0273-2289/1559-0291 (Online)/06/135/0219/$30.00

Complete Bioconversion of Hemicellulosic Sugars From Agricultural Residues Into Lactic Acid by *Lactobacillus pentosus*

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Received December 13, 2005; Revised March 3, 2006; Accepted March 31, 2006

Abstract

On the basis of previous knowledge, different agroindustrial wastes were submitted to dilute-acid hydrolysis with H_2SO_4 to obtain hemicellulosic sugars and then employed for lactic acid production by *Lactobacillus pentosus*. Toxic compounds released from lignin did not affect lactic acid fermentation when hydrolysates from trimming vine shoots, barley bran husks, or corncobs were employed as carbon source, and complete bioconversion of hemicellulosic sugars was achieved. Nevertheless, *Eucalyptus globulus* hydrolysates had to be submitted to a detoxification process with activated charcoal. Maximum lactic acid concentration (33 g/L) was reached employing barley bran hydrolysates, whereas corncobs, trimming vine shoots, and detoxified *E. globulus* hydrolysates yielded 26, 24, and 14.5 g/L of lactic acid, respectively. The maximum product yield from pentoses (0.76 g/g) was achieved using hydrolysates from trimming vine shoots, followed by hydrolysates from detoxified *E. globulus* (0.70 g/g), barley bran (0.57 g/g), and corncob (0.53 g/g). These results confirm that *L. pentosus* can be employed to ferment hemicellulosic sugars (mainly xylose, glucose, and arabinose) from acid hydrolysates of most agricultural residues without appreciable substrate inhibition.

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Index Entries: Trimming vine shoots; barley bran husks; corncobs; *Eucalyptus globulus*; lactic acid; *Lactobacillus pentosus.*

Introduction

Lignocellulosic agroindustrial residues such as trimming vine shoots, barley bran husks, corncobs, and *Eucalyptus globulus* chips are an abundant renewable energy source to obtain hemicellulosic sugars *(1–4)*. Some microorganisms can ferment hemicellulosic sugars into food additives such as lactic acid and bacteria *(4–6)*. Before bioconversion, a pretreatment is usually necessary to break down the complex structure of the three major polymeric constituents—cellulose, hemicellulose, and lignin—in which lignin has the function of binding cellulose and hemicellulose together in the vascular tissue of the plant. Different pretreatment methods have been proposed for the fractionation of lignocellulose, and dilute-acid hydrolysis is one of the processes of major concern *(4–9)*. During acid hydrolysis of lignocellulosic materials, it is important to select an adequate pretreatment to solubilize the hemicellulosic sugars, thus avoiding the formation of toxic compounds such as furfural and hydroxymethylfurfural (HMF). For this reason, it is usually performed with mineral acids such as dilute H_2SO_4 or HCl at concentrations in the range of 2–5% and temperatures lower than 160°C *(9)*.

Bioconversion of cellulosic sugars into lactic acid by different *Lactobacilli* has been described widely. Some investigators have proposed the fermentation of different agricultural residues into lactic acid employing *Lactobacillus rhamnosus (10,11)*. Others have proposed the bioconversion of *E. globulus* wood into lactic acid through simultaneous saccharification and fermentation and have achieved good yields and productivities *(12)*. Most studies of lactic acid production from lignocelluosic materials utilize only the cellulosic fraction, because there are few microorganisms capable of fermenting hemicellulosic sugars. In addition, some investigators have reported that the presence of glucose at concentrations greater than 5 g/L in the fermentation medium repressed the bioconversion of xylose into lactic acid by *Lactobacillus xylosus (5)*. This is an additional drawback affecting the effective bioconversion of hemicellulosic sugars from agricultural residues, because hemicellulosic liquids are usually made up of mixtures of glucose, xylose, and arabinose.

The present study focused on the bioconversion of hemicellulosic sugars from different agroindustrial wastes into lactic acid by *Lactobacillus pentosus*.

Materials and Methods

Hydrolysis of Lignocellulosics

Samples of trimming vine shoots, barley bran husks, corncobs, and *E. globulus* chips were dried; milled to a particle size of less than 1 mm; homogenized in a single lot, to avoid compositional differences; and stored until use.

