

Optimization of Japanese beech hydrolysis treated with batch hot-compressed water by response surface methodology

Lü Xin^{1,2}, Shiro Saka²

(1. College of Food Science and Technology, Nanjing Agricultural University, China;

2. Graduate School of Energy Science, Kyoto University, Kyoto 615-8510, Japan)

Abstract: In the present work, the combined effects of temperature and reaction time on hydrolysis of Japanese beech as treated by batch hot-compressed water were investigated by response surface methodology. A two-factor and three-level full factorial design was used for experimental design. The statistical models were established to configure the relationship between process conditions (temperature and reaction time) and water-soluble, saccharides from hemicelluloses (including arabinose, galactose, mannose, rhamnose, xylose and xylooligosaccharides) as well as decomposition compounds (5-HMF, furfural and organic acids). As a result of multiple response numerical optimization by applying desirability function method, the optimal hydrolysis conditions were obtained to be temperature of 200°C and reaction time of 3 min. At this optimum point, water-soluble, saccharides from hemicelluloses, 5-HMF, furfural and organic acids were 31.5%, 19.5%, 0.28%, 0.70%, and 0.59%, respectively.

Keywords: hydrolysis of Japanese beech, bioenergy, biomass, hot-compressed water, response surface methodology, optimization

DOI: 10.3965/j.issn.1934-6344.2008.02.039-045

Citation: Lü Xin, Shiro Saka. Optimization of Japanese beech hydrolysis treated with batch hot-compressed water by response surface methodology. *Int J Agric & Biol Eng.* 2008; 1(2): 39–45.

1 Introduction

As the depletion of fossil fuels is concerned, the renewable energy attracts much attention. Bioenergy is viewed as an important contributor to sustainable energy resources, in which bioethanol now is widely used in USA, Brazil, China and other countries as gasoline additive or substitute^[1,2]. However, the main raw materials for ethanol production now are corn and sugarcane; substitutively the low-cost lignocellulosic materials have been highlighted in recent twenty years because of food crisis concern^[3].

Conversion of lignocellulosic materials to bioethanol or other value-added chemicals required fractionation and/or hydrolysis of the raw biomass which contains hemicelluloses, cellulose and lignin. Various methods

for the fractionation and hydrolysis of biomass were studied, such as acid method, lime method, ammonia method, etc^[4]. Among these methods, water alone could also be used to degrade and fractionate lignocellulosic materials. Bobleter O, et al.^[5] pioneered the technique of hydrothermolysis mainly as a pretreatment of subsequent enzymatic hydrolysis.

Both batch and continuous flow hot-compressed water processes were used for hydrolysis of lignocellulosic materials, in which higher level of hemicelluloses and lignin removal can be obtained by continuous flow method, however, the process costs more water and energy than batch method^[6]. As treated by batch hot-compressed water, it has been found that water-soluble increased with temperature and reaction time. Hemicelluloses of Japanese beech could be hydrolyzed in the form of water solubles at temperatures from 170°C to 210°C within 15 minutes^[7, 8]. However, the optimal hydrolysis conditions of batch hot-compressed water, especially considering the hydrolysis and decomposition together, had remained elusive. The goal of this work was to optimize the batch hot-compressed water hydrolysis of Japanese beech.

Response Surface Methodology (RSM) is widely used to optimize different processes, which was successfully

Received date: 2008-07-30 Accepted date: 2008-12-17

Biographies: Lü Xin, Ph.D, Lecturer. College of Food Science and Technology, Nanjing Agricultural University. Shiro Saka, Professor, Graduate School of Energy Science, Kyoto University, Kyoto, Japan. Email: saka@energy.kyoto-u.ac.jp

Corresponding author: Lü Xin, College of Food Science and Technology, Nanjing Agricultural University, Weigang No.1, Tongwei Road, Xuanwu District, Nanjing City, Jiangsu Province, 210095, China. Email: xinlu_syu@yahoo.com.cn

applied for the optimization of medium composition, culture conditions and fermentation^[9-11]. This method can deal with two or more factors at several levels, yielding results that are valid over a wide range of experimental conditions. The present study undertook the optimization of water-soluble, saccharides from hemicelluloses and decomposition products for hydrolysis of Japanese beech as treated by batch hot-compressed water. Under optimal conditions it was possible to obtain maximum water-soluble and saccharides from hemicelluloses as well as minimum decomposition products. A two-factor and three-level full factorial design and RSM had been used to determine the effects of temperature and reaction time as well as to identify the optimal hydrolysis conditions.

