Appl Biochem Biotechnol (2009) 153:163–170
DOI 10.1007/s12010-009-8548-5

Biotechnological Production of Xylitol: Enhancement of Monosaccharide Production by Post-Hydrolysis of Dilute Acid Sugarcane Hydrolysate

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Received: 6 May 2008 / Accepted: 21 January 2009 /
Published online: 12 February 2009

Abstract Dilute-acid hydrolysis pretreatment of sugarcane bagasse resulted in release of 48% (18.4 g/L) of the xylan in the hemicellulose fraction into the hydrolysate as monomeric xylose. In order to enhance the recuperation of this monomer, a post-hydrolysis stage consisted of thermal treatment was carried out. This treatment resulted in an increase in xylose release of 62% (23.5 g/L) of the hemicellulose fraction. Original and post-hydrolysates were concentrated to the same levels of monomeric xylose in the fermentor feed. During the fermentation process, cellular growth was observed to be higher in the post-hydrolysate (3.5 g/L, $Y_{x/s}$=0.075 g cells/g xylose) than in the original hydrolysate (2.9 g/L, $Y_{x/s}$=0.068 g cells/g xylose). The post-treated hydrolysate required less concentration of sugars resulting in a lower concentration of fermentation inhibitors, which were formed primarily in the dilute acid hydrolysis step. Post-hydrolysis step led to a high xylose–xylitol conversion efficiency of 76% (0.7 g xylitol/g xylose) and volumetric productivity of 0.68 g xylitol/L h when compared to 71% (0.65 g xylitol/g xylose and productivity of 0.61 g xylitol/L h) for the original hemicellulosic hydrolysate.

Keywords Dilute acid hydrolysis · Post-hydrolysis · Xylose monomers · Inhibitors · Xylitol

Introduction

Hemicelluloses are a plant cell wall polysaccharide and the third most abundant renewable polymer in nature. In some plants, it comprises up to 40% of the total dry material. Hemicellulose is consisted of short-branched heteropolysaccharides chain of mixed hexosans and pentosans that are easily hydrolyzed using dilute acids [1].

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The most common form of hemicelluloses is xylose polymer (xylan). Extensive research has been undertaken to convert hemicellulose-derived carbohydrates, particularly xylose, into useful products since it can be used as a carbon and energy source in fermentation processes [1–4]. Hydrolysis of hemicellulose yields glucose, D-xylose, L-arabinose, and other minor sugars. During acid hydrolysis, obtained sugars are degraded rapidly to fermentation inhibitors include: furfural, hydroxymethylfurfural, and other condensation byproducts [3].

The bioconversion of xylose to xylitol is of interest as it is a polyol with important applications as a sweetener. Xylitol has important advantages over glucose, saccharose, and other polyols such as anticariogenicity, low caloric value, and negative heat of dissolution [5–7].

Economic interest in xylitol production by fermentation can be enhanced if the required xylose solutions can be obtained from the hydrolysis of low-cost lignocellulosic wastes. Sugarcane bagasse is a renewable, cheap, and widely available waste. The hydrolysis of sugarcane bagasse to obtain xylose solutions has two important advantages—the elimination of a waste and the production of a value-added product that enhances the economics of the process [8]. The hydrolysates obtained after the acid hydrolysis need to be processed in order to eliminate degradation products that are toxic for cellular growth. Such compounds with inhibitory effect are furfural, 5-hydroxymethyl furfural (HMF), acetic acid, hydroxybenzaldehyde, syringaldehyde, and vanillin [9].

More severe hydrolysis conditions not only lead to a higher release of monomeric sugars available for fermentation but can also lead to loss of sugars in the form of degradation products. Mild conditions can be used for hydrolysis of soluble oligomers to monomeric sugars without significant sugar degradation. According to du Toit et al. [8], working with softwoods, they observed that to achieve maximum monomer yield required more severe conditions than was needed to achieve maximum total hemicellulose recovery. Milder conditions caused less sugar degradation resulting in enhanced hemicellulose recovery. However, the mild conditions produced a sugar solution rich in monosaccharide (xylose) and at the same time containing considerable amounts of low molecular weight oligosaccharides of the same sugar [1]. Since yeasts cannot assimilate these resulted sugar oligomers, a post-hydrolysis stage (represented by thermal, acidic, or enzymatic treatment) is needed to produce the corresponding sugars monomers [10]. Such conversion will ensure an increase of the xylose content in the obtained hydrolysate, thus, leading to a reduction in the operation time and energy consumption of the hydrolysate concentration process.

