

# Wood Hydrolysis and Hydrolysate Detoxification for Subsequent Xylitol Production

By Attilio Converti, José Manuel Domínguez, Patrizia Perego, Silvio Silvério da Silva, and Mario Zilli\*

This study deals with two different aspects of the transformation of lignocellulosics into xylitol: the optimization of conditions for wood hydrolysis and the setting-up of an adequate hydrolyzate detoxification procedure necessary to obtain high xylitol yields in the successive fermentation process. A comparison between the processes of wood auto-hydrolysis (steam explosion) and pre-hydrolysis with dilute sulfuric acid, carried out batch-wise in laboratory scale, shows comparable yields, either in terms of final concentrations of xylose and pentose sugars in the hydrolyzate or of solubilised fraction of wood. On the other hand, notwithstanding the longer time required, the pre-hydrolysis with dilute sulfuric acid produced acid hydrolyzates with lower contents of inhibiting substances (furfural, acetic acid, etc.). In order to obtain satisfactory xylitol yields from the hydrolysate produced by steam explosion, samples of this hydrolyzate were submitted to different detoxification techniques and then fermented batch-wise by a *Pachysolen tannophilus* strain previously adapted to this substrate. The best detoxification was performed by adding to the traditional overliming with  $\text{Ca}(\text{OH})_2$  and sulfite reduction, three steps of a) filtration to remove insoluble substances, b) stripping of acetic acid and furfural, and c) lignin-derived compounds removal by adsorption on charcoal. The fermentation of this hydrolyzate was very effective, achieving a final xylitol concentration of 39.5 g/l from 89.0 g/l xylose after 96 h, corresponding to a volumetric productivity of 0.41 g/lh and a product yield of 0.63 g/g.

## 1 Introduction

A great deal of work has been done during the last decade to develop alternative processes for the integral utilization and revaluation of vegetable biomass. Hardwoods (in particular *Eucalyptus globulus*) demonstrated to be of particular interest, because of their rapid growth as well as of the excellent quality of the wood pulp that can be obtained (1).

Only the cellulose fraction is today utilized for paper manufacture from wood pulp, whereas the lignin and hemicellulose fractions are burnt to produce heat or wasted.

This process could be performed in a more profitable way by fractionating the woody material into these fractions and separately utilizing them in different processes. To this purpose, a wood acid hydrolysis can be performed which leads to: a) a liquid fraction containing sugars (xylose, glucose, mannose, galactose, arabinose, and rhamnose), degradation products (furfural, hydroxymethylfurfural, and phenolic compounds coming from lignin degradation), and acetic and uronic acids (2–6); this portion can be fermented to produce different compounds (mainly ethanol and xylitol) (2–5, 7–12); b) a solid waste, mainly composed by the cellulose and lignin fractions, that can be employed in other different biotransformations (13, 14).

The complete utilisation of the hemicellulose fraction of agro-industrial and forest wastes is one of the main goals of the bioprocesses involved in lignocellulosics conversion. All yeasts are able to ferment glucose, which is the product of cellulose and starch hydrolysis, while xylose, the main component of hemicellulose, can be fermented very slowly by specific yeasts, with low yield and after effective acid hydrolysis (15–21).

Hemicellulose, that can account for up to 50 % by weight of vegetable biomass, contains a mixture of pentoses (mainly xylose) and hexoses (mainly glucose), but several inhibiting by-products are also released during its hydrolysis (22), therefore hemicellulose hydrolyzates require subsequent treatments before fermentation. The growing interest in the bioconversions of these hydrolyzates comes from the possibility of transforming materials, up to now considered undesirable wastes, into high added value-products. Among them, xylitol, because of its food and health-care applications (23–26), is today considered a compound of high commercial interest.

Hemicellulose hydrolyzates can be prepared from wood chips, adequately pre-treated by mechanical methods, by the steam explosion process (17, 27–30) or the pre-hydrolysis with dilute acids (17, 31–34).