2 Materials and methods

2.1 Feedstock materials

Japanese beech (*Fagus crenata*) was used as raw material in all experiments. Wood flour (18 mesh pass) was extracted by ethanol-benzene (1:2) and dried at 105°C for 24 h before experiment. On average, extractive-free wood contains 45 % (wt) cellulose, 29 % (wt) hemicellulose, and 26 % (wt) lignin^[12].

The reagents used for HPLC/ICS3000 analysis were as follows: monosaccharides (xylose, rhamnose, galactose 98%, arabinose and mannose), fufural, 5-HMF(5-hydroxymethyl-2-furfural), formic acid 98%, glycolic acid 98%, lactic acid and acetic acid 99% from Nacalai tesque Inc., Kyoto, Japan. Xylooligosaccharides (xylobiose 99%+, xylotrisose 95%+, xylotetraose 90%+, xylopentaose 95%+ and xylohexaose 95%+), from Wako Pure Chemical Industries, Ltd. Osaka, Japan.

2.2 Hot-compressed water treatment

The reactor with 5mL working volume was made of Inconel-625 and thermocouple was used to assay the temperature inside the tube. Approximately, 150 mg extractive-free Japanese beech flour and 4.9 mL water were added to the above reactor. Distilled water in high performance liquid chromatography (HPLC) grade was used in all experiments. After addition of wood flour and water, the sealed reactor was immersed into oil bath for hydrolysis of wood flour. Profiles of temperature in reactor is illustrated in Figure 1. The reaction time was accounted after the temperature inside the tube reached designed temperature, that is, reaction time for 170°C, 190°C and 210°C was counted after 7 min, 12 min and 12 min, respectively. After reaction, the reactor vessel was

immediately immersed in tap water to quench the reaction. All the experiments were performed in duplicate, with the average value reported.

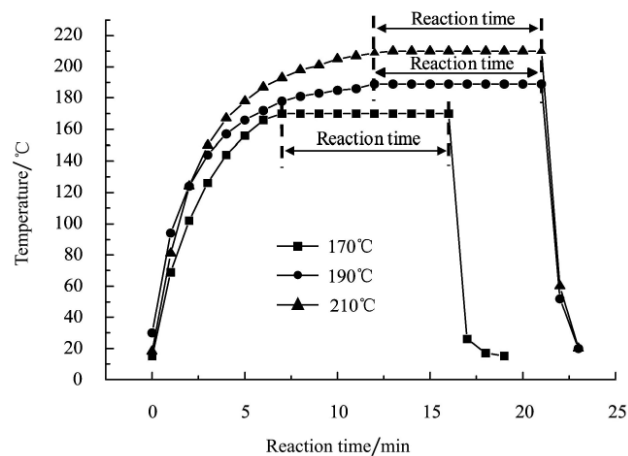


Figure 1 Temperature profile of preheating-time and definition of reaction-time

2.3 Optimization of experiment design

A two-factor and three-level full factorial experimental design was employed to collect the data necessary for optimization of the batch treatment procedure. One central point was added to estimate the experimental error as well as to investigate the suitability of the proposed model. Coding of the independent variables was done according to the following equation:

$$x_i = \frac{(X_i - X_0)}{\Delta X_i} \quad (1)$$

Where x_i and X_i were the dimensionless and the actual values of the independent variable i , X_0 was the actual value of the independent variable i at the central point and ΔX_i was the step change of X_i .

The temperature and reaction time were selected as the independent variables. The range and the levels of both coded and actual values of the independent variables were listed in Table 1. The performance of the process could be described by the following quadratic polynomial equation:

$$Y = \beta_0 + \sum_{i=1}^n \beta_i x_i + \sum_{i=1}^n \beta_{ii} x_i^2 + \sum_{i=1}^n \sum_{j>i}^n \beta_{ij} x_i x_j \quad (2)$$

Where Y represented response variable, β_0 , β_i , β_{ii} , β_{ij} , were intercept coefficient, linear term, quadratic term and interaction term, respectively. x_i , x_j were coded levels of independent variables. Y_1 , Y_2 , Y_3 , Y_4 and Y_5 represented water-soluble, (1/saccharides from hemicelluloses), 5-HMF, furfural and organic acids, respectively. The Design Expert 7.00(trial) software

was used for the regression analysis and numerical optimization.

