ACADEMIA ROMÂNĂ
Revue Roumaine de Chimie
http://web.icf.ro/rrch/

Rev. Roum. Chim.,
2012, *57*(11), 915-920

MATHEMATICAL MODELLING OF THE PROCESS OF SUGAR BEET PULP VALORISATION BY ACID HYDROLYSIS

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Received November 1, 2011

A new method to recover reducing sugars from sugar beet pulp, useful for various bioprocesses, was developed in this study via an acid hydrolysis. The process was conducted in two stages with yields of 50% for the first stage and 80% for the second one and led to a final concentration of 8.8 g/L of reducing sugars starting from wet beet pulp. Response surface methodology was employed to optimize the parameters and to describe and predict the amount of reducing sugar obtained from the hydrolysis stages.

INTRODUCTION

World sugar production is around 160 Mt yearly with a consumption of about 23 kg per capita. Total utilization is increasing by approximately 1.4% annually thanks to the improved standard of living in densely populated countries like China and India. About one-quarter of the world production is extracted from beet (*Beta vulgaris* L. ssp. *vulgaris*).[1]

The excessive resource use and devastating change in global environment led to the increased pressure on sugar-beet processing industry, considered as being one of the most polluting industries. Environmental problems associated with high energy consumption and production of large amounts of waste led to a series of restrictions in order to achieve waste minimization and sustainable production.[2,3]

Sugar beet pulp is the most important by-product of the sugar industry. Due to its richness in pectin (24–32%), cellulose (22–30%) and hemicelluloses (22–30%) and to its low lignin content (1–3%)[4,5] it is particularly suitable for the production of biofuels and bioproducts by means of enzymatic hydrolysis[6-9] and microbial fermentation.[10]

The beet-pulp is generally used as fodder in cattle-raising industry[11] and, more recently, it can serve as a substrate for bio-hydrogen production,[12,13] in obtaining pectin extract[14,15] and biodegradable plastics[16], antioxidants[17-19] or sugar beet cellulose nanofibril-reinforced composites.[20]

This study presents a new way of valorisation by the conversion of hemicelluloses and cellulose from the beet pulp in reducing sugars, useful for various bioprocesses. Instead of using the well known enzymatic method, this work chooses to perform the hydrolysis with sulphuric acid, at different concentrations.

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RESULTS AND DISCUSSION

Chemical composition of sugar beet pulp

The chemical composition of the sugar beet pulp determined by HPLC analysis is presented in Table 1. The data obtained indicate a high amount of hemicelluloses (over 30%) and cellulose confirming the hypothesis that beet pulp can be a viable source of reducing sugars.

The humidity of the beet pulp samples was around 86.4%. The sugar pulp used in this study contains an amount of 276.43 μg/g Fe, 21.34 μg/g Pb and 0.01 μg/g Hg.

Table 1

Chemical composition of sugar beet pulp

Component	%, w/w dry matter	Component	%, w/w dry matter
cellulose	22 – 30%	lignin	3 – 7%
hemicelluloses	24 – 32%	galactose	4.9 – 5.1%
pectin	24 – 32%	rhamnose	1.7 – 2.5%
glucose	20.3 – 22.1%	xylose	1.2 – 1.9%
galacturonic acid	20.2 – 21.1%	methanol	1.3 – 1.8%
arabinose	19.7 – 20.8%	mannose	0.8 – 1.3%
protein	10,4 – 11.2%	ash	3.4 – 3,9%

Table 2

BBD test and the response for beet pulp hydrolysis
with the observed responses and predicted values for the amount of reducing sugars

