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Comparison between Different Hydrolysis Processes of Vine-Trimming Waste to Obtain Hemicellulosic Sugars for Further Lactic Acid Conversion

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Abstract Trimming vine shoot samples were treated with water under selected operational conditions (autohydrolysis reaction) to obtain a liquid phase containing hemicellulose-decomposition products. In a further acid-catalyzed step (posthydrolysis reaction), xylooligosaccharides were converted into single sugars for the biotechnological production of lactic acid using *Lactobacillus pentosus*. A wide range of temperatures, reaction times, and acid concentrations were tested during the autohydrolysis–posthydrolysis process to investigate their influence on hemicellulose solubilization and reaction products. The maximum concentration of hemicellulosic sugars was achieved using autohydrolysis at 210 °C followed by posthydrolysis with 1% H_2SO_4 during 2 h. Data from autohydrolysis–posthydrolysis were compared with the results obtained at the optima conditions assayed for prehydrolysis (3% H_2SO_4 at 130 °C during 15 min) based on previous works. Prehydrolysis extracted more hemicellulosic sugars from trimming vine shoots; however, the protein content in the hydrolysates from autohydrolysis–posthydrolysis was higher. The harsher conditions assayed during the autohydrolysis process and the higher content of protein after this treatment could induce Maillard reactions decreasing consequently the concentration of hemicellulosic sugars in the hydrolysates. Therefore, despite the several advantages of autohydrolysis (less equipment caused by the absence of mineral acid, less generation of neutralized sludges, and low cost of reagents) the poor results obtained in this work with no detoxified hydrolysates (Q_P=0.36 g/L h, Q_S=0.79 g/L h, $Y_{P/S}$=0.45 g/g, $Y_{P/Sth}$=61.5 %) or charcoal-treated hydrolysates (Q_P=0.76 g/L h, Q_S=1.47 g/L h, $Y_{P/S}$=0.52 g/g, $Y_{P/Sth}$=71.5 %) suggest that prehydrolysis of trimming vine shoots with diluted H_2SO_4 is more attractive than autohydrolysis-posthydrolysis for obtaining lactic acid through fermentation of hemicellulosic sugars with *L. pentosus*. Besides the higher hemicellulosic sugars concentration

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achieved when using the prehydrolysis technology, no detoxification steps are required to produce efficiently lactic acid (Q_P=1.14 g/L h; Q_S=1.64 g/L h; $Y_{P/S}$=0.70 g/g; $Y_{P/Sth}$=92.6 %), even when vinification lees are used as nutrients (Q_P=0.89 g/L h; Q_S=1.54 g/L h; $Y_{P/S}$= 0.58 g/g; $Y_{P/Sth}$=76.1 %).

Keywords Trimming vine shoots · Autohydrolysis · Prehydrolysis · *L. pentosus* · Lactic acid

Introduction

When lignocellulosic residues like trimming vine shoots are field burned, polycyclic aromatic hydrocarbon compounds such as benzopyrene, catechol, hydroquinone, phenanthrene, and naphthalene are released [1]. Tsutsui et al. [2] have reported that all these compounds can inhibit DNA synthesis and induce cancerous tumors in animals and humans. Moreover, Wen-Tien and Jih-Ming [3] reported that during the field burning of agricultural residues, anthropogenic N_2O and other gases are emitted to the atmosphere contributing to the global warming. Consequently, it would be interesting to find alternative processes to the field burning of this kind of materials. Some authors [4–9] have reported the fractionation of lignocellulosic materials to obtain a variety of marketable chemicals from the polymeric fractions of the raw materials (cellulose, hemicellulose, and lignin). In this way, hydrolysis to break down polymers can be conceived as a possible first processing step for an integral use of biomass. In this kind of treatment, hemicellulose can be almost completely solubilized [10–13], whereas little alteration is caused in both lignin and cellulose, which are recovered in the solid phase. In this work, two different hydrolysis processes have been studied: autohydrolysis and prehydrolysis. In comparison with prehydrolysis, where a mineral acid is added to the reaction media [4, 14–18] in autohydrolysis reactions, the catalytic species (hydronium ions) come from water autoionization and from the acetic acid generated from acetyl groups [11–13]. Owing to this, autohydrolysis causes low environmental impact (no neutralization sludges are generated) with limited equipment corrosion [19]. Under mild operational conditions, the reaction is very selective, avoiding cellulose decomposition and lignin repolymerization.

Lactic acid (LA) is a commodity chemical with a wide range of applications, mainly in the food industry (which utilizes about 80% of the total production, especially as a microbial preservative or as an agent for acidification or buffering), but also in the production of pharmaceuticals and cosmetics, as well as in the polymer industry, where it is used for making biodegradable plastics. The industrial production of lactic acid can be carried out by two alternative technologies: chemical synthesis from fossil fuels and biotechnological processes, the latter being based on the utilization of biomass as raw materials. Nowadays, the fermentative production of lactic acid is the world's leading technology. Besides, natural products are preferred by consumers, and the tendency is to replace chemical processes with biotechnological ones. Nevertheless, to produce biological food additives at an industrial scale, fermentation technologies must be cost competitive with chemical synthesis. The Mercier medium [20] includes, among others, yeast extract and peptone, which are very expensive, the total price of nutrients being able to account for almost 30% of the final cost of the process [21]. Consequently, it is particularly interesting to find out alternative, financially competitive nutrient sources. The use of vinification lees has turned out to be a useful manner to reduce costs, as lees are basically dead yeasts, representing a potential source of nutrients [22].