In this work, it was proposed a post-hydrolysis stage that consisted of a thermal treatment of the obtained acid hydrolysate, aiming to convert these oligosaccharides, found in the hemicellulosic hydrolysate, to their respective monosaccharide. In addition, the paper presents comparative fermentation results for dilute acid hydrolysis with and without post-hydrolysis treatment for fermentation feeds concentrated to equal concentration of xylose.

Materials and Methods

Chemical Characterization of Sugarcane Bagasse

Sugarcane bagasse used in this work was chemically characterized for the determination of its content (cellulose, hemicellulose, and lignin) based on the methodologies described by Browning [11]. All the analyses were done in triplicates.

Preparation of the Hemicellulosic Hydrolysate and Post-Hydrolysate

A pilot plant 250-L reactor was loaded with 20 Kg of sugarcane bagasse and 0.50% sulfuric acid solution. The reactor was operated at 121 °C for 10 min using a bagasse/acid solution ratio of 1:10 (*w*/*v*). The resulting hemicellulosic hydrolysate was submitted to a posterior post-hydrolysis stage (thermal treatment), where it was heated at 121 °C for 10 min in the same pilot plant reactor employed for the acid hydrolysis.

Both hemicellulosic hydrolysate and post-hydrolysate were vacuum concentrated at 70 °C (using a concentration factor of 2.6× and 2.0×, respectively) in order to attain the same initial xylose concentration at the beginning of the fermentation process for better results comparison.

The concentrated hydrolysates were detoxified using the procedure "overliming", where the pH of the hydrolysates was neutralized by the addition of CaO, lowered to 5.5 by the addition of H_3PO_4, and then treated by activated charcoal for 1 h [12]. Finally, the detoxified hydrolysates were autoclaved at 110 °C for 15 min.

Inoculum Preparation

Candida guilliermondii FTI 20037, described by Barbosa et al. [13], was used in the experiments. A loopful of a slant culture was transferred to 125-mL Erlenmeyer flasks containing 50 mL of the growth medium: 3.0 g/L $(NH_4)_2SO_4$, 0.10 g/L $CaCl_2.2\ H_2O$, 10% (*v*/*v*) rice bran extract, and 30 g/L xylose. The flasks were maintained under agitation of 200 rpm at 30 °C for 24 h. After centrifugation at 2,200×*g* for 20 min, the cells were rinsed twice with sterile water and added to the fermentation medium in order to reach an initial concentration of 1 g/L.

Medium and Fermentation Conditions

The fermentation medium was prepared by supplementing both concentrated sugarcane bagasse hydrolysate and post-hydrolysate with 3.0 g/L $(NH_4)_2SO_4$, 0.10 g/L $CaCl_2.2\ H_2O$, 10% (*v*/*v*) rice bran extract and 1 g/L of *C. guilliermondii* as the fermenting yeast. The fermentation was performed in 125-mL Erlenmeyer flasks containing 50 mL of fermentation medium operating at a temperature of 30 °C, agitation of 200 rpm and pH of 5.5.

Calculations

Xylitol Yield

$$Y_{P/S} = {}^{(\Delta P)}\Big/_{(\Delta S)} = {}^{(\mathrm{Pf}\ -\ \mathrm{Pi})}\Big/_{(\mathrm{So}\ -\ \mathrm{Sf})} \tag{1}$$

Where, $Y_{P/S}$: xylitol yield based on xylose consumption (g xylitol/g xylose)

Pf final xylitol concentration (g/L)
Pi initial xylitol concentration (g/L)
So initial xylose concentration (g/L)
Sf final xylose concentration (g/L)