Table 1 Experimental ranges and levels of both coded and actual values of the independent variables

Independent variables	Variables	Levels		
		-1	0	1
x_1	Temperature/°C	170	190	210
x_2	Time/min	3	9	15

2.4 Analytical methods

After hot-compressed water treatment, 200 μ L water-soluble sample was retrieved by centrifugation using 0.45 μ m Millipore filter tube. The solid residues were filtrated and dried at 105°C for 24 h.

$$\text{Water-soluble(\%)} = \frac{\text{Wood flour} - \text{Residue}}{\text{Wood flour}} \times 100\% \quad (3)$$

HPLC (ICS 3000, Dionex, USA) with CarboPac PA1 column and electrochemical detector was used to assay monosaccharides (rhamnose, arabinose, galactose, mannose and xylose) and xylooligosaccharides (xylobiose, xylotriose, xylotetraose, xylopentaose and xylohexaose). The system was operated at 35°C with a gradient program mixture of water, 0.2 mol/L sodium hydroxide and 1 mol/L sodium acetate.

HPLC (Shimadzu, LC-10A, Japan) equipped with a Shodex Sugar KS-801 (Showadenk) column and refractive index detector/uv-vis detector was used to determine 5-HMF and furfural. The carrier solvent was distilled water at a flow rate of 1.0 mL/min, column temperature was set at 80°C.

CE (capillary electrophoresis) (HP3D, Agilent, USA) was used to assay the formic acid, acetic acid, glycolic acid and lactic acid. A fused-silica capillary (75 μ m id, 104 cm total length, 95.5 cm effective length) from Agilent was used. The temperature was set at 15°C for analysis.

All weight percentages of saccharides from hemicelluloses, 5-HMF, furfural and organic acids were calculated from the mass of these compounds divided by the mass of dry extractive-free wood which was added in the reactor initially.

3 Results and discussion

3.1 Response surface for water-soluble

The matrix of experimental design and experimental results of two-factor and three-level full factorial experiments are shown in Table 2. Neglecting the

statistically insignificant terms, step-wise regression model of response surface for water-soluble is represented by the equation:

$$Y_1(\text{Water-soluble}) = 32.8 + 4.8x_1 + 10.5x_2 - 1.3x_1x_2 - 3.05x_1^2 - 2.7x_2^2 \quad (4)$$

The results were analyzed by using analysis of variance (ANOVA) whose results are shown in Table 3. The F -value (126.28) of model means that the regression model is statistically significant ($p=0.0002$). R^2 and $Adj-R^2$ were calculated to check the model adequacy. High proportion of variability ($R^2 = 0.99$) in the response model can be explained successfully by the model. However, a large value of R^2 does not always imply that the regression model is good one because R^2 will increase with adding a variable regardless of whether the additional variable is statistically significant or not. Thus, it is preferred to use an $Adj-R^2$ to evaluate the model adequacy and should be over 90%. Table 3 shows that R^2 and $Adj-R^2$ values for the model did not differ dramatically indicating insignificant terms have not been included in the model. The significance of each term after step-wise removal of insignificant terms is also shown in Table 3 based on p -value. The smaller the magnitude of p -value, the more significant is the corresponding term. According to the p -value of all significant terms in the model, temperature produces largest effect on water-soluble, followed by terms of reaction time, quadratic temperature and interaction.

To visualize the combined effects of temperature and time on water-soluble, the response surface and contour plots were generated for the models in function of two variables. Figure 2 shows the three dimensional graph representing the response surface for water-soluble. It is shown that an increase of temperature and time leads to an increase of water-soluble.