Conditions selected for the acidic hydrolysis of the different lignocellulosic residues were based on previous works *(1–4,13)*. Trimming vine shoots and barley bran husks were treated with 3% H_2SO_4 for 15 min, corncobs with 2% H_2SO_4 for 15 min, and *E. globulus* chips with 3% H_2SO_4 for 60 min. In all cases, hydrolysates were obtained by thermal treatment in an autoclave at 130°C using a liquid/solid ratio of 8 g/g, and prehydrolysis was assumed to start at the beginning of the isothermal operation.

Detoxification of Hemicellulosic Hydrolysates of E. globulus

Neutralized hydrolysates obtained from *E. globulus* chips were mixed with 15% weight of charcoal (Probus, Madrid, Spain) and stirred for 1 d at room temperature. The liquors were recovered by filtration.

Aliquots of the same hydrolysates (50 mL) were alternately extracted in duplicate with 150 mL of ethyl acetate in 500-mL baffled Erlenmeyer flasks placed on an orbital shaker at 300 rpm and at a temperature in the range of 40–50°C. The pH of the hydrolysates was adjusted to 3.0 with powdered $Ca(OH)_2$. Organic phases were vacuum evaporated at temperatures lower than 40°C. Aqueous phases from extraction were used to prepare culture media.

Microorganism

L. pentosus CECT-4023T (ATCC-8041) was obtained from the Spanish Collection of Type Cultures (Valencia, Spain). The strain was grown at 31°C for 24 h on plates using the complete medium proposed by lactic acid bacteria containing 20 g/L of glucose, 5 g/L of yeast extract, 10 g/L of peptone, 5 g/L of sodium acetate, 2 g/L of sodium citrate, 2 g/L of K_2HPO_4, 0.58 g/L of $MgSO_4 \cdot 7H_2O$, 0.12 g/L of $MnSO_4 \cdot H_2O$, 0.05 g/L of $FeSO_4 \cdot 7H_2O$, and 20 g/L of agar *(14)*. Inocula were prepared by preliminary suspension of cells from plates in 5 mL of sterile hydrolysates. Biomass concentration was determined by optical density measurements at 600 nm, and final dilution with sterile hydrolysates to the selected inoculum concentration (4.0 g/L) was done.

Fermentations

Hydrolysates selected for fermentations were neutralized with powdered $CaCO_3$ to a final pH of 6.0, and the $CaSO_4$ precipitated was separated from the supernatant by filtration. The clarified liquors were supplemented with 10 g of yeast extract/L and 10 g of corn steep liquor/L, sterilized in an autoclave for 15 min at 121°C, and used directly as fermentation media. Experiments were carried out at 31°C in a 2-L Biostat B batch reactor (Braun, Melsungen, Germany) with a 1.0-L working volume and at 150 rpm. During fermentation, the pH was controlled at 6.0 by the addition of 4 *M* NaOH. Samples (2 mL) were taken at given fermentation times and centrifuged at 6000 rpm for 3 min. The supernatants were stored for analysis.

Fermentations were carried out in triplicate, and the corresponding results were reported as mean values. Standard deviations were less than 2.6% of the mean.

Analytical Methods

Glucose, xylose, arabinose, acetic acid, furfural, HMF, and lactic acid were measured by a high-performance liquid chromatograph (model 1100; Agilent, Palo Alto, CA) equipped with a refractive index detector and a column model ION-300 (Transgenomic, San Jose, CA) eluted with 0.02 *M* H_2SO_4 at a flow rate of 0.4 mL/min at 50°C.

Results and Discussion

Lactic Acid Production From Hemicellulosic Sugars

Lignocellulosic biomass wastes are potential substrates for lactic acid fermentation with microorganisms *(4,10–12)*. The process requires a pretreatment of biomass to open up the lignocellulosic polymers, in order to allow their hydrolysis by either enzymes or acids, followed by fermentation of the hexose and pentose sugars derived from the cellulose and hemicellulose fractions, respectively. As is well known, the hemicellulosic sugars can be obtained either by enzymatic hydrolysis, which is slow and requires a large amount of enzymes, or by employing acid hydrolysis, which is faster but results in many degradation products that can inhibit the bioprocess. When some investigators submitted wheat straw to acidic or enzymatic hydrolysis, the content of glucose and arabinose in hydrolysates after hydrolysis was nearly the same in both cases, but xylose concentration was 10-fold higher in acidic hydrolysates *(6)*. Owing to xylose isomerase activity of the enzyme preparation, such an enzymatic treatment led to the release of xylulose instead of xylose; therefore, because *Lactobacillus brevis* is unable to ferment xylulose, higher lactic acid yield was obtained using acidic treatment.