In a previous work [15], the optimum operating conditions for prehydrolysis of trimming vine shoots were studied and selected on the basis of both the generation of hemicellulosic sugars (glucose, arabinose, and mainly xylose) and the concentration of reaction byproducts (furfural, hydroxymethylfurfural [HMF], and acetic acid) which can inhibit lactic acid fermentation by *Lactobacillus pentosus*. This work deals with the study of the autohydrolysis–posthydrolysis process under different conditions. The results were compared with those obtained after prehydrolysis. Finally, under the optimum conditions in terms of solubilized sugars and generated byproducts, the fermentation into lactic acid by *L. pentosus* was considered for both processes using the best hydrolysates as carbon source supplemented with a general medium of *Lactobacillus* or vinification lees as nutrients.

Materials and Methods

Raw Materials and Analysis

Locally collected vine-trimming waste samples were dried, milled (to a particle size less than 1 mm), homogenized into a single lot (to avoid compositional differences), and stored until use. Portions from the homogenized lot were submitted to moisture content determination and to a quantitative hydrolysis in a two-stage acid treatment (the first step with 72% H_2SO_4 at 30 °C during 1 h, and the second one after dilution of the media to 3% H_2SO_4 and heating at 121 °C during 1 h) for cellulose, hemicellulose, and lignin determination. The solid residue obtained after hydrolysis was weighed and considered as Klason lignin [15]. The analysis of the supernatants and solid residue gave the following composition: 34.1 % of cellulose, 19.0 % of hemicellulose, and 27.1% of lignin.

Autohydrolysis

Trimming wastes were subjected to non-isothermal autohydrolysis in a 600-mL Parr reactor reaching different conditions of temperature (190, 210, 230, 235, and 242 °C, respectively) with a liquid to dry-solid ratio of 8:1. After autohydrolysis, the solid and liquid phases were separated by filtration. Hydrolysis was carried out in triplicate, and mean values and their standard deviations are reported in Tables 1, 2, and 3.

Acid Posthydrolysis of Autohydrolysis Liquors

Different acid posthydrolysis assays of autohydrolysis liquors were carried out to select the best conditions to obtain the maximum yield of hemicellulosic sugars. The acid

Table 1 Composition (g/L) of hydrolysates obtained after autohydrolysis processes at different temperatures.

T (°C)	Glucose	Xylose	Arabinose	Formic acid	Acetic acid	Furfural
190	2.4±0.13	2.1±0.11	1.1±0.08	0.90±0.19	0.9±0.05	0.14±0.03
210	2.0±0.21	2.4±0.19	1.2±0.06	0.90±0.03	3.6±0.07	0.09±0.01
230	1.9±0.14	2.7±0.07	1.3±0.12	0.89±0.06	5.6±0.33	0.88±0.01
235	2.3±0.33	2.8±0.45	1.7±0.13	2.2±0.29	7.5±0.20	1.9±0.01
242	3.9±0.21	3.2±0.32	1.9±0.35	2.6±0.05	8.3±0.12	2.2±0.02

Table 2 Composition (g/L) of hydrolysates after autohydrolysis at 190 °C followed by posthydrolysis with 0.5 % H_2SO_4 at different reaction times.

t (min.)	Glucose	Xylose	Arabinose	Formic acid	Acetic acid	Furfural
30	2.6±0.14	2.9±0.01	1.4±0.14	0.86±0.29	1.7±0.08	0.11±0.01
75	3.5±0.19	5.7±0.41	1.7±0.13	0.74±0.06	2.0±0.11	0.07±0.04
120	3.9±0.11	6.8±0.19	1.9±0.09	0.58±0.00	2.2±0.04	0.07±0.02
165	5.7±0.21	9.6±0.24	2.0±0.17	0.57±0.26	2.8±0.20	0.07±0.01
210	6.5±0.11	10.6±0.24	2.0±0.13	0.57±0.01	2.7±0.02	0.35±0.04

posthydrolysis reactions were carried out in an autoclave STERILCLAV-75 (RAYPA, Barcelona, Spain) using 0.5–4.0% H_2SO_4 at 130 °C and applying different reaction times. The liquid phase from posthydrolysis was neutralized with $CaCO_3$ to a final pH of 6.5 and the $CaSO_4$ precipitated was separated from the supernatant by filtration.

Table 3 Composition of hydrolysates after autohydrolysis at different temperatures followed by posthydrolysis for 120 min using different concentrations of H_2SO_4.

