

Producing Fermentable Glucose by Acid Hydrolysis of Lignocelluloses Material

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ABSTRACT

Due to the difficulty in finding a strict mechanism for hydrolysis reactions, it is usual to use simplified models to determine the kinetics of the hydrolysis of lignocelluloses material to obtain sugar solutions (xylose and glucose) with low concentrations of inhibitor (furfural and acetic acid). Modeling of concentrate sulfuric acid hydrolysis of Poplar tree pruning has been achieved using quadratic equation and response surface methodology (RSM). A L9 table of Taguchi method was applied to design of hydrolysis experiment and produced sugar was detected by HPLC. A second-order polynomial equation was applied to relate temperature, time, solid content and acid concentration to sugar concentration. Hydrolysis and fermentation are two important and major steps in bioethanol production from lignocelluloses materials. During hydrolysis process, cellulose, hemicelluloses and lignin are broken down to mainly glucose, xylose, and phenolic compounds. It is important to determine the fermentable sugar concentration because sugars are the main carbon source for most microorganisms. In this study, the concentrated acid hydrolysis of Poplar tree pruning in form of total sugar production was investigated as a function of 3 variables: acid concentration, temperature and solid content. A quadratic equation was applied to relate temperature, solid content and acid concentration to sugar concentration.

KEYWORDS: Kinetic modeling, Fermentable sugar, Acid hydrolysis, Poplar, Response surface model

1. INTRODUCTION

Bioethanol is a liquid biofuel which can be produced from several different biomass feedstocks. In compare with gasoline this fuel has a higher octane number, broader flammability limits, higher flame speeds and higher heat of vaporization. Fuel ethanol production from lignocellulosic biomass is emerging as one of the most important technologies for sustainable production of renewable transportation fuels. The production of ethanol from this lignocellulosic biomass involves different steps of pretreatment, hydrolysis (saccharification), fermentation and ethanol recovery [1]. Pretreatment affects the structure of biomass by solubilizing cellulose, reducing crystallinity and increasing the available surface area and pore volume of the substrate. Hydrolysis of biomass is essential for generation of fermentable sugars which are then converted to ethanol by microbial action [1].

Hydrolysis (saccharification) breaks down the hydrogen bonds in the hemicelluloses and cellulose fraction into their sugar components, pentose and hexose [2]. Besides process time, Sugar production by saccharification was affected by 3 more parameters (acid concentration, solid content and temperature). Due to the difficulty in finding a strict mechanism for hydrolysis reactions, it is usual to use simplified models to determine the kinetics of the hydrolysis of lignocellulosic material to obtain the best condition for highest sugar production. One of these models was response surface methodology used by some authors for optimization of acid pretreatment. The surfaces generated can be used to obtain the optimal conditions. However, in some experiment this optimal condition was valid only for inside the studied experimental condition [3].

This model was used by Castru [4] to optimize dilute acid pretreatment of rapeseed straw. Similar optimizing was used by Jeong [5] for extraction of hemicelluloses. Zhou [6] used the same investigation for efficient hydrolysis of steam exploded of corn Stover. Optimization of H₂SO₄- catalyzed hydrothermal pretreatment of rapeseed straw was other research used by Xuebin lu and his colleague [7].

In this study, a batch type hydrolysis system using concentrated sulfuric acid was used for fermentable sugar production from Poplar tree pruning waste. Taguchi method was used for the design of experiment and a quadratic equation evaluated the effect of acid concentration, solid content, and temperature on the hydrolysis.

2. MATERIAL AND METHODS

Poplar tree pruning (PTP) was gathered in August of 2011 from city of Mashhad in Iran. It was washed by distilled water, air dried, milled using vibratory disc mill (Retsch RS 100) to particle size smaller than 50 micrometers and stored in sealed plastic bags at room temperature.

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Hydrolysis experiments were performed in screw-capped laboratory bottles (Pyrex bottles) as batch reactor placed in a hot water jacket with an electric heater and a temperature controller (Figure 1). The reactor was loaded with dried PTP and sulfuric acid solution. The process variables were acid concentration (15, 25, 35 wt %) and temperature (60, 70, 80°C). The reaction time is 2 hours. Once the temperature of reaction mixture reached to the designed point, pretreatment time was started. At the end of each experiment the bottle was removed from the heating jacket and putted in a cool water bath, PH value reached 7 by Sodium hydroxide (NaOH). Solid residue was separated from solution by filtration, washed with distilled water and final solution reached to 1000 ml. A 200 ml sample of solution was analyzed by HPLC.

