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Dilute Sulfuric Acid Pretreatment of Agricultural and Agro-Industrial Residues for Ethanol Production

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Abstract

The potential of dilute-acid prehydrolysis as a pretreatment method for sugarcane bagasse, rice hulls, peanut shells, and cassava stalks was investigated. The prehydrolysis was performed at 122°C during 20, 40, or 60 min using 2% H_2SO_4 at a solid-to-liquid ratio of 1 : 10. Sugar formation increased with increasing reaction time. Xylose, glucose, arabinose, and galactose were detected in all of the prehydrolysates, whereas mannose was found only in the prehydrolysates of peanut shells and cassava stalks. The hemicelluloses of bagasse were hydrolyzed to a high-extent yielding concentrations of xylose and arabinose of 19.1 and 2.2 g/L, respectively, and a xylan conversion of more than 80%. High-glucose concentrations (26–33.5 g/L) were found in the prehydrolysates of rice hulls, probably because of hydrolysis of starch of grain remains in the hulls. Peanut shells and cassava stalks rendered low amounts of sugars on prehydrolysis, indicating that the conditions were not severe enough to hydrolyze the hemicelluloses in these materials quantitatively. All prehydrolysates were readily fermentable by *Saccharomyces cerevisiae*. The dilute-acid prehydrolysis resulted in a 2.7- to 3.7-fold increase of the enzymatic convertibility of bagasse, but was not efficient for improving the enzymatic hydrolysis of peanut shells, cassava stalks, or rice hulls.

Index Entries: Bagasse; ethanol; acid hydrolysis; pretreatment; enzymatic hydrolysis; agricultural residues.

Introduction

Concerns about exhaustion of the world's reserves of fossil fuels and about the greenhouse effect have resulted in an increasing worldwide interest in using fuels from renewable resources, for instance ethanol. However, a reduction of the ethanol production cost is desirable to improve the

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competitiveness. As the sugar- and starch-containing feedstocks traditionally used for ethanol production represent the largest share of the total production cost *(1)*, the use of cheaper and more abundant raw materials is desirable for increasing the production.

Lignocellulosic materials are the world's most widely available low-cost renewable resources to be considered for ethanol production. A huge diversity of lignocellulosic wastes is available around the world. Sugarcane bagasse, rice hulls, peanut shells, and cassava stalks are agricultural and agro-industrial residues that could be considered for bioconversion in tropical countries *(2)*. These lignocellulosic residues are available on a renewable basis as they are generated by the harvest and processing of sugar cane (*Saccharum officinarum*), rice (*Oryza sativa*), peanut (*Arachis hypogaea*), and cassava (*Manihot dulcis*), which are regularly cultivated crops.

Potential applications for these materials include production of activated charcoal *(3)*, energy generation *(4)*, and pulp production *(5)*. However, except bagasse, which is used for energy generation to run sugar mills, pulp and paper production, and cattle feed manufacturing *(6)*, the other materials are of low-economic value and cause environmental problems. Therefore, they can be considered for bioethanol production.

Although lignocellulosic residues provide cheap raw material, cost-intensive hydrolysis processes are required to obtain fermentable sugars. The hydrolysis can be catalyzed by acids, either concentrated or diluted, or by enzymes. Hydrolysis of cellulose with diluted acid is performed at high-temperature; whereas hydrolysis with either concentrated acids or enzymes is performed at low-temperature *(7,8)*. Dilute acid can also be used for prehydrolysis of hemicelluloses, which is a process performed at relatively low temperatures *(9,10)*. After acid-catalyzed hydrolysis of hemicelluloses, a solid residue consisting of cellulose and lignin is obtained and the cellulose can then be hydrolyzed either by using acid under harsher conditions or by using cellulases. Dilute-acid prehydrolysis can be used as a pretreatment method for increasing the reactivity of cellulose toward cellulases *(11)*.