Conversion Efficiency of Xylose to Xylitol

$$E(\%) = {}^{(YP/S\ \text{experimental})}\Big/_{(YP/S\ \text{theoretical})} {}^{*100} \tag{2}$$

Where, *E*: conversion efficiency of xylose to xylitol

$Y_{P/S}$ theoretical—theoretical conversion efficiency of xylose to xylitol (0.917 g xylitol/g xylose according to Barbosa et al. [13]

Volumetric Productivity of Xylitol

$$QP = {(Pf - Pi)}/{t} \quad (3)$$

Where, QP: overall xylitol production rate (g xylitol/L h)

t: fermentation time (h)

Analytical Methods

Xylose, glucose, arabinose, acetic acid, ethanol, xylitol, and arabitol concentrations were measured by high-pressure liquid chromatography (Waters Ltda, Kyoto, Japan) provided with a Bio-Rad HPX87 H (300×7.8 mm) column (maintained at a temperature of 45 °C) and a refractive index detector. A solution of H_2SO_4 (0.01 N) was used as the mobile phase at a flow rate of 0.6 mL/min.

Biomass Assay

Glucose was found to be consumed at the first fermentation minutes due to law concentration in the fermentation medium. In this work, xylose was considered the only carbon source for biomass determination.

The liquid phase of the samples taken during the fermentation runs was centrifuged (2,000×g, 15 min) to separate the obtained biomass. Cell concentration in the resulting suspension was determined by optical density (OD) measurements at 640 nm, using a spectrophotometer, model DU 640 B (Beckman Coulter, Fullerton, CA, USA). A previously constructed calibration curve was used to relate the OD measurements to dry cell concentration in taken samples.

Determination of Phenolic Compounds (Toxic Compounds)

The concentration of phenolic compounds was determined by the method described by Rocha [14]. A sample of 2 mL of the hydrolysate was alkalinized with NaOH 6 mol/L until pH 12 and then diluted to 50 mL. The absorbance of this solution was then analyzed using a spectrophotometer (Beckmam DU 640 B, CA, USA) at 280 nm. Distilled water was used as a blank. Soluble lignin concentration was calculated by the Eq. 7, as described in previous studies by Rocha [14]:

$$C_{lig}(g/L) = \alpha^{*}(A_{lig280} - A_{pd280}) - \beta \quad (4)$$

where:

C_{lig} Lignin concentration (g/L)

A_{lig280} Lignin Absorbance at 280 nm

A_{pd280} Absorbance corresponding to the sugar decomposition products (furfural and hydroxymethylfurfural). In this case, the A_{pd280} was ≈0 (since furfural and HMF concentrations were very low after the detoxification procedure, as shown in Table 2);

α and β are the equation coefficients, $\alpha = 3,279 \times 10^{-4}$ and $\beta = 4,187 \times 10^{-2}$

Table 1 Chemical composition of sugarcane bagasse.

Components	% Composition
Hemicellulose (Xylan)	25.8
Cellulose (Glucane)	42.8
Lignin	22.1
Extractives	6.1
Ashes	1.4
Total	98.2

Results obtained by Eq. 7 were validated by using the Folin–Ciocalteu method [15] with vanillin as calibration standard.

Results and Discussion

Hydrolysis and Post-Hydrolysis

The chemical composition of the sugarcane bagasse used in this work is presented in Table 1.

A hemicellulosic hydrolysate was obtained at the end of the acid hydrolysis stage with an initial xylose concentration of 18.4 g/L (corresponding to 2.3 kg of xylose in 125 L of obtained hydrolysate). The acid hydrolysis represented a conversion efficiency (hemicellulose to xylose) of 48%, considering that the maximum theoretical xylose concentration found in the sugarcane bagasse used in this work was 38.4 g/L (corresponding to 4.8 kg of xylose in 125 L of hydrolysate) [16]. In order to increase the xylose concentration found in the obtained hemicellulosic hydrolysate, a post-hydrolysis stage was carried out. At the end of the post-hydrolysis stage, almost a complete conversion of xylose-oligosaccharides to xylose-monosaccharide took place, resulting in a 22% increase in the total xylose concentration (23.5 g/L) corresponding to 61.2 % of conversion efficiency. Results are shown in Table 2. Sarrouh et al. [1], working with concentrated acid hydrolysis for the

Table 2 Characterization of the hemicellulosic hydrolysate and post-hydrolysate during the three stages of the process (hydrolysis, concentration and detoxification).