H_2SO_4					
T (°C)	0.5%	1%	2%	3%	4%
Glucose (g/L)					
190	3.9±0.11	6.8±0.07	6.9±0.12	7.0±0.02	6.7±0.02
210	4.1±0.01	7.3±0.72	7.7±0.29	7.3±0.54	7.2±0.02
230	3.7±0.15	5.0±0.15	5.3 ±0.02	5.4±0.01	6.7±0.11
242	1.3±0.03	1.4±0.00	1.3±0.08	1.5±0.01	1.3±0.03
Xylose (g/L)					
190	6.8±0.19	10.0±0.09	6.9±0.12	9.3±0.00	8.7±0.00
210	10.6±0.46	14.8±0.69	14.0±0.31	12.6±0.32	11.8±0.07
230	10.7±0.70	13.2±0.11	12.5±0.11	11.9±0.07	10.3±0.24
242	1.1±0.16	0.84±0.08	0.73±0.17	0.89±0.01	0.89±0.01
Arabinose (g/L)					
190	1.9±0.09	2.1 ±0.09	1.8±0.03	1.8±0.09	1.6±0.00
210	1.7±0.18	1.8 ±0.26	2.0±0.11	1.0±0.51	1.2±0.08
230	0.60±0.04	0.38±0.07	0.49±0.00	0.47±0.01	1.9±0.12
242	1.3±0.03	0.37±0.34	0.63±0.49	1.2±0.03	1.5±0.01
Formic acid (g/L)					
190	0.58±0.00	1.1±0.23	0.66±0.01	0.72±0.02	0.78±0.00
210	2.0±0.44	1.9±0.69	2.9±0.10	2.0±0.65	1.2±0.04
230	2.8±0.00	2.3±0.01	2.5±0.13	2.7±0.25	0.62±0.01
242	2.9±0.01	2.8±0.03	2.3±0.57	2.9±0.04	2.3±0.57
Acetic acid (g/L)					
190	2.2±0.04	3.0±0.22	2.9±0.04	2.8±0.62	2.9±0.00
210	4.3±0.16	5.4±0.31	5.2±0.05	5.1±0.19	4.5±0.01
230	5.0±0.03	5.9±0.14	5.9±0.04	6.0±0.14	2.8±0.03
242	7.4±0.02	7.5±0.01	6.2±0.79	6.8±0.01	6.2±0.79
Furfural (g/L)					
190	0.07±0.02	0.38±0.16	0.46±0.00	0.42±0.03	0.54±0.14
210	0.00±0.00	0.17±0.17	0.44±0.04	0.36± 0.03	0.38±0.03
230	0.47±0.11	1.0±0.02	1.3±0.01	1.5±0.04	0.24±0.02
242	1.2±0.04	1.4±0.03	1.1±0.05	0.82±0.13	1.3±0.08

Prehydrolysis

Based on previous works [15] acid prehydrolysis of prunings of vine-stocks were carried out in autoclave STERILCLAV-75 (RAYPA, Barcelona, Spain) at 130 °C with 3 % sulfuric acid for 15 min using a liquid/dry-solid ratio of 8 g/g.

Charcoal Detoxification

Powdered charcoal (Probus, Madrid, Spain) was activated with hot water and dried at room temperature. Charcoal detoxification of hydrolysates was carried out by contacting hydrolysates obtained after autohydrolysis–posthydrolysis treatments and charcoal with a liquid to dry-solid ratio of 10:1 at room temperature under stirring for 1 h. The liquid phase was recovered by filtration and used for making the culture media for the microorganism; 98.7% of the initial color was removed using this procedure.

Microorganism

Lactobacillus pentosus CECT-4023T (ATCC-8041) was obtained from the Spanish Collection of Type Cultures (Valencia, Spain). The strain was grown on plates using the complete media proposed by Mercier et al. [20], which consists of glucose 20 g/L, yeast extract 5 g/L, peptone 10 g/L, sodium acetate 5 g/L, sodium citrate/2 g L, K_2HPO_4 2 g/L, $MgSO_4{\cdot}7H_2O$ 0.58 g/L, $MnSO_4{\cdot}H_2O$ 0.12 g/L, $FeSO_4{\cdot}7H_2O$ 0.05 g/L, and agar 20 g/L, and incubated at 31 °C for 24 h. Inocula were prepared by solubilization of cells from plates in 5 mL of sterile hydrolysates. Biomass concentration in inocula was measured by optical density at 600 nm and adjusted by dilution with hydrolysates, to reach a final concentration in the inocula of 4.0 g/L.

Fermentation

Hydrolysates selected for fermentation were neutralized with $CaCO_3$ to a final pH of 6.5, and the $CaSO_4$ precipitated was separated from the supernatant by filtration. The clarified liquors were supplemented with the Mercier medium [20] or 20 g/L of distilled vinification lees, sterilized and used directly as fermentation media. Lactic acid fermentations were carried out in triplicate in 250-mL Erlenmeyer flasks with a final volume of 100 mL. Twenty grams per liter of calcium carbonate were added to the fermentation media to neutralize the lactic acid produced. Fermentations were carried out in orbital shakers at 150 rpm and 31 °C. Samples (2 mL) were taken at given fermentation times and centrifuged at 6,000 rpm for 3 min. The supernatants were stored for glucose, xylose, arabinose, acetic acid, and lactic acid analysis.