A drawback of acid hydrolysis is the formation of byproducts, which can negatively affect the fermentability of the hydrolysates *(12,13)*. The fermentation inhibitors include acetic acid, released by deacetylation of hemicelluloses, formic, and levulinic acids, which are sugar degradation products; phenolic compounds that are mainly formed by the partial degradation of lignin, and the furan aldehydes furfural and 5-hydroxymethylfurfural (HMF), which are formed by the degradation of pentoses and hexoses, respectively *(14)*. In order to have an efficient fermentation process, it is desirable to reduce the formation of inhibitors during hydrolysis as much as possible.

Considering sugarcane bagasse, rice hulls, peanut shells, and cassava stalks, only dilute-acid prehydrolysis of sugarcane bagasse has been extensively studied previously *(9,10,15,16)*. However, much of the previous work concerning dilute-acid hydrolysis of bagasse has been focused on

obtaining high-yields of xylose rather than on the enzymatic convertibility of the pretreated bagasse. In this investigation, the dilute-acid prehydrolysis of these four different agricultural and agro-industrial materials was investigated with respect to the formation of sugars, the fermentability of the prehydrolysates, and the enzymatic convertibility of the pretreated solid materials.

Materials and Methods

Raw Material

Sugarcane bagasse from the 2004 harvest was generously donated by "Horacio Rodríguez" sugar mill (Matanzas, Cuba). Cassava stalks, peanut shells, and rice hulls were acquired from local producers (Matanzas Provincial Delegation of the Cuban Ministry of Agriculture, Matanzas, Cuba). The rice hulls were obtained from a low-efficiency artisan rice mill. The materials were air-dried to a dry matter (DM) content of 90–92%, milled to pass a 2-mm screen and stored in plastic bags in a dark chamber at room temperature.

Dilute-Acid Prehydrolysis

Thirty-five grams of dried raw material were mixed with a diluted H_2SO_4 (Merck, Darmstadt, Germany) solution giving a final concentration of 2 g of acid per 100 g of slurry. The liquid-to-solid ratio was 10 g/g. Treatments were performed at 122°C during 20, 40, or 60 min. Stainless steel cylinders with a total volume of 500 mL were used as reaction vessels. The cylinders were mounted in a rotor and immersed in a polyethylene glycol heating bath, which allowed a relatively rapid heating of the slurries to the work temperatures. A control panel (Jaako Pöyry AB, Karlstad, Sweden) was used for a careful control of the temperature of the bath. The pretreatment was performed in duplicates. When the reaction time had elapsed, the reactors were cooled to room temperature in water-baths and the pretreated slurries were separated by vacuum filtration (Edwards RV8 pump, BOC Ltd., Crawley Sussex, England) into a liquid fraction, hereafter referred to as prehydrolysate, and a solid residue, hereafter referred to as filter cake. The filter cake was washed with two volumes of deionized water. The prehydrolysates were stored in a cold chamber at 4°C until further use. The filter cakes were dried under mild conditions, weighed, and stored in plastic bags in a cold chamber. Samples of the prehydrolysates and the filter cakes were taken for analysis.

Analysis of the Solid Fraction

The DM content was determined using a moisture analyzer (MA40, Sartorius AG, Göttingen, Germany). Extractives were determined gravimetrically after a Soxhlet extraction with 96% (v/v) ethanol during 24 h. For determination of the chemical composition of raw and pretreated

materials, duplicate samples were hydrolyzed first with 72% H_2SO_4 during 1 h at 30°C and then for another hour with 4% H_2SO_4 at 121°C. The mixture was separated by vacuum filtration through previously weighed filter crucibles and the lignin content was determined gravimetrically (Mettler AE260 Delta Range, Mettler Toledo, Switzerland). The sugar content in the obtained filtrate was analyzed by anion-exchange chromatography using a DX 500 system (Dionex, Sunnyvale, CA) equipped with a CarboPac PA-1 column. The column was eluted with Milli-Q water (Millipore, Billerica, MA) at a flow rate of 1 mL/min. Before the analysis of each sample, the column was activated by a mixture of 200 m*M* NaOH and 70 m*M* NaOAc. A postcolumn addition of 300 m*M* NaOH was applied before the pulse amperometric detection (Dionex ED 40).