Composition	Acid hydrolysate		Concentrated hydrolysate		Detoxified hydrolysate	
	Original hydrolysate	Post-hydrolysate	Hydrolysate X 2.6[a]	Post-hydrolysate X 2.0[b]	Hydrolysate	Post-hydrolysate
Xylose (g/L)	18.3	23.9	48.0	48.0	34.0	34.0
Glucose (g/L)	1.2	1.5	2.7	3.0	0.0	0.0
Arabinose (g/L)	1.1	1.5	3.3	3.4	2.8	2.7
Furfural (g/L)	0.08	0.08	0.2	0.1	0.002	0.001
HMF (g/L)	0.07	0.07	0.2	0.1	0.002	0.001
Acetic acid (g/L)	1.5	1.5	3.9	3.0	1.4	0.80
Total phenols (g/L)	3.7	3.9	9.0	7.2	0.81	0.60

[a] X 2.6 concentrated to 1/2.6 of its initial volume

[b] X 2.0 concentrated 1/2 of its initial volume

obtaining of fermentable sugars, observed that during the dilute acid hydrolysis of the hemicellulosic fraction of sugarcane bagasse, not all hemicellulose is degraded to monosaccharide sugars, presence of oligosaccharides were also observed. Such phenomenon is explained due to the partial hydrolysis of the hemicellulosic fraction (xylan polymer) during the dilute acid hydrolysis of the sugarcane bagasse [1].

The post-hydrolysis stage consisted in a thermal treatment of the obtained hemicellulosic hydrolysate. Such treatment aims to degrade the present oligosaccharides and leading to an optimum xylose extraction from the hemicellulosic fraction of sugarcane bagasse. Such fact was also observed in the work of Shevchenko et al. [17], where the authors explained that when Douglas wood chips were used as the substrate, sulfuric acid-catalyzed post-hydrolysis at 120 °C for 1 h allowed most of the original hemicellulose to be recovered in the monomeric form with no noticeable losses due to sugar degradation.

Inhibitors Formation and Hydrolysates Bioconversion

An important point to consider is the possible formation of inhibitors (toxic substances for the cellular metabolism) during chemical or thermal pretreatment of sugarcane bagasse. Such inhibitors will negatively influence the fermentability of the obtained sugars and, as a result, will turn the post-hydrolysis stage not viable. According to results shown in Table 2, the difference in the concentration factor between the original and post-hydrolysates influenced significantly inhibitors production. Such fact led to lower concentrations of the phenolic toxic compounds in the post-hydrolysate (7.2 g/L) at a concentration factor of 2× in comparison with the original hydrolysate (9.0 g/L) when concentrated 2.6 times in order to obtain the same initial xylose concentration for the fermentation process.

In order to evaluate the fermentability of the obtained post-hydrolysate, for the biotechnological production of xylitol, comparative fermentations were carried through using the post-hydrolysate and the original hemicellulosic hydrolysate as substrates rich in xylose. It was observed that after 36 fermentation hours, both hydrolysate and post-hydrolysate had shown almost a complete consumption of xylose and a consequent production of xylitol (Fig. 1). On the other hand, the cellular growth was slightly higher for the post-hydrolysate (3.5 g/L, $Y_{x/s}$=0.070 g cells/g xylose) than for the original hydrolysate (2.9 g/L, $Y_{x/s}$=0.068 g cells/g xylose). Such behavior indicated that the cells preferred the post-hydrolysate medium than the original medium for their growth. This is likely due to lower toxic substances presence (inhibitors) in the post-hydrolysate [16].

