

Application of Statistical Experimental Design for the Optimisation of Dilute Sulphuric Acid Hydrolysis of Cassava Bagasse

Nosakhare Andrew Amenaghawon, Samuel Enoroghoe Ogbeide, Charity Ovbromoka Okieimen

University of Benin, Faculty of Engineering, Department of Chemical Engineering, PMB 1154, Ugbowo, Benin City, Edo State, Nigeria
andrew.amenaghawon@uniben.edu, enoroghoe.ogbeide@uniben.edu, cookieimen@uniben.edu

Abstract: The dilute sulphuric acid hydrolysis of cassava bagasse was investigated in this study under the following conditions: acid concentration (0.5-2.5%w/w), temperature (100-130 °C), time (5-40 minutes) and liquid to solid ratio (10-30 mL/g). Results of chemical composition analysis of the bagasse showed that the major components of the cassava bagasse used in this study were cellulose, hemicellulose and lignin which accounted for 36.6, 21.3 and 17.3% of the weight of the material respectively. A statistical model was developed and validated to predict the yield of fermentable sugars during hydrolysis. Response Surface Methodology (RSM) was used to optimise the hydrolysis conditions. The maximum sugar yield of 81.63% was obtained at the optimum temperature, acid concentration, hydrolysis time and liquid to solid ratio (i.e. 122.5 °C, 2 %w/w, 20.5 minutes and 25 mL/g respectively).

Keywords: Cassava bagasse; Fermentable sugar; Central composite design; Response surface methodology; Optimisation

1 Introduction

Cassava (*Manihot spp*) is a shrubby and tuberous plant which is typically cultivated in tropical countries such as Nigeria, Brazil, Thailand etc [1, 2]. It contains about 94% carbohydrate with low fractions of protein and vitamins making it one of the most important economic root crops grown in West Africa [3]. With a production capacity of 45 million tonnes per annum, Nigeria is by far the world's largest producer of cassava [4]. Cassava is typically processed into consumable food materials such as garri, fufu, cassava chips, cassava flour, starch, farina, tapioca, macaroni, cassava bread and pudding [5]. During this process, cassava bagasse, a renewable solid waste material is generated. The bagasse is

usually disposed in the environment surrounding the processing plants thereby contributing to environmental pollution as a result of its high organic content and biodegradability [6]. As a result of its relatively high cellulose and low ash content, cassava bagasse has been identified as a potentially ideal substrate for the biotechnological production of value added products such as organic acids, biopolymers, xylanase enzyme, antibiotics and bioethanol [6, 7].

The hydrolysis of cassava bagasse produces a glucose rich hydrolysate which could be fermented to produce bioethanol by a suitable microorganism [2]. Producing bioethanol from a lignocellulosic feedstock such as cassava bagasse appears to be potentially sustainable especially for countries like Nigeria and Brazil in which the plant is massively cultivated. The use of cassava bagasse as feedstock in ethanol production not only reduces waste and its associated environmental problems but also reduces the cost of ethanol production as the bagasse is abundantly available at little or no cost.

It is important to optimise the variables upon which the yield of fermentable sugars is dependent in order to maximise the yield during hydrolysis. Response surface methodology based on statistically designed experiments has been found to be very useful in optimising multivariable processes. According to Montgomery [8], it is employed for multiple regression analysis of quantitative data obtained from statistically designed experiments.

The aim of this study was to optimise the effect of acid concentration, hydrolysis temperature, hydrolysis time and liquid to solid ratio on the hydrolysis of cassava bagasse to produce fermentable sugars. A four variable central composite design was adopted to design the hydrolysis experiments.

2 Materials and Methods

2.1 Lignocellulosic Feedstock Preparation

Fresh cassava bagasse was obtained from a local cassava processing facility in Benin City, Edo State, Nigeria. It was sun dried to reduce moisture and prevent biodeterioration. The dried bagasse was milled to a particle size of about 1.5 mm, homogenised in a single lot and stored under dry conditions prior to use. The moisture content of the bagasse was determined gravimetrically after drying at 105°C for 20 minutes [9].