Analysis of the Liquid Fraction

Sugars were determined as described under "Analysis of the Solid Fraction." Carboxylic acids were quantified using a Dionex ICS-2000 chromatography system equipped with a conductivity detector. Separation was performed on an IonPac AS 15 (250 × 4 mm) column with an IonPac AG15 (50 × 4 mm) precolumn (Dionex, Sunnyvale, CA), using isocratic elution with 35 m*M* NaOH supplied at a rate of 1.2 mL/min.

The furan aldehydes HMF and furfural were determined by high-performance liquid chromatography using a Shimadzu VP series system (Shimadzu, Kyoto, Japan) with ultraviolet (UV) detection at 282 nm. Separation was performed using an XTerra MS C_{18} column (5 µm, 2.1 × 150 mm) (Waters, Milford, MA) eluted at a flow rate of 0.4 mL/min with a gradient of Milli-Q water and acetonitrile containing 0.016% (v/v) trifluoroacetic acid. The gradient scheme consisted of four steps with a combined time of 26 min:

1. Ten percent acetonitrile was applied for 8 min.
2. The concentration of acetonitrile was increased linearly to 100% during 8 min.
3. Hundred percent acetonitrile was applied for 6 min.
4. The concentration of acetonitrile was decreased linearly to 10% during 4 min.

The total content of phenolic compounds was determined colorimetrically (Unicam UV-visible spectrophotometer, Cambridge, UK) using the Folin-Ciocalteu method *(17)*. Vanillin was used as the calibration standard.

Fermentability of the Prehydrolysates

The pH of the prehydrolysates was adjusted from around 1 to 5.5 with 8 *M* NaOH using a pH meter (WPA Linton, Cambridge, UK). The prehydrolysates were supplemented with 0.5 g/L of $(NH_4)_2HPO_4$, 0.025 g/L of $MgSO_4 \cdot 7H_2O$, 1.38 g/L of $NaH_2PO_4 \cdot H_2O$, and 1 g/L of yeast extract. All prehydrolysates, except the ones from rice hulls, were supplemented with

20 g/L of glucose. The chemicals were supplied by Sigma-Aldrich Chemie GmbH (Steinheim, Germany). A reference solution containing 20 g/L of glucose and supplemented with the same nutrients was also prepared.

The fermentations were carried out in 50-mL flasks, sealed with rubber stoppers and equipped with cannulas for CO_2 removal. The flasks were inoculated with baker's yeast (Jästbolaget AB, Rotebro, Sweden) to an initial biomass concentration of 1 g/L (dry weight), and incubated at 30°C in a water-bath with magnetic stirring (IKA-Werke, Staufen, Germany) for 24 h. Samples were withdrawn after 2, 3, 4, 6, 8, 10, and 12 h. Fermentations were performed in duplicates and the mean values were given as results.

Glucose was monitored during the fermentations using a glucometer (Glucometer Elite XL, Bayer AG, Leverkusen, Germany). The final glucose concentration was determined by ion chromatography as described under "Analysis of the Solid Fraction." Ethanol was analyzed with an ethanol kit (Ethanol UV-test, R-Biopharm AG, Darmstadt, Germany). The ethanol yield (g/g), the volumetric productivity of ethanol (g/[L·h]), and the glucose consumption rate (g/[L·h]) were used as criteria of fermentability. For calculation of the yield, the ethanol concentration after 12 h was divided by the initial concentration of glucose. The productivity was based on the ethanol concentration achieved after 3 h of fermentation. The inhibition of the ethanol yield was calculated according to the expression:

$$\text{Yield inhibition (\%)} = [(Y_{ref} - Y_{preh})/Y_{ref}] \times 100$$

where Y_{ref} is the ethanol yield in the reference fermentation and Y_{preh} is the ethanol yield in the fermentation of the prehydrolysate. The inhibition of the volumetric productivity was calculated in an analogous way.