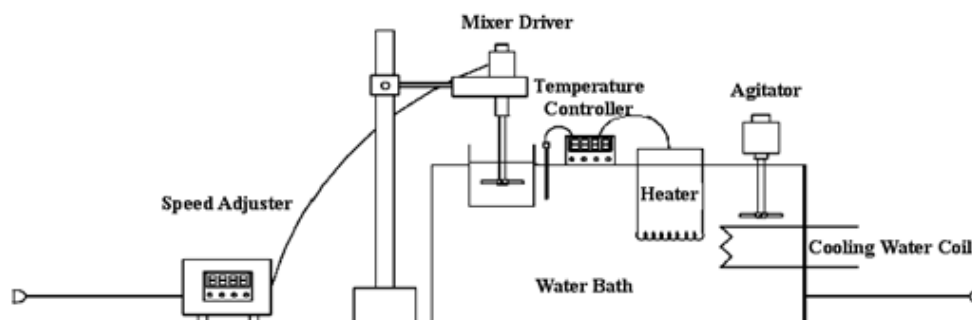


Fig.1. A scheme of Experimental Setup of Hydrolysis reactor

2.1. Analysis

The composition of the total sugar from acid hydrolysis (Glucose, Xylose, Mannose, Arabinose, Galactose) was determined by high performance liquid chromatography (HPLC- model JASCO) by Biorad column Aminex HPX-87P and detected by RI detector in temperature 40°C.

2.2. The mathematical model, Factors and responses

The study of pretreatment performance of PTP by concentrated sulfuric acid was addressed by performing the experimental design in which temperature ($T^{\circ}\text{C}$), solid content (S , %) and acid concentration (C , %) were selected as factors and total sugar concentration (TS) was considered as response. To construct the response surface model, a second order polynomial equation was fitted to the data using multiple regressions. The response of tested variables can then predicted by following a second order model:

$$TS = A_0 + A_1.C + A_2.T + A_3.S + A_4.C^2 + A_5.T^2 + A_6.S^2 + A_7.C.T + A_8.C.S + A_9.T.S \quad (1)$$

Where A_0 is the intercept, A_1, A_2, A_3 are the linear coefficient, A_4, A_5, A_6 are the square coefficients and A_7, A_8, A_9 are the interaction coefficients. The statistical significance of regression coefficient and effects were checked by analysis of variance (ANOVA) using the software Qualitec-4 (Bloomfield, Michigan, USA)

This equation has 10 constant coefficients and at least 10 equations were needed to determine these coefficients by least square methods. Based on previous experience with concentrated acid hydrolysis to ensure a broad range of response (Arastehnodeh, 2012), three levels for each factor were considered and PTP was pretreated at 9 different operational conditions according to L9 Taguchi matrix and one trail in severest condition performed at the end. Table 1 shows the selected conditions. Every experiment was carried out for 3 times and nearest results was averaged.

Table 1: Experimental design, Experimental factor and code levels

Run	Acid concentrate. (code)	Temperature (code)	Solid content (code)	Acid concentrat. (%)	Temperature (°C)	Solid content (%)
1	1	1	1	25	60	15
2	1	2	2	25	70	25
3	1	3	3	25	80	35
4	2	1	2	30	60	25
5	2	2	3	30	70	35
6	2	3	1	30	80	15
7	3	1	3	35	60	35
8	3	2	1	35	70	15
9	3	3	2	35	80	25
10	3	3	3	35	80	35

3. RESULT AND DISCUSSION

After pretreatment of PPT using condition described in table1, the extracted sugar content are presented in Table 2.

Table 2. Experimental and predicted values of total sugar produced

Run	Total sugar(mg/lit) (Experimental)	Total sugar(mg/lit) (Predicted)
1	0.07	0.07
2	0.11	0.1
3	0.18	0.18
4	0.14	0.14
5	0.22	0.22
6	0.79	0.78
7	0.24	0.24
8	0.84	0.83
9	0.57	0.57
10	0.52	0.52

From these results, second-order regression model was fitted to experimental data and A_n coefficients were calculated by Sigmaplot 12 software.

