ACID HYDROLYSIS OF OLIVE-PRUNING DEBRIS FOR D-XYLOSE PRODUCTION

Juan F. García[a1,*], Sebastián Sánchez[a2], Vicente Bravo[b], Luc Rigal[c] and Manuel Cuevas[a3]

[a] *Department of Chemical, Environmental and Materials Engineering, University of Jaén, Campus Las Lagunillas, 23071 Jaén, Spain; e-mail: [1] jfgarmar@ujaen.es, [2] ssanchez@ujaen.es, [3] mcuevas@ujaen.es*

[b] *Department of Chemical Engineering, University of Granada, Campus Universitario de Fuentenueva, 18071 Granada, Spain; e-mail: vbravo@ugr.es*

[c] *Laboratoire de Chimie Agro-Industrielle, Ecole Nationale Supérieure d'Ingénieurs en Arts Chimiques et Technologiques (Unité Mixte de Recherche 1010), 118 route de Narbonne, 31077 Toulouse Cedex 4, France; e-mail: luc.rigal@ensiacet.fr*

Received March 5, 2008
Accepted June 4, 2008
Published online July 25, 2008

Selective hydrolysis of the hemicellulose fraction of olive-pruning debris was attempted in order to achieve a maximum yield of fermentable D-xylose without degrading the cellulose fraction. To this end, hydrolysis with a dilute acid was conducted in a heterogeneous stirred tank reactor at mild temperature (70–90 °C) and sulfuric acid concentration (0.0–0.5 mol l^{-1}) for 300 min. Temperature was the determining factor in sugar formation and fiber conversion. The highest hemicellulose and cellulose conversion at 90 °C were 92.9 and 16.0%, respectively. From 100 g of the raw material, 35.8 g of sugars were recovered. At this temperature, using 0.5 M H_2SO_4 for 4.5 h, D-glucose and D-xylose yields of 13.1 and 10.4%, respectively, were obtained.

Keywords: Dilute acid hydrolysis; Hemicellulose; Olive-pruning debris; D-Xylose.

Olive-pruning debris is a renewable, low-cost, readily available agricultural waste, especially in Mediterranean countries. At present the land devoted to olive-tree cultivation in the European Union makes ca. 5.4 million hectare, which is roughly about 4% of the arable land[1]. Since olive-pruning (in traditional and intensive cultivation) generates on average 3×10^3 kg of lignocellulose residues per year[2], it can be estimated that the total annual production exceeds 1.6×10^{10} kg. Olive-pruning debris consists of leaves, thin branches and wood in different proportions depending on cultivation conditions, production and local uses.

doi:10.1135/cccc20080637

A number of new technological applications have been found for this debris, but their economic viability is yet to be demonstrated. In most cases, the debris is left in the fields and then burnt or ploughed into the soil (after grinding). This process involves additional costs and environmental drawbacks. Basically, there are two ways of the valorisation of olive-pruning debris: thermochemical and biochemical conversion. Bioconversion is a two-stage process: hydrolysis of cellulose and hemicellulose fractions and fermentation of the resulting sugar solution (hydrolysate). However, it is very difficult to hydrolyse both fractions simultaneously. When the goal is bioethanol production, two methods are proposed: enzymatic hydrolysis and simultaneous saccharification and fermentation[3]. The methods have two disadvantages: the cost of enzymes and the loss of hemicellulose sugars in the pretreatment, which may account for up to 35% of the total carbohydrate content in residues[4]. Therefore, alternative methods are needed if the main objective of the hydrolysis is to recover the D-xylose contained in the hemicellulose in order to produce xylitol by fermentation. One of the alternative methods is the enzymatic hydrolysis pretreatments for D-xylose production. However, many of such methods, like hydrothermal treatment or extrusion, produce remarkable results only in achieving total or partial solubilization of hemicellulose and lignin fractions and most of the sugars occur as oligosaccharide chains[5].

Acid hydrolysis of cellulose seems to be difficult. First, cellulose is associated with other carbohydrates of the lignocelullose substrate. Second, the crystalline structure of cellulose constitutes another problem. Cellulose consists of linear 1,4-linked β-D-glucopyranose chains that are linked by hydrogen bonds (four intramolecular and two intermolecular bonds) into crystalline structures[6]. Thus, acid hydrolysis requires high temperatures or high acid concentrations. This implies higher costs of reagents, higher equipment corrosion[7] and the formation of potential yeast growth inhibitors from lignin and others sugar degradation products (furfural, hydroxymethyl furfural), which affects the fermentation performance[8].