Enzymatic Convertibility

For evaluating the enzymatic convertibility of cellulose, approx 110 mg of the washed pretreated solid fraction was placed in a Falcon tube, and 0.04 *M* acetate buffer (pH 4.8) was added giving a total volume of 5 mL and a DM content of 2%. A commercial preparation of *Trichoderma reesei* cellulases (Celluclast 1.5L) and a β-glycosidase preparation (Novozym 188), both produced by Novozymes A/S (Bagsværd, Denmark), were added at a loading of 25 filter paper units/g DM and 0.46 cellobiose units/mL, respectively. The reaction mixture was incubated in a rotating incubator (New Brunswick Scientific, Edison, NJ) at 50°C and 150 rpm for 24 h. By the end of the hydrolysis, the liquid was separated from the solids by centrifugation, the glucose concentration was determined by ion chromatography, and the results were used for calculating the enzymatic convertibility of cellulose. In a parallel experiment, the enzymatic convertibility of the untreated raw materials was also assayed. The experiments were performed in triplicates.

Table 1
Main Components of the Raw Materials in Percentage

Material	Glucan	Xylan	Arabinan	Ethanol extractives	Klason lignin	Ash
Bagasse	36.1	20.8	2.8	6.1	17.8	2.0
Rice hulls	49.1	8.3	1.3	6.4	12.9	15.2
Peanut shells	22.1	10.7	1.4	8.5	35.2	7.2
Cassava stalks	31.0	12.3	0.9	7.6	24.8	8.0

Results and Discussion

Composition of the Materials

The composition of the raw materials used in this investigation is shown in Table 1. Sugarcane bagasse and rice hulls had the highest carbohydrate content. The high contents of glucan, which were attributed to remaining starch and ash in the rice hulls, are noteworthy. In the other materials, the most notable was the high-lignin content of peanut shells, which is in agreement with previous results *(18)*.

Effect of the Prehydrolysis on the Formation of Sugars

The prehydrolysis conditions studied were selected because they have been successfully used for materials such as sugarcane bagasse *(10)* and sorghum straw *(19)*. As a result of the partial hydrolysis of polysaccharides, sugars were formed during the dilute-acid prehydrolysis. Xylose, derived from hemicelluloses, and glucose, mainly derived from cellulose and starch, were the major sugars found in the prehydrolysates of all the materials (Table 2). The standard deviation of the sugar analyses was 5.7%. Arabinose was the third most abundant sugar, whereas the galactose content was less relevant and mannose was detected only in the prehydrolysates of peanut shells and cassava stalks. The different sugar content of the prehydrolysates indicates that the hemicelluloses of the investigated materials have different composition. However, it is obvious that the different sugar yield is also a consequence of the different susceptibility to dilute-acid prehydrolysis displayed by the different materials.

For all the materials, the glucose and xylose content generally increased with increasing length of the prehydrolysis. Thus, no extensive degradation of monosaccharides was observed, although the furan aldehydes are products of acid-catalyzed degradation. Most of the arabinose and galactose were formed after the shortest prehydrolysis time (Table 2). The high-degree of arabinose release under mild pretreatment conditions has previously been observed for sugarcane bagasse (Martín C. unpublished, *[16]*). The ease of arabinose hydrolysis is supposedly owing to its

Table 2
Sugar Composition of the Prehydrolysates Obtained After Dilute Sulfuric Acid Prehydrolysis of Sugarcane Bagasse, Rice Hulls, Peanut Shells, and Cassava Stalks During 20, 40, and 60 min

Material	Prehydrolysis time (min)	Glucose (g/L)	Xylose (g/L)	Arabinose (g/L)	Galactose (g/L)	Mannose (g/L)
Bagasse	20	2.1	17.2	2.0	0.7	ND
Bagasse	40	3.7	18.9	2.1	0.8	ND
Bagasse	60	4.0	19.1	2.2	0.8	ND
Rice hulls	20	26	4.8	1.0	0.5	ND
Rice hulls	40	29	5.6	1.2	0.5	ND
Rice hulls	60	33.5	6.9	1.4	0.6	ND
Peanut shells	20	1.3	1.7	1.5	0.7	0.1
Peanut shells	40	1.4	4.1	1.5	0.9	0.1
Peanut shells	60	1.5	5.3	1.5	1.0	0.2
Cassava stalks	20	3.5	2.1	0.8	1.2	0.2
Cassava stalks	40	5.1	4.9	0.8	1.5	0.5
Cassava stalks	60	5.2	6.3	0.8	1.6	0.7

ND, not detected.

location in the branches of arabinoxylan, where the cleavage of the glycosidic bonds is easier than in the backbone of the macromolecule *(20,21)*.

