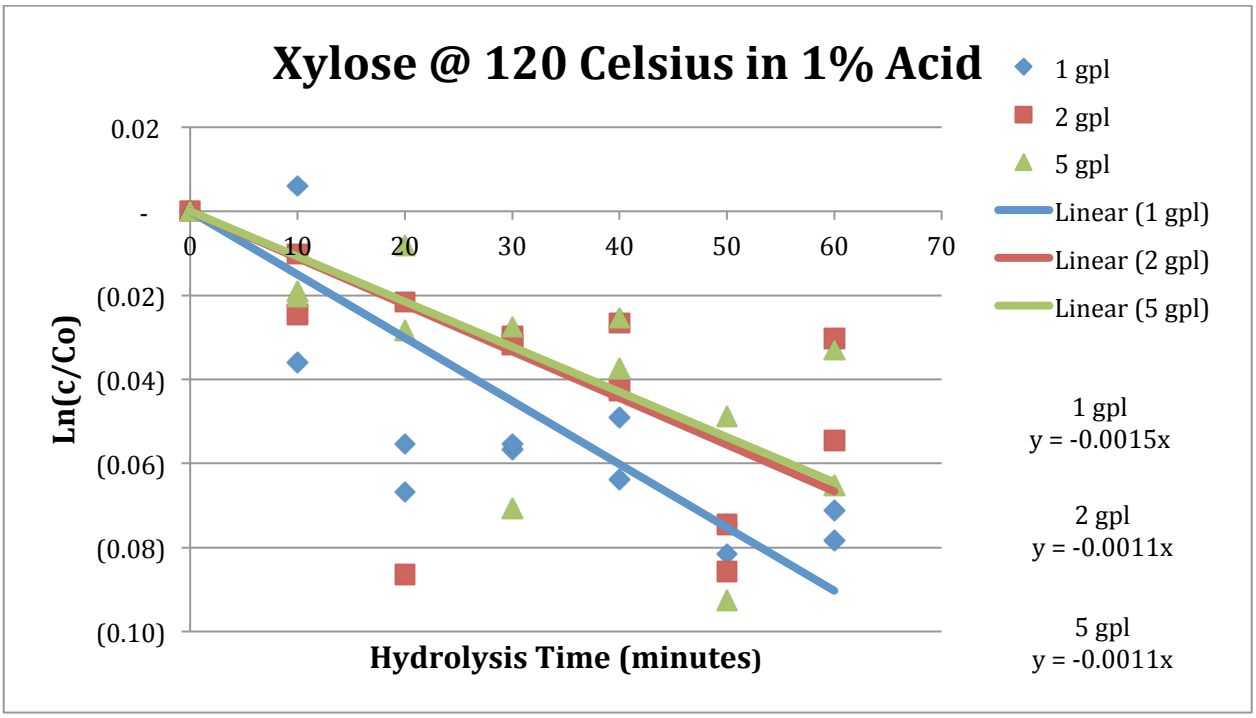


Figure 3: Degradation Profile of Xylose at 120°C in 1% Acid

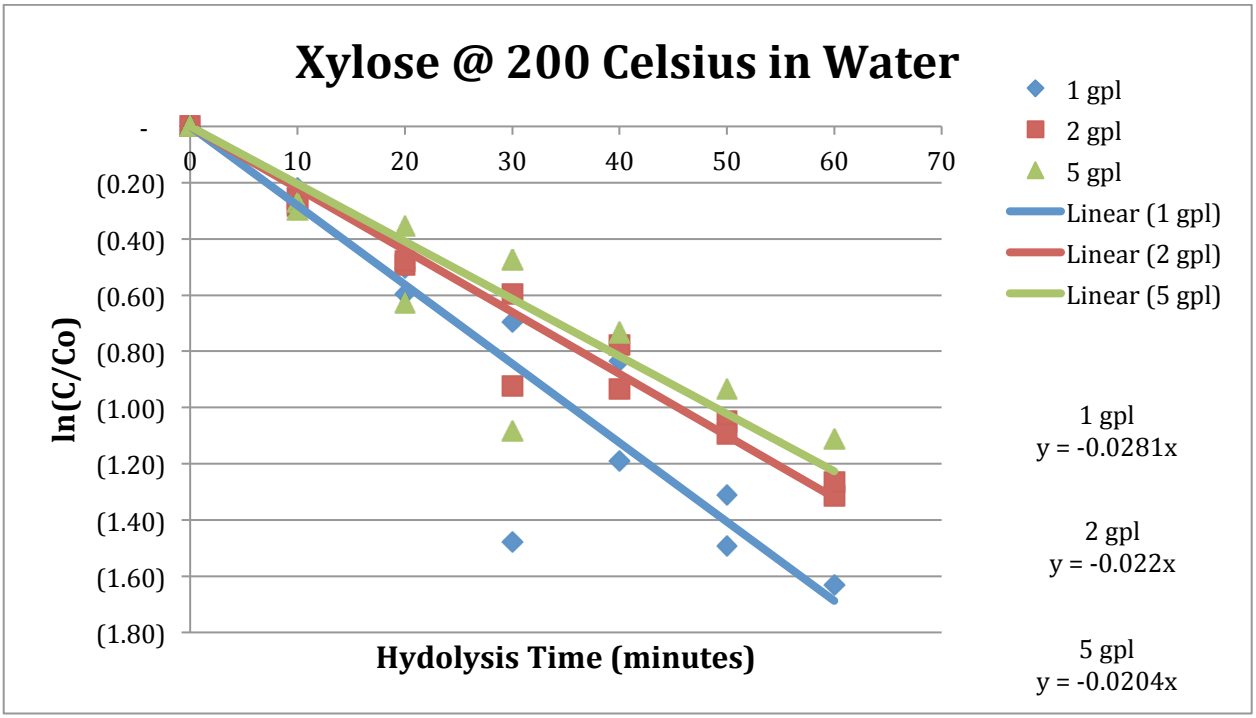


Figure 4: Degradation Profile of Xylose at 200°C in Water

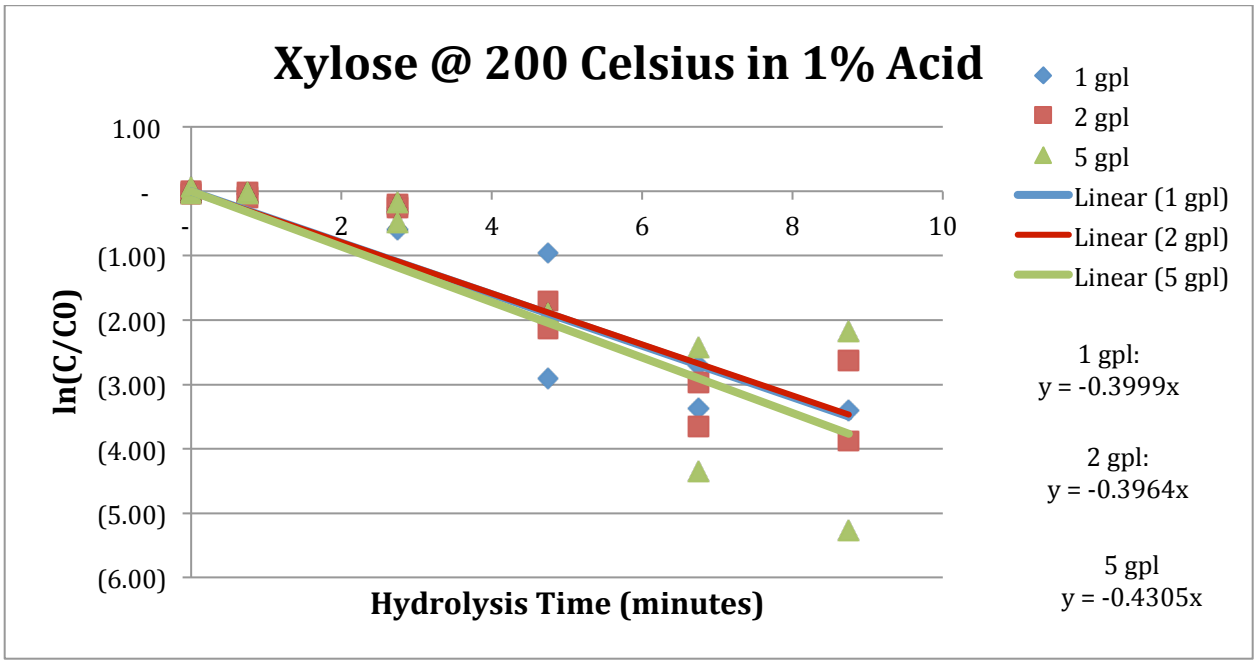


Figure 5: Degradation Profile of Xylose at 200°C in 1% Acid

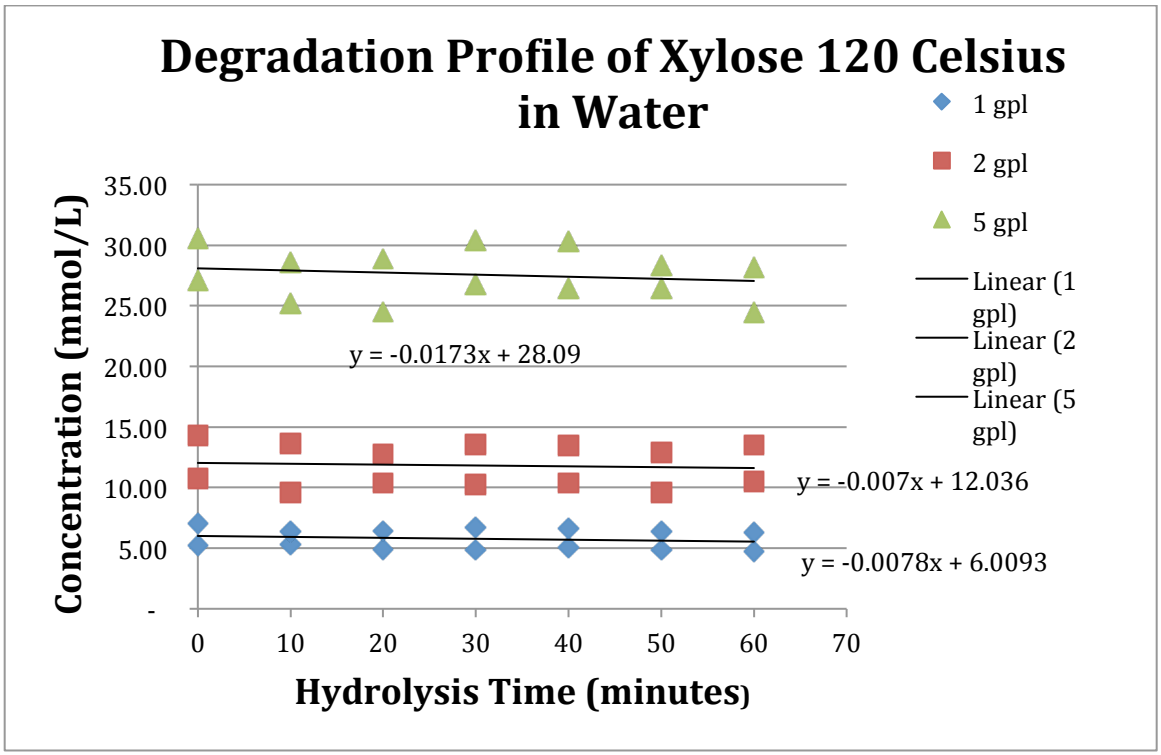


Figure 6: Raw Data of Degradation Profile of Xylose at 120°C in Water

