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OPTIMIZATION OF BARLEY HUSKS ACID HYDROLYSIS PROCESS USING THE RESPONSE SURFACE METHODOLOGY

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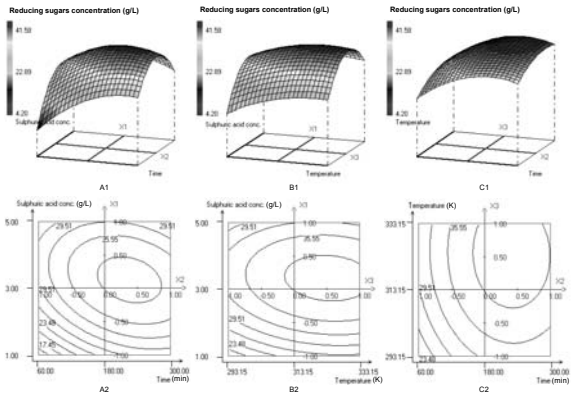
Barley husks were submitted to a two-step acid hydrolysis process able to conduct to a material with high content of fermentable sugar (31.77 g/L). The first hydrolysis stage was conducted in "mild" conditions with diluted sulphuric acid and aimed to recover monosaccharides from hemicellulose content. More "aggressive" conditions were employed in the second stage of hydrolysis targeting the cellulose content in order to obtain glucose which is known as highly suitable to be transformed in biomass. The most important parameters of the developed process (temperature, sulphuric acid concentration, hydrolysis time) were optimized using the Response Surface Methodology. According to the obtained results the first hydrolysis step can be properly conducted at 323.35 K with diluted sulphuric acid (3.02 g/L) for 282 min. For the second hydrolysis step, the temperature set at 368.65 K and the use of concentrated sulphuric acid (75.2 g/L) for 26.7 min seemed to be adequate.



INTRODUCTION

Lignocellulosic materials, especially those of residual nature generated in agricultural and industrial activities, represent a renewable, abundant and cheap source of raw materials for multiple purposes. Barley husks belong to this type of materials. They are separated from barley grains by pneumatic conveying in malt industries and collected together with grain fragments and low-density spent grains.[1] Barley husks are composed mainly of lignocellulosic cell walls.[2] They contain high amounts of polysaccharides[3,4] (cellulose: 39%, hemicelluloses: 12%, lignin: 22%, starch: 11% reported at the solid material[5]) but also different quantities of proteins,[6] fibers,[7] resins[8] and antioxidants.[9] They are used in various fields such as: dyes removal from aqueous effluents,[10-12] antioxidant recovery,[13,14] arabinoxylan recuperation,[15-17] reinforced composites production,[5] bioethanol production[18] and so on. Due to their important content of sugars, barley husks can be also used as carbon sources for microorganisms' development process. Many different researches were focused on this direction. Species such as *Agrocybe*, *Lentinus*,[19] *Pleurotus ostreatus*[20] or

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Streptomyces[21] were successfully cultivated on substrate containing fermentable sugars from barley spent grain or barley husks. In order to obtain these sugars several steps are required[22] the main two being represented by the raw material pretreatment and by the hydrolysis of celluloses and hemicelluloses existing in barley husks. The pretreatment phase aims to improve the rate of production as well as the total yield of liberated sugars in hydrolysis step. It includes different combinations of physical (milling, grinding), chemical (ozonolysis, wet oxidation) and biological processes (treatment with different species of fungi) able to reduce the particle size, to insure lignin degradation and to solubilize the lignocellulose components.[23] The hydrolysis step can be conducted by direct dilute-acid hydrolysis,[24] steam explosion,[25] autohydrolysis[26] etc. The latter two are less severe than the first one, and present several advantages, namely limited solubilisation of lignin, low generation of degradation products, and low usage of chemicals, all of which are positive economic and environmental factors. However, steam explosion and autohydrolysis lead to large amounts of oligomeric saccharides, which have to be converted into monosaccharides (by acid or enzymatic catalysis) before use. The acid process is faster, and the catalyst is cheap and allows high monosaccharide recovery. To make an adequate choice, the performance of the raw material under consideration must be studied for each process. The main factors affecting monosaccharides recovery in dilute-acid hydrolysis are catalyst concentration, reaction time, and temperature, whereas enzymatic hydrolysis is also dependent on additional factors such as substrate structure, and type and ratio of enzymatic activities present in the commercial enzyme preparations.[24]

The present work proposes a new method for barley husks polysaccharides hydrolysis. It involves the use of sulphuric acid of different concentrations and it was directed to the optimization of three different parameters known as affecting the process: temperature, sulphuric acid concentration and hydrolysis time. Their influence was followed by applying the response surface methodology (RSM) known as an efficient way to achieve a proper optimization by analysing and modelling the effects of multiple variables and their responses. This method has been widely used for the optimization of various processes in many different fields such as food chemistry,[27-29] chemical engineering[30-32] or biotechnology.[33-36] In our study, a central composite design with three variables and three levels of variation was employed to evaluate the coefficients in a quadratic mathematical model in order to obtain the best possible combination of the above mentioned parameters suitable to conduct to a hydrolysed material with high content of fermentable sugars.