The steam explosion process consists of an auto-hydrolysis of wood promoted by the release of organic acids as a consequence of wood chips heating with steam at high pressure. This process, which is able to emphasize the recovery of the hemicellulose fraction, can be improved by hydrolytic pre-treatment with dilute sulfuric acid under vacuum. After water steam treatment at high pressure, the pressure is suddenly released by opening a valve. The wood chips are then subjected to an explosive decompression that causes the separation of the hemicellulose as well as of a little quantity of lignin. After water extraction of hemicellulose sugars, the remaining insoluble residue, which consists mainly of cellulose

[\*] Prof. A. Converti, Dr. P. Perego, Dr. M. Zilli, Università degli Studi di Genova, Dipartimento di Ingegneria Chimica e di Processo “G. B. Bonino”, via Opera Pia 15, 16145 Genova, Italy; Prof. J. M. Domínguez, Universidad de Vigo, Campus de Ourense, Departamento de Ingeniería Química, Edificio Politécnico, As Lagoas, 32004 Ourense, Spain; Prof. S. S. da Silva, Departamento de Biotecnologia, Faculdade de Engenharia Química de Lorena (FAENQUIL), Campus 1, Rodovia Itaiubá-Lorena, km 74,5, 12600-000 Lorena-SP, Brasil.

and lignin, is separated for the successive enzymatic hydrolysis. If necessary, the pentose sugars can be concentrated by a counter-current washing scheme, involving several wood batches. The most important variables are temperature, pressure, and residence time, which are mutually dependent. The main benefits are: a) the production of a solid waste easy to be enzymatically hydrolyzed (35); b) a reduced energy demand with respect to mechanical processes; and c) the lack of environmental and acid recycling costs with respect to chemical treatments (17).

The pre-hydrolysis with dilute acids, which ensures an effective removal of the hemicellulose fraction alone, consists of an acid pre-treatment of the wood, previously reduced into very small particles. This process, which can be carried out either at normal (36) or high pressure (37), implies, if compared with the steam explosion process, higher costs and longer times, but lower amounts of inhibitors are released.

The results of batch tests of *Eucalyptus globulus* wood hydrolysis, carried out under different operative conditions by these two techniques (the second only at atmospheric pressure) are compared in this study and discussed. In addition, a satisfactory detoxification of the hydrolyzate produced by the steam explosion process has been performed by filtration, stripping of acetic acid and furfural, and adsorption of lignin-derived compounds on charcoal. The resulting detoxified hydrolyzate was fermented by an adapted strain of *Pachysolen tannophilus*, giving xylitol yields and productivities comparable with those obtained with the hydrolyzate produced by dilute sulfuric acid pre-hydrolysis.

## 2 Materials and Methods

### 2.1 Steam Explosion Process

To determine the percentages of solubilized wood (SW) as well as of xylose (X) and reducing sugars (RS) released by the *Eucalyptus globulus* wood, finely crushed chips were solubilised under extreme conditions (long hydrolysis time, high sulfuric acid concentration, and high temperature) and then submitted to quantitative analysis, whose results are: 36–39% glucan, 28–35% araban, and 17–20% xylan.

According to the two stage-hydrolysis process suggested by the Tennessee Valley Authority (TVA), samples of wood, previously reduced to 2.5x2.5x0.5 cm average size, were treated under vacuum with dilute sulfuric acid solutions for 30 min (27). Afterwards, the chips were drained and exposed to high pressure steam.

The pressure was then suddenly released, by opening a blowing-off valve and the residues were washed to extract the sugars. To select the optimal hydrolysis conditions, three different operating parameters were varied: the sulfuric acid concentration (5–25 g/l), the hydrolysis time (0.5–5.0 min), and the pressure (9.5–13.5 atm).

### 2.2 Pre-hydrolysis with Dilute Sulfuric Acid

Wood samples, previously milled to give particles smaller than 1 mm average size, were dried at room temperature, mixed with water in a 1 liter-working volume reactor up to a concentration of 100 g/l, and boiled. After addition of concentrated sulfuric acid until the desired concentration, the reaction was carried out under reflux at normal pressure. In order to select the optimal hydrolysis conditions, two different operative parameters were varied: the sulfuric acid concentration (10–150 g/l) and the hydrolysis time (0–22 h).