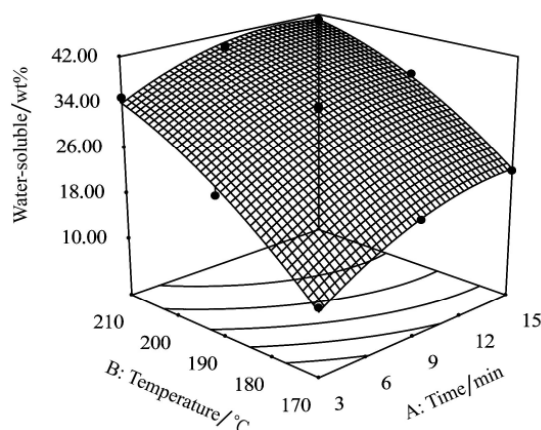


Figure 2 Response surface plot of water-soluble as a function of time and temperature

Table 2 Experimental design of 3² full factorial batch hot-compressed water treatment and corresponding experimental results

Run	Independent variables		Water-soluble / % (wt)	Saccharides from hemicelluloses ¹⁾ / % (wt)	5-HMF / % (wt)	Furfural / (wt)%	Organic acids ²⁾ / % (wt)
	x ₁ : time	x ₂ : temperature					
1	1	-1	22.2	5.7	0.08	0.06	0.28
2	0	1	39.8	8.2	2.08	2.5	1.12
3	0	0	33.5	19.8	0.22	0.37	0.52
4	-1	-1	11.1	1.3	0.04	0.01	0.05
5	-1	1	35.0	20.9	0.38	0.75	0.69
6	0	-1	19.3	2.2	0.05	0.03	0.39
7	1	1	41.0	4.9	4.13	3.01	0.96
8	1	0	34.9	19.5	0.46	0.9	0.66
9	-1	0	23.4	6.4	0.07	0.07	0.32
10	0	0	33.1	19.5	0.18	0.39	0.55

Note: 1) Sacchrides from hemicelluloses: rhamnose, arabinose, galactose, mannose, xylose, xylooligosaccharides (xylobiose, xylotriose, xylotetraose, xylopentaose, xylohexaose); 2) Organic acids: formic acid, acetic acid, glycolic acid, lactic acid.

Table 3 Results of ANOVA of water-soluble

Source	Sum of Squares	DF	Mean Square	F value	p-value
Model	854.87	5	170.84	126.28	0.0002
x ₁ -time	136.33	1	136.33	100.69	0.0006
x ₂ -temperature	665.71	1	665.71	491.70	<0.0001
x ₁ x ₂	6.50	1	6.50	4.80	0.0935
x ₁ ²	22.01	1	22.01	16.26	0.0157
x ₂ ²	16.65	1	16.65	12.30	0.0247
R ²	0.99				
Adj-R ²	0.98				

3.3 Response surface for saccharides from hemicelluloses

Hemicelluloses of lignocellulosic materials could be hydrolyzed efficiently by hot-compressed water^[4,8,13]. The hydrolyzed compounds from hemicelluloses, especially pentoses and other saccharides could be further utilized for fermentation^[14, 15].

The saccharides from hemicelluloses was coded inversely to give the best model to satisfy the real data. The relationship between percent of saccharides from hemicelluloses) and treatment conditions is described as:

$$Y_2(\text{Saccharides from hemicelluloses, \%}) = 0.08 - 0.09 x_1 - 0.2 x_2 + 0.19 x_1 x_2 + 0.22 x_2^2 \quad (5)$$

The results of ANOVA for saccharides from hemicelluloses(%) are listed in Table 4 which demonstrate that the model is statistically significant. The *F*-value of 81.66 means that the regression model is statistically significant ($p < 0.0001$). R^2 ($R^2=0.98$) and $Adj-R^2$ ($Adj-R^2=0.97$) values for the model did not differ dramatically which indicate that insignificant terms have not been included in the model and the model is adequate. Also, it is shown that the most significant term is temperature because the smallest *p*-value of temperature term ($p=0.0001$). Interaction term, quadratic temperature

and reaction time are arranged in significance order according to *p*-values of these terms.