RESULTS AND DISCUSSION

Chemical composition of barley husks hydrolysed

The HPLC analysis of sugars released by complete acid hydrolysis (after the two process stages and for the optimized values for hydrolysis time, temperature and sulphuric acid concentration) indicates: 44.74 % glucose, 32.26% xylose, 5.32% arabinose, 16.74%, galacturonic acid, 0.91% galactose and 1.1% traces of other monosaccharides (w/w total reducing sugar). The reducing sugar represents 75.4% (± 2%) reported at hydrolysed dry matter content.

Hydrolysed barley husks dry matter content was established at 88.8%. The ash amount was of 5.74% (w/w dry matter) and metal contents were (mg/kg of hydrolysed product): 65.4 mg/kg K^+, 77.08 mg/kg Na^+, 219.7 mg/kg Ca^{2+}, 88.3 mg/kg Mg^{2+}, 222.95 mg/kg Fe^{2+}, 1.87 mg/kg Cu^{2+}, 17.39 mg/kg Zn^{2+}, 20.56 mg/kg Mn^+.

Optimization of acid hydrolysis conditions

The Response Surface Methodology (RSM) design was used to optimize the barley husks acid hydrolysis in order to establish the best combination of the process parameters for high fermentable sugar content in the experimental hydrolysed mix. The effect of the independent variables X_1 (sulphuric acid concentration, g/L), X_2 (hydrolysis time, min), X_3 (temperature, K) at three variation levels (Table 1) in the hydrolysis process is shown in Table 2.

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

$$x_i = (X_i - X_0)/\Delta X \qquad (1)$$

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.

The behaviour of the chosen parameters involved in the acid hydrolysis process of barley husks was predicted by a second-order polynomial model which was fitted to correlate the relationship between the independent variables for each response function (reducing sugars for each hydrolysis stage). For the three studied factors, the general equation form can be written as follows:

$$ {}_nY = {}_nA_0 + {}_n\sum_{i=1}^{3} A_i X_i + {}_n\sum_{i=1}^{3} A_{ii} X_i^2 + {}_n\sum_{i=1}^{2}\sum_{j=i+1}^{3} A_{ij} X_i X_j \quad (2) $$

where the values of n are between 1 and 2, ${}_nY$ is the response (${}_1Y$, reducing sugars content after the 1st hydrolysis stage; ${}_2Y$, reducing sugars content after the 2nd hydrolysis stage), ${}_nA_0$, ${}_nA_i$, ${}_nA_j$, and ${}_nA_{ij}$ are the regression coefficients of variables for the intercept, linear, quadratic and interaction terms, respectively, X_i and X_j are the independent variables ($i \neq j$).

The RSM was applied to build up an empirical relation in order to express the content of reducing sugars obtained by barley husks acid hydrolysis, leading to the following response equations:

1st stage of acid hydrolysis:

$$ {}_1Y = 38.64 + 3.889 \cdot x_1 + 4.278 \cdot x_2 + 2.929 \cdot x_3 - 3.555 \cdot x_1 \cdot x_2 - 0.966 \cdot x_1 \cdot x_3 - \\ -0.238 \cdot x_2 \cdot x_3 - 10.086 \cdot x_1^2 - 5.076 \cdot x_2^2 - 2.656 \cdot x_3^2 \quad (3) $$

2nd stage of acid hydrolysis:

$$ {}_2Y = 24.173 + 0.224 \cdot x_1 + 0.047 \cdot x_2 + 0.051 \cdot x_3 - 0.087 \cdot x_1 \cdot x_2 - 0.058 \cdot x_1 \cdot x_3 - \\ -0.118 \cdot x_2 \cdot x_3 - 0.180 \cdot x_1^2 - 0.125 \cdot x_2^2 - 0.017 \cdot x_3^2 \quad (4) $$

Table 1

Code and level of independent variable chosen for the RSM test for barley husks hydrolysis

Variables	Symbol		1st stage hydrolysis				2nd stage hydrolysis			
			Levels			ΔX	Levels			ΔX
	Coded	Uncoded	-1	0	1		-1	0	1	
			Real values				Reall values			
Sulphuric acid concentration, g/L	x_1	X_1	1	3	5	2	60	70	80	10
Hydrolysis time, min	x_2	X_2	60	180	300	120	15	25	35	10
Temperature, K	x_3	X_3	293.15	313.15	333.15	20	363.15	373.15	383.15	10

Table 2

RSM algorithm and experimental and predicted values for the amounts of reducing sugar obtained after barley husks acid hydrolysis