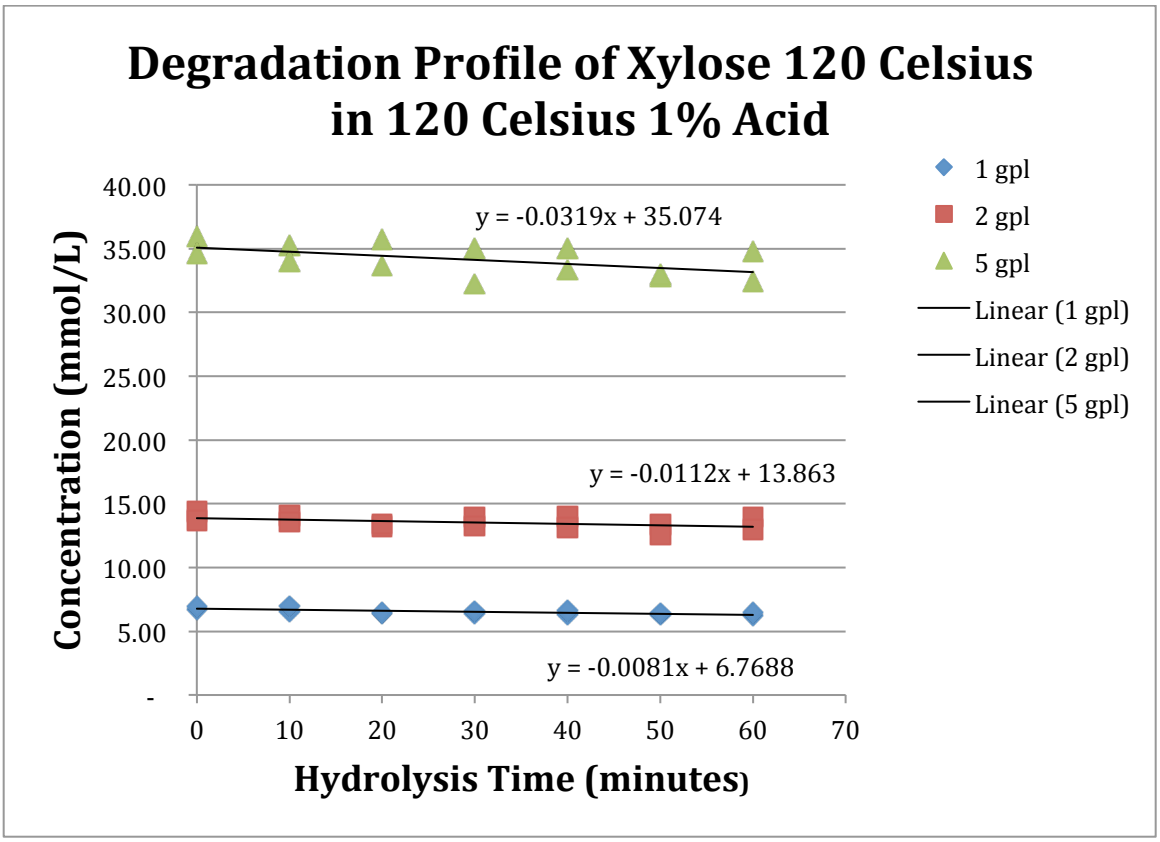


Figure 7: Raw Data of Degradation Profile of Xylose at 120°C in 1% Acid

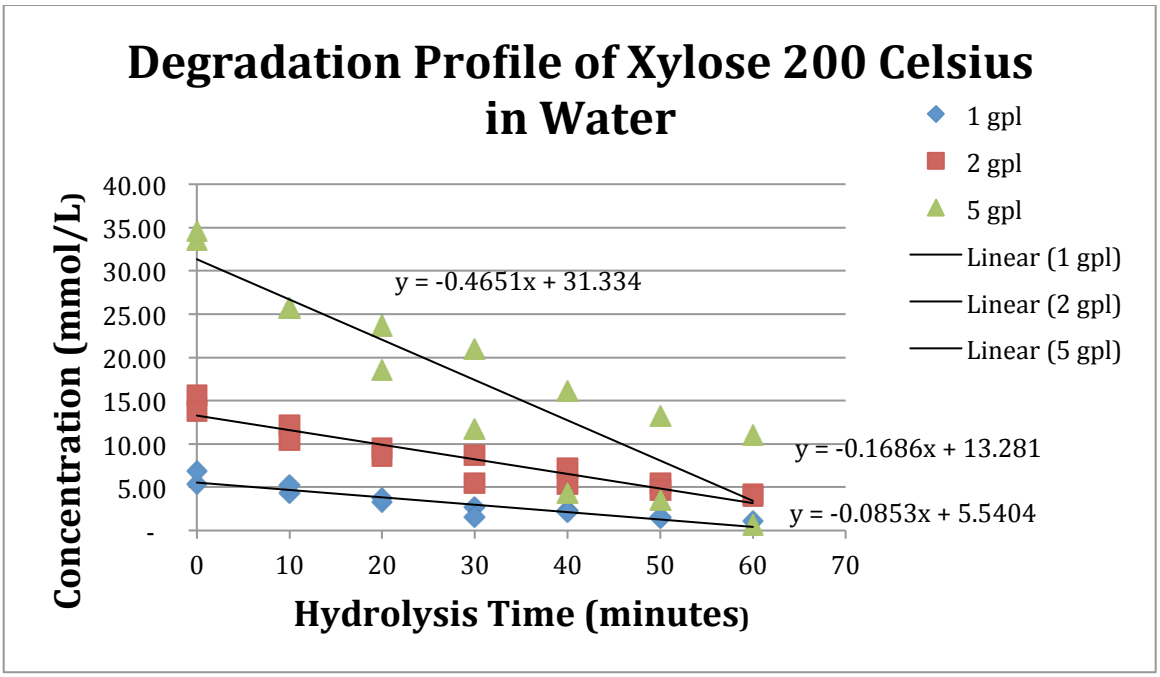


Figure 8: Raw Data of Degradation Profile of Xylose at 200°C in Water

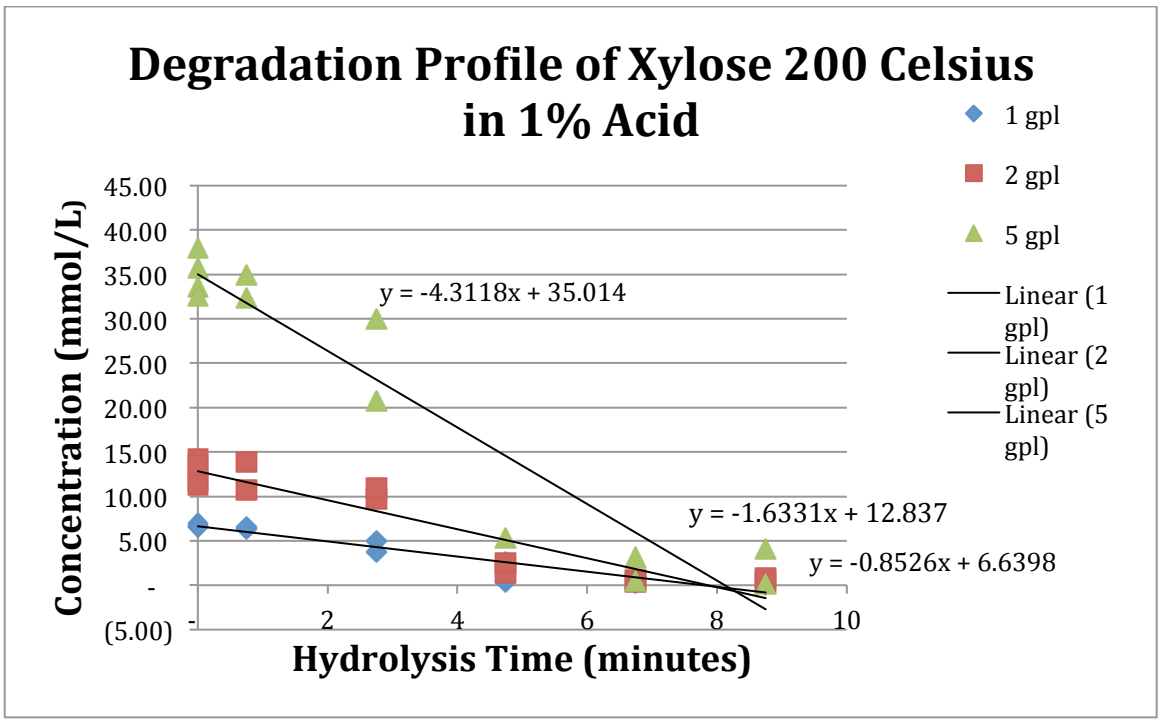


Figure 9: Raw Data of Degradation Profile of Xylose at 200°C in 1% Acid

Appendix 1

Reduced Model

SUMMARY OUTPUT

Regression Statistics	