On the basis of previous works *(1–4,6,14)* the hemicellulosic sugars were obtained by acid hydrolysis rather than by enzymatic hydrolysis. It should be noted that the maximum temperature (130°C) and H_2SO_4 concentration (3%) employed in our study were lower than those proposed by other investigators *(9)*, whereas Garde et al. *(6)* employed *L. pentosus* and *L. brevis* for lactic acid production from straw hemicellulosic hydrolysates after treatment with 4% H_2SO_4 at 100°C for 2 h.

Figure 1A–C shows the kinetic profile during lactic acid fermentation of barley bran husk, corncob, and trimming vine shoot hemicellulosic hydrolysates, respectively. In these cases, *L. pentosus* first fermented glucose through the homofermentative pathway, and only lactic acid was produced. When glucose was depleted, *L. pentosus* started to ferment xylose and arabinose, and all the sugars were completely consumed. Table 1 shows the kinetic parameters calculated in each run after a fermentation time

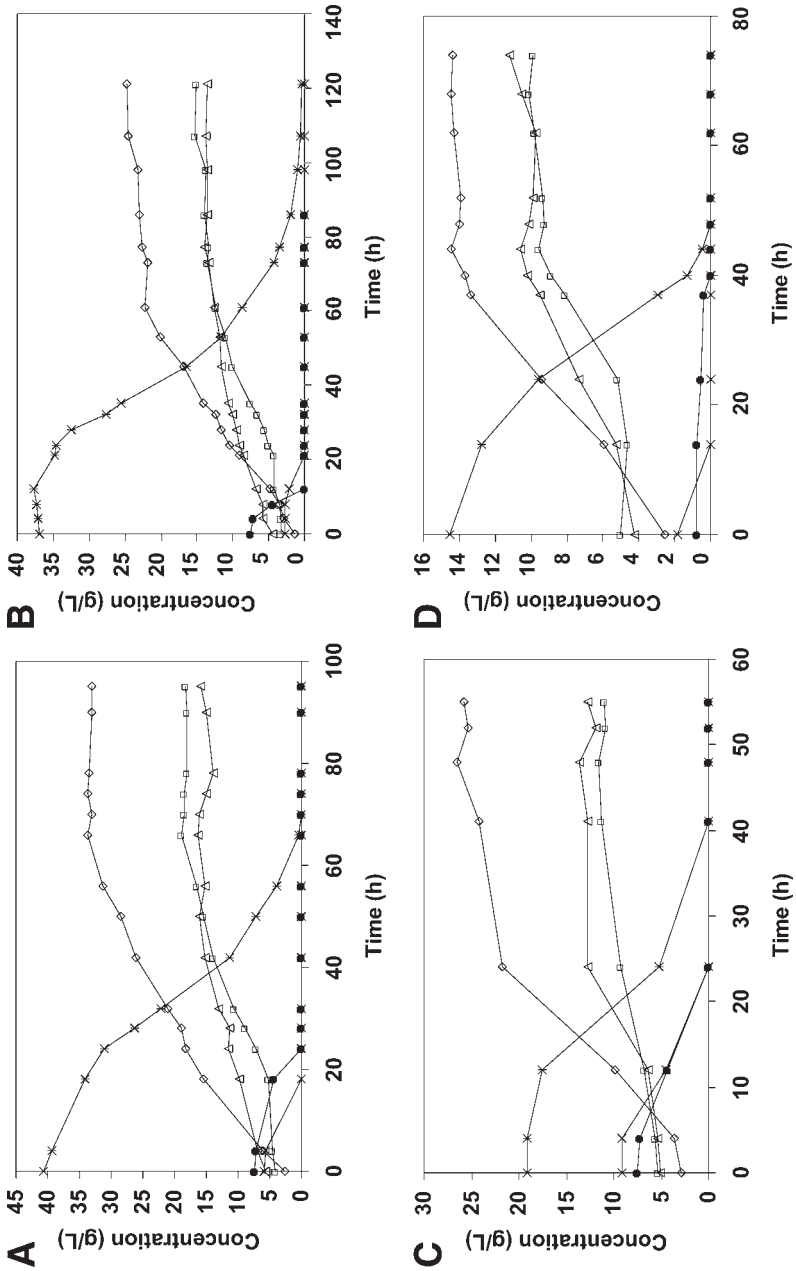

Fig. 1. Kinetic profiles achieved during lactic acid fermentation of **(A)** hemicellulosic hydrolysates of barley bran husks, **(B)** hemicellulosic hydrolysates of corncobs, **(C)** hemicellulosic hydrolysates of trimming vine shoots, and **(D)** hemicellulosic hydrolysates from *E. globulus* detoxified with activated charcoal using *L. pentosus*: (◇) lactic acid; (□) acetic acid; (△) biomass; (×) glucose; (*) xylose; (●) arabinose.

Table 1
Final Product Concentrations, Productivities, and Yields
of Fermentations of Hemicellulosic Fractions of Different Acidic Hydrolysates by *L. pentosus*[a]

Residue	Lac_{max} (g/L)	Ac_{max} (g/L)	X_{max} (g/L)	Q_{Lac} (g/[L·h])	Q_{Ac} (g/[L·h])	Qx (g/[L·h])	$Y_{Lac/S}$	$Y_{Ac/S}$	$Y_{X/S}$
Barley bran husks	33.7	19.0	16.4	0.60	0.18	0.22	0.57	0.27	0.20
Corncobs	24.7	15.3	13.8	0.34	0.10	0.18	0.53	0.27	0.21
Vine shoots	26.5	11.6	13.6	0.51	0.12	0.16	0.76	0.20	0.27
E. globulus chips	3.79	5.67	2.02	0.10	0.002	0.033	0.069	0.007	0.032
E. globulus chips extracted with ethyl acetate	3.58	5.20	1.98	0.081	0.001	0.028	0.046	0.003	0.021