Run	x_1	x_2	x_3	Reducing sugars concentration (g/L)				
				1st stage hydrolysis			2nd stage hydrolysis	
				Observed	Predicted		Observed	Predicted
1	-1	-1	-1	8.40	4.97		23.40	23.27
2	-1	-1	0	13.07	11.76		23.57	23.51
3	-1	-1	1	14.53	13.23		23.67	23.72
4	-1	0	-1	16.00	18.12		23.49	23.64
5	-1	0	0	21.60	24.67		23.71	23.77
6	-1	0	1	25.07	25.90		23.80	23.86
7	-1	1	-1	22.13	21.11		23.75	23.77
8	-1	1	0	25.47	27.42		23.81	23.78
9	-1	1	1	29.33	28.42		23.87	23.75
10	0	-1	0	22.27	23.46		23.79	23.82
11	0	-1	0	24.13	29.29		23.95	24.00
12	0	-1	0	27.13	29.80		24.16	24.15

Table 2 (continued)

13	0	0	0	33.73	33.06		24.13	24.11
14	0	0	0	41.20	38.64		24.20	24.17
15	0	0	0	41.58	38.91		24.27	24.21
16	0	1	0	32.00	32.50		24.21	24.15
17	0	1	0	40.53	37.84		24.09	24.10
18	0	1	0	38.80	37.88		23.91	24.01
19	1	-1	1	22.80	21.79		23.95	24.00
20	1	-1	0	25.33	26.65		24.09	24.13
21	1	-1	-1	29.47	26.19		24.25	24.23
22	1	0	1	24.93	27.82		24.23	24.21
23	1	0	0	38.93	32.44		24.31	24.22
24	1	0	-1	28.27	31.75		24.24	24.20
25	1	1	1	24.27	23.71		24.17	24.16
26	1	1	0	26.53	28.09		24.00	24.05
27	1	1	-1	25.07	27.16		23.87	23.91

The predicted values agreed well with the experimental ones obtained from the RSM design. Analysis of variance (ANOVA) showed that the resultant quadratic polynomial models adequately represented the experimental data with the determination coefficients (R^2) with values of 0.907 and 0.933, respectively and the adjusted determination coefficient (Adj. R^2) with values of 0.858 and 0.897. This indicates that the quadratic polynomial models obtained were adequate to describe the influence of the independent variables studied on the reducing sugar amount of the obtained hydrolysable.

ANOVA was used also to evaluate the significance of the coefficients of the quadratic polynomial models. For any of the terms in the model, a small *p*-value would indicate a more significant effect on the respective response variables. For the 1st stage of barley husks acid hydrolysis, the variables with the largest effect on the reducing sugar content were the sulphuric acid concentration and the hydrolysis time; the other parameter (hydrolysis temperature) did not show a significant effect $p = 0.132$ (Table 3). The quadratic term of sulphuric acid concentration also had a significant effect ($p < 0.01$). However, the effect of the other two quadratic terms was less significant ($p = 0.131$ and $p = 5.7$ respectively).

For the 2nd stage of hydrolysis, the variable having the largest effect on the response function was the linear term of sulphuric acid concentration, followed by the quadratic term of the same parameter and the interaction between hydrolysis time and hydrolysis temperature ($p < 0.01$ in both cases) (Table 4). The quadratic term of hydrolysis time ($p = 0.17$) and the interaction between hydrolysis time and hydrolysis temperature ($p = 0.19$) also had a significant effect on the reducing sugar content of hydrolysed material, while the effect of the remaining terms was insignificant.

The fitted polynomial equations were expressed as surfaces (3D) and contour plots (2D) and are illustrated in Figs. 1 and 2. They permit to visualize the relationship between the response and experimental levels of each factor and to deduce the optimum conditions. 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.

Table 3

1st stage of acid hydrolysis equation 3 coefficients significance

Coefficient	Value	Significance %	Coefficient	Value	Significance %
$_1A_0$	38.640	< 0.01	$_1A_{22}$	-5.076	0.131
$_1A_1$	3.889	0.0104	$_1A_{33}$	-2.656	5.700
$_1A_2$	4.278	< 0.01	$_1A_{12}$	-3.555	0.142
$_1A_3$	2.929	0.132	$_1A_{13}$	-0.966	31.40
$_1A_{11}$	-10.086	< 0.01	$_1A_{23}$	-0.238	79.60

Table 4

2^{nd} stage of acid hydrolysis equation 4 coefficients significance

Coefficient	Value	Significance %	Coefficient	Value	Significance %
$_2A_0$	24.173	< 0.01	$_2A_{22}$	-0.125	0.170
$_2A_1$	0.224	< 0.01	$_2A_{33}$	-0.017	62.9
$_2A_2$	0.047	2.45	$_2A_{12}$	-0.087	0.198
$_2A_3$	0.051	1.63	$_2A_{13}$	-0.058	2.52
$_2A_{11}$	-0.180	< 0.01	$_2A_{23}$	-0.118	0.0131