Analytical Methods

Glucose, xylose, arabinose, formic acid, acetic acid, furfural, HMF, and lactic acid were measured by high-performance liquid chromatography (HPLC) (Agilent, model 1100, Palo Alto, CA, USA) with a RI detector using a Transgenomic ION-300 column (Transgenomic Inc., San Jose, CA, USA) eluted with 0.02 M H_2SO_4 at a flow rate of 0.4 mL/min. Soluble protein content was measured following the Lowry method [23].

Qualitative GS/MS Analysis

Samples were derivatized to confirm the identity of the sugars and avoid problems derived from the same retention time of some compounds in analysis by HPLC. Around 50 mg of standard reagent or dry sample was weighed into a 25-mL round-bottomed flash and trimethylsilylated by adding 200 mL of pyridine, 1 ml of bis(trimethylsilyl)trifluoroacetamide (BSTFA) and 50 ml of trimethylchlorosilane (TMCS). The flask was sealed, shaken vigorously, kept at 60 °C under stirring for 30 min in a water bath and cooled to room temperature before GC/MS analysis (injection volume 1.5 mL). The identity of the compounds was confirmed by comparing both the retention time and the mass spectral data with those of pure compounds (similarity percentage higher than 85 %).

Results and Discussion

Autohydrolysis–Posthydrolysis of Trimming Vine Shoots

Trimming vine shoots are field burned or simply used as a landfill. Nevertheless, it is a lignocellulosic material and could be better used, as its composition is rich in oligo- and polysaccharides. Thus, upon hydrolytical stages, the polymeric sugars can be fractionated to obtain sugar-containing solutions, which, after nutrient supplementation, could be used as fermentation media for producing food additives like lactic acid [15]. The most important hydrolytical processes for obtaining hemicellulosic sugars from lignocellulosic residues consist of autohydrolysis–posthydrolysis [11, 13] or prehydrolyisis [15, 16, 18]. First of all, in this work, trimming vine wastes were submitted to autohydrolysis stages at different temperatures: 190, 210, 230, 235, and 242 °C. Table 1 shows the composition of the resulting liquors, which contain a mixture of low molecular weight sugars (glucose, xylose, and arabinose), soluble sugar oligomers (not quantified), and sugar-decomposition products (formic acid, acetic acid, and furfural). It can be observed that temperatures above 235 °C increased considerably the concentration of degradation products. Thus, at 242 °C, the concentration of hemicellulosic sugars increased slightly, whereas, the degradation products increased drastically, achieving concentrations of formic acid, acetic acid, and furfural in the vicinity of 2.6, 8.3, and 2.2 g/L respectively, which makes no viable a further fermentation process. Consequently, to reduce the presence of these inhibitors, temperatures in the vicinity of 190 °C should be employed to carry out the autohydrolysis stage.

As the sugar oligomers (mostly xylooligosaccharides) produced during autohydrolysis cannot be assimilated by bacteria, a posthydrolysis stage (catalyzed by acids or enzymes) is needed to produce the corresponding low molecular weight sugars. The lower cost, simplicity and faster kinetics of the acid posthydrolysis makes it preferable over enzymatic hydrolysis [11]. Under selected conditions, the acid posthydrolysis of oligomers can be carried out with minimal decomposition into formic acid, acetic acid, and furfural, which are also posthydrolysis byproducts affecting the fermentability of the liquors. Thus, Table 2 shows the composition of hemicellulosic solutions from autohydrolysis at 190 °C, followed by posthydrolysis with 0.5 % H_2SO_4 at different reaction times. It can be observed that the concentration of glucose and xylose increased directly with time from 2.6 g/L up to 6.5 g/L and from 2.9 g/L up to 10.6 g/L respectively, whereas the arabinose concentration remained unchanged (around 2 g/L). In relation to the content of inhibitors, the posthydrolysis stage almost did not change their concentration comparing with autohydrolysis at 190 °C. So, to obtain the highest sugar concentrations, a posthydrolysis should be carried out with 0.5 % of H_2SO_4 during 165–210 min.

From the above study, the optima conditions recommended for posthydrolysis are too time-consuming. This time can be shortened assaying different concentrations of H_2SO_4 for posthydrolysis carried out during 120 min (an intermediate value as harsher conditions would lead to higher values of sugars with higher contents of inhibitors). Table 3 shows the operating conditions assayed as well as the amounts of hemicellulosic sugars and inhibitors obtained. It can be observed that the concentration of glucose and xylose increased with temperature during autohydrolysis up to 210 °C and with H_2SO_4 concentration up to 1% (for xylose) or 2–3% (for glucose). Higher values of both temperature and catalyst decreased the concentrations of glucose, xylose, and arabinose increasing significantly the concentration of inhibitors (formic acid and furfural).

Consequently, according to the data of Table 3, xylose-containing fermentation media prepared from trimming vine shoots can be successfully assayed from the sequence autohydrolysis at 210 °C posthydrolysis with 1% H_2SO_4 during 2 h. Under these conditions, the medium contains around 15 g/L of xylose; 7 g/L of glucose and 2 g/L of arabinose.