Results in Table 3 clearly show that the post-hydrolysis stage had a positive impact on the fermentation process. According to the obtained results, it was evident that xylitol production using post-hydrolysate lead to a xylose–xylitol conversion efficiency 4% (corresponding to 0.5 g xylitol/L) higher than that obtained for original hydrolysate. Furthermore, an improvement of almost 13% (corresponding to 0.8 g xylitol/L h) was observed in the volumetric productivity of xylitol using the post-hydrolysate as a substrate instead of the original hemicellulosic hydrolysate, as shown in Fig. 1. Such results were expected since the inhibitors concentrations (acetic acid and phenolic compounds) were higher for the concentrated original hydrolysate than for the concentrated post-hydrolysate, leading to a negative impact on the fermentation process yield and productivity (Table 3). Rivas et al. [10], working with corn cob hemicellulosic hydrolysate for xylitol production, observed that in comparison with the conventional acid hydrolysis (pre-hydrolysis), the post-hydrolysis increased the process productivity by 18% and the product yield by 25%.

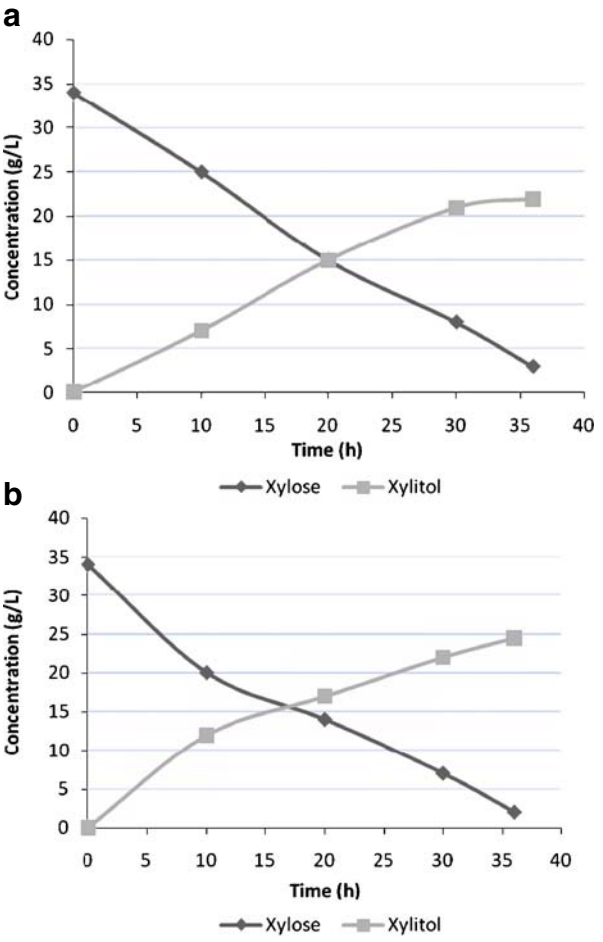

Fig. 1 Fermentation profile with xylose consumption and xylitol formation for original hydrolysate (**a**) and post-hydrolysate (**b**) during 36 h of fermentation

Conclusions

The post-hydrolysis stage resulted in increasing the xylose concentration from 18.4 to 23.5 g/L. In other words, the post-hydrolysate required less concentration than the untreated

Table 3 Evaluation of fermentation process parameters for original hydrolysate and post-hydrolysate for xylitol production after 36 h of fermentation.

Bioprocess parameters	Original hydrolysate	Post-hydrolysate
So (g/L)	34.0	34.0
Pf (g/L)	22.0	24.5
$Y_{P/S}$ (g/g)	0.65	0.70
E (%)	71	76
QP (g/L h)	0.61	0.68
$Y_{x/s}$ (g/g)	0.068	0.075

So initial concentration of xylose, *Pf* final concentration of xylitol, $Y_{P/S}$ xylitol yield, *E*% bioconversion efficiency, *QP* process productivity, $Y_{x/s}$ process yield in biomass

hydrolysate to provide fermentation feeds of equal xylose concentration. This resulted in lower inhibitors concentration (acetic acid and phenolic compounds) in the post-hydrolysate feed and positively influenced the bioconversion process by increasing its productivity by 13% and xylitol yield by 7%.

Acknowledgement The authors would like to thank FAPÈSP for its financial support.

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