Table 4 Results of ANOVA of (Saccharides from hemicelluloses, %)

Source	Sum of Squares	DF	Mean Square	F value	p-value
Model	0.48	4	0.12	81.66	<0.0001
x ₁ -time	0.049	1	0.049	33.44	0.0022
x ₂ -temperature	0.18	1	0.18	119.44	0.0001
x ₁ x ₂	0.14	1	0.14	95.86	0.0002
x ₂ ²	0.11	1	0.11	77.88	0.0004
R ²	0.98				
Adj-R ²	0.97				

Figure 3 shows the three dimensional graph representing the response for saccharides from hemicelluloses(%). It is shown that the increase of temperature leads to a decrease of saccharides from hemicelluloses(%) when treated in shorter time, while saccharides from hemicelluloses(%) increase when treated in longer time. On the other hand, saccharides from hemicelluloses(%) decreased with reaction time when treated at lower temperature, while saccharides from hemicelluloses(%) increased with reaction time when treated at higher temperature. Since the inverse transformation was done for saccharides from

hemicellulose, the smaller the saccharides from hemicelluloses(%) is, the larger is the saccharides from hemicelluloses.

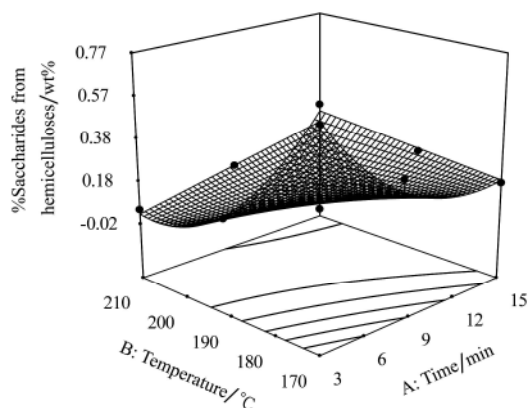


Figure 3 Response surface plot of Saccharides from hemicelluloses (%) as a function of time and temperature

3.4 Response surface for decomposition products

It is well known that decomposition of saccharides would occur when treated by hot-compressed water. The decomposition products from saccharides, e.g. furfural and 5-HMF, are known inhibitors to microbial growth^[16-19]. Besides, these two typical decomposition agents could be further decomposed to small molecules, such as formic acid, acetic acid, etc., which are possible inhibitory agents too^[19]. Moreover, these degradation products would lower the recovery rate of saccharides. Although techniques could be adopted for removing these inhibitory compounds^[20], the prevention of these inhibitory compounds formation from beginning was expected.

Stepwise regression models for 5-HMF, furfural and organic acids are described by the equations as follows:

$$Y_3 (5\text{-HMF}) = 0.2 + 0.7x_1 + 1.1x_2 + 0.9x_1x_2 + 0.9x_2^2 \quad (6)$$

$$Y_4 (\text{Furfural}) = 0.4 + 0.5x_1 + 1.0x_2 + 0.6x_1x_2 + 0.6x_2^2 \quad (7)$$

$$Y_5 (\text{Organic acids}) = 0.7 + 0.1x_1 + 0.3x_2 - 0.2x_1^2 \quad (8)$$

The results of ANOVA for 5-HMF, furfural and organic acids are listed in Table 5, Table 6, Table 7, which demonstrate that these models are statistically significant. R^2 of regression models for 5-HMF, furfural and organic acids are 0.95, 0.97 and 0.94, respectively, while $Adj-R^2$ are 0.91, 0.95 and 0.91, respectively. The values of R^2 and for $Adj-R^2$ show that the models are adequate and insignificant terms have not been included in the models. In the models of all decomposition compounds, the temperature is the most significant term since the p -values of temperature term are smallest term among all terms. This implied that temperature played most important role in the formation of decomposition

compounds.

Table 5 Results of ANOVA of 5-HMF

Source	Sum of Squares	DF	Mean Square	F value	p-value
Model	15.14	4	3.79	24.25	0.0018
x_1 -time	2.91	1	2.91	18.65	0.0076
x_2 -temperature	6.87	1	6.87	44.01	0.0012
x_1x_2	3.44	1	3.44	22.04	0.0054
x_2^2	1.92	1	1.92	12.29	0.0172
R^2	0.95				
$Adj-R^2$	0.91				

Table 6 Results of ANOVA of Furfural

Source	Sum of Squares	DF	Mean Square	F value	p-value
Model	10.13	4	2.53	41.84	0.0005
x_1 -time	1.64	1	1.64	27.14	0.0034
x_2 -temperature	6.32	1	6.32	104.46	0.0002
x_1x_2	1.22	1	1.22	20.17	0.0065
x_2^2	0.95	1	0.95	15.61	0.0108
R^2	0.97				
$Adj-R^2$	0.95				