2.2 Chemical Composition Analysis

The cassava bagasse was analysed to determine the percentage of cellulose, hemicellulose, lignin, ash and extractives according to the Renewable Energy Laboratory (NREL) standard analytical procedures. Cellulose and hemicellulose were determined by quantitative acid hydrolysis of the extractive-free material using a High Performance Liquid Chromatography (HPLC) system equipped with an Aminex HPX-87 P column (Bio-Rad, USA) and refractive index (RI) detector (Refracto Monitor[®] III, Model 1109, LDC/Milton Roy, USA) [10]. Lignin content (Klason and acid-soluble) was determined following the quantitative acid hydrolysis step [10]. The total extractives present in the biomass was determined by ethanol extraction in a Soxhlet extraction apparatus [11]. The ash content was determined after combustion at 575°C [12].

2.3 Acid Hydrolysis

Acid hydrolysis of cassava bagasse was carried out in an autoclave using dilute sulphuric acid concentration in the range 0.5-2.5% w/w. The operating conditions of the hydrolysis reaction were as follows: temperature (100-130°C), time (5-40 minutes) and liquid to solid ratio (10-30 mL/g). At the end of the hydrolysis reaction, the solid residue was separated by centrifugation and the pH of the resulting supernatant was adjusted to 10 using 2N Ca(OH)₂. The resulting precipitate was removed by centrifugation and the supernatant was adjusted to a pH of 6.5 using 10% H₂SO₄ [13].

2.4 Analytical Methods

The fermentable sugars recovered from the cassava bagasse during hydrolysis were quantified using a UV-Vis spectrophotometer (PG Instruments model T70) at a wavelength of 540 nm according to Miller [14].

2.5 Experimental Design

A four variable central composite design (CCD) for response surface methodology was used to develop a statistical model for the hydrolysis process. The range of the variables that were optimised is shown in Table 1. The CCD is a design that combines the vertices of the hypercube whose coordinates are given by a 2ⁿ factorial design with star points [15]. The star points provide the estimation of curvature of the nonlinear response surface. The experimental design made up of 30 runs was developed using Design Expert[®] 7.0.0 (Stat-ease, Inc. Minneapolis, USA). The coded and actual values of the independent variables were calculated as follows.

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

where x_i and X_i are the coded and actual values of the independent variable respectively. X_o is the actual value of the independent variable at the centre point and ΔX_i is the step change in the actual value of the independent variable. The following generalised second order polynomial equation was used to estimate the response of the dependent variable.

$$Y_i = b_o + \sum b_i X_j + \sum b_{ij} X_i X_j + \sum b_{ii} X_i^2 + e_i \quad (2)$$

where Y_i is the dependent variable or predicted response, X_i and X_j are the independent variables, b_o is offset term, b_i and b_{ij} are the single and interaction effect coefficients and e_i is the error term.

Table 1
Experimental range and levels of independent variables

Independent Variable	Symbols	Coded and Actual Levels				
		-2	-1	0	+1	+2
Temperature(⁰ C) Acid	X_1	100	107.5	115	122.5	130
Concentration (w/w %)	X_2	0.5	1.0	1.5	2.0	2.5
Time (min)	X_3	5.0	13.25	22.5	31.25	40
Liquid/Solid ratio (mL/g)	X_4	10	15	20	25	30

3 Results and Discussion

3.1 Chemical Composition Analysis of Cassava Bagasse

Table 2 shows the result of chemical composition analysis of the cassava bagasse biomass. The major components of the raw material were cellulose, hemicellulose and lignin each accounting for 36.6, 21.3 and 17.3% respectively. The cellulose content of the cassava bagasse used in this study was comparable to that of other lignocellulosic biomass typically used for bioethanol production [16-18]. The relatively high percentage of cellulose in cassava bagasse makes it a suitable feedstock for producing second generation bioethanol after taking it through necessary pretreatment steps. The relatively low percentage of lignin in cassava bagasse improves the ease with which hydrolysis of the cellulose and hemicellulose fractions can be carried out by acids or enzymes [19].