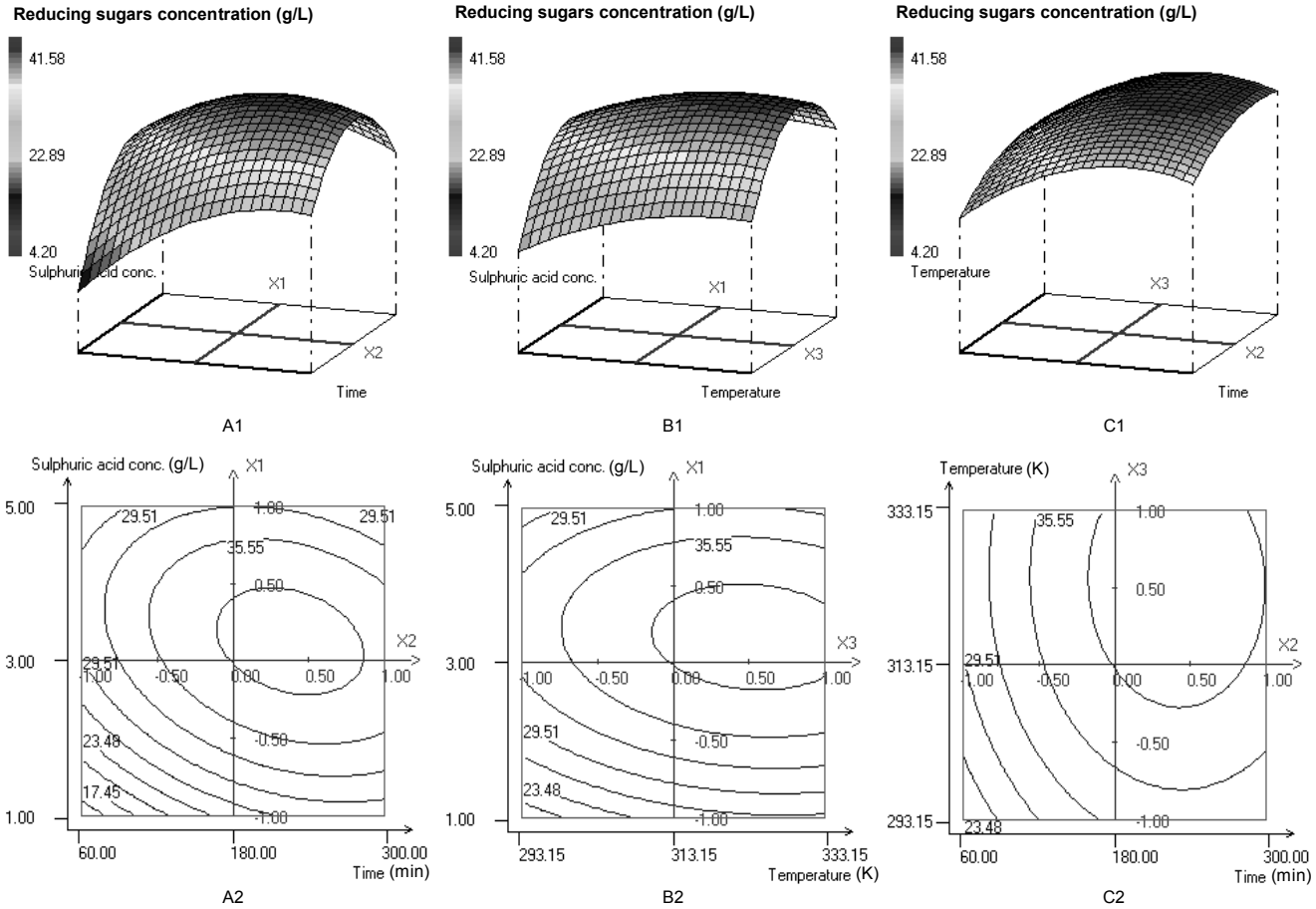

Fig. 1 – Response surface plots (1) and contour plots (2) for the effects of (A) sulphuric acid concentration and hydrolysis time; (B) sulphuric acid concentration and hydrolysis temperature; (C) hydrolysis temperature and hydrolysis time on the reducing sugar content of hydrolysed barley husks after the 1^{st} stage of hydrolysis.

Testing of optimal process conditions

The real values of the independent variables for the optimum results were calculated targeting the maximum values for all the response variables. The obtained values are presented in Table 5.

In order to validate the adequacy of the model equations (3 and 4), a verification of the experiment was carried out in 10 replicates (5 for each stage) under the above mentioned conditions.

The standard t-Test showed minor differences (39.31 g/L ± 2.2% reducing sugar content for the 1^{st} stage hydrolysis and 24.23 g/L ± 1.5% reducing sugar content for the 2^{nd} stage) between response variable and the experimental results. Combining the products from both hydrolysis stages, an amount of 31.77 g/L ± 1.9% reducing sugars was obtained. The analyses of the conducted experiments in these conditions indicate an overall hydrolysis yield of 65.76 ± 2% (if the monosaccharides traces are taken in consideration), hence 9.062 g (75.4% w/w hydrolysed dry matter) of reducing sugars from 15 g of raw material (8.13 humidity).