E. globulus chips treated with activated charcoal	14.5	10.1	11.2	0.28	0.13	0.072	0.70	0.30	0.40

[a]Results refer to fermentation times corresponding to maximum lactic acid concentrations. Lac_{max} = maximum lactic acid concentration; Ac_{max} = maximum acetic acid concentration; X_{max} = maximum biomass concentration; Q_{Lac} = lactic acid volumetric productivity; Q_{Ac} = acetic acid volumetric productivity; Q_x = biomass volumetric productivity; $Y_{Lac/S}$ = lactic acid yield from sugars; $Y_{Ac/S}$ = acetic acid yield from sugars; $Y_{X/S}$ = biomass yield from sugars.

corresponding to maximum product formation. The maximum lactic acid concentration (33.7 g/L) was achieved employing barley bran hydrolysates, whereas corncob and trimming vine shoot hydrolysates yielded only 26.5 and 24.7 g/L of lactic acid, respectively. Nevertheless, these values are higher than those obtained in other works using wheat straw hydrolysates, in which concentrations of about 8 g/L were achieved using a coculture of *L. pentosus* and *L. brevis*, whereas it was as low as 6.7 g/L when fermentation was performed using *L. pentosus* alone *(6)*.

In the present work, the theoretical yield of lactic acid from pentoses varied between 0.53 g/g using corncob hydrolysates and 0.76 g/g using trimming vine shoot hydrolysates. These values are similar to those obtained in other work *(6)*, in which a yield of 0.6 g/g and phosphoketolase pathway behavior were reported. *L. pentosus* does in fact belong to the facultative, heterofermentative *Lactobacillus* species that are characterized by the ability to ferment hexoses through the Embden-Mayerhof-Parnas pathway and use the phosphoketolase pathway for pentose uptake *(4,15)*. This means that *L. pentosus* can produce under optimal conditions 2 mol of lactate/mol from glucose and 1 mol of lactate/mol plus 1 mol of acetate/mol from xylose or arabinose. It must be pointed out that *L. pentosus* also showed good lactic acid volumetric productivities (between 0.34 g/[L·h] using corncob hydrolysates and 0.60 g/[L·h] using barley bran hydrolysates) when fermenting xylose and arabinose independently of the amount of the glucose present in the media.