Table 3 summarizes the model constant coefficients obtained from ANOVA table for total sugar. Following equation (Eq.2) represents empirical relationship between responses and the test variable (temperature, solid content and acid concentration):

$$TS = -16.1554 + 0.535C + 0.27187T - 0.17433S - 0.00413C^2 - 0.0011T^2 + 0.00015S^2 - 0.00407TC + 0.002067CS + 0.0011TS \quad (2)$$

Study t test and p value, which determine the significance of each coefficient, were presented in table 3. The corresponding variable will be more significant if the absolute t value increases and p value decreases. The F value was used to determine whether the observed relationship between the dependent and independent variables occurs by chance. The degrees of freedom were calculated to determine a confidence level for the model. The larger effect had the linear term of Acid concentration (C). The multiple coefficient of correlation (R=0.985) and total determination coefficient (R²=0.97) indicate good agreement between experimental and predicted values of TS. The three dimensional response surface graphs were plotted to illustrate the effect of the independent variable on TS (Figure 2).

Table 3. Significance of regression equation for k_1

Coefficient	Std. Error	t	P	
A ₀	-16.155	4.3578	-1.5924	0.1297
A ₁	0.535	0.0321	12.1954	<0.0001
A ₂	0.278	0.1096	0.3156	0.7562
A ₃	-0.1743	0.1285	-1.2386	0.2323
A ₄	-0.00413	0.0003	-17.2598	<0.0001
A ₅	-0.0011	0.0007	0.1532	0.8801
A ₆	0.00015	0.0042	2.1583	0.0455
A ₇	-0.004	0.0003	-2.2010	0.0418
A ₈	0.00206	0.0007	-2.1281	0.0483
A ₉	0.0011	0.0012	1.2528	0.2272
R	Rsqr	Adj Rsqr	Standard Error of Estimate	
0.9852	0.9706	0.9550	0.2562	

Corrected for the mean of the observations:

	DF	SS	MS	F	P
Regression	9	2.1000	0.21000	000	<0.0001
Residual	0	1.19E-29	0.0656		

The coefficient of sulfuric acid (C) and temperature (T) were positive, indicating that higher levels of these two variables result in higher sugar production and the coefficient of solid content(S) is negative, indicating that lower levels of this variable result in more sugar production.

According to the obtained P-values, it is indicated that each variable has direct effect on total sugar production. The interactive effect of these components on total sugar production was analyzed by non linear regression which is shown in table 3. It was determined that by increasing the acid concentration, temperature, and time of process, the amount of total produced sugar was increased as well, while by increasing the amount of each gram of wood in acid solution, the total amount of sugar decreased.

The response surface has a result similar to what was observed about coefficient. As can be seen in Fig2.A, keeping fixed temperature at 70 °C the maximum value of sugar produced is obtained when solid content is in minimum and acid concentration is in maximum. Similarly, at fixed acid concentration (C=25%) higher value of sugar is obtained at minimum value of solid and higher value of temperature (Fig 2.C). Fig(2.B) shows at fixed amount of solid, a maximum sugar zone can be seen around maximum value of temperature and acid concentration. Fig2 shows the 3D response surfaces plots on the basis of Eq2 and illustrate the modeled effects on independent variation.

4.3. Evaluation of model validity

To evaluate the model validity, two different experiments were designed. Experiments and results are shown in table 5. Table 5 shows this model is in good comprise with experimental when error was less than 5% except for furfural.