Sugarcane Bagasse

Sugarcane bagasse was the most susceptible material to the prehydrolysis conditions used in this work. The hemicellulose fraction of bagasse was hydrolyzed to a high-extent as indicated by the high-concentrations of xylose, arabinose (Table 2), and acetic acid (Table 3) in the prehydrolysates. The conversion of the xylan of the raw bagasse was 73–81% (Fig. 1). The concentrations of hemicellulose degradation products such as xylose, arabinose, and acetic acid increased with less than 30% when the reaction time increased from 40 to 60 min. The relatively low-glucose concentration (Table 2) indicates that cellulose was only marginally hydrolyzed. Even under the harshest conditions, the conversion was no more than 10% (Fig. 2). Evidently, only the noncrystalline part of the cellulose was hydrolyzed, as 72% sulfuric acid was needed to obtain complete hydrolysis. These results on dilute sulfuric acid hydrolysis of Cuban bagasse are comparable with previous reports using bagasse from Australia *(15)*, Brazil *(9)*, and Mexico *(10)*.

Rice Hulls

Although the hydrolysis of the hemicellulose fraction of rice hulls was substantial, the high-degree of glucan hydrolysis was more remarkable. The glucose concentration in the prehydrolysates ranged from 26 to 33.5 g/L (Table 2). The glucose yield ranged between 46.9 and 61.4% (Fig. 2). Because

Table 3
Content of Fermentation Inhibitors in the Prehydrolysates

Material	Prehydrolysis time (min)	Acetic acid (g/L)	Formic acid (g/L)	Furfural (g/L)	HMF (g/L)	Phenolic compounds (g/L)
Bagasse	20	2.5	0.16	0.10	0.03	0.28
Bagasse	40	2.8	0.18	0.29	0.06	0.22
Bagasse	60	2.7	0.20	0.36	0.07	0.24
Rice hulls	20	0.9	0.09	0.05	0.10	0.23
Rice hulls	40	1.0	0.12	0.11	0.17	0.23
Rice hulls	60	1.1	0.15	0.17	0.21	0.23
Peanut shells	20	1.1	0.11	0.02	0.10	0.11
Peanut shells	40	1.7	0.19	0.05	0.15	0.12
Peanut shells	60	1.9	0.24	0.10	0.17	0.12
Cassava stalks	20	1.5	0.10	0.01	0.01	0.04
Cassava stalks	40	1.9	0.14	0.04	0.02	0.07
Cassava stalks	60	2.0	0.16	0.10	0.03	0.11

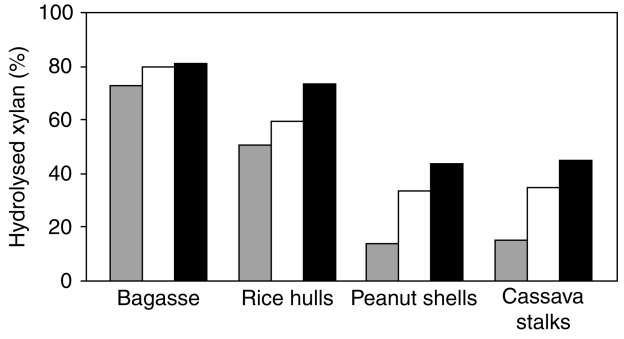

Fig. 1. Xylan converted to xylose during dilute acid prehydrolysis of sugarcane bagasse, rice hulls, peanut shells, and cassava stalks. Prehydrolysis time: 20 min (gray bars), 40 min (white bars), and 60 min (black bars).