On the other hand, negligible amounts of lactic acid were produced when *L. pentosus* fermented *E. globulus* chip hydrolysates, giving a lactic acid productivity of only 0.10 g/(L·h). Because the reaction time during acid hydrolysis was four times longer than for the other agricultural residues, a strong inhibitory effect on the fermentation was likely exerted by toxic compounds present in these hydrolysates at high levels. Severe conditions are in fact reported in the literature for the acid hydrolysis of *Eucalyptus*, owing to particularly stronger links among cellulose, hemicellulose, and lignin than in other lignocellulosic residues *(4)*. Consequently, to ferment these media, the hydrolysate had to be submitted to a detoxification step.

Detoxification of Hemicellulosic Hydrolysates From E. globulus *Chips*

Temperature, pressure, and acid concentrations play important roles in the formation of toxic compounds during acid hydrolysis of lignocellulosic materials. At high temperatures and pressures, glucose and xylose can in fact be degraded to furfural and HMF, respectively. Further degradation of these byproducts can lead to the formation of formic and levulinic acids, whereas phenolic compounds can be generated from partial breakdown of lignin *(9)*. Besides these compounds, other substances are formed during hydrolysis that are toxic to fermenting microorganisms *(7,9,16–18)*.

To remove inhibitory compounds, *E. globulus* hydrolysates were submitted to detoxification processes using either ethyl acetate or activated charcoal. Other investigators have successfully employed these detoxifica-

tion methods to remove toxic elements from hemicellulosic hydrolysates. For instance, in some works *(16)* the bioconversion of hemicellulosic sugars into xylitol with *Debaryomyces hansenii* was improved by treating *E. globulus* hydrolysates with activated charcoal, whereas in others cases the utilization of organic solvents for the extraction of phenolic compounds from different agricultural residues was proposed *(18)*.

When hydrolysates were detoxified with ethyl acetate, *L. pentosus* was not able to ferment the hemicelluosic sugars, and almost the same fermentation profile as in undetoxified hydrolysates was observed (Table 1). Thus, at the end of the fermentation, the lactic acid concentration was only 3.58 g/L with a volumetric productivity of 0.081 g/(L·h) and a product yield of 0.046 g/g. This could be owing to the fact that ethyl acetate did not entirely remove the toxic compounds released from this kind of hydrolysate. On the contrary, when hemicellulosic hydrolysates from *E. globulus* were detoxified with activated charcoal, interesting fermentation profiles were obtained and hemicellulosic sugars were completely consumed (Fig. 1D). Maximum lactic acid production from hemicellulosic sugars was 0.70 g/g after detoxification; this value is comparable with that (0.76 g of lactate/g of sugars) obtained from the fermentation of nondetoxified barley bran hemicellulosic hydrolysates.

Conclusion

Hemicellulosic sugar solutions obtained by acid prehydrolysis of raw trimming vine shoot, corncob, and barley bran husk samples were used for making fermentation media useful for lactic acid production by *L. pentosus* CECT-4023T (ATCC-8041). Lignin-derived byproducts were likely released at very low levels using this technique, and the fermentations were effectively performed. Nevertheless, the hydrolysates obtained by acidic hydrolysis of *E. globulus* chips had a toxic effect on the microorganism, and physicochemical treatments were needed to detoxify the hydrolysates. Extraction of these inhibitory substances with ethyl acetate was not effective, but detoxification with activated charcoal provided suitable culture media to carry out properly fermentation by *L. pentosus*.

Finally, it is important to stress that *L. pentosus* provided excellent results in terms of overall productivity and product yield when fermenting hemicellulosic sugars, such as xylose and arabinose, independently of the amount of the glucose present in the media.

Acknowledgments

We are grateful to the following institutions for financial support of this work: the Spanish Ministry of Science and Technology (project PPQ2003-02802, which has partial financial support from the FEDER funds of the European Union), Xunta de Galicia (PGIDIT04PXIC38302PN), and the Isidro Parga Pondal Program financed by the Xunta de Galicia of Spain.

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