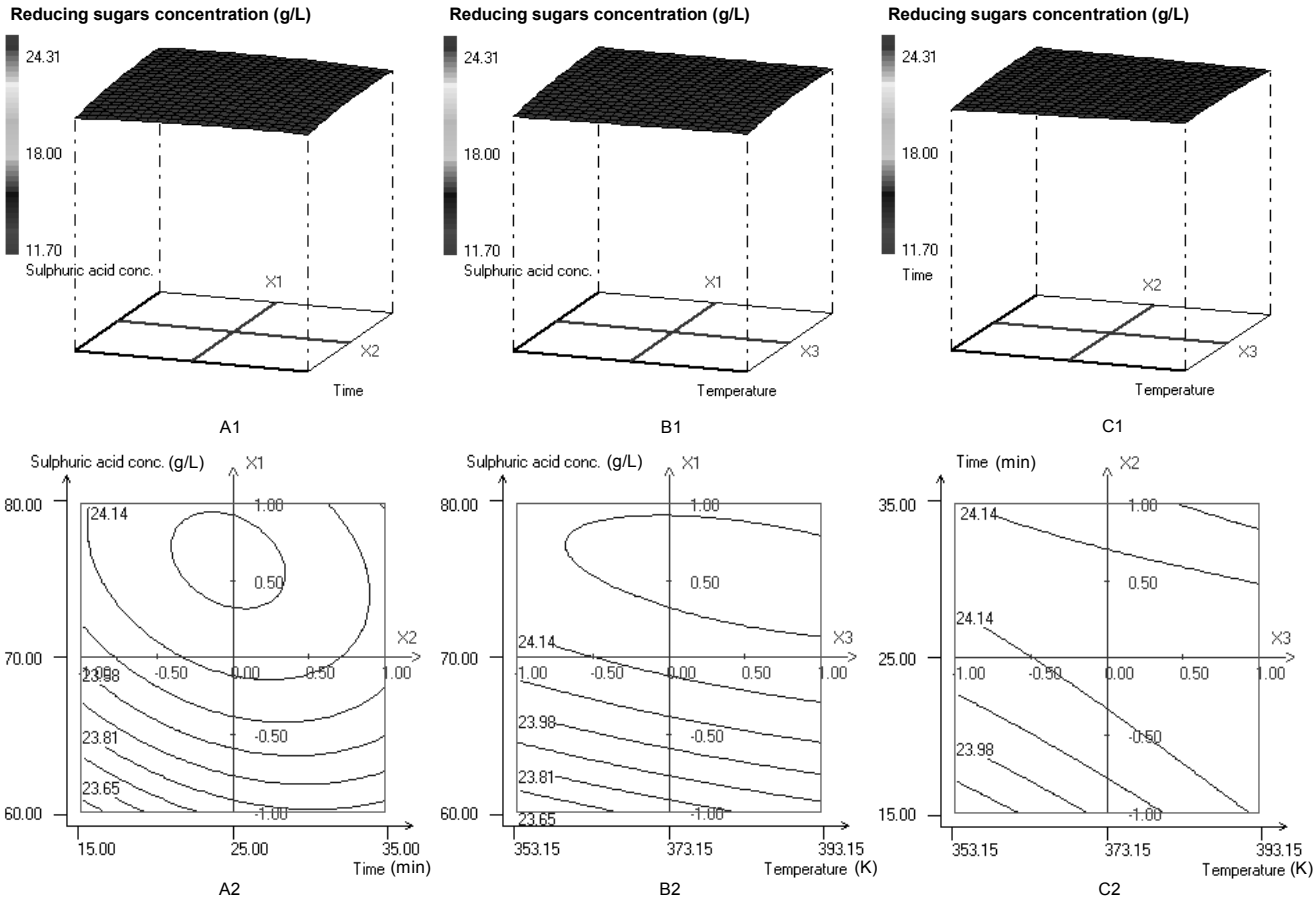

Fig. 2 – Response surface plots (1) and contour plots (2) for the effects of (A) sulphuric acid concentration and hydrolysis time; (B) sulphuric acid concentration and hydrolysis temperature; (C) hydrolysis temperature and hydrolysis time on the reducing sugar content of hydrolysed barley husks after the 2nd stage of hydrolysis.