Table 2
Chemical composition of cassava bagasse compared with other biomass resources

Component	Weight percent			
	This study	Sugar cane bagasse [17]	Corn stover (NREL)	Eucalyptus chips [16]
Cellulose	36.6	39.5	36.1	40
Hemicellulose	21.3	22.1	21.4	16.1
Lignin	17.3	17.1	17.2	23.1
Proteins	2.4	1	1.1	3.8
Ash	7.0	2.3	7.1	2

3.2 Statistical Modelling

The central composite design resulted in 30 experimental runs as shown in Table 3. The response or dependent variable was chosen as the yield of fermentable sugars $Y\%$. Equation (3) is the quadratic statistical model in terms of actual variables that was obtained after applying multiple regression analysis to the experimental data presented in Table 3.

$$Y = 44.633 - 1.761X_1 - 2.829X_2 + 6.028X_3 + 3.020X_4 + 0.170X_1X_2 - 0.00615X_1X_3 - 0.679X_2X_3 - 0.00423X_3X_4 + 0.00646X_1^2 + 1.454X_2^2 - 0.0697X_3^2 + 0.0145X_4^2 \quad (3)$$

The values of total sugar yield as predicted by Equation (3) are also presented in Table 3. The results of analysis of variance (ANOVA) carried out to determine the fit of the statistical model are presented in Tables 4 and 5.

The model F value of 29.79 and very low p value (<0.0001) showed that the model was significant. The "Lack of Fit" F value of 2.52 implies that there was insignificant lack of fit. The coefficient of variation (CV) obtained was 6.53% (Table 5). This value indicates the degree of precision with which the treatments were compared [20]. The relatively low value of CV obtained showed that the treatments were carried out with high precision and reliability [21]. An Adequate precision value of 21.82 was obtained. Cao et al. [22] reported that the Adeq. precision gives an indication of the signal to noise ratio and suggested that a value greater than 4 is generally desired. The value of 21.82 obtained indicates an adequate signal and the model can be used to navigate the design space. The coefficient of determination (R^2) was obtained as 0.955. This indicates that 95.5% of the variability in the response could be explained by the statistical model while 4.5% could not be accounted for by the independent variables [13]. The R^2 value indicates the degree to which the model was able to predict the response. The closer the R^2 value is unity, the better the model can predict the response [20].

Table 3
Central composite design matrix for the optimisation of variables and the response values

Run No	Factors								Response	
	Coded levels				Actual values				Sugar Yield (%)	
	X ₁	X ₂	X ₃	X ₄	X ₁	X ₂	X ₃	X ₄	Observed	Predicted
1	1	1	-1	1	122.5	2.0	13.75	25	84.91	78.46
2	1	-1	1	1	122.5	1.0	31.25	25	68.99	72.42
3	-2	0	0	0	100.0	1.5	22.50	20	64.10	68.47
4	-1	-1	1	-1	107.5	1.0	31.25	15	57.31	54.07
5	-1	-1	1	1	107.5	1.0	31.25	25	74.78	76.87
6	0	0	0	0	115.0	1.5	22.50	20	64.65	64.65
7	-1	-1	-1	-1	107.5	1.0	13.75	15	45.22	38.04
8	0	2	0	0	115.0	2.5	22.50	20	69.16	71.93
9	-1	1	-1	-1	107.5	2.0	13.75	15	52.33	48.54
10	1	-1	1	-1	122.5	1.0	31.25	15	52.19	49.62
11	0	0	0	-2	115.0	1.5	22.50	10	35.23	39.59
12	0	0	0	2	115.0	1.5	22.50	30	94.06	92.61
13	1	1	1	1	122.5	2.0	31.25	25	77.01	73.58
14	1	1	1	-1	122.5	2.0	31.25	15	46.91	50.77
15	-1	1	1	1	107.5	2.0	31.25	25	76.29	75.48
16	0	0	-2	0	115.0	1.5	5.00	20	34.97	37.72
17	0	0	0	0	115.0	1.5	22.50	20	64.65	64.65
18	-1	-1	-1	1	107.5	1.0	13.75	25	64.41	68.25
19	1	1	-1	-1	122.5	2.0	13.75	15	43.55	48.25
20	0	0	2	0	115.0	1.5	40.00	20	48.70	48.87
21	1	-1	-1	-1	122.5	1.0	13.75	15	36.05	35.21
22	0	0	0	0	115.0	1.5	22.50	20	64.65	64.65
23	2	0	0	0	130.0	1.5	22.50	20	65.19	63.74
24	0	0	0	0	115.0	1.5	22.50	20	64.65	64.65
25	0	0	0	0	115.0	1.5	22.50	20	64.65	64.65
26	-1	1	-1	1	107.5	2.0	13.75	25	78.71	78.74
27	0	0	0	0	115.0	1.5	22.50	20	64.65	64.65
28	0	-2	0	0	115.0	0.5	22.50	20	60.14	60.28
29	1	-1	-1	1	122.5	1.0	13.75	25	64.11	65.41
30	-1	1	1	-1	107.5	2.0	31.25	15	55.23	52.67