Nevertheless, acid hydrolysis of lignocelullose materials carried out at mild temperatures (100–150 °C) and acid concentrations (0.025–1.0 mol l^{-1}) allows selective hemicellulose hydrolysis. Cellulose is hardly attacked and remains in the solid phase with lignin because its crystalline structure prevents the access of an acid to polymer chains[9,10]. If the objective is D-xylose production, the conditions should be milder in order to avoid its degradation.

Our aim was to hydrolyse the hemicellulose fraction of the olive-pruning debris with dilute sulfuric acid at low temperatures (70–90 °C) and low acid

concentrations (0.0–0.5 mol l^{-1}). This method could lead to higher D-xylose production while the sugar degradation and formation of microbial inhibitors would be avoided.

EXPERIMENTALS

Raw Material

The olive-pruning debris was collected on-site after fruit-harvesting. It consisted of thin branches (usually < 5 cm diameter) and leaves. Leaves were removed from the debris using a densimeter machine. The debris was air-dried at room temperature in laboratory, milled and screened. The size of the fraction selected was between 30 and 40 mesh (0.425–0.600 mm), according to ASTM guidelines.

Hydrolysis

Acid hydrolysis process was carried out in a 1-l discontinuous stirred tank reactor at atmospheric pressure. Temperature and sulfuric acid concentration were varied between 70 and 90 °C, and between 0 and 0.5 mol l^{-1}, respectively. The hydrolysis time was set to 5 h and stirring was kept at 250 rpm. The acid mixture was heated until it reached the hydrolysis temperature. Then, 50 g of dried debris were added in the 1/20 solid/liquid ratio. The course of hydrolysis was followed by sampling at fixed intervals. The 5-ml samples were centrifuged and their sugar and acetic acid contents were measured. After hydrolysis, the reactor content was cooled to room temperature and the wet material was filtered to give solid and liquid fractions. Finally, the water-insoluble solid was washed, air-dried and characterized.

Characterization of Solid Residues

The raw material and the solid obtained after hydrolysis were characterized by moisture (TAPPI T 12 os-75), acid-insoluble lignin (TAPPI T 222 os-74) and ashes (TAPPI T 15 os-58). Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were quantified[11]. The percentages of hemicellulose and cellulose were calculated using the relationships:

$$\% \text{ hemicellulose} = \% \text{ NDF} - \% \text{ ADF}$$

$$\% \text{ cellulose} = \% \text{ ADF} - \% \text{ lignin}$$

The hemicellulose (X_H), cellulose (X_C) and lignin (X_L) fractional conversions were determined from the fiber contents using the relationships:

$$X_H = \frac{\text{g hemicellulose reacted}}{\text{g dry matter fed}} 100$$

$$X_C = \frac{\text{g cellulose reacted}}{\text{g dry matter fed}} 100$$

$$X_L = \frac{\text{g lignin reacted}}{\text{g dry matter fed}} 100$$

Hydrolysate Characterization

The concentrations of total reducing sugars and acetic acid in the hydrolysate samples were quantified following Miller's method[12] and the enzymatic method described by Bergmeyer and Möllering[13]. Concentrations of D-arabinose, D-fructose, D-galactose, D-glucose and D-xylose were determined by HPLC. A Dionex Biolc DX300 system with a pulsed amperometer detector equipped with a gold electrode was used for analysis. Chromatography was performed on a CarboPac PA1 anion-exchange column (4.6 mm × 250 mm) equipped with a guard column. Elution was performed at a flow rate of 1 ml min^{-1} at 20–22 °C with an increasing gradient of NaOH from 0 to 1 mol l^{-1}. The sample volume injected was 25 µl. Analyse was completed in a run time of 50 min and the post-run time was 15 min.

D-Glucose (Y_G), D-xylose (Y_X), acetic acid (Y_A) and total reducing sugars (Y_S) yields were determined from the resulting concentrations using the relationships:

$$Y_G = \frac{\text{g D-glucose formed}}{\text{g dry matter fed}} 100$$

$$Y_X = \frac{\text{g D-xylose formed}}{\text{g dry matter fed}} 100$$

$$Y_A = \frac{\text{g acetic acid formed}}{\text{g dry matter fed}} 100$$

$$Y_S = \frac{\text{g total reducing sugars formed}}{\text{g dry matter fed}} 100 .$$

RESULTS AND DISCUSSION

First of all, the raw material was characterized as follows: moisture 8.3%, ash 2.3%, cellulose 36.4%, hemicellulose 21.5%, lignin 17.1%. These values are similar to those obtained in previous works[2,5].