### 2.3 Detoxification Procedure

The content of inhibiting substances in the hydrolysates was reduced by overliming to pH 10.0 with  $\text{Ca}(\text{OH})_2$ , filtering, acidifying to pH 5.5 with concentrated  $\text{H}_2\text{SO}_4$ , adding sodium sulfite (1 g/l), and finally filtering to remove the precipitate. The pH was then adjusted to 5.5. This TVA detoxification procedure (15) was improved making additional steps aiming at removing specific residual inhibitors.

To remove acetic acid and furfural, a known volume of hydrolyzate, detoxified following the TVA method, was heated at 100 °C for variable time, replacing volume losses with distilled water.

The lignin substances were removed by 1 h treatment with powdered charcoal (Probus, Madrid, Spain) in the ratios 1/205 and 1/10 g/g. The supernatants were recovered by filtration and treated again at the same way with the same amount of charcoal. The liquid phase was recovered by filtration and used for making culture media. The charcoal was activated by boiling in distilled water for 3 h, filtering, and subsequently removing the excess of water by evaporation at room temperature. The exhausted charcoal was regenerated following the same procedure.

### 2.4 Microorganism and Culture Conditions

A *Pachysolen tannophilus* strain (NRRL Y-7426), previously adapted to hemicellulose hydrolyzate, was used in all the fermentations.

The cells were grown in fermentation media containing 10 g/l pure xylose, 3 g/l yeast extract, 3 g/l malt extract, and 5 g/l peptone. The microorganism was maintained in agar slant tubes containing a medium formulated with the same components and concentrations as the previous one plus 20 g/l of agar. The yeast cells were adapted to the detoxified hydrolyzates by carrying out six successive batch cultures using as inoculum the cells recovered from the previous experiment. Before the fermentation, the hydrolyzates were supplemented with 3 g/l yeast extract, 3 g/l malt extract, and 5 g/l peptone, and sterilized in autoclave. The fermentations were conducted at 30 °C under microaerophilic conditions in 100 ml Erlenmeyers flasks (containing 50 ml of culture media) placed in an orbital shaker at 200 rpm.

## 2.5 Analytical Techniques

A semi-quantitative estimation of the removal of lignin-derived compounds was done measuring the hydrolyzate absorbance at 279 nm before and after adsorption on charcoal (38).

At given fermentation times, samples from the fermentation media were taken, centrifuged, filtered through 0.45  $\mu\text{m}$ -membranes, and analyzed by HPLC, using two Shodex SH columns (mobile phase,  $\text{H}_2\text{SO}_4$  0.01 M; flow rate, 0.7 ml/min; IR and UV detection). This method allowed the determination of glucose, xylose, arabinose, acetic acid, ethanol, xylitol, and furfural.

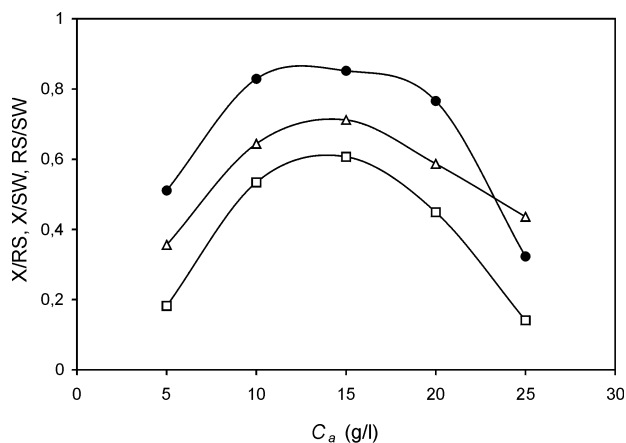
Biomass concentration was determined by dry weight, filtering known volumes of samples of the fermentation broth.

## 3 Results and Discussion

### 3.1 Wood Hydrolysis by the Steam Explosion Process

A series of auto-hydrolysis tests was performed according to the steam explosion process, employing low amounts of dilute sulfuric acid as catalyst. In order to optimize the procedure for preparing the hydrolyzate to be fermented, the acid concentration, the hydrolysis time, and the pressure were parametrically varied.