Table 4
Analysis of variance (ANOVA) for quadratic model

Sources	Sum of Squares	df	Mean Squares	F value	p value
Model	5711.13	10	475.93	29.79	< 0.0001
X ₁	33.56	1	33.56	2.10	0.1654
X ₂	203.63	1	203.63	12.75	0.0024
X ₃	186.37	1	186.37	11.67	0.0033
X ₄	4215.57	1	4215.57	263.88	< 0.0001
X ₁ X ₂	6.52	1	6.52	0.41	0.5315
X ₁ X ₃	2.61	1	2.61	0.16	0.6912
X ₂ X ₃	141.28	1	141.28	8.84	0.0085
X ₃ X ₄	54.69	1	54.69	3.42	0.0817
X ₁ ²	3.63	1	3.63	0.23	0.6399
X ₂ ²	3.63	1	3.63	0.23	0.6399
X ₃ ²	781.67	1	781.67	48.93	< 0.0001
X ₄ ²	3.63	1	3.63	0.23	0.6399
Residual	271.66	17	15.98		
Lack of	233.15	12	19.43	2.52	0.1582
Pure Error	38.51	5	7.70		
Cor Total	5982.72	29			

Table 5
Statistical information for ANOVA

Source	Response
R ²	0.955
Adjusted R ²	0.923
CV %	6.53
Adeq. Precision	21.82

3.3 Optimisation of Dilute Acid Hydrolysis of Cassava Bagasse

Response surface curves were plotted to examine the effect of the interaction between the independent variables and to determine the optimum levels of the variables. The effect of temperature and time on the hydrolysis yield is shown in Figure 1. At high reaction times, the hydrolysis yield increased slowly with an increase in temperature from 108 to 123 °C. This observation might be attributed to the increase in the rate of collision of the molecules of the reacting species during the reaction. Hence the higher the temperature, the more frequent the molecules will collide with each other resulting in reaction. For the range of temperatures investigated, intermediate levels of time were needed to obtain a high hydrolysis yield, and any further increase in time resulted in a decrease in hydrolysis yield. This might have resulted from the degradation of fermentable sugars to by-products such as furfural and hydroxyl methyl furfural [23]. The

maximum sugar yield of about 82% was obtained at a temperature of about 122 °C and a time of about 21 minutes. Lu *et al.* [24] reported the optimum yield of dilute acid hydrolysed corn stover as 85.4% using a sulphuric acid concentration of 2.0%, at a temperature of 120 °C and a reaction time of 43 minutes.

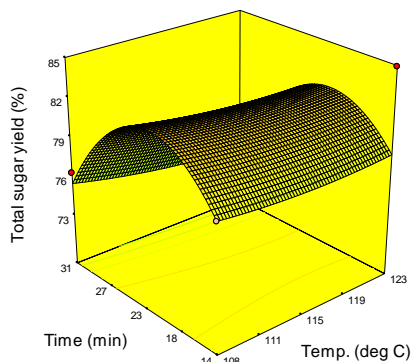


Figure 1
Effect of temperature and time on total sugar yield

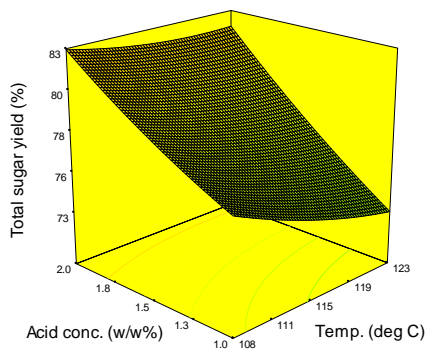


Figure 2
Effect of acid concentration and temperature on total sugar yield

Figure 2 shows the effect of acid concentration and hydrolysis temperature when time and liquid to solid ratio were maintained at their centre points. It was observed that at low temperatures, there was a rapid and progressive increase in the sugar yield when the acid concentration was increased from 1 to 2 w/w%. The same observation was recorded at high temperatures. The trend observed may be attributed to the catalytic activity of the acid. A high concentration of acid implies a high concentration of hydrogen ions in solution. Since, the hydrogen ions are responsible for the catalytic activity of the acid, it should be expected that an increase in the concentration of acid will result in an increase in the catalytic activity of the acid during hydrolysis. Hence the rate at which the glycosidic bonds are cleaved will also increase thereby resulting in the release of more fermentable sugars [25]. At high concentration of acid, increasing the temperature did not result in any significant change in the total sugar yield. However, at low concentration of acid, the total sugar yield was observed to decrease slightly with increase in temperature.