Table 5

Values for optimum results

	X_1 (sulphuric acid concentration, g/L)	X_2 (time, min)	X_3 (temperature, K)
1st stage of hydrolysis	3.02	282	323.35
2nd stage of hydrolysis	75.2	26.7	368.65

EXPERIMENTAL

Chemical, reagents and materials

Sulphuric acid (95-97%) used for the hydrolysis process was provided by Merck Chemicals Romania. Sodium potassium tartrate, sodium metabisulfite, sodium hydroxide, calcium oxide, calcium carbonate, Amberlite IR-118 resin, glucose, xylose, arabinose, galacturonic acid, galactose, nitric acid and 3-5-dinitrosalicylic acid (DNS) reagent with a concentration of 98% were supplied by Sigma-Aldrich (Redox Lab Supplies Bucharest, Romania).

The preparation of DNS reagent was realised according to the method reported by Chu and Feng (2012).[37] Briefly, 300 g of sodium potassium tartrate and 8 g of sodium metabisulfite were dissolved with approximately 500 mL of distilled water. Sodium metabisulfite was added to absorb dissolved oxygen that may interfere with glucose oxidation. Another solution was prepared using 16 g of sodium hydroxide and 10 g of 3,5-dinitrosalicylic acid in about 200 mL of distilled water. The two solutions were then combined and distilled water was added to obtain 1 L of DNS reagent solution.

All other standard solutions were prepared using distilled water or adequate solvents.

The barley husks used in this study were kindly offered by SC Albrau SA Oneşti, Roumania. The samples were collected from the dump stock of the company, shortly after they emerged from the technological process of grain sorting, packed in plastic bags and stored at room temperature.

Hydrolysis process

In order to recover the biggest amount of fermentable sugar from the barley husks the reactor presented in Fig. 3 was used for developing a two stage hydrolysis process (Fig. 4).

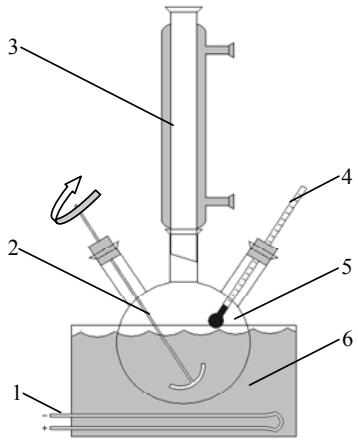

Fig. 3 – Hydrolysis reactor:
1. Electrical heater, 2. Stirrer, 3. Condenser, 4. Thermometer, 5. 3-necks flask, 6. Oil-bath.

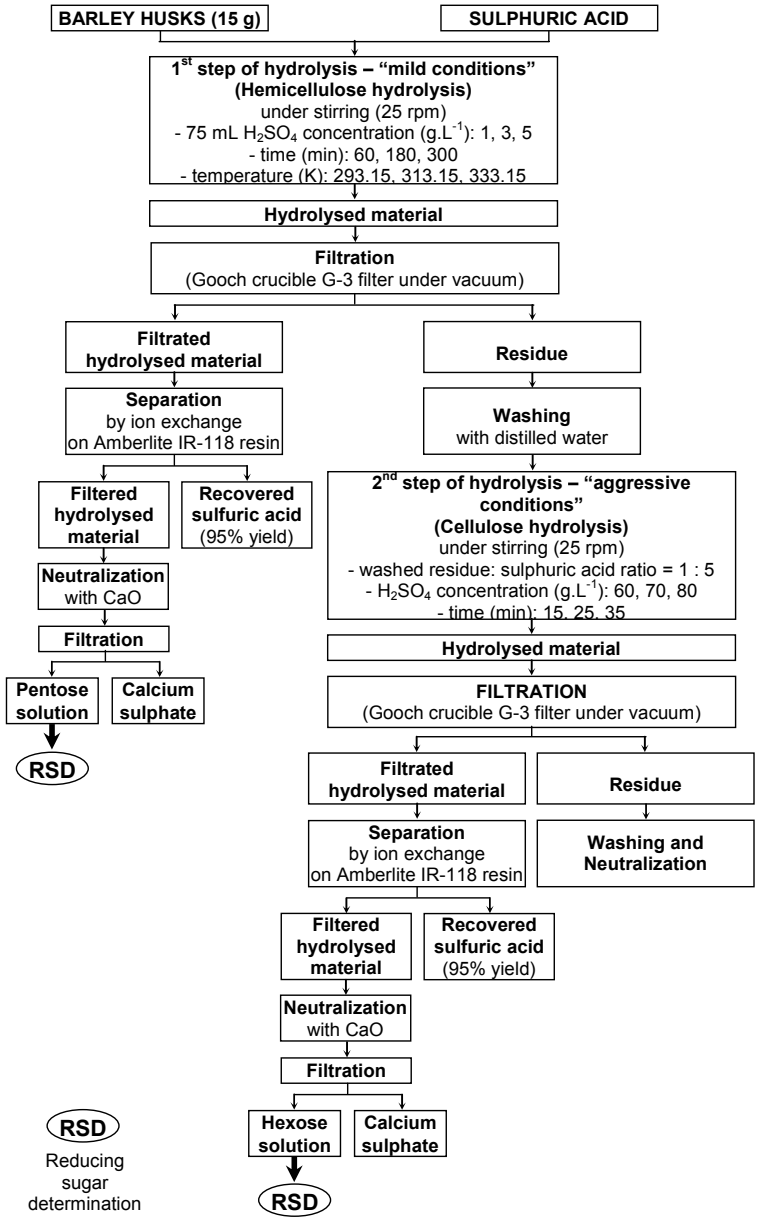

Fig. 4 – Schematic representation of barley husks hydrolysis process.