To study the effect of the sulfuric acid concentration ( $C_a$ ) on the steam explosion process, five tests were carried out exposing the *Eucalyptus globulus* wood for 2 min at a pressure of 12.5 atm and varying  $C_a$  within the range 5–25  $\text{g/l}^1$ . In accordance with the results of Beck and Strickland, who studied the hydrolysis of oak wood [27], the weight percentages (referred to 100 g of raw woody material) of xylose (X), reducing sugars (RS), and solubilized wood (SW) increased with the acid concentration and achieved maximum values at  $C_a = 15 \text{ g/l}$ . Above this threshold, on the contrary, a progressive decrease of them took place, which can be justified by an inhibition of the catalytic phenomenon due to excess acid conditions. The results depicted in Fig. 1 demonstrate that also the hydrolyzate composition was strongly influenced by the catalyst concentration. It can be observed that  $C_a = 15 \text{ g/l}$  was the optimal concentration in terms of hydrolyzate composition, being able to ensure maximum values of the xylose fraction with respect either to total reducing sugars (X/RS) (0.85) or to solubilized wood (X/SW) (0.61) as well as of the reducing sugar fraction with respect to solubilized wood (RS/SW) (0.71). In other words, at this catalyst concentration, not only the fraction of wood which can be hydrolyzed was maximum, but even the hydrolyzate itself was richer in xylose, the sugar used for the successive biotransformation into xylitol.



**Figure 1.** Influence of sulfuric acid concentration on the composition of wood hydrolyzate obtained by the steam explosion process; (●) X/RS; (□) X/SW; (△) RS/SW.

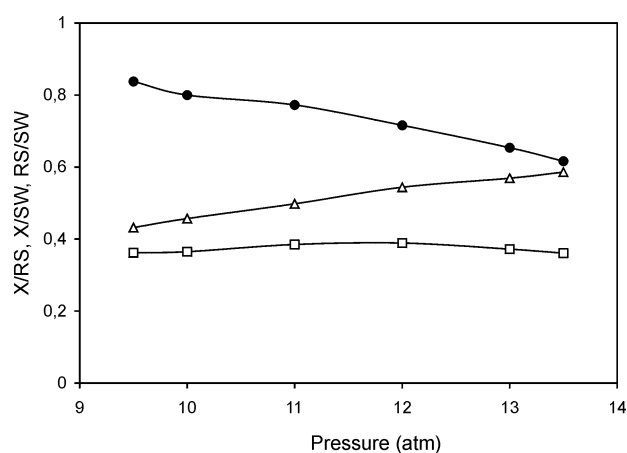
Tab. 1 summarizes the results of hydrolysis tests carried out varying the exposure time at 12.5 atm from 0.5 to 5.0 min and keeping constant the sulfuric acid concentration at the above value. As for the sulfuric acid concentration, also the exposure time to a given pressure played a significant role in wood solubilization. An increase of the exposure time from 0.5 to 3 min made the solubilization progressively more effective (up to about 33%), while longer exposures seemed to stop the process, probably because of a compaction of the woody material which hindered the free penetration of the matrix by the catalyst. The xylose and reducing sugar fractions too followed similar trends. On the other hand, the influence of the exposure time to a given pressure on the hydrolyzate composition was less important than that of sulfuric acid concentration. The xylose fraction with respect to total reducing sugars (X/RS) oscillated around 0.80, with maximum value of 0.85 after only 1.5 min, while the fractions of xylose (X/SW) and reducing sugars (RS/SW) with respect to the solubilized wood kept nearly constant, around 0.55 and 0.68, respectively.

Finally, the percentage of reducing sugars increased almost linearly with the pressure, while the percentage of xylose achieved a nearly constant threshold (12.5%). This means that, contrary to the effect of exposure time, a pressure increase did not affect the catalyst ability to penetrate the matrix but likely favoured the hydrolysis of the cellulose rather than the hemicellulose fraction, thus worsening the process selectivity. As better highlighted in Fig. 2, although a pressure increase did not significantly influence the xylose fraction with respect to the solubilized wood (X/SW), it was the cause of a marked decrease of xylose fraction with respect to total reducing sugars (X/RS) as well as of a corresponding increase of reducing sugar fraction in solubilised wood (RS/SW). Since a greater solubilization of both cellulose and lignin fractions usually leads to a larger release of inhibitors [17, 22], it would be possible to reach an optimal compromise, between the opposite requirements of obtaining hydrolyzates as rich in xylose and devoid of inhibitors as possible, working at an intermediate pressure of 11–12 atm.