Comparative Study: Autohydrolysis–Posthydrolysis and Prehydrolysis

To obtain directly fermentable hydrolysates, the optima conditions given above for autohydrolysis–posthydrolysis were compared with the optima prehydrolysis conditions obtained in a previous work [15]. It has to be considered that the thermal hydrolysis of vine-trimming wastes leads not only to the solubilization of hemicelullosic sugars, but also to the generation of a complex mixture of compounds, which can inhibit further fermentation steps.

Bustos et al. [15] assayed different reaction times and catalyst concentrations during prehydrolyis experiments, maintaining the reaction temperature at 130 °C. Under optimal conditions (3% H_2SO_4 and 15 min), 18 g/L of xylose, 11 g/L of glucose and 5 g/L of arabinose were produced. This hemicellulosic sugar concentration is slightly higher than that obtained when using the sequence of autohydrolysis–posthydrolysis. These results agree with those achieved by Rivas et al. [11] for the generation of fermentable hydrolysates from corn cobs during the biotechnological production of xylitol by using sequential autohydrolysis (at 202 °C) and posthydrolysis (in the presence of 0.5% H_2SO_4, at 125 °C for 165 min) steps or treating corn cobs by prehydrolysis (2% H_2SO_4, 130 °C, and 15 min). The concentration of hemicellulosic sugars from corn cobs was higher in hydrolysates obtained by prehydrolysis (35 g/L of xylose, 3.2 g/L of glucose, and 4.6 g/L of arabinose) than in hydrolysates coming from autohydrolysis–posthydrolysis (28.2 g/L of xylose, 2.1 g/L of glucose, and 3.2 g/L arabinose). However, in spite of the fact that autohydrolysis–posthydrolysis gave lower hemicellulosic sugar concentrations, some authors [11] point out that in comparison with the conventional acid hydrolysis (prehydrolysis), the autohydrolysis–posthydrolysis increases the productivity and product yield of some biotechnological processes.

Fermentation of Hemicellulosic Sugars for Lactic Acid Production

The main aim of this study was to analyze the productivities and yields achieved during the lactic acid fermentation from xylose-containing media obtained after prehydrolysis or autohydrolysis–posthydrolysis treatments. Rivas et al. [11], comparing both treatments, found that the productivity and the product yield of the biotechnological production of xylitol can be increased by 18% and 25%, respectively, using hemicellulosic hydrolysates from autohydrolysis–posthydrolysis of corn cobs. This fact suggests that the mildly acidic conditions used by Rivas et al. [11] during autohydrolysis–posthydrolysis resulted in liquors with limited inhibitor concentrations that do not affect the biotechnological production of xylitol by *Debaryomyces hansenii*, particularly those coming from sugar decomposition and acid-soluble lignin. So, on the basis of this work, hemicellulosic hydrolysates from trimming vine shoots obtained by prehydrolysis or autohydrolysis–posthydrolysis were assayed to carry out the fermentation into lactic acid by *L. pentosus*.

Figures 1a, b and 2 show the kinetic profiles of lactic and acetic acid production as well as the glucose and xylose consumption of hemicelullosic sugars coming from prehydrolysis and autohydrolysis-posthydrolysis, respectively. Fermentations were carried out using the complete medium for lactic acid bacteria proposed by Mercier et al. [20]. It can be observed in both cases that glucose was consumed within 7–8 h, whereas xylose was consumed slowly, with 26% of xylose remaining unconverted at the time where the maximum concentrations of lactic acid were obtained. Additionally, scarce arabinose was consumed at the end of the process (data not shown), and consequently it was not considered when yields were calculated. Table 4 shows the stoichiometric parameters, productivities, and yields during lactic acid fermentation of hydrolysates coming from autohydrolysis and prehydrolysis by *L. pentosus*. Lower volumetric productivities (Q_P=0.36 g/L h), volumetric

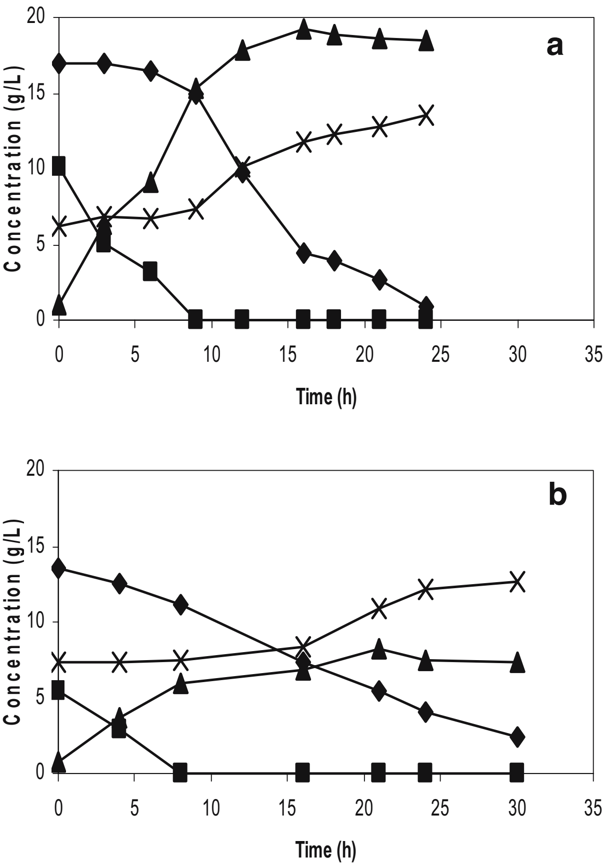
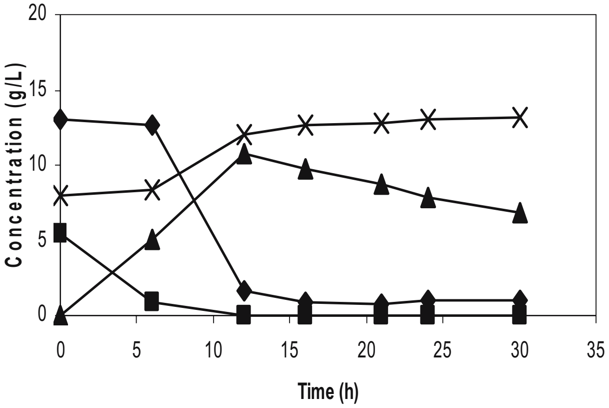