Fermentable sugar content determination

The sugar composition of the hydrolysed husks was determined on a Biorad Aminex HPX-87P chromatographic column using a Shimadzu LC-10 ATV HPLC Chromatograph coupled with a refractometric detector RID - 10A following NREL Chemical Analysis and Testing Standard Procedures.[38] Each sample was run in duplicate.

The total reducing sugars content of the supernatant was measured using the 3,5-dinitrosalicylic acid reagent (DNS) method[39] based on a calibration curve developed from standard glucose.[40] An FT-IR Bruker Tensor 27 spectrophotometer set at 540 nm was employed.

Dry matter content determination

The dry matter content of hydrolysed barley husks was evaluated by drying the material for 1 hour at 135 ± 5 °C in a Kern MLB 50-3 Moisture Balance.

Ash content determination

The ash content was determinate at 575 ± 25 °C, 24 h with a Caloris L 1003 Muffle Furnace according to the standard procedure 942.05 described in AOAC.[41]

Heavy metal content determination

The heavy metal content was determined using a VARIAN AA Duo AAS Simultaneous Flame/Graphite Furnace Atomic Absorption (K^+, Na^+, Ca^{2+}, Mg^{2+}, Fe^{2+}, Cu^{2+}, Zn^{2+}, Mn^+) starting from the mineralized samples obtained in a Milestone START E Microwave Reactor (the mineralization was realized in a mixture of HNO_3 65% and H_2O_2 30% at 200 °C, 30 minutes).

Experimental design

The Response Surface Methodology was used for three factors with replicates at the center point. The variables were the temperature, sulphuric acid concentration and hydrolysis time each at three coded levels (-1, 0, +1). A total of 30 experimental trials that included 3 trials for the replication of the central points were performed. The response value was represented by the fermentable sugar content (Y).

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

For characterization of the samples, at least five replicates measurements were performed, unless otherwise specified. The results were analysed by using ANOVA (analysis of variance) and the t-Test was used to examine the differences in XLSTAT-Pro 7.5 version. Results with a corresponding probability value of $p < 0.05$ were considered to be statistically significant.

CONCLUSIONS

The current study showed that the second-order polynomial model was sufficient to describe and predict the amount of reducing sugar from barley husks submitted to a two steps hydrolysis process within the experimental ranges.

The RSM was adopted to find the optimum hydrolysis conditions for obtaining a significant quantity of reducing sugar. These conditions were established to be 323.35 K with diluted sulphuric acid (3.02 g/L) for 282 min for the 1st stage of hydrolysis and 368.65 K with concentrated sulphuric acid (75.2 g/L) for 26.7 min for the 2nd adopted hydrolysis step.

The conducted experiments revealed that the hydrolysed barley husks can contain reducing sugars in amounts 31.77 g/L for the entire developed process hence 9.062 g of reducing sugars from 15 g of raw material when the overall hydrolysis yield is 65.76. Hydrolysed barley husks dry matter content was established at 88.8% with 75.4% reducing sugar and 5.74% ash.

Our investigation showed that the barley husks can be successfully applied for the recovery of fermentable sugars. Once recovered, these sugars can be employed in various directions being interesting especially in biomass production where adequate dilutions can ensure the necessary intake for the development process of different types of microorganisms.