Run	x_1	x_2	x_3	Reducing sugars concentration (g/L)			
				1^{st} stage hydrolysis		2^{nd} stage hydrolysis	
				Observed	Predicted	Observed	Predicted
1	-1	-1	-1	2.28	2.309	4.48	4.454
2	-1	-1	0	2.51	2.455	4.58	4.549
3	-1	-1	1	2.64	2.584	4.70	4.623
4	-1	0	-1	2.53	2.565	4.60	4.728
5	-1	0	0	2.68	2.704	4.75	4.840
6	-1	0	1	2.79	2.825	4.85	4.931
7	-1	1	-1	2.76	2.718	4.82	4.727
8	-1	1	0	2.86	2.849	4.93	4.855
9	-1	1	1	2.92	2.962	4.96	4.964
10	0	-1	0	2.77	2.982	4.86	5.095
11	0	-1	0	2.96	2.982	5.05	5.095
12	0	-1	0	3.12	2.982	5.32	5.095
13	0	0	0	3.08	3.202	5.21	5.280
14	0	0	0	3.23	3.202	5.44	5.280
15	0	0	0	3.35	3.202	5.36	5.280
16	0	1	0	3.23	3.319	5.29	5.188
17	0	1	0	3.40	3.319	5.16	5.188
18	0	1	0	3.37	3.319	5.00	5.188
19	1	-1	1	3.00	3.060	5.06	5.150
20	1	-1	0	3.16	3.161	5.21	5.253
21	1	-1	-1	3.32	3.245	5.39	5.336
22	1	0	1	3.29	3.243	5.34	5.245
23	1	0	0	3.40	3.352	5.41	5.331

Table 2 (continued)

24	1	0	-1	3.39	3.444	5.35	5.396
25	1	1	1	3.36	3.323	5.29	5.063
26	1	1	0	3.35	3.440	5.08	5.132
27	1	1	-1	3.32	3.323	4.84	5.063

Fitting the models

By applying the multiple regression analysis on the experimental data on the 1st and the 2nd stages hydrolysis of sugar beet pulp, the response variable and the test variable were related through the following second-order polynomial equations:

1st stage equation:

$$y = 3.202 + 0.324 \cdot x_1 + 0.168 \cdot x_2 + 0.015 \cdot x_3 - 0.029 \cdot x_1 \cdot x_2 - 0.115 \cdot x_1 \cdot x_3 + \\ + 0.008 \cdot x_2 \cdot x_3 - 0.174 \cdot x_1^2 - 0.052 \cdot x_2^2 - 0.009 \cdot x_3^2 \quad (1)$$

2nd stage equation:

$$y = 5.280 + 0.2459 \cdot x_1 + 0.047 \cdot x_2 + 0.013 \cdot x_3 - 0.107 \cdot x_1 \cdot x_2 - 0.089 \cdot x_1 \cdot x_3 + \\ + 0.017 \cdot x_2 \cdot x_3 - 0.194 \cdot x_1^2 - 0.138 \cdot x_2^2 - 0.010 \cdot x_3^2 \quad (2)$$

In the 1st stage of the hydrolysis, the ANOVA of the quadratic regression model showed that the values of the determination coefficient (R^2) and the adjusted determination coefficient (Adj. R^2) were 0.943 and 0.912, respectively, which suggested a high degree of correlation between the observed and the predicted values.

The p values are used to check the significance of each coefficient, which in turn may indicate the pattern of the interaction between the variables. The results showed that the sulphuric acid concentration and the hydrolysis time were the most significant factors, which influenced the amount of reducing sugars in the 1st stage ($p < 0.01$).

The analysis of variance (ANOVA) for the 2nd stage of hydrolysis indicated that the coefficient of determination (R^2) of the predicted model was 0.818 and that the adjusted determination coefficient (Adj. R^2) was 0.721, suggesting a good fit; the predicted model seemed to reasonably represent the observed values.

The results showed that the sulphuric acid concentration was the most significant factor, which influenced the amount of reducing sugars in the 2nd stage ($p < 0.01$).

In Figs. 1 and 2 the response functions for each hydrolysis stage are plotted in 3D (response surface plot) and 2D (contour plot) using the NemrodW v. 2000 software, allowing us to visualize the maximum value determined. Two variables within the experimental rang were depicted in 3D surface plots, while the third variable was kept constant at zero level. The shapes of contour plots, circular or elliptical, indicated whether the mutual interactions between the variables were significant or not.

Optimization

The real values of the independent variables for the optimum results were calculated, and are presented in Table 3.

In order to validate the adequacy of the model equations (1 and 2), a verification of the experiment was carried out under the optimal conditions mentioned above. Under the optimal conditions, the model predicted a maximum response of 3.4 g/L for the 1st stage of hydrolysis and a maximum response of 5.4 g/L for the 2nd stage of hydrolysis.