The solids obtained after hydrolysis were mainly compound of cellulose and lignin, which could also be used afterwards (e.g., pellets for heating boiler). For the solids obtained from the hydrolysis carried out at 90 °C (except for C_A= 0), the percentages obtained in cellulose, hemicellulose and lignin were in the range 54–56, 3–6 and 28–31%, respectively. For lower temperatures, the percentage of hemicellulose increases up to 12.6% for the obtained one with 0.25 M H_2SO_4 at 70 °C.

The main monosaccharides generated were D-glucose and D-xylose. D-Galactose was hardly obtained. However, its concentration increased slightly through the hydrolysis process. Maximum concentrations of D-arabinose (<1.8 g l^{-1}) and of D-fructose (<1.0 g l^{-1}) were obtained at the beginning of the process (Fig. 1) and they remained constant throughout the process. Even though this pentose may have come only from the hemicellulosic fraction, there must exist a small fraction of amorphous cellulose or water-soluble extracts from which the hexose may have been obtained. D-Arabinose seems to be easier to obtain than D-xylose by hydrolysis. The highest D-arabinose yield (3.5%) was reached at a time of 15 min using 0.5 M H_2SO_4 at 90 °C. However, D-xylose is the main pentose in the hemicellulose. Normally, reaction time was not excessive, except in the experiment conducted under the most severe conditions. In this experiment, sugars underwent degradation in the period between 4.5 and 5 h: D-xylose and D-glucose yields decreased from 10.4 to 9.4% and from 13.1 to 12.5%, respectively.

In order to study the kinetics of the total reducing sugars formation, the experimental concentrations (*s*) were introduced into the empirical equation (Eq. (*1*))

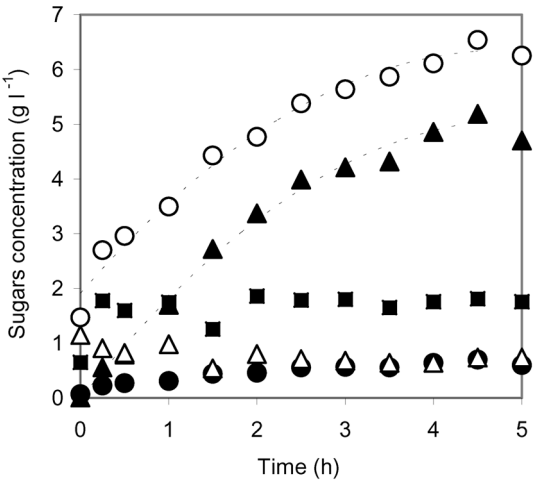

FIG. 1
D-Arabinose (■), D-galactose (●), D-glucose (○), D-xylose (▲) and D-fructose (△) concentrations in the course of hydrolysis with 0.5 M H_2SO_4 at 90 °C

$$(s - s_0) = \frac{s_m t}{\frac{s_m}{r_0} + t} \qquad (1)$$

where r_0 represents the apparent initial hydrolysis rate, s_0 is the initial sugar concentration and s_m is the maximum substrate concentration. Its value is approximately the same as that obtained for total reducing sugars at the end of the hydrolysis (t = 5 h). This empirical equation meets the condition $t = 0$ min $\rightarrow s = s_0$.

Equation (*2*) was obtained by linearization of Eq. (*1*) in order to calculate the parameters r_0 and s_m by plotting $1/(s - s_0)$ versus $1/t$ (Fig. 2). This method was adopted for the differential method of kinetic data treatment to be applied.

$$\frac{1}{s - s_0} = \frac{1}{s_m} + \frac{1}{r_0}\frac{1}{t} \qquad (2)$$

Table I shows the r_0 and s_m values determined. It can be seen that the highest apparent initial hydrolysis rate was obtained at 90 °C, at a sulfuric