Table 7 Results of ANOVA of Organic acids

Source	Sum of Squares	DF	Mean Square	F value	p-value
Model	0.87	3	0.29	32.57	0.0004
x_1 -time	0.12	1	0.12	13.16	0.0110
x_2 -temperature	0.70	1	0.70	78.38	0.0001
x_1^2	0.055	1	0.055	6.18	0.0474
R^2	0.94				
$Adj-R^2$	0.91				

Figure 4, Figure 5 and Figure 6 show the three-dimensional graphs representing the response surface for 5-HMF, furfural and organic acids. It is shown that an increase of temperature and time leads to an increase of 5-HMF, furfural and organic acids, which indicate that decomposition of saccharides will increase with temperature and reaction time.

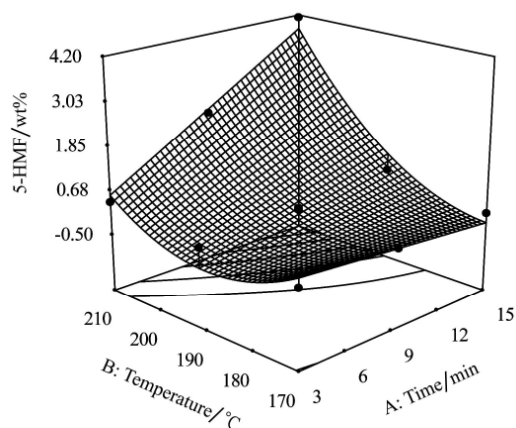


Figure 4 Response surface plot of 5-HMF as a function of time and temperature

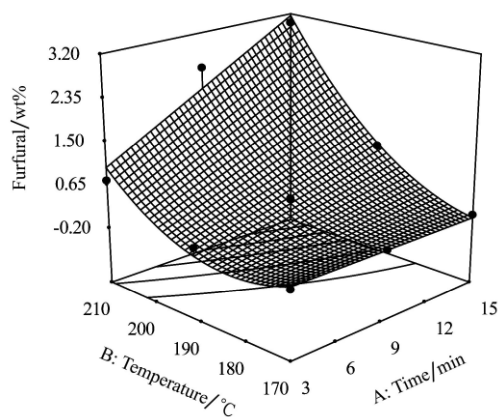


Figure 5 Response surface plot of furfural as a function of time and temperature

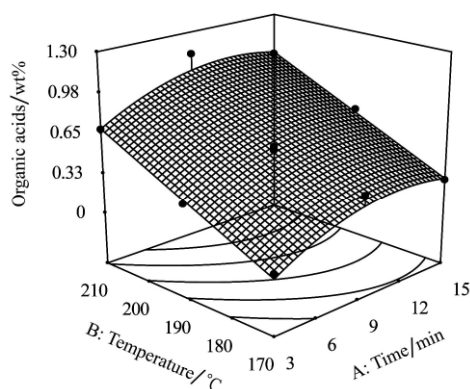


Figure 6 Response surface plot of organic acids as a function of time and temperature

3.5 Numerical optimization

Optimum conditions for batch hot-compressed water treatment were determined to obtain maximum water-soluble and saccharides from hemicelluloses as well as minimum 5-HMF, furfural and organic acids. Quadratic polynomial models for each response obtained in this study were utilized to determine the specified optimal conditions by using Design Expert 7.00 (trial) software. Desirability function method was used to obtain optimal conditions^[21-23]. It is shown in Figure 7 that desirability increased with reaction time at lower temperature while decreased with reaction time at higher temperature. When treated at certain reaction time, the desirability increased firstly with temperature and decreased then. That is, optimal desirability could be obtained at high temperature/short reaction time or low temperature/long reaction time. The higher the desirability is, the better is the optimal solution. Two solutions were obtained after numerical optimization based on desirability, the one is 200°C/3min; second one is 178°C/15min. Because desirability of (200°C/3 min) is higher than the one of (178°C/15 min), the optimal solution (200°C, 3min) with highest desirability was

selected according to above analysis for the maximum water-soluble and saccharides from hemicelluloses as well as minimum 5-HMF, furfural and organic acids.