Fig. 1 Kinetic profile of lactic acid and acetic acid production as well as glucose and xylose consumption using media made from prehydrolysis (**a**) or autohydrolysis-posthydrolysis (**b**) liquors. The experimental results are the average of three assays, with average deviation lower than 2.6 %. Xylose (♦), glucose (■), lactic acid (▲), acetic acid (*)

Fig. 2 Kinetic profile of lactic acid and acetic acid production as well as glucose and xylose consumption using charcoal-detoxified media made from autohydrolysis-posthydrolysis liquors. The experimental results are the average of three assays, with average deviation between assays lower than 2.2 %. Xylose (♦), glucose (■), lactic acid (▲), acetic acid (*)



sugars consumption (Q_S=0.79 g/L h), product yield ($Y_{P/S}$=0.45 g/g), and especially theoretical product yield ($Y_{P/Sth}$=61.5 %) were obtained when hydrolysates coming from the autohydrolysis–posthydrolysis were fermented in comparison with the fermentation of hydrolysates coming from the prehydrolysis treatment (Q_P=1.14 g/L h; Q_S=1.64 g/L h; $Y_{P/S}$=0.70 g/g; $Y_{P/Sth}$=92.6 %). During the autohydrolysis treatment, high temperatures at prolonged times are applied. This fact could increase the concentration of inhibitors, particularly furfural. Furthermore, it could be supposed that some Maillard reactions could be induced between proteins and sugars, which affect the fermentability of hemicellulosic sugars by *L. pentosus*. The Maillard reaction (MR) takes place between amino groups and reducing compounds during the home and industrial processing of foods [24]. Maillard reaction products (MRP) have not yet been fully characterized, although it is known that

Table 4 Stoichiometric parameters, productivities, and yields obtained during lactic acid fermentations of hemicellulosic hydrolysates from prehydrolysis and autohydrolysis-posthydrolysis using general medium of *Lactobacillus* as nutrients (results calculated for fermentation times leading to maximum sugar consumption).

	Prehydrolysis	Auto-posthydrolysis	Auto-posthydrolysis (detoxified hydrolysates)
Xylose$_{t=0}$ (g/L)	17.0±1.3	13.5±0.9	13.1±0.7
Xylose$_{t=\text{final}}$ (g/L)	0.90±0.2	2.4±0.1	0.9±0.2
Glucose$_{t=0}$ (g/L)	10.1±0.5	5.5±0.7	5.4±0.4
Glucose$_{t=\text{final}}$ (g/L)	0.0±0.0	0.0±0.0	0.0±0.0
Lactic acid$_{t=0}$ (g/L)	0.9±0.4	0.72±0.2	0.9±0.3
Lactic acid$_{t=\text{final}}$ (g/L)	19.2±0.5	8.2±0.5	10.0±0.7
Acetic acid$_{t=0}$ (g/L)	6.2±0.4	7.1±0.6	8.0±0.7
Acetic acid$_{t=\text{final}}$ (g/L)	11.8±0.7	12.2±1.0	13.2±1.1
Time (h)	16	21	12
Q_P (g/L h)^a	1.14	0.36	0.76
Q_S (g/L h)^a	1.64	0.79	1.47
$Y_{P/S}$ (g/g)^a	0.70	0.45	0.52
Theoretical yield (%)^b	92.6	61.5	71.5

[a] Nomenclature: Q_P, global volumetric productivity of lactic acid; Q_S, volumetric rate of substrates (xylose and glucose) consumption; $Y_{P/S}$, sugars to lactic acid yield [g lactic acid produced/(g xylose consumed + g glucose consumed)].

[b] (g lactic acid produced * 100)/[(g xylose consumed * 0.6) + (g glucose consumed)].

they have two different components: a low molecular weight (LMW) fraction and a high molecular weight (HMW) fraction or melanoidin [25]. Most investigations focused on the effect of MRP on microorganisms have been carried out in specific microbial growth media, which show that MRP can stimulate microbial growth [26] or inhibit it [27]. In this way, Einarsson et al. [28] described the inhibition of *L. plantarum* in the presence of MRP.