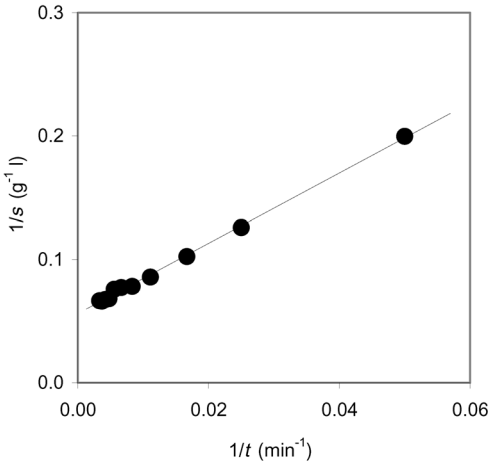

FIG. 2
Calculation of the apparent initial hydrolysis rate (r_0) and maximum sugar concentration (s_m) for hydrolysis with 0.37 M H_2SO_4 at 90 °C using Eq. (*2*)

acid concentration of 0.37 mol l^{-1}. The r_0 values for 0.5 M H_2SO_4 were smaller than those for 0.37 M H_2SO_4 both at 80 and 90 °C. This is probably due to partial degradation of the sugars formed from the beginning of the hydrolysis.

On the basis of these r_0 values, adjustments were tested for each temperature by means of the kinetic equation

$$r_0 - (r_0)_0 = kC_A^n \qquad (3)$$

where $(r_0)_0$ corresponds to the r_0 value for $C_A = 0$. In order to determine the reaction order (n) and the rate constant (k), Eq. (*3*) was linearized:

$$\ln [r_0 - (r_0)_0] = \ln k + n \ln C_A . \qquad (4)$$

Figure 3 shows that at 80 °C and especially at 90 °C partial degradation of total reducing sugars occurred to a considerable extend. This made it im-

TABLE I
Apparent initial hydrolysis rate (r_0) and maximum sugar concentration (s_m) obtained of different temperatures (T) and sulfuric acid concentrations (C_A)

T °C	C_A mol l^{-1}	r_0 g l^{-1} min^{-1}	r^2	s_m g l^{-1}
70	0.50	0.137	0.976	8.01
70	0.37	0.0795	0.997	8.09
70	0.25	0.0512	0.987	6.30
70	0.00	0.0198	0.947	–
80	0.50	0.151	0.998	16.72
80	0.37	0.158	0.973	17.67
80	0.25	0.116	0.995	10.56
80	0.00	0.0238	0.937	–
90	0.50	0.239	0.980	15.50
90	0.37	0.351	0.998	17.86
90	0.25	0.164	0.995	18.38
90	0.00	0.0498	0.945	–

possible to obtain a straight line when adjusting the values of r_0 to Eq. (*3*). At a milder temperature of 70 °C, the degradation of the hydrolysis products must be small and the adjustment was acceptable (r^2 = 0.988) leading to Eq. (*5*)

$$\ln [r_0 - (r_0)_0]_{70\,^\circ C} = 1.9\, C_A^{1.9}. \qquad (5)$$

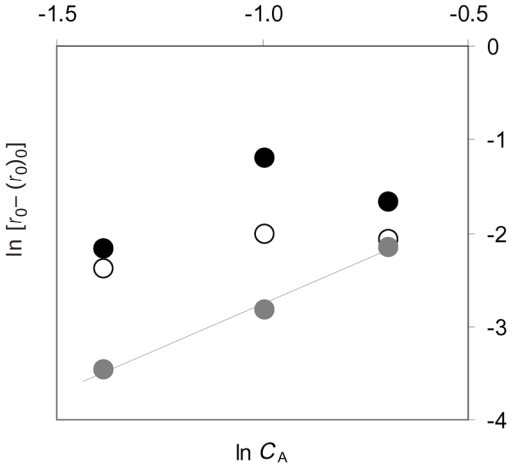

FIG. 3
Application of the linearized form of Eq. (*3*) in the reaction order and kinetic constant calculation to hydrolysis carried out at 70 (●), 80 (○) and 90 °C (●)

Effect of Sulfuric Acid Concentration

An increase in the concentration of H_2SO_4 led to a general increase in the conversion of fibers and in the D-glucose and D-xylose yields (Table II). However, the total reducing sugars yields were very similar for 0.5 and 0.37 M H_2SO_4, the apparent initial hydrolysis rate (r_0) being higher for 0.37 M H_2SO_4. On the other hand, the process conducted at 90 °C with 0.5 M H_2SO_4 was the only one in which a decrease in sugar yields was observed at the end of the process (Fig. 1). As the acid concentration increased, sugar formation and degradation rates increased, too. This is in line with Yat's findings[14]. Therefore, even though an increase in acid concentration led to an increase in sugar production, it also involved an increase in sugar degradation and in the formation of microbial inhibitor compounds.