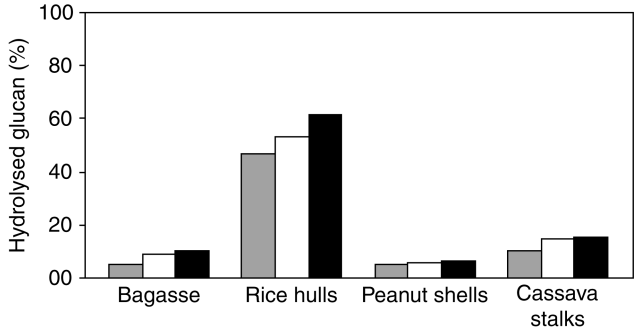

Fig. 2. Glucan converted to glucose during dilute acid prehydrolysis of sugarcane bagasse, rice hulls, peanut shells, and cassava stalks. Prehydrolysis time: 20 min (gray bars), 40 min (white bars), and 60 min (black bars).

the conditions used for prehydrolysis should be too weak for cellulose hydrolysis, the glucose should likely be derived from starch in grain remains in the hulls and probably from glucans in the hemicellulose fraction.

Peanut Shells and Cassava Stalks

Peanut shells and cassava stalks rendered low amounts of sugars on prehydrolysis (Table 2) indicating that complete hydrolysis of hemicelluloses would require more severe conditions. Even though xylan conversion increased noticeably with increasing prehydrolysis time, the highest conversion was only 43.6 and 45.1% for peanut shells and cassava stalks, respectively. This is considerably lower than what was achieved for sugarcane bagasse and rice hulls (Fig. 1). The low degree of xylan hydrolysis observed for peanut shells and cassava stalks might be linked to their high-lignin content (Table 1). Although the conditions used for pretreatments were too weak for complete hydrolysis of xylan, they were strong enough for complete hydrolysis of arabinan, even with the shortest prehydrolysis time.

In the prehydrolysates of cassava stalks, including those obtained under mild conditions, glucose was rather abundant. Taking into account that the conditions were far too weak for cellulose hydrolysis, it appears reasonable to assume that glucose is an important component of cassava stalk hemicelluloses. The considerable amounts of galactose and mannose found in the prehydrolysates indicate that those sugars also are important constituents of cassava stalk hemicelluloses. These findings suggest that hemicelluloses of cassava stalks differ considerably from hemicelluloses of other agricultural residues, such as wheat straw *(22)*, rice straw *(23)*, and sugarcane bagasse *(21)*, and are closer to wood hemicelluloses, which contain mannose and galactose heteropolymers *(20)*.

Formation of Fermentation Inhibitors

The data from the pretreatment experiments indicate how the different conditions used influence the formation of inhibitory compounds for each of the different raw materials. As expected, the concentration of most of the inhibitors, except the phenolic compounds, increased with increasing severity of the treatment. However, even under the harshest conditions the inhibitor content of the prehydrolysates was relatively low (Table 3). This is a consequence of the mild prehydrolysis conditions used, which did not lead to any major degradation of the released sugars.

Acetic acid, generated by hydrolysis of hemicelluloses, was the most abundant inhibiting compound found in the prehydrolysates. The highest concentrations were found in bagasse prehydrolysates, wherein hemicelluloses were hydrolyzed to a higher degree. However, in all the prehydrolysates, the concentrations of acetic acid were below the inhibiting limit *(24)*. The low concentration of formic acid and the absence of levulinic

acid in the prehydrolysates indicate that the degradation of furan aldehydes was modest. Higher formation of formic and levulinic acids could be expected if the hydrolysis conditions would be more severe.

The concentrations of furan aldehydes were relatively low, but increased with increasing pretreatment time (Table 3). In the bagasse and cassava stalk prehydrolysates, the concentrations of furfural were higher than the concentrations of HMF, whereas the situation was different in the rice hull and peanut shell prehydrolysates (Table 3). Higher concentrations of furfural could possibly be related to the relatively high-xylan content of bagasse and cassava stalks (Table 1).