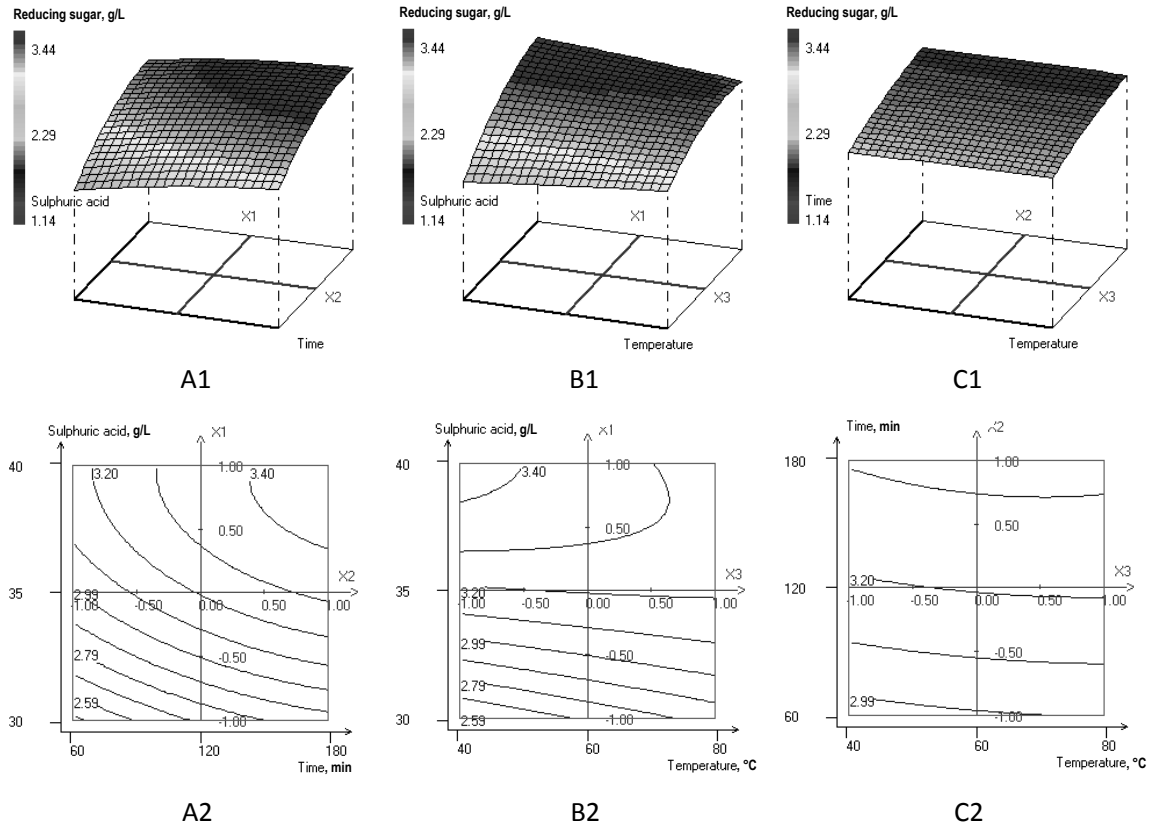

Fig. 1 – Response surface plots (1) and contour plots (2) for the effects of (A) sulphuric acid concentration and time; (B) sulphuric acid concentration and temperature; (C) time and temperature on the amount of the reducing sugars in the 1st stage of hydrolysis.