Figure 3 shows the effect of acid concentration and time on the total sugar yield. The hydrolysis yield increased with increase in acid concentration. This observation is similar to that shown in Figure 2. However, the rate of the hydrolysis reaction was not as fast as the case shown in Figure 2. A quadratic response similar to that shown in Figure 1 was observed for the total sugar yield with respect to the hydrolysis time. A similar quadratic behaviour was reported by Lenihan *et al.* [26] for the dilute acid hydrolysis of potato peels. The maximum sugar yield was recorded as 88% and this was obtained at an acid concentration of 2 % w/w and a time of about 23 minutes.

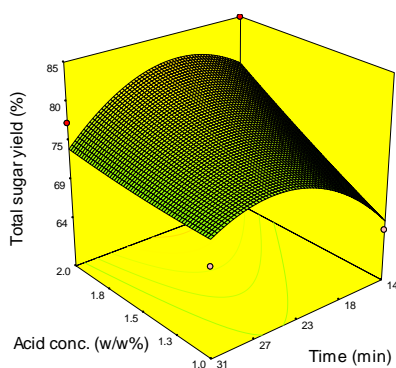


Figure 3
Effect of acid concentration and time on total sugar yield

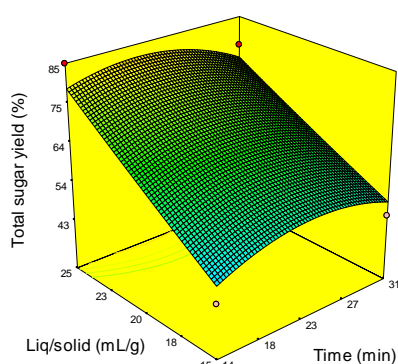


Figure 4
Effect of liquid to solid ratio and time on total sugar yield

Figure 4 shows the effect of liquid to solid ratio and hydrolysis time on the total sugar yield. The plot showed that the liquid to solid ratio had a significant effect on the total sugar yield within the range of time investigated. The maximum sugar yield was obtained at a liquid to solid ratio of 25 mL/g and a time of about 23 minutes. A similar result was reported by Mansilla et al. [27] who reported an optimum liquid to solid ratio of 25 mL/g for the production of furfural from rice hull via acid hydrolysis. Similarly, Zhang et al. [28] reported an optimum liquid to solid ratio of 20 mL/g during a reaction time 10 minutes to obtain a sugar yield of 91.3% from dilute phosphoric acid hydrolysed oil palm empty fruit bunch.

The values of acid concentration, hydrolysis temperature, hydrolysis time and liquid to solid ratio were optimised based on the statistical model (Equation 3) and the top five results are shown in Table 6. Optimisation results show that the maximum sugar yield was obtained at a temperature of 122.5 °C, an acid concentration of 2 % w/w, a time of 20.5 minutes and liquid to solid ratio of 25 mL/g. The maximum sugar yield obtain at these optimised conditions was 81.63%.

Table 6
Solutions for optimum conditions of hydrolysis

Solution Number	Temp. (°C)	Acid Conc. (% w/w)	Time (min)	Liquid/Solid (mL/g)	Sugar Yield (%)	Desirability
1	122.5	2	20.5	25	81.63	0.946
2	122.5	2	21.4	25	81.58	0.946
3	122.5	2	19.3	25	81.54	0.946
4	122.5	2	19.8	25	81.57	0.945
5	122.4	2	19.9	25	81.58	0.945

3.4 Validation of Statistical Model

In order to confirm the validity of the statistical model, three confirmation experimental runs were performed at the chosen optimum hydrolysis conditions indicated in Table 6. The result shows that the maximum experimental sugar yield of 81.14% obtained was close to the predicted value of 81.63%. The excellent correlation between the predicted and measured values of these experiments shows the validity of statistical model.