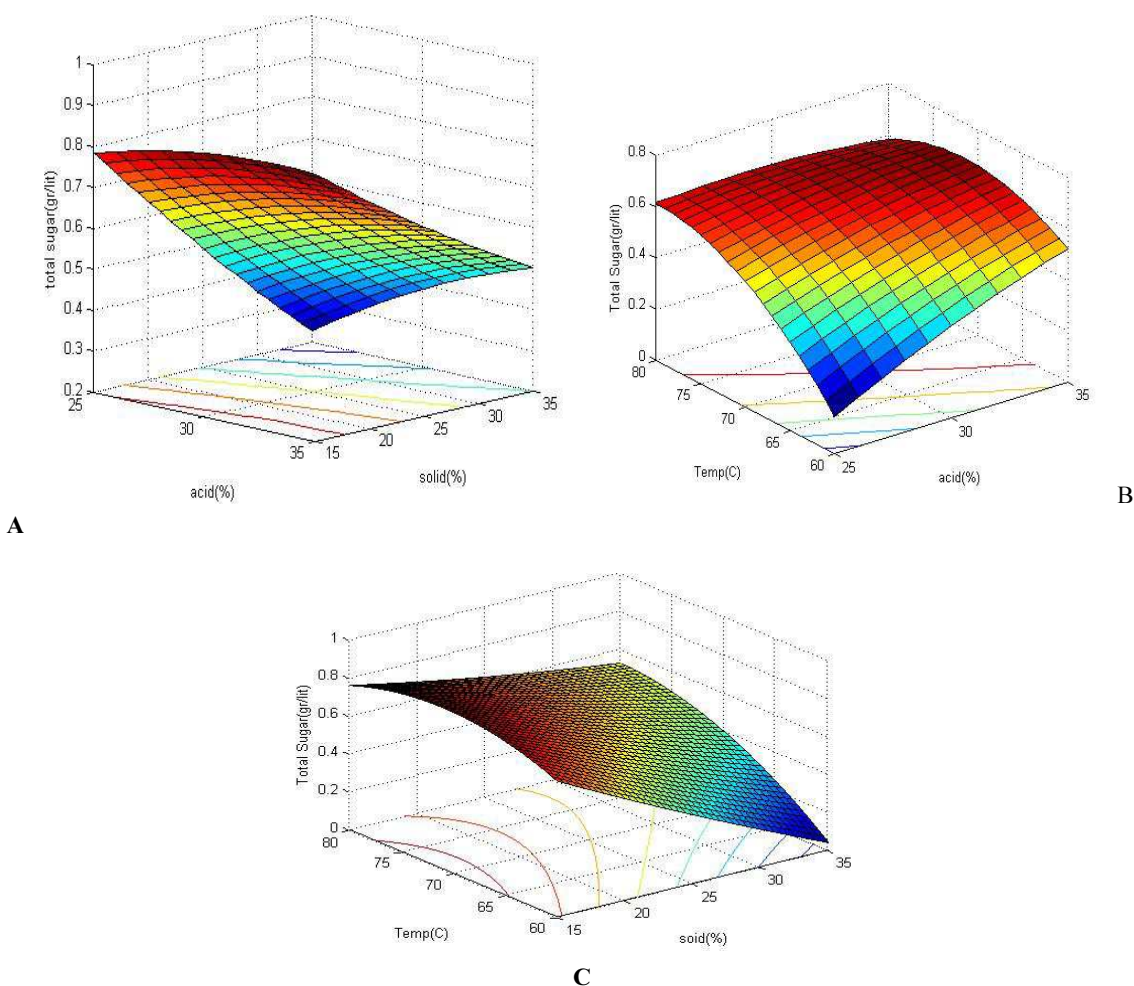


Fig2. Response surface curves representing the interactive effect of concentration of sulfuric acid, solid content and temperature on total sugar production.

A. the effect of acid concentration and solid content in 70°C with reaction time 2hr.

B. effect of acid concentration and temperature in solid content 25% and reaction time 2hr.

C. effect of acid temperature and solid content in acid concentration 25% and reaction time 2hr.

Table 2: validity of the model (eq.2)

Acid concentration (%)	Temperature (°C)	Solid content (%)	Time (min)	Comparision	Glucose (%)
35	80	15	120	Model	0.65
				Real	0.68
				Error	5%
30	80	35	120	Model	0.45
				Real	0.461
				Error	0.25

4. Conclusion

This work confirms that Poplar tree pruning can be considered as a suitable feed stock for sugar production in bioethanol process. Concentrated acid hydrolysis helps to reach the fermentable sugar in normal process conditions. Besides process time, this process could be modeled by a 3 variables quadratic equation. These 3 variables were temperature, acid concentration and solid content. Model allows adjusting these variables to reach optimum condition for maximum glucose concentrations. The optimal and best results was obtained when the experiment was carried out with acid sulfuric concentration of 35% , temperature of 80 °C process time of 2 hours and amount of wood in acid solution of 15% , which is the same as conditions in the model experiment. This modeling can have a key role in choosing the best experimental conditions and reducing the economical risks and also to improving the results.

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