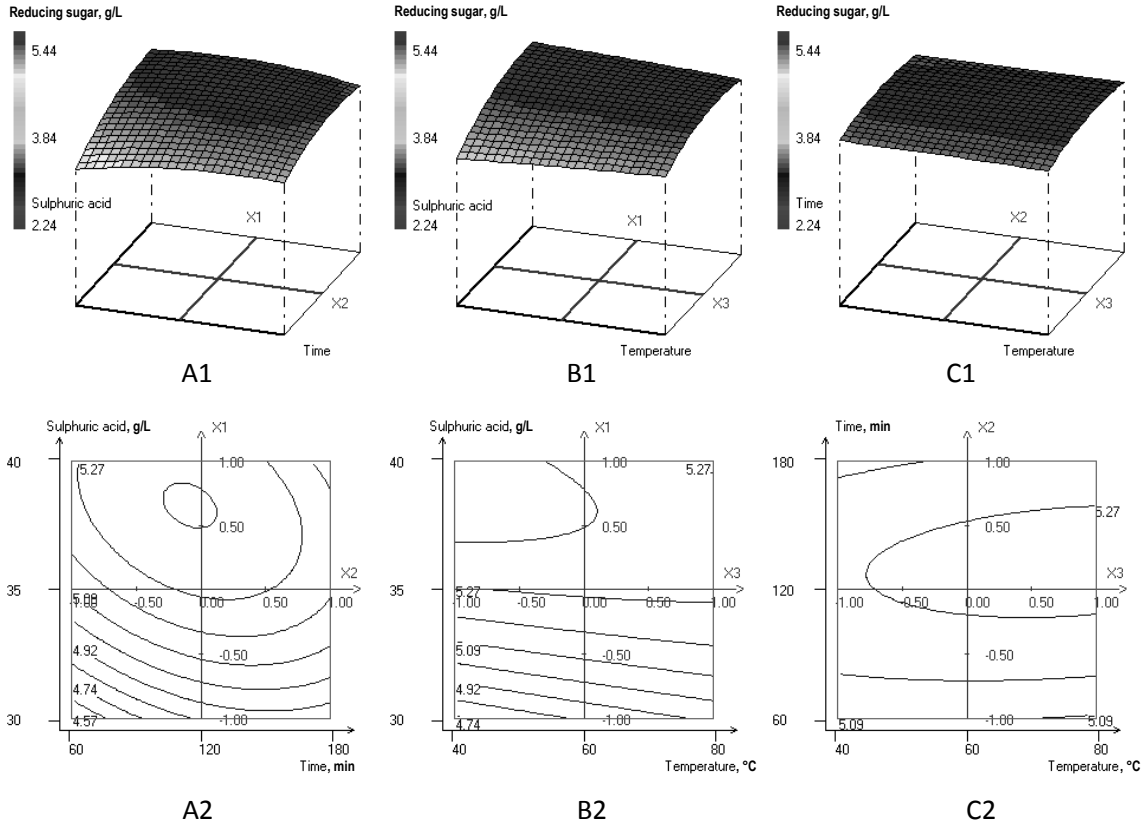

Fig. 2 – Response surface plots (1) and contour plots (2) for the effects of (A) sulphuric acid concentration and time; (B) sulphuric acid concentration and temperature; (C) time and temperature on the amount of the reducing sugars in the 2nd stage of hydrolysis.

Table 3

Values for optimum results

	X_1 (sulphuric acid concentration, g/L)	X_2 (time, min)	X_3 (temperature, °C)
1st stage of hydrolysis	38.5	170.4	65.4
2nd stage of hydrolysis	106.9	25.2	63.5

EXPERIMENTAL

Materials, chemical and reagents

The sugar beet pulp used in this study was provided by one of the most important Roumanian sugar producers. The samples were collected from the dump stock of the company, shortly after they emerged from the process, packed in plastic bags and stored at -20 °C.

The sulphuric acid 95-97% was provided by Merck Chemicals Roumania, the 3,5-Dinitrosalicylic acid (DNS) reagent 98% and Amberlite IR-118 resin were provided by Sigma-Aldrich.

Determination of sugar beet composition and reducing sugars

The chemical composition of the sugar beet pulp was determined on a 5 mm chromatographic column, 25 cm x 4.6 mm using a Shimadzu LC-10 ATV HPLC Chromatograph coupled with a refractometric detector RID - 10A.

The heavy metal content was determined using a VARIAN AA Duo AAS Simultaneous Flame / Graphite Furnace Atomic Absorption starting from the mineralized samples obtained in a Milestone START E Microwave Reactor.

An FT-IR Bruker Tensor 27 spectrophotometer set at 540 nm and 3,5-Dinitrosalicylic acid (DNS) reagent were used for the determination of the amount of reducing sugars in both of the solutions obtained.

Hydrolysis process

Preliminary tests have shown the need to run experiments in two stages.