Experiments at optimal condition were done to confirm the prediction. The water-soluble, saccharides from hemicelluloses, 5-HMF, furfural and organic acids were 31.5%, 19.5%, 0.28%, 0.70%, and 0.59%, respectively. Although the formation of decomposition compounds could not be prevented completely in optimal conditions, these inhibitors may have little effects on later fermentation because microorganism can endure certain extent of these inhibitory agents^[24, 25].

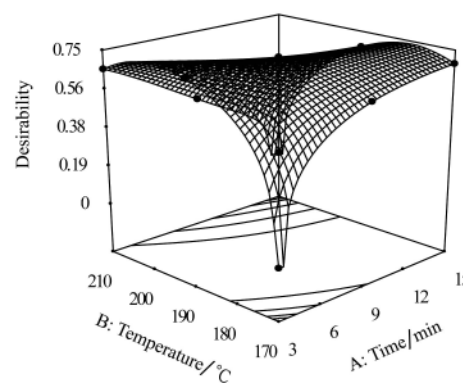


Figure 7 Variation of desirability in numerical optimization process

4 Conclusions

The response surface methodology (RSM) for modeling the influence of temperature and reaction time on the performance of hydrolysis of Japanese beech when treated by batch hot-compressed water was used to determine the optimum conditions which yield maximum water-soluble and saccharides from hemicelluloses as well as minimum 5-HMF, furfural and organic acids. Quadratic polynomial models were obtained for predicting water-soluble, saccharides from hemicelluloses, 5-HMF, furfural and organic acids. Based on the models, multiple response optimization by applying desirability function method was done to predict the optimal conditions for maximum water-soluble and saccharides from hemicelluloses as well as minimum 5-HMF, furfural and organic acids. The optimal conditions were found to be temperature of 200°C and reaction time of 3min. At this optimum condition, water-soluble 31.5%, saccharides from hemicelluloses 19.5%, 5-HMF 0.28%, furfural 0.70% and organic acids 0.59% were obtained.

[References]

- [1] Arthur J Ragauskas, Charlotte K Williams, Brian H. Davison, George Britovsek, John Cairney, Charles A