1) List of symbols at the end of the paper.

**Table 1.** Effect of the exposure time ( $t$ ) to a pressure of 12.5 atm on the percentages of xylose (X), reducing sugars (RS), and solubilised wood (SW) released during the steam explosion process.  $C_a = 15$  g/l.

$t$ (min)	X (%)	RS (%)	SW (%)
0.5	9.5	12.2	17.5
1.0	12.8	15.4	22.7
1.5	15.8	18.5	27.1
2.0	17.7	20.8	30.1
3.0	17.8	22.1	32.7
4.0	17.9	22.5	33.0
5.0	18.0	22.8	33.2



**Figure 2.** Influence of the pressure on the composition of wood hydrolyzate obtained by the steam explosion process; (●) X/RS; (□) X/SW; (Δ) RS/SW.

The final step of this former part of the work has been focused on the release of inhibitors during the steam explosion process. No significant variation of the inhibitors concentrations was observed varying the three operating variables, with the only exceptions being the tests carried out at high pressure (13–14 atm) which evidenced excessive release of inhibitors. The average values of acetic acid and furfural concentrations in hemicellulose hydrolyzates prepared by steam explosion and final counter-current operation were 31.2 and 1.2 g/l, respectively, after 4 min exposure to 12.5 atm and using a 10 g/l sulfuric acid solution.

### 3.2 Wood Hydrolysis by Dilute Sulfuric Acid Pre-hydrolysis

Wood hydrolysis with dilute acids is under investigation since a very long time. A good deal of research was addressed to different types of woody materials and different acids, sometimes ensuring solubilization yields close to the theoretical values [3, 39–41]. In a similar study, performed using sulfuric acid solutions and hydrolysis times lower than the ones explored in this work, Parajó and co-workers

suggested  $C_a = 10\%$  and  $t = 10$  h as the optimal values of these parameters for the solubilization of *Eucalyptus globulus* wood [36].

Tab. 2 shows the results of two hydrolysis tests carried out for 22 h using two different sulfuric acid concentrations ( $C_a = 40$  and 70 g/l). As for steam explosion, a longer hydrolysis led to a more effective wood solubilization as well as to an hydrolyzate with higher xylose and reducing sugar levels, but the time required by the pre-hydrolysis at normal pressure was much longer, even if the sulfuric acid solution was much more concentrated. It was demonstrated that the release of reducing sugars from a lot of different woody materials can kinetically be described by a two-step mechanism [36, 42–45], with a first step (referred to as an easily-degradable hemicellulose fraction) which is more than one order of magnitude faster than the second (referred to on the other hand, as a recalcitrant hemicellulose fraction). The data of Tab. 2 appear to be consistent with this mechanism as well, showing a starting rapid growth of all the three percentages (X, RS, and SW) up to 6 h, followed by a marked deceleration.

The sugar composition of the hydrolyzate obtained with  $C_a = 70$  g/l was similar to that of the hydrolyzate obtained by steam explosion, with the X/RS, X/SW, and RS/SW ratios ranging between 0.75–0.84, 0.45–0.61, and 0.59–0.73, respectively. On the other hand, the hydrolyzate obtained with  $C_a = 40$  g/l contained less xylose, thus evidencing a worse solubilization of the hemicellulose fraction as well as a lower hydrolysis selectivity. In addition, all these fractions progressively increased with the time, which means that a longer hydrolysis improved wood solubilization and gave a hydrolyzate richer in xylose, that is more suited for the subsequent biotransformation. In spite of this advantage, since an increase of the hydrolysis time reduces the productivity, an optimum hydrolysis duration could be close to the value (10 h) suggested by Parajó and co-workers [36].