The reactor used for the hydrolysis is described in Fig. 3.

In the first stage, 15 g of defrosted beet-pulp were milled and mixed in the reactor with 75 mL of diluted sulphuric acid (Table 4). Stirrer speed was set at 25 rev/min and the temperature was set according to the experimental algorithm (Tables 1 and 4). The mixture resulted from the experiment was filtered under vacuum and the sulphuric acid was separated by column chromatography on Amberlite IR-118. The residual sulphuric acid was neutralized with CaO and the remaining solution was refiltered.

The residue from the first filtration was repeatedly washed with distilled water and then mixed in the reactor with concentrated sulphuric acid in a ratio of 1:5 (Table 4). The resulting mixture was filtered and neutralized as described in the previous case.

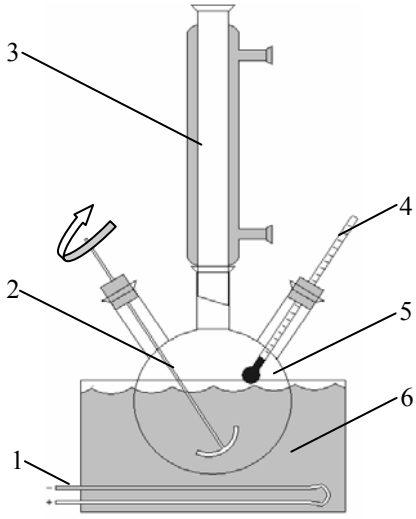

Fig. 3 – Hydrolysis reactor:
1. Electrical heater, 2. Stirrer, 3. Condenser, 4. Thermometer 5. 3-necks flask, 6. Oil-bath.

Experimental design

The Box-Behnken (BBD) design was used to statistically optimize the formulation parameters and to evaluate the main effects, interaction effects and quadratic effects of the formulation ingredients on reducing sugars obtained from hemicelluloses and cellulose hydrolysis from beet pulp. The complete design consisted of 27 experimental points and three replications of the centre points. The effect of the independent variables X_1 (sulphuric acid concentration, g/L), X_2 (hydrolysis time, min), X_3 (experimental temperature, °C) at three variation levels (Table 4) in the beet pulp hydrolysis process is shown in Table 1.

Table 4

Code and level of independent variable chosen for the BBD test for beet pulp hydrolysis

Variables	Symbol		1st stage hydrolysis Levels				2nd stage hydrolysis Levels			
	Coded	Uncoded	-1	0	1	ΔX	-1	0	1	ΔX
			Actual values				Actual values			
Sulphuric acid concentration, g/L	x_1	X_1	30	35	40	5	60	70	80	10
Hydrolysis time, min	x_2	X_2	60	120	180	60	15	25	35	10
Temperature, °C	x_3	X_3	40	60	80	20	90	100	110	10

The correspondence between the coded and the uncoded values can be obtained using the following formula:

$$x_i \quad X_i \quad X_0 \ / \ X \tag{3}$$

where x_i is the coded value of an independent variable; X_i is the actual value of an independent variable at the centre point and ΔX is the step change value of an independent variable. For predicting the optimal point, a second-order polynomial model was fitted to correlate the relationship between the independent variables and the response (reducing sugars obtained in the two stages of hydrolysis). For the three factors, the equation was:

$$Y = A_0 + \sum_{i=1}^{3} A_i X_i + \sum_{i=1}^{3} A_{ii} X_i^2 + \sum_{i=1}^{2} \sum_{j=i+1}^{3} A_{ij} X_i X_j \tag{4}$$

where Y is the response (reducing sugars obtained in each stage of hydrolysis). A_0, A_i, A_j, and A_{ij} are the regression coefficients of variables for the intercept, linear, quadratic and interaction terms, respectively. X_i and X_j are the independent variables ($i \neq j$).[21, 22]

Analyses of the experimental design and data were carried out using the NemrodW v. 2000 software.

CONCLUSIONS

Beet pulp is a good material for acid hydrolysis, due to its high content of hemicelluloses and cellulose. The experiments led to a good yield of reducing sugars for the two stages of the hydrolysis, the recorded values were around 50% for the first stage and 80% for the second one.