The formation of phenolic compounds was most apparent in prehydrolysates of bagasse and rice hulls. Some phenols may originate from low-molecular weight compounds, such as lignans that are soluble in water. Partial degradation of lignin is generally the main source of phenols, but some phenols, such as phenolic acids from gramineous plants, are derived from the hemicellulose fraction *(25,26)*. As the conditions used in this work were too mild to cause extensive lignin degradation, and the highest formation of phenols was observed in the prehydrolysates of residues of sugarcane and rice, two plants belonging to the *Gramineae* family, it might be expected that a considerable part of the phenols found in the prehydrolysates of bagasse and rice hulls result from hydrolysis of lignin-like substituents in hemicelluloses.

Fermentability of the Prehydrolysates

The fermentability of the prehydrolysates was assessed using baker's yeast. The pattern of glucose consumption during fermentation of the prehydrolysates is shown in Fig. 3. Although glucose consumption in the prehydrolysates was slower than in the reference fermentation, all the prehydrolysates fermented relatively rapidly without any detoxification. However, there were some differences between the different raw materials. The highest glucose consumption rates were achieved in prehydrolysates of rice hulls and cassava stalks, where glucose was depleted within 6 h (Fig. 3B,D). The glucose consumption in the 20-min prehydrolysate of cassava stalks was very close to that observed in the reference fermentation. No inhibition of the volumetric ethanol productivity was observed in that prehydrolysate or in the prehydrolysates of rice hulls (Fig. 4). The good fermentability of prehydrolysates of rice hulls, combined with their high-glucose concentration, make them especially attractive for ethanolic fermentation.

In the fermentation of the prehydrolysates of bagasse and peanut shells the consumption of glucose was slower (Fig. 3A,C), and the inhibition of the ethanol productivity was more noticeable (Fig. 4). The slower fermentation rates may be linked to the higher concentrations of inhibitory compounds. However, because the concentrations of all the inhibitors

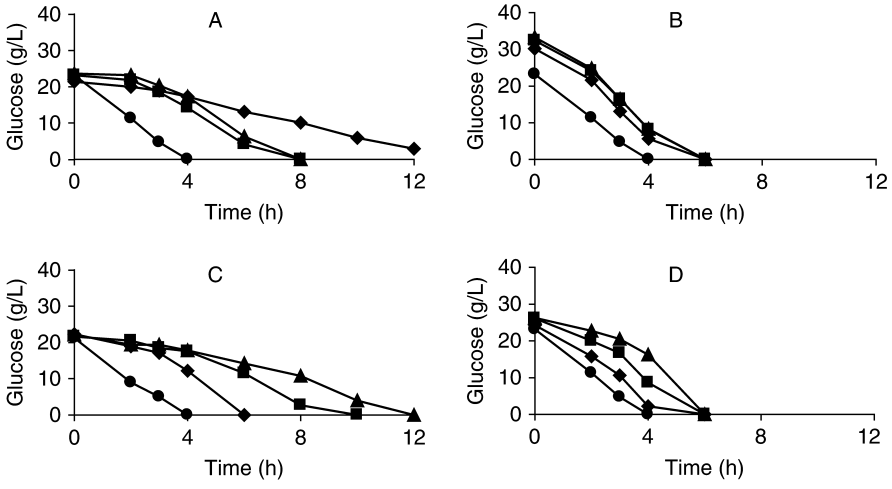

Fig. 3. Glucose consumption during fermentation of the prehydrolysates of sugarcane bagasse **(A)**, rice hulls **(B)**, peanut shells **(C)**, and cassava stalks **(D)** obtained by dilute sulfuric acid prehydrolysis at 122°C during 20 (◆), 40 (■), and 60 min (▲). Reference fermentation (●).

Fig. 4. Inhibition of the volumetric productivity of ethanol. Gray bars, 20-min prehydrolysis; white bars, 40-min prehydrolysis; black bars, 60-min prehydrolysis.

were rather low, the inhibitory effect did not last long and all fermentations were completed within 12 h (Fig. 3).

For most materials, the fermentation rate decreased with increasing prehydrolysis time (Fig. 3). This was obviously because of increasing inhibitor content. However, for bagasse, the prehydrolysate obtained with the shortest prehydrolysis time appeared to be most inhibitory (Figs. 3A and 4). One reason could be the higher content of phenolic compounds in that prehydrolysate (Table 3). Another explanation might be related to acetic acid. Assuming that acetic acid exerts a stimulatory effect on the ethanolic fermentations at the concentrations observed in this study, a faster fermentation of the prehydrolysates obtained after 40 and 60 min treatment may be expected.