Sugar yields were similar to those obtained by other authors using other lignocellulosic biomasses. For example, in the research[15] on hydrolysis of *Eucalyptus grandis* chips the best results were obtained when working with 0.5% H_2SO_4 at 140 °C for 10 min. They achieved yields of 0.85 in D-glucose, 8.5% in D-xylose and 2.5% in acetic acid. Parajó et al.[16] used pine debris at the 1/10 solid/liquid ratio in the boiling reaction mixture. After 5-h hydrolysis of 100 g of debris with 5% (ca. 0.5 M) H_2SO_4, they obtained a concentration of 17.5 g l^{-1} of hemicellulosic sugars and a concentration of D-glucose of about 2 g l^{-1}. In our research, a 5-h hydrolysis of 50 g of dry debris with a solid/liquid ratio of 1/20 was conducted under similar conditions (90 °C, 0.5 M H_2SO_4). After 4.5 h, the concentrations of total reducing sugars obtained were 17.4 g l^{-1}, 5.2 g l^{-1} of D-xylose and 6.5 g l^{-1} of D-glucose. The D-glucose concentrations and the D-xylose yields were much higher than those obtained by the above-mentioned authors. This seems to indicate that olive-tree pruning debris is more prone to acid hydrolysis.

TABLE II
Effect of temperature (T) and the sulfuric acid concentration (C_A) on the yields of total reducing sugars (Y_S), D-glucose (Y_G), D-xylose (Y_X) and acetic acid (Y_A), and on hemicellulose (X_H), cellulose (X_C) and lignin (X_L) conversions

T °C	C_A mol l^{-1}	Y_S %	Y_G %	Y_X %	Y_A %	X_H %	X_C %	X_L %
70	0.00	2.5	0.7	0.0	0.0	5.7	0.0	0.0
70	0.25	16.0	2.9	0.5	1.4	60.8	5.1	0.0
70	0.37	18.0	3.2	0.4	1.8	65.0	6.6	0.0
70	0.50	20.3	3.6	0.7	2.3	65.8	11.8	0.0
80	0.00	2.7	0.8	0.0	0.1	8.2	0.0	0.0
80	0.25	23.7	4.7	1.5	2.3	73.4	10.9	0.0
80	0.37	30.9	6.5	3.4	3.1	82.1	11.4	0.0
80	0.50	31.3	7.9	4.7	3.0	83.0	13.3	3.3
90	0.00	3.4	1.0	0.0	0.1	11.0	0.3	0.0
90	0.25	30.3	10.1	7.8	2.9	82.5	15.0	0.1
90	0.37	35.8	12.9	8.5	3.0	92.6	16.0	2.1
90	0.50	34.9	12.5	9.4	3.3	92.9	15.6	5.4

Influence of Temperature

Temperature was the key factor for sugar yields and fiber conversion. This has been reported by other authors with respect to acid hydrolysis of other lignocellulosic biomass[14,17,18]. At 90 °C, all sugar yields were over 30% and D-glucose yields were over 10%, except in hydrolysis conducted without acid (Table II). D-Xylose yields were perhaps most outstanding: at 70 °C, D-xylose production was virtually nil, at 80 °C a small quantity was produced and at 90 °C there was a substantial increase in yields with 0.5 M H_2SO_4 up to 9.4% (Table II). The effect of temperature on pentose degradation was smaller than that of the acid concentration.

As to fiber conversion, the use of 0.37 or 0.5 M H_2SO_4 made no difference. This is because the conversion depended largely on temperature, particularly the hemicellulose conversion (Fig. 4). Acid hydrolysis at 70 °C was not feasible at any acid concentration: the hemicellulose conversion was low and D-xylose production negligible. Cellulose conversions showed the same behavior. Finally, the percentages of lignin conversion were not significant (maximum 5.4% with 0.5 M H_2SO_4 at 90 °C) if we take into account the error of the methods employed for fiber determination.