The optimum hydrolysis parameters were generated, and the response surface methodology was sufficient to describe and predict the amount of reducing sugars obtained from the hydrolysis stages.

By mixing the two solutions obtained in the process of wet sugar beet pulp hydrolysis (86.4% water) a concentration of maximum of 8.8 g/L of reducing sugars can be obtained, after parameter optimisation.

The reducing sugars obtained this way can be used as main source of carbon in the biochemical processes that do not include the biosynthesis of products, which can be used in the food industry due to the hydrolysis performed with concentrated mineral acid. Compared with the enzymatic hydrolysis, the acid one has the advantages of using moderate temperatures and giving the opportunity to recover and reuse the sulphuric acid via chromatographic separation (over 90%).

REFERENCES

1. E. Biancardi, J. M. McGrath, L. W. Panella, R. T. Lewellen and P. Stevanato, *Root and Tuber Crops*, **2010,** J.E. Bradshaw (Ed.).
2. Y. Akcay and M. Uzunoz, *J. of Appl. Sci.*, **2006**, *6*, 1123-1127.
3. E. Alkaya and G. N. Demirer, *Biomass and bioenerg.*, **2011**, *35*, 32-39.
4. M. Hutnan, M. Drtil, J. Derco and L. Mrafkova, *Biodegradation*, **2000**, *11*, 203-211.
5. A. R. Kirby, A. J. MacDougall and V. J. Morris, *Food Biophy.*, **2006**, *1*, 51-56.
6. Y. Kato and M. Kobayashi, *J. of Applied Glycoscience*, **2000**, *1*, 45-48.
7. M. Spagnuolo, C. Crecchio, M. D. R. Pizzigallo and P. Ruggiero, *Biotech. and Bioeng.*, **1999**, *64*, 685-691.
8. M. F. Gailing, A. Guibert and D. Combes, *Bioproc. and Biosyst. Eng.*, **2000**, *22*, 69-74.
9. L. Sidi Ali, N. Cochet, T.K. Ghose and J.M. Lebeault, *Biotech. Lett.*, **1984**, *11*, 723-728.
10. Y. Zheng, C. Yu, Y. S. Cheng, R. Zhang, B. Jenkins and J. S. VanderGheynst, *Biores. Tech.*, **2011**, *102*, 1489-1495.
11. J. C. Pereira and J. González, *Anim. Res.*, **2004**, *53*, 99-110.
12. M. Hutnan, M. Drtil, J. Derco, L. Mrafkova, M. Hornak and S. Mico, *Pl. J. of Env. Stud.*, **2001**, *10*, 237-243.
13. L. Ozkan, H. Erguder and T. Demirer, *Int. J. of Hydrogen Energ.*, **2010**, *36,* 382-389.
14. E. Favela-Torres, T. Volke-Sepúlveda and G. Viniegra-González, *Food Tech. and Biotech.*, **2006**, *44*, 221-227.
15. C. K. Siew and P. A. Williams, *J. of Agr. and Food Chem.*, **2008**, *56*, 4164-4171.
16. L. Liu, M. L. Fishman, K. B. Hicks and C. Liu, *J. of Agr. and Food Che.*, **2005**, *53*, 9017-9022.
17. M. Sakac, J. Gyura, A. Mišan, Z. Šereš and B. Pajin., *Sugar Ind.*, **2011**, *136*, 151-157.
18. M. Sakac, J. Gyura, A. Mišan, Z. Šereš and B. Pajin., *Sugar Ind.*, **2009**, *134*, 418-425.
19. A. A. Mohdaly, M. A. Sarhan, I. Smetanska and A. Mahmoud, *J. of the Sci. of Food and Agr.*, **2010**, *90*, 218-226.
20. J. Leitner, B. Hinterstoisser, M. Wastyn. J. Keckes and W. Gindl, *Cellulose*, **2007**, *14*, 419-425.
21. X. L. Bai, T. Li. Yue, Y. H. Yuan and H. W. Zhang, *J. of Sep. Sci.*, **2010**, *33*, 1-8.
22. S. Wang, F. Chen, J. Wu, Z. Wang, X. Liao and X. Hu, *J. of Food Eng.*, **2007**, *78*, 693-700.