The results of hydrolysis tests carried out for 4 and 10 h with sulfuric acid concentrations increasing from 10 to 150 g/l are gathered in Tab. 3. All three percentages progressively increased with sulfuric acid concentration after a given hydrolysis time. In particular, it should be noted that the solubilized fraction of the wood with  $C_a = 150$  g/l was higher than that obtained by steam explosion and approached the theoretical value. An increase of sulfuric acid concentration influenced the hydrolyzate composition less significantly than the hydrolysis time. In fact, both xylose fractions with respect to total reducing sugars (X/RS) and solubilized wood (X/SW) slowly increased, while no relevant effect was observed on the reducing sugar content of the hydrolyzate (RS/SW). On the analogy to the hydrolysis time, these results would suggest to use high sulfuric acid concentrations in order to ensure a more effective wood solubilisation and to obtain an hydrolyzate having a higher xylose content. However, also in this case it should be considered that the acid cost is one of the most important items of the total cost of the pre-hydrolysis process, and then using acid concentrations higher than 100 g/l could be very expensive. In addition, the higher the acid concentration,

**Table 2.** Dependence of the percentages of xylose (X), reducing sugars (RS), and solubilised wood (SW) on the duration of wood pre-hydrolysis with dilute sulfuric acid.

Time (h)	$C_a = 40 \text{ g/l}$			$C_a = 70 \text{ g/l}$		
	X (%)	RS (%)	SW (%)	X (%)	RS (%)	SW (%)
0.5	2.4	4.4	7.0	5.4	7.2	12.1
1	5.6	7.6	11.8	8.2	10.5	17.0
2	8.4	10.8	16.7	10.3	13.5	21.5
4	10.6	13.6	20.9	11.9	15.8	24.2
6	11.5	15.0	22.5	12.9	16.4	25.1
8	12.1	15.9	23.2	13.6	17.5	25.6
10	12.7	16.5	23.8	14.2	18.0	25.9
14	13.1	17.5	24.7	15.2	18.6	26.2
18	13.6	18.3	25.2	15.8	19.0	26.4
22	13.8	18.8	25.7	16.3	19.3	26.6

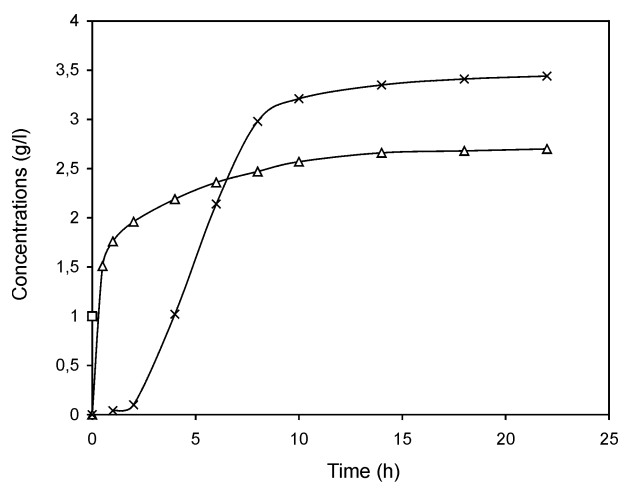
**Table 3.** Dependence of the percentages of xylose (X), reducing sugars (RS), and solubilised wood (SW) on the concentration of sulfuric acid used for wood pre-hydrolysis.

$C_a$ (g/l)	$t = 4 \text{ h}$			$t = 10 \text{ h}$		
	X (%)	RS (%)	SW (%)	X (%)	RS (%)	SW (%)
10	9.3	12.7	19.8	11.7	15.5	21.9
20	9.8	13.0	20.2	11.9	15.8	22.3
40	10.7	13.7	21.3	12.5	16.4	23.4
70	12.5	15.8	24.1	14.0	18.0	25.7
100	14.9	19.9	28.5	16.6	20.6	30.0
125	17.3	21.6	33.0	19.3	23.3	34.5
150	19.7	24.4	38.1	22.2	26.7	40.0

the higher the amounts of  $\text{Ca}(\text{OH})_2$  necessary for overliming and of sludge produced by this operation.

An additional test of wood pre-hydrolysis was carried out with  $C_a = 100 \text{ g/l}$  to study the release of substances able to inhibit the fermentation process. Like xylose and the other reducing sugars, also the acetic acid, coming from the acetyl groups associated to the hemicellulose fraction, was released following two-stage kinetics (Fig. 3). At the end of hydrolysis, an acetic acid concentration (2.7 g/l) lower than that (7.0 g/l) proposed by Watson and co-workers as an inhibiting threshold for ethanol production by *Pachysolen tannophilus* was reached [46], which means that the hydrolyzate produced with dilute acid pre-hydrolysis did not require any additional treatment to remove this inhibitor.