Furthermore, to improve both productivities and yields, the liquors from autohydrolysis–posthydrolysis were detoxified with activated charcoal. During this stage, the volatile compounds present in the reaction media (such as acetic acid or furfural) were partially removed, whereas the concentrations of xylose-degradation and nonvolatile byproducts (namely, the lignin-derived fraction, which can act as a fermentation inhibitor) increased proportionally [11]. Table 4 also shows the productivities and yields during the bioconversion of charcoal-treated (detoxified) hydrolysates obtained by autohydrolysis–posthydrolysis of trimming vine shoots into lactic acid. It can be observed that using detoxified hydrolysates, the global volumetric productivity (0.76 g/L h), volumetric sugar consumption (1.47 g/L h), product yield (0.52 g/g), and theoretical product yield (71.5 %) improved significantly. Moreover, Fig. 2 shows the kinetic profile of lactic and acetic acid production, as well as glucose and xylose consumption using media made from detoxified autohydrolysis-posthydrolysis liquors. The final lactic acid concentration (10.0 g/L) was higher than that achieved by Garde et al. [29] using hemicellulosic hydrolysates obtained after treating straw with 4% H_2SO_4 at 100 °C for 2 h. These authors achieved around 8.0 g/L of lactic acid when using a co-culture of *L. pentosus* and *L. brevis* or 6.7 g/L when *L. pentosus* was employed as the only microorganism.

To check the viability of vinification lees as economic nutrients, assays were performed using media made from prehydrolysis or detoxified autohydrolysis-posthydrolysis liquors and 20 g/L of vinification lees as the only nutrients. Table 5 compares the results obtained under both conditions and Fig. 3 shows the time evolution of representative fermentation assays.

Table 5 Stoichiometric parameters, productivities, and yields obtained during lactic acid fermentations of hemicellulosic hydrolysates obtained from prehydrolysis, or autohydrolysis-posthydrolysis followed by detoxification with activated charcoal, using 20 g/L of vinification lees as nutrients.

	Prehydrolysis	Auto-posthydrolysis + detoxification
$\text{Xylose}_{t=0}$ (g/L)	17.3±0.9	13.6±0.5
$\text{Xylose}_{t=\text{final}}$ (g/L)	2.5±0.4	11.4±0.2
$\text{Glucose}_{t=0}$ (g/L)	9.9±0.5	5.7±0.6
$\text{Glucose}_{t=\text{final}}$ (g/L)	0.0±0.0	0.0±0.0
$\text{Lactic acid}_{t=0}$ (g/L)	1.3±0.6	0.5±0.3
$\text{Lactic acid}_{t=\text{final}}$ (g/L)	15.6±0.7	7.2±0.8
$\text{Acetic acid}_{t=0}$ (g/L)	4.6±0.2	5.4±0.4
$\text{Acetic acid}_{t=\text{final}}$ (g/L)	9.1±0.9	6.2±0.7
Time (h)	16	21
Q_P (g/L h)[a]	0.89	0.32
Q_S (g/L h)[a]	1.54	0.38
$Y_{P/S}$ (g/g)[a]	0.58	0.85
$Y_{P/Sth}$ (%)[b]	76.1	95.4

[a] Nomenclature: Q_P, global volumetric productivity of lactic acid; Q_S, volumetric rate of substrates (xylose and glucose) consumption; $Y_{P/S}$, sugars to lactic acid yield [g lactic acid produced/(g xylose consumed + g glucose consumed)].

[b] $Y_{P/Sth}$ = theoretical yield: (g lactic acid produced * 100)/[(g xylose consumed * 0.6) + (g glucose consumed)].