Multiple F	0.91683
R Square	0.840577
Adjusted I	0.800577
Standard I	0.803833
Observati	26

ANOVA

	df	*SS*	*MS*	*F*	*ynificance F*
Regressio	1	85.1724201	85.1724	131.815863	3.1E-11
Residual	25	16.1536742	0.64615		
Total	26	101.326094			

	Coefficients	*tandard Erro*	*t Stat*	*P-value*	*ower 95%*	*Upper 95%*	*ver 95.0%*	*Upper 95.0%*
Intercept	0	#N/A	#N/A	#N/A	#N/A	#N/A	#N/A	#N/A
X Variable	-0.33744	0.02939103	-11.481	1.8417E-11	-0.398	-0.276909691	-0.4	-0.27691

F Critical	=	3.42213221
Top F	=	0.88622677
Bottom F	=	0.62527046
F	=	1.4173495

1 vs. 5 gpl

X1	X2	X3	Y	
0	0	0	-	1 gpl
1	0	0	0.00	
2	0	0	(0.05)	
4	0	0	(0.28)	
6	0	0	(2.90)	
8	0	0	(3.37)	
10	0	0	(3.40)	
0	0	0	-	
6	0	0	(0.96)	
8	0	0	(2.71)	
1	0	0	(0.04)	
2	0	0	(0.04)	
4	0	0	(0.60)	
0	1	0	-	
1	1	1	(0.03)	5 gpl
2	1	2	(0.04)	
4	1	4	(0.48)	
8	1	8	(4.36)	
10	1	10	(5.27)	
0	1	0	-	
6	1	6	(1.90)	
8	1	8	(2.43)	
10	1	10	(2.18)	
1	1	1	0.06	
2	1	2	(0.02)	
4	1	4	(0.17)	

Full Model

SUMMARY OUTPUT

Regression Statistics	
Multiple F	0.926321
R Square	0.85807
Adjusted I	0.80225
Standard I	0.79074
Observati	26

ANOVA

	df	*SS*	*MS*	*F*	*gnificance F*
Regressio	3	86.94487365	28.98162	46.35054	1.12E-09
Residual	23	14.38122062	0.62527		
Total	26	101.3260943			

	Coefficients	*tandard Erro*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*ower 95.0%*	*pper 95.0%*
Intercept	0	#N/A	#N/A	#N/A	#N/A	#N/A	#N/A	#N/A
X Variable	-0.32026	0.042758347	-7.49007	1.3E-07	-0.40872	-0.23181	-0.40872	-0.23181
X Variable	0.548372	0.344260535	1.592897	0.124835	-0.16379	1.260529	-0.16379	1.260529
X Variable	-0.10729	0.074987343	-1.43073	0.165954	-0.26241	0.047836	-0.26241	0.047836

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