Furfural is another powerful inhibitor of the fermentation, therefore a lot of procedures were set up in the past to reduce its negative effects, such as increase of inoculum, microbial adaptation to the substrate, and different chemical and


**Figure 3.** Release of inhibitors during the pre-hydrolysis of *Eucalyptus globulus* wood carried out at  $C_a = 100 \text{ g/l}$ . ( $\Delta$ ) Acetic acid concentration (g/l); ( $\times$ ) Furfural concentration  $\times 10$  (g/l).

physical treatments [5, 9, 11, 12, 46]. Although the inhibition effects depend on the other components of the hydrolyzate as well as on the selected microorganism, Ackerson and co-workers proposed, as inhibition threshold for ethanol-producing yeasts, furfural concentrations around 0.030–0.046% [39], a range which is quite higher than the values detected in this study in hydrolyzates prepared by dilute sulfuric acid pre-hydrolysis of wood (Fig. 3).

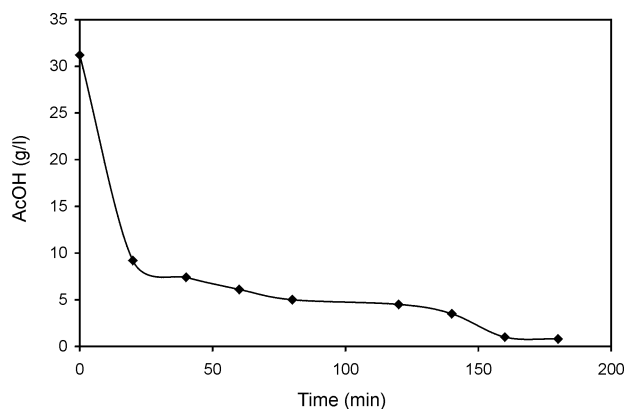
### 3.3 Detoxification of Hemicellulose Hydrolyzates Produced by Steam Explosion

The hydrolyzates produced by steam explosion, containing high concentrations of fermentation inhibitors, have been diluted up to a half of the starting concentration (to reduce the power of inhibitors) and detoxified by overliming. It is known, in fact, that this procedure is able to ensure positive effects on the hydrolyzate fermentation, among which a partial removal of acid compounds (acetic and tannic acids) and phenolic compounds [47], an effective precipitation of heavy metal ions [48], and the conversion of furfural into furfuryl acid [15, 48, 49].

Although the overliming ensured the removal of more than 60% of the lignin-derived compounds (LDC), the yeast *P. tannophilus* was able to metabolize xylose only partially, producing negligible amounts of xylitol at a volumetric productivity only of 0.02 g/lh (Tab. 4).

To overcome the inhibiting effect of LDC, the hydrolyzates were treated with charcoal as described by Parajó and co-workers [50]. The results of Tab. 4 show that the higher the amount of charcoal used to treat a given volume of hydrolyzate, the higher the fraction of removed LDC. At a charcoal/hydrolyzate ratio of 1/10 g/g more than 95% of LDC were removed, while no significant additional elimination of LDC (97.7% removal) was observed increasing this ratio up to 1/5 g/g, in spite of a redoubling of the treatment costs.

The elimination of acetic acid by evaporation was the last detoxification procedure tested in this study. The inhibiting power of this acid depends on the concentration of its undissociated form, which is function of both total concentration ( $C_a$ ) and pH and varies from one species to the other. The evaporation can accelerate the fermentation process [51] by removing acetic acid, furfural, and some other volatile compounds [12, 15, 47]. To remove acetic acid from the fermentation broth, the hydrolyzate was boiled for 3 h, during which samples were drawn every 20 min. As shown in Figure 4, a boiling time of 160 min was sufficient to reduce the acetic acid concentration in the hydrolyzate from 31.2 to 1.0 g/l, that is to a value even lower than the threshold of complete *P. tannophilus* growth inhibition (1.45 g/l) [46]. At the same time, furfural concentration decreased from 1.2 to less than 0.5 g/l.



**Figure 4.** Decrease of acetic acid concentration as function of stripping time in hemicellulose hydrolyzate obtained by steam explosion.