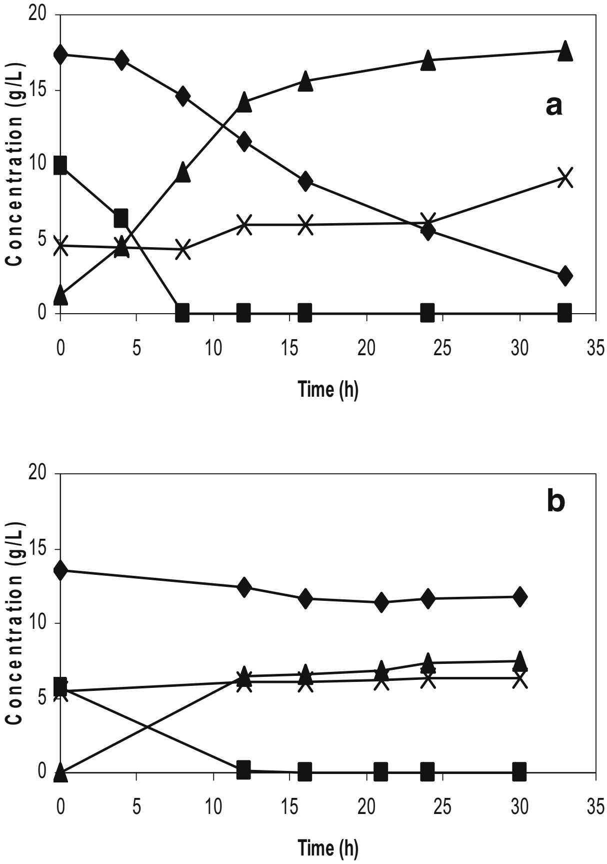


Fig. 3 Kinetic profile of lactic acid and acetic acid production as well as glucose and xylose consumption using media made from prehydrolysis (**a**) or autohydrolysis-posthydrolysis (**b**) liquors, containing 20 g/L of vinification lees as nutrients. The experimental results are the average of three assays, with average deviation lower than 2.3 %. Xylose (♦), glucose (■), lactic acid (▲), acetic acid (*)

When using liquors from prehydrolysis with both the general medium of *Lactobacillus* (Fig. 1a) or vinification lees (Fig. 3a) as nutrients, the results show that no significant differences were observed. The lactic acid concentration decreased only 18.7 % using vinification lees as nutrients (Q_P=0.89 g/L h; Q_S=1.54 g/L h; $Y_{P/S}$=0.58 g/g; $Y_{P/Sth}$=76.1%). These parameters suggest that vinification lees can be used as economic nutrients without affecting strongly the parameters of the process. On the contrary, when using vinification lees as nutrients in detoxified hydrolysates coming from the autohydrolysis-posthydrolysis technology, as it can be in Fig. 3b, negligible xylose consumption was observed, reaching a final lactic acid concentration of only 7.2 g/L, coming from the consume of glucose (Q_P= 0.32 g/L h; Q_S=0.38 g/L h; $Y_{P/S}$=0.85 g/g; $Y_{P/Sth}$=95.4%).

Table 6 Soluble protein content (g/L) in hydrolysates coming from prehydrolysis or autohydrolysis-posthydrolysis, before nutrient supplementation (B) and after nutrient supplementation with Mercier medium (M) or vinification lees (V).

	Hydrolysates from prehydrolysis	Hydrolysates from auto-posthydrolysis	Detoxified hydrolysates from auto-posthydrolysis
B	3.2±0.4	7.1±0.3	1.6 ±0.03
M	11.5±1.1	15.4±1.2	8.2±0.5
V	4.8±0.2	9.6±0.9	2.6±0.1

On the basis of the lower lactic acid concentrations and global volumetric productivities obtained using detoxified hydrolysates from autohydrolysis and vinification lees as nutrients, it can be speculated that vinification lees could contain some compounds that produce a negative synergic effect on lactic acid fermentation.

Finally, Table 6 shows the protein concentration of liquors obtained by prehydrolysis or autohydrolysis-posthydrolysis, before supplementation or after addition of the Mercier Medium or vinification lees. It can be observed that liquors coming from autohydrolysis contain more proteins than liquors coming from prehydrolysis, 7.1 and 3.2 g/L, respectively. This fact, joined to the higher reaction temperature employed during autohydrolysis at prolonged times, could confirm the idea that Maillard reactions are produced during the autohydrolysis treatment, thus reducing the sugar concentration obtained in the hemicellulosic liquors. On the other hand, when hydrolysates coming from autohydrolysis were treated with activated carbon, the protein concentration of liquors decreased from 7.1 g/L to 1.6 g/L, meaning that detoxification can be partially negative in terms of protein removal.

In addition, in spite of the lower protein concentration achieved after supplementation with vinification lees than the one obtained using the Mercier medium, this concentration was enough in experiments conducted with synthetic media and the same microorganism [22]. Thus, lees can be considered an inexpensive source of essential microbial nutrients for *L. pentosus* when using liquors coming from the prehydrolysis treatment, probably because vinification lees provide not only proteins, but also minerals, including Mg, Mn, or Fe, which are present in the Mercier medium [30]. Oppositely, the benefits derived from the detoxification of liquors coming from the autohydrolysis–posthydrolysis with activated charcoal were counteracted with the drastic removal of proteins. This fall was not covered by the addition of lees, explaining consequently the low fermentation parameters achieved under these conditions.

Conclusions

In conclusion, and taking into account the results obtained in this work, prehydrolyis treatment should be recommended for obtaining hemicellulosic sugars from trimming vine shoots compared with autohydrolysis–posthydrolysis process. Prehydrolysis extracted more hemicellulosic sugars from trimming vine shoots. Furthermore, the harsher conditions assayed during the autohydrolysis process and the higher content of protein after this treatment could induce Maillard reactions, which not only decrease the concentration of hemicellulosic sugars in hydrolysates, but also generate Maillard reaction products that could limit the fermentability of hydrolysates.

Besides, fermentations carried out with hemicellulose-containing liquors coming from prehydrolysis can be more efficiently converted into lactic acid by *Lactobacillus pentosus*, even when vinification lees are used as economic nutrients. On the contrary, the results achieved from hemicellulose-containing liquors coming from autohydrolysis–posthydrolysis were worse even if phenolic compounds were removed by detoxification with activated charcoal.

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