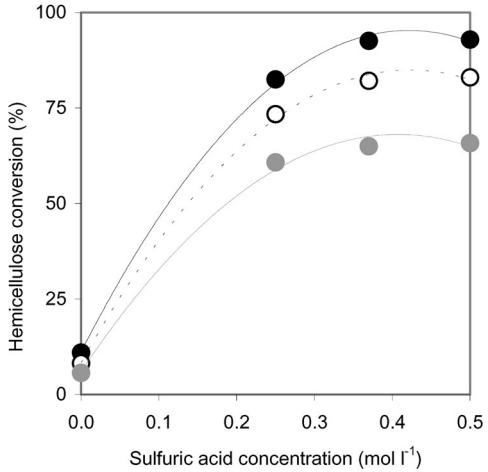

FIG. 4
Dependence of hemicellulose conversion on sulfuric acid concentration in hydrolysis carried out at 70 (●), 80 (○) and 90 °C (●)

CONCLUSIONS

From the results obtained in this work it can be concluded that the hydrolysis with sulfuric acid is an interesting option for using olive-pruning debris in a D-xylose scheme, for subsequent xylitol production by fermentation. D-Glucose was also produced in large quantities. Its conversion into bioethanol would improve the economic viability of the process. Negligible lignin degradation was observed. At 90 °C, using 0.5 M H_2SO_4 for 4.5 h, D-glucose and D-xylose yields of 13.1 and 10.4%, respectively, were obtained. Even though the three variables studied (process time, sulfuric acid concentration and temperature) are very closely linked, temperature was the variable with the greatest influence on the conversion of fibers into the sugars. The maximum values of hemicellulose conversion increased from 65.8% at 70 °C to 92.9% at 90 °C. In general, the highest hydrolysis rates were obtained with 0.37 M H_2SO_4. Under these conditions (90 °C, 0.37 M H_2SO_4), 35.8 g of sugars were recovered from 100 g of raw material. For xylitol and ethanol production the fermentation of the hydrolysates obtained at 90 °C will be carried out with non-traditional yeast.

The authors are grateful to the Spanish "Dirección General de Investigación, Ciencia y Tecnología" for funding this research in the framework of project PB98-0305. Dr J. F. García Martín acknowledges the grant of the "Junta de Andalucía".

REFERENCES

1. Olistat (2006) Dataset. http://mars.jrc.it/marspac/olivine/Olistat.htm. Accessed 1 April 2006.
2. Sánchez S., Bravo V., Moya A. J., Moya M., Romero I., Torrero R., San Miguel M. P.: *Ing. Quim.* **2002**, *391*, 194.
3. Ogier J. C., Ballerini J. P., Leygue J. P., Rigal L., Pourquié J.: *Oil Gas Sci. Technol.* **1999**, *54*, 67.
4. Clausen E. C., Gaddy J. L.: *Ann. N.Y. Acad. Sci.* **1983**, *413*, 435.
5. García J. F., Sánchez S., Bravo V., Cruz N., Cuevas M., Rigal L., Doumeng C.: *Afinidad* **2008**, *65*, 39.
6. Zhbankov R. G.: *J. Mol. Struct.* **1992**, *270*, 523.
7. Silvers M. V., Zacchi G.: *Bioresour. Technol.* **1995**, *51*, 43.
8. Larsson S., Palmqvist E., Hahn-Hägerdal B., Tenborg C., Stenberg K., Zacchi G., Nilvebrant N. O.: *Enzyme Microb. Technol.* **1999**, *24*, 151.
9. Weil J., Westgate P. J., Kolhmann K., Ladisch M. R.: *Enzyme Microb. Technol.* **1994**, *16*, 1002.
10. Rahman S. H. A., Choudhury J. P., Ahmad A. L., Kamaruddin A. H.: *Bioresour. Technol.* **2007**, *98*, 554.
11. Van Soest P. J., Wine R. H.: *J. Assoc. Off. Anal. Chem.* **1967**, *50*, 50.
12. Miller G. L.: *Anal. Chem.* **1959**, *31*, 426.

13. Bergmeyer H. U., Möllering H. in: *Methods of Enzymatic Analysis* (H. U. Bergmeyer, Ed.), p. 1520. Verlag Chemie, Weinheim/Academic Press, New York 1974.
14. Yat S. C., Berger A., Shonnard D. R.: *Bioresour. Technol.* **2008**, *99*, 3855.
15. Silva S. S., Felipe M. G. A, Silva J. B. A., Prata A. M. R.: *Process Biochem.* **1998**, *33*, 63.
16. Parajó J. C., Santos V., Del Río F.: *Afinidad* **1995**, *52*, 161.
17. Lis M. J., Carrillo F., Colom X., Martínez D., Nogués F.: *Ing. Quim.* **2000**, *369*, 181.
18. Roberto I. C., Mussatto S. I., Rodríguez R. L. C. B.: *Ind. Crops Prod.* **2003**, *31*, 431.