Table 4
Enzymatic Convertibility (EC) of Sugarcane Bagasse, Rice Hulls, Peanut Shells, and Cassava Stalks Pretreated by Using Dilute Sulfuric Acid

Material	Prehydrolysis time (min)	Enzymatic convertibility[a] (%)	EC_{PM}/EC_{UM}
Bagasse	20	45.6	2.7
Bagasse	40	59.8	3.4
Bagasse	60	66.0	3.7
Rice hulls	20	14.2	<1
Rice hulls	40	13.5	<1
Rice hulls	60	22.2	<1
Peanut shells	20	15.3	1.20
Peanut shells	40	15.4	1.20
Peanut shells	60	17.3	1.33
Cassava stalks	20	12.6	<1
Cassava stalks	40	12.9	<1
Cassava stalks	60	14.8	<1

EC_{PM}/EC_{UM}, relative convertibility; PM, pretreated material; UM, unpretreated material.
[a]The enzymatic convertibility is related to the glucan contained in the filter cakes.

Enzymatic Convertibility of the Pretreated Materials

In order to investigate dilute acid prehydrolysis as a pretreatment method for enzymatic hydrolysis of cellulose, the filter cakes obtained after separation of the prehydrolysates were subjected to hydrolysis using cellulolytic enzymes. The best enzymatic convertibilities were achieved for bagasse, whereas the other materials were converted to a lesser extent (Table 4). A *t*-test at 95% confidence level indicated that the differences between the enzymatic convertibility of different materials were statistically significant. The highest degree of conversion was achieved for bagasse pretreated during 60 min, for which 66% of the cellulose of the filter cake was hydrolyzed to glucose. However, taking into account the losses occurring during the pretreatment, the conversion equals only 40% of the cellulose of the raw bagasse. That is relatively low considering the conversion achieved in enzymatic hydrolysis of bagasse pretreated by steam-explosion or wet-oxidation *(27,28)*. Therefore, the optimization of dilute sulfuric acid pretreatment conditions of sugarcane bagasse deserves more attention.

The enzymatic convertibility of the untreated raw materials was also assayed. The relative convertibility indicates how many times higher the enzymatic convertibility of the pretreated materials was compared with that of the unpretreated materials. As can be seen in Table 4, dilute sulfuric acid prehydrolysis improved the enzymatic convertibility of bagasse 2.7–3.7 times. For the rest of the materials, the relative convertibility was low, indicating that the prehydrolysis was not efficient for improving the

enzymatic convertibility. An additional experiment, using a higher enzyme load, did not lead to significant improvements of the enzymatic convertibility of any of the materials investigated (data not shown). More severe conditions have to be considered in future experiments.

The enzymatic convertibility of pretreated rice hulls and cassava stalks was unexpectedly lower than that of the unpretreated materials. This indicates that either the pretreatment used for those materials was inadequate or that easily hydrolysable glucans were present. Rice hulls and cassava stalks contain noncellulose glucans that were not further characterized. Perhaps those glucans can be hydrolyzed by the enzyme preparation used without pretreatment. More severe conditions of dilute sulfuric acid prehydrolysis of rice hulls and cassava stalks appear to be needed for improving the subsequent enzymatic hydrolysis of cellulose.

Conclusions

Under the conditions tested, prehydrolysis using dilute sulfuric acid was efficient for obtaining sugars from sugarcane bagasse and rice hulls hemicelluloses, and for improving the enzymatic convertibility of bagasse cellulose, but it was not efficient for the other materials. This work demonstrates the potential in using dilute sulfuric acid for pretreatment before enzymatic hydrolysis of bagasse, but further optimization of the conditions is desirable. For rice hulls, peanut shells, and cassava stalks, more severe conditions need to be studied. It is also of interest to investigate rice hulls free of grain remains. An investigation of that issue is underway.

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