REFERENCES

1. E. Conde, P. Gullón, A. Moure, H. Domínguez and J. C. Parajó, *Food Bioprod. Process.*, **2009**, *87*, 208-214.
2. C. Sánchez, *Biotechnol. Adv.*, **2009**, *27*, 185-194.
3. A. Lazaridou, T. Chornick, C. G. Biliaderis and M. S. Izydorczyk, *J. Cereal Sci.*, **2008**, *48*, 304-318.
4. P. Gullón, M. J. González-Muñoz and J. C. Parajó, *Bioresource Technol.*, **2011**, *102*, 6112-6119.
5. A. K. Bledzki, A. A. Mamun and J. Volk, *Compos. Sci. Technol.*, **2010**, *70*, 840-846.
6. S. Kalra and S. Jood, *Food Chem.*, **1998**, *61*, 35-39.
7. S. H. Knutsen and A. K. Holtekjølen, *Food Chem.*, **2007**, *102*, 707-715.
8. E. Pehlivan, T. Altun and S. Parlayıcı, *J. Hazard. Mater.*, **2009**, *164*, 982-986.
9. P. Sharma, H. S. Gujral and B. Singh, *Food Chem.*, **2012**, *131*, 1406-1413.
10. T. Robinson, B. Chandran and P. Nigam, *Environ. Int.*, **2002**, *28*, 29-33.
11. T. Robinson, B. Chandran and P. Nigam, *Bioresource Technol.*, **2002**, *85*, 119-124.
12. G. Crini, *Bioresource Technol.*, **2006**, *97*, 1061-1085.
13. D. A. Pereira de Abreu, P. P. Losada, J. Maroto and J. M. Cruz, *Food Res. Int.*, **2010**, *43*, 1277-1282.
14. D. A. Pereira de Abreu, K. V. Rodriguez and J. M. Cruz, *Innov. Food Sci. Emerg.*, **2012**, *13*, 134-141.
15. A. Höije, C. Sandström, J. P. Roubroeks, R. Andersson, S. Gohil and P. Gatenholm, *Carbohyd. Res.*, **2006**, *341*, 2959-2966.
16. A. Höije, M. Gröndahl, K. Tømmeraas and P. Gatenholm, *Carbohyd. Polym.*, **2005**, *61*, 266-275.
17. T. Persson, E. Dinh and A. S. Jönsson, *Food Bioprod. Process.*, **2009**, *87*, 228-233.
18. T. H. Kim, F. Taylor and K. B. Hicks, *Bioresource Technol.*, **2008**, *99*, 5694-5702.

19. S. I. Mussatto, G. Dragone and I. C. Roberto, *J. Cereal Sci.*, **2006**, *43*, 1-14.
20. D. Wang, A. Sakoda and M. Suzuki, *Bioresource Technol.*, **2001**, *78*, 293-300.
21. B. Szponar, K. Pawlik, A. Gamian and E. Dey, *Biotechnol. Lett.*, **2003**, *25*, 1717-1721.
22. S. N. Naik, V. V. Goud, P. K. Rout and A. K. Dalai, *Renew. Sust. Energ. Rev.*, **2010**, 14, 578-597.
23. F. Talebnia, D. Karakashev and I. Angelidaki, *Bioresource Technol.*, **2010**, 201, 4744-4753.
24. L. Duarte, F. Carvalheiro, S. Lopes, S. Marques, J. Parajo and F. Gírio, *Appl. Biochem. Biotech.*, **2004**, *115*, 1041-1058.
25. H. Krawczyk, T. Persson, A. Andersson and A. S. Jönsson, *Food Bioprod. Process.*, **2008**, *86*, 31-36.
26. G. Garrote, J. M. Cruz, H. Domínguez and J. C. Parajó, *Journal of Food Engineering*, **2008**, *84*, 544-552.
27. S. Jeong, Kwak B. M., J. H. Ahn and S. H. Jeong, *Food Chem.*, **2012**, *133*, 473-481.
28. Y. Yuan, Y. Gao, L. Mao and J. Zhao, *Food Chem.*, **2008**, *107*, 1300-1306.
29. C. Zhu and X. Liu, *Carbohyd. Polym.*, **2013**, *92*, 1197-1202.
30. W. Arbia, L. Adour, A. Amrane and H. Lounici, *Food Hydrocolloid.*, **2013**, *31*, 392-403.
31. S. Wu, X. Yu, Z. Hu, L. Zhang and J. Chen, *J. Environ. Sci.*, **2009**, *21*, 1276-1283.
32. Z. Zhang, J. Peng, W. Qu, L. Zhang, Z. Zhang, W. Li and R. Wan, *J. Taiwan Inst. Chem. E.*, **2009**, *40*, 541-548.
33. J. Zhou, X. Yu, C. Ding, Z. Wang, Q. Zhou, H. Pao and W. Cai, *J. Environ. Sci.*, **2011**, *23*, 22-30.
34. N. Aktaş, İ. H. Boyacı, M. Mutlu and A. Tanyolaç, *Bioresource Technol.*, **2006**, *97*, 2252-2259.
35. Y. Yücel, *Fuel Process. Technol.*, **2012**, *99*, 97-102.
36. G.-T. Jeong, Yang H.-S., Park D.-H., *Bioresource Technol.*, **2009**, *100*, 25-30.
37. K. H. Chu and X. Feng, *Process. Saf. Environ.,* **2012**, *in press.*
38. A. Sluiter, B. Hames, R. Ruiz, C. Scarlata, J. Sluiter, D. Templeton, and D. Crocker, *Chemical Analysis and Testing (CAT) Standard Procedure. NREL, Golden, CO*, **2011**.
39. G. L. Miller, *Anal. Chem.*, **1959**, *31*, 420-428.
40. Y. N. Liang, J. Yesuf., S. Schmitt, K. Bender and J. Bozzola, *J. Ind. Microb. Biot.*, **2009**, *36*, 961-970.
41. Association of Official Analytical Chemists International, **1995**: AOAC. Official methods of analysis. 16th edition, Arlington, VA, USA.