Conclusions

The hydrolysis of cassava bagasse was carried out using dilute sulphuric acid according to a four variable central composite design. The major components of cassava bagasse as obtained from chemical composition analysis are cellulose, hemicellulose and lignin. The optimum hydrolysis conditions are a temperature of 122.5 °C, acid concentration of 2 %w/w, reaction time of 20.5 minutes and a liquid to solid ratio of 25 mL/g. The maximum sugar yield obtained at these optimised conditions was 81.63%. RSM with central composite design has proved to be a useful tool in identifying the important factors that influence the dilute acid hydrolysis of cassava bagasse as well as their optimised levels.

Acknowledgement

This work was supported by the University of Benin Research and Publications Committee (URPC) under the Tertiary Education Trust Fund (TETFUND) grant.

References

- [1] A. Nebiyu, E. Getachew: Soaking and Drying of Cassava Roots Reduced Cyanogenic Potential of Three Cassava Varieties at Jimma, Southwest Ethiopia, *African Journal of Biotechnology*, Vol. 10, No. 62, 2011, pp. 13465-13469
- [2] A. L. Woiciechowski, S. Nitsche, A. Pandey, C. R. Soccol: Acid and Enzymatic Hydrolysis to Recover Reducing Sugars from Cassava Bagasse: an Economic Study, *Brazilian Archives of Biology and Technology*, Vol. 45, No. 3, 2002, pp. 393-400
- [3] L. Uyigue, M. Omotioma: Modelling the Reduction Mechanism of Hydrogen Cyanide Contained in Processed Cassava Wastewater, *Journal of the Nigerian Society of Chemical Engineers*, Vol. 26, No. 2, 2011, pp. 70-80
- [4] FAO. *Food Outlook: Global Market Analysis*. Food and Agriculture Organisation, Rome, Italy, 2009, 28p
- [5] Authors N. A. Amenaghawon, F. A. Aisien, S. E. Ogbeide: Bioethanol Production from Pretreated Cassava Bagasse using combined Acid and Enzymatic Hydrolysis, *University of Benin Journal of Science and Technology*, Vol. 1, No. 2, 2013, pp. 48-53

- [6] K. R. Sugumaran, S. P. Chakravarthi, V. Ponnusami: Cassava Bagasse: A Potential and Low Cost Substrate for Cellulase Production in an Economical Fermentation, *Research Journal of Pharmaceutical Biology and Chemical Science*, Vol. 4, No. 2, 2013, pp. 1168-1175
- [7] A. Pandey, C. R. Soccol, P. Nigam, V. T. Soccol, L. P. S. Vandenberghe, R. Mohan: Biotechnological Potential of Agro-Industrial Residues. II: Cassav Bagasse, *Bioresource Technology*, Vol. 74, 2000, pp. 81-87
- [8] D. C. Montgomery: *Design and Analysis of Experiments*. 6. ed. New York: John Wiley & Sons, Inc., 2005
- [9] A. Sluiter, B. Hames, D. Hyman, C. Payne, R. Ruiz, C. Scarlata, J. Sluiter, D. Templeton, J. Wolfe: Determination of Total Solids in Biomass and Total Dissolved Solids in Liquid Process Samples, Technical Report NREL/TP-510-42621. National Renewable Energy Laboratory, Golden, Colorado. 2008a
- [10] A. Sluiter, B. Hames, R. Ruiz, C. Scarlata, J. Sluiter, D. Templeton, D. Crocker. Determination of Structural Carbohydrates and Lignin in Biomass. Technical Report NREL/TP-510-42618, National Renewable Energy Laboratory, Golden, Colorado. 2008b
- [11] A. Sluiter, R. Ruiz, C. Scarlata, J. Sluiter, D. Templeton: Determination of Extractives in Biomass, Technical Report NREL/TP-510-42619. National Renewable Energy Laboratory, Golden, Colorado. 2008c
- [12] A. Sluiter, B. Hames, D. Hyman, C. Payne, R. Ruiz, C. Scarlata, J. Sluiter, D. Templeton, J. Wolfe: Determination of ash in biomass, Technical Report NREL/TP-510-42622. National Renewable Energy Laboratory, Golden, Colorado. 2008d
- [13] N. A. Amenaghawon, C. O. Okieimen, S. E. Ogbeide: Modelling and Statistical Optimization of Dilute Acid Hydrolysis of Eucalyptus Wood Chips using Response Surface Methodology, *Pacific Journal of Science and Technology*, Vol. 15, No. 1, 2014, pp. 245-256
- [14] G. L. Miller: Use of Dinitrosalicylic Acid Reagent for Determination of Reducing Sugar. *Analytical Chemistry*, Vol. 31, No. 3, 1959, pp. 426-428
- [15] G. E. P. Box, Hunter, J. S. Hunter: *Statistics for Experimenters: An Introduction to Design, Data Analysis and Model Building*. New York: John Wiley & Sons Inc., 1978. p. 653
- [16] E. V. Canettieri, G. J. M. Rocha, J. A. Carvalho, J. B. A. Silver. Optimization of Acid Hydrolysis from the Hemicellulosic Fraction of Eucalyptus Grandis Residue using Response Surface Methodology, *Bioresource Technology*, Vol. 98, No. 2, 2007, pp. 422-428
- [17] C. Martin, Y. Lopez, Y. Plasencia, E. Hernandez: Characterisation of Agricultural and Agro-Industrial Residues as Raw Materials for Ethanol