These promising results suggested to test the effects of additional detoxification treatments on the fermentability of the concentrated hydrolyzate (without any preliminary dilution or acetic acid stripping). A set of final batch tests was then carried out with and without treating the hydrolyzate with charcoal, in order to evaluate separately the inhibiting effects of acetic acid and of acetic acid together with LDC, whose results are summarized in Table 5. Notwithstanding the absence of acetic acid, the lignin-derived compounds strongly inhibited the bioconversion, either without adsorption on charcoal or using a very low charcoal/hydrolyzate ratio (1/205 g/g). On the contrary, when using a charcoal/hydrolyzate ratio of 1/10 g/g, the fermentation was very effective, achieving a final xylitol concentration of 39.5 g/l from 89.0 g/l xylose after 96 h, corresponding to a volumetric productivity of 0.41 g/lh and a product yield of 0.63 g/g.

## 4 Conclusion

The results of *Eucalyptus globulus* wood hydrolysis by steam explosion and by dilute sulfuric acid at atmospheric pressure are compared and discussed in this work. In the light of the results obtained, it can be concluded that the steam explosion process is able to ensure a quicker wood hydrolysis, but the hydrolyzates produced by this way require stronger detoxification treatments.

Among the detoxification treatments tested to enhance the fermentability of steam explosion hydrolyzates, the over-

**Table 4.** Parameters of xylitol production by *P. tannophilus* from hemicellulose hydrolyzates obtained by steam explosion and detoxified with overliming and subsequent adsorption on charcoal.

Charcoal/Hydrolyzate Ratio (g/g)	$S_0$ (g/l)	Time (h)	$S$ (g/l)	Xyt (g/l)	$Q$ (g/lh)	$Y_{P/S}$ (g/g)	Removed LDC Fraction
0	53.6	158.3	45.7	3.1	0.02	0.46	0.611
1/205	59.5	115.3	12.0	12.5	0.11	0.26	0.660
1/10	44.3	115.3	3.5	19.9	0.17	0.49	0.954

**Table 5.** Parameters of xylitol production by *P. tannophilus* from hemicellulose hydrolysates obtained by steam explosion and detoxified in different ways. OL = Overliming; ST = Acetic acid stripping; AC = Adsorption on charcoal.

Treatment	$S_0$ (g/l)	$S$ (g/l)	Xyt (g/l)	$Q$ (g/lh)	$Y_{P/S}$ (g/g)
OL	92.0	84.1	3.1	0.018	0.392
OL + ST	92.0	45.6	20.2	0.122	0.436
OL + ST + AC (1/205)	91.0	40.0	21.2	0.128	0.415
OL + ST + AC (1/10)	89.0	15.6	39.5	0.411	0.627

liming and the treatment on charcoal are more suitable for the removal of lignin-derived compounds, while the simple boiling is sufficient to reduce the acetic acid and furfural concentrations below inhibitory levels. Combining these detoxification techniques allowed to perform a fermentation to xylitol to be performed with yields comparable to those which can be obtained with hydrolysates produced by dilute sulfuric acid at atmospheric pressure.

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## Symbols used

$C_a$	[g/l]	Concentration of sulfuric acid
$P$	[atm]	Pressure
$Q$	[g/lh]	Volumetric productivity
RS	[%]	Percentage of reducing sugars in the hydrolyzate referred to 100 g of wood
RS/SW		Fraction of reducing sugars in the hydrolyzate referred to the solubilised fraction of wood
$S$	[g/l]	Substrate (xylose) concentration
$S_0$	[g/l]	Starting substrate (xylose) concentration
SW	[%]	Percentage of solubilized wood referred to 100 g of wood
$t$	[min or h]	Time
X	[%]	Percentage of xylose in the hydrolyzate referred to 100 g of wood
X/RS		Fraction of xylose in the hydrolyzate referred to total reducing sugars
X/SW		Fraction of xylose in the hydrolyzate referred to the solubilized fraction of wood
Xyt	[g/l]	Xylitol concentration
$Y_{P/S}$	[g/g]	Yield of product on substrate

## Abbreviations

AC	Adsorption on charcoal
AcOH	Acetic acid
LDC	Lignin-derived compounds
OL	Overliming
ST	Acetic acid stripping
TVA	Tennessee Valley Authority

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