- Eckert, et al. The path forward for biofuels and biomaterials. *Science*, 2006; 311(27): 484–489.
- [2] Alexander E Farrell, Richard J Pelvin, Brian T Turner, Andrew D Jones, Michael O'Hare, Daniel M Kammen. Ethanol can contribute to energy and environmental goals. *Science*, 2006; 311(27): 506–508.
- [3] Jens R Rostrup-Nielsen. Making fuels from biomass. *Science*, 2005; 308(3): 1421–1422.
- [4] Nathan Mosier, Charles Wyman, Bruce Dale, Richard Elander, Lee Y Y, Mark Holtzapple, et al. Features of promising technologies for pretreatment of lignocellulosic biomass. *Bioresource Technology*, 2005; 96(6): 673–686.
- [5] Bobleter O, Niesner R, Rohr M. The hydrothermal degradation of cellulosic matter to sugars and their fermentative conversion to protein. *Journal of Applied Polymer Science*, 1976; 20(8): 2083–2093.
- [6] Chaogang Liu, Wyman Charles E. The effect of flow rate of compressed hot water on xylan, lignin, and total mass removal from corn stover. *Industrial and Engineering Chemistry Research*, 2003; 42(21): 5409–5416.
- [7] Lu Xin, Shiro Saka, Dong Mingsheng, Chen Xiaohong, Jiang Mei. Preliminary investigation on hot-compressed water pretreatment of Japanese beech powder for fuel-ethanol production. *Transactions of the Chinese Society of Agricultural Engineering*, 2008; 24(3): 219–222 (in Chinese).
- [8] William Shu-lai Mok, Michael Jerry Antal JR. Biomass pretreatment by hot-compressed liquid water. *Advances in Thermochemical Biomass Conversion*, 1994; 12: 1572–1582.
- [9] Pardeep Kumar, Satyanarayana T. Optimization of culture variables for improving glucoamylase production by alginate-entrapped *Thermomucor indicaeaeudaticae* using statistical methods. *Bioresource Technology*, 2007; 98(6): 1252–1259.
- [10] Sen Ramkrishna, Swaminathan T. Response surface modeling and optimization to elucidate and analyze the effects of inoculum age and size on surfactin production. *Biochemical Engineering Journal*, 2004; 21(2): 141–148.
- [11] Bazaraa W A, Hassan E E. Response surface optimization for the continuous glucose isomerization process. *Journal of Industrial Microbiology*, 1996; 17(2): 100–103.
- [12] Ero Sjostrom, Raimo Alen. *Analytical methods in wood chemistry, pulping and papermaking*. Germany: Springer-Verlag. 1999.
- [13] Van walsum G. Peter, Allen Stephen G, Spencer Mark J, Laser M. S, Antal M J, Lynd L R. Conversion of lignocellulosics pretreated with liquid hot water to ethanol. *Applied Biochemistry and Biotechnology*, 1996; 57/5(17): 157–170.
- [14] Perego P, Converti A, Zilli M, Del Borghi M. Acid hemicellulose hydrolysates: Physical treatments and continuous immobilized-cell fermentations. *Bioprocess Engineering*, 1994; 10(1): 35–41.
- [15] Patrizia Perego, Attilio Converti, Emilio Palazzi, Marco Del Borghi, Giuseppe Ferraiolo. Fermentation of hardwood hemicellulose hydrolysate by *Pachysolen tannophilus*, *Candida shehatae* and *Pichia stipitis*. *Journal of Industrial Microbiology and Biotechnology*, 1990; 6(3): 157–164.
- [16] Ando S, Arai I, Kiyoto K, Hanai S. Identification of aromatic monomers in steam-exploded poplar and their influence on ethanol fermentation by *Saccharomyces cerevisiae*. *Journal of Fermentation and Technology*, 1986; 64(6), 567–570.
- [17] Jose M Oliva, Marya J Negro, Felicia Saez, Ignacio Ballesteros, Paloma Manzanares, Alberto Gonzalez, et al. Effects of acetic acid, furfural and catechol combinations on ethanol fermentation of *Kluyveromyces marxianus*. *Process Biochemistry*, 2006; 41(5): 1223–1228.
- [18] Helle Steve, Cameron David, Lam Janet, White Ben, Duff Sheldon. Effect of inhibitory compounds found in biomass hydrolysates on growth and xylose fermentation by a genetically engineered strain of *S. cerevisiae*. *Enzyme and Microbial Technology*, 2003; 33(6): 786–792.
- [19] Klinke H B, Thomsen A B, Ahring B K. Inhibition of ethanol-producing yeast and bacteria by degradation products produced during pre-treatment of biomass. *Applied Microbiology and Biotechnology*, 2004; 66(1): 10–26.
- [20] Hisashi Miyafuji, Toshiki Nakata, Katsunobu Ehara, Shiro Saka. Fermentability of water-soluble portion to ethanol obtained by supercritical water treatment of lignocellulosics. *Applied Biochemistry and Biotechnology*, 2005; 124(1-3): 963–972.
- [21] Derringer G., Suich R. Simultaneous optimization of several response variables. *Journal of Quality Technology*, 1980; 12(4): 214–219.
- [22] Harrington E C. The desirability function. *Industrial Quality Control*, 1965; 21(10): 494–498.
- [23] Pizarro C, Gonzalez-Saiz J M, Perez-del-Notario N. Multiple response optimization based on desirability functions of a microwave-assisted extraction method for the simultaneous determination of chloroanisoles and chlorophenols in oak barrel sawdust. *Journal of Chromatography A*, 2006; 1132(1-2): 8–14.
- [24] Berta Sanchez, Juan Bautita. Effects of furfural and 5-hydroxymethylfurfural on the fermentation of *Saccharomyces cerevisiae* and biomass production from *Candida guilliermondii*. *Enzyme and Microbial Technology*, 1988; 10(5): 315–318.
- [25] Boyer L J, Vega J L, Klasson K T, Clausen E C, Gaddy J L. The effects of furfural on ethanol production by *Saccharomyces cerevisiae* in batch culture. *Biomass and Bioenergy*, 1992; 3 (1): 41–48.