- Production, Chemical and Biochemical Engineering Quarterly, Vol. 20, No. 4, 2006, pp. 443-446
- [18] S. H. A. Rahman, J. P. Choudhury, A. L. Ahmad, A. H. Kamaruddin: Optimisation Studies on Acid Hydrolysis of Oil Palm Empty Fruit Bunch Fiber for Production of Xylose, *Bioresource Technology*, Vol. 98, No. 3, 2007, pp. 554-559
- [19] D. Piarpuzán, J. A. Quintero, C. A. Cardona: Empty Fruit Bunches from Oil Palm as a Potential Raw Material for Fuel Ethanol Production, *Biomass Bioenergy*, Vol. 35, No. 3, 2011, pp. 1130-1137
- [20] B. Qi, X. Chen, F. Shen, Y. Wan: Optimization of Enzymatic Hydrolysis of Wheat Straw Pretreated by Alkaline Peroxide Using Response Surface Methodology. *Industrial and Engineering Chemistry Research*, Vol. 48, 2009, pp. 7346-7353
- [21] X. J. Hou, W. Chen: Optimization of Extraction Process of Crude Polysaccharides from Wild Edible Bachu Mushroom by Response Surface Methodology, *Carbohydrate Polymers*, Vol. 72, 2008, pp. 67-74
- [22] G. Cao, N. Ren, A. Wang, D. J. Lee, W. Guo, B. Liu, Y. Feng, Q. Zhao: Acid Hydrolysis of Corn Stover for Biohydrogen Production using *Thermoanaerobacterium Thermosaccharolyticum* W16, *International Journal of Hydrogen Energy*, Vol. 34, 2009, pp. 7182-7188
- [23] G. Najafpour, A. Ideris, S. Salmanpour, M. Norouzi: Acid Hydrolysis of Pretreated Palm Oil Lignocellulosic Wastes, *IJE Transactions*, Vol. 20, 2007, pp. 147-156
- [24] X. B. Lu, Y. M. Zhang, J. Yang, Y. Liang: Enzymatic Hydrolysis of Corn Stover after Pretreatment with Dilute Sulfuric Acid, *Chemical Engineering Technology*, Vol. 30, No. 7, 2007, pp. 938-944
- [25] R. Hu, L. Lin, T. Liu, S. Liu: Dilute Sulphuric Acid Hydrolysis of Sugar Maple Wood Extract at Atmospheric Pressure, *Bioresource Technology*, Vol. 101, No. 10, 2010, pp. 3586-3594
- [26] P. Lenihan, A. Orozco, E. O'neill, M. N. M. Ahmad, D. W. Rooney, G. M. Walker: Dilute Acid Hydrolysis of Lignocellulosic Biomass, *Chemical Engineering Journal*, Vol. 156, No. 2, 2010, pp. 395-403
- [27] H. D. Mansilla, J. Baeza, S. Urzúa, G. Maturana, J. Villaseñor, N. Durán: Acid-catalysed Hydrolysis of Rice Hull: Evaluation of Furfural Production, *Bioresource Technology*, Vol. 66, No. 3, 1998, pp. 189-193
- [28] D. Zhang, Y. L. Ong, Z. Li, J. C. Wu: Optimisation of Dilute Acid-catalysed Hydrolysis of Oil Palm Empty Fruit Bunch for High Yield Production of Xylose, *Chemical Engineering Journal*, Vol. 181, 2012, pp. 636-642