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STEAM PRETREATMENT ASSISTED BY ACID OR ALKALINE IMPREGNATION FOR ETHANOL PRODUCTION FROM BAGASSE AND SUGARCANE TRASH

PRETRATAMIENTO AL VAPOR ASISTIDO POR IMPREGNACIÓN ÁCIDA O ALCALINA PARA LA PRODUCCIÓN DE ETANOL A PARTIR DE BAGAZO Y PAJA DE CAÑA DE AZÚCAR

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Resumen

El bagazo y la paja de caña de azúcar pueden ser convertidos en etanol por hidrólisis catalizada por ácidos o por enzimas seguida de fermentación de los monosacáridos formados. Antes de la hidrólisis enzimática se requiere un pretratamiento. La explosión con vapor asistida por impregnación de la biomasa con ácido diluido o con álcali promete ser una tecnología viable en el futuro. En el presente trabajo se evalúa la eficiencia de la impregnación de la biomasa residual de la caña de azúcar con ácido sulfúrico, dióxido de azufre y cal antes de la explosión con vapor. La impregnación con dióxido de azufre previo a la explosión con vapor condujo a conversiones de aproximadamente 70% de la celulosa y 57% de los xilanos residuales en la hidrólisis enzimática y a un rendimiento de 53 g por 100 g de bagazo. En el tratamiento asistido con impregnación con cal, las conversiones enzimáticas de la celulosa y los xilanos fueron, respectivamente, 80 y 50%, y el rendimiento de azúcares fue 40 g/100 g.

Palabras clave: bagazo de caña de azúcar, etanol, pretratamiento, hidrólisis.

Abstract

Sugarcane bagasse and trash, the left-over residue of leaves and tops, can be converted to ethanol by enzymatic or acid catalyzed hydrolysis and fermentation of the released monosaccharides. Prior to enzymatic hydrolysis, pretreatment is required, and steam explosion enhanced by pre-impregnation with dilute acid or alkali promises to be a viable technology in the future. In the present work, the efficiency of the impregnation of sugarcane residual biomass with sulphuric acid, sulphur dioxide and lime prior to steam explosion is evaluated. Sulphur dioxide impregnation prior to steam explosion led to enzymatic conversions of around 70% of cellulose and 57% of the xylan remaining in the fibres and a total sugar yield of 53 g per 100 g bagasse. In the lime-assisted pretreatment the enzymatic conversions of cellulose and xylan were approximately 80 and 50%, respectively, and the sugar yield was 40%.

Keywords: sugarcane bagasse, ethanol, pretreatment, hydrolysis.

Introduction

Due to increasing concern about the greenhouse effect, crude oil prices and other factors, ethanol is considered a viable alternative to gasoline as a vehicle fuel. Lignocellulosic materials are abundant and promising feedstocks for industrial production of low-cost ethanol[24]. One of the major lignocellulosic materials in tropical countries is sugarcane bagasse, the fibrous residue obtained after extracting the juice from sugar cane (*Saccharum officinarum*) in the sugar and ethanol production processes. Most of the bagasse produced in the sugar industry is used as a fuel for generating the energy required by the sugar mills. However, it is recognized that the energetic demands of the factories could be satisfied with 30-50% of the produced bagasse provided that the thermal efficiency of combustion units would be improved. Therefore, a surplus of bagasse would become available for different alternative uses[6], including ethanol production. Bagasse is an interesting raw material for industrial bioconversion processes since it is rich in carbohydrates, it is readily available at the sugar mill site, and the cost for harvest and transportation is borne by the sugar production[1]. Sugarcane trash, the left-over residue of leaves and tops, which is usually incorporated to the soil or burnt after harvesting, is another potential raw material, and about equal in quantity to bagasse. A review on the status and perspectives of ethanol production from sugarcane bagasse was recently published[3].

In a lignocellulose-to-ethanol process, hydrolysis is required for converting the polysaccharides into fermentable sugars. Enzymatic hydrolysis is promising for improving the technology so that cellulosic ethanol would become competitive on an industrial scale. However, since the access of native cellulose to the enzymes is limited by its close association with hemicelluloses and lignin, a pretreatment is required to improve the enzymatic convertibility and to lower the cost of the process[23]. The pretreatment is aimed to remove lignin and hemicelluloses and reduce cellulose crystallinity[22], thus enhancing the enzymatic susceptibility of cellulose. An effective pretreatment must (i) produce easily hydrolysable fibres; (ii) preserve the utility of the hemicelluloses; and (iii) avoid the formation of inhibitory by-products[8]. An economical pretreatment should use inexpensive chemicals and require simple equipment and procedures. Several chemical, physical and biological pre-treatment methods have been investigated for different lignocellulosic materials[22]. Recent work related to conversion of sugarcane biomass to ethanol has involved pretreatment tests with lime[7,19], steam explosion[9,11], dilute-acid hydrolysis[17], wet oxidation[9], liquid hot water[8], as well as organosolv using ethanol[14].

Steam pretreatment, with or without explosion, has been claimed as one of the most efficient and economic options for different lignocellulosic materials[18].

Steam pretreatment involves heating lignocellulose at high temperatures and pressures, followed by mechanical disruption of the pretreated material either by violent discharge (explosion) into a collecting tank or by mild blending after bleeding the steam pressure down to atmospheric (no explosion). The high-pressure steam modifies the cell wall structure, yielding a slurry from which hemicelluloses are easily recovered by water-washing, leaving a water-insoluble fraction composed of cellulose, residual hemicelluloses and a chemically modified lignin that can be further recovered by different chemical treatments. The efficiency of the pretreatment can be enhanced if biomass materials are impregnated with acids or alkalis prior to high-pressure steaming.

In the present work, the efficiency of the impregnation of sugarcane residual biomass with acidic (sulphur dioxide and sulphuric acid) and alkaline (lime) agents prior to steam explosion is evaluated.

Materials and methods

Raw material

Sugarcane bagasse from M. Muñoz sugar mill (Matanzas, Cuba) were used in the experiments of steam explosion assisted by impregnation with acidic agents. In the treatments with lime impregnation sugarcane trash and bagasse pith originated from Louisiana (USA) were used.

Steam explosion with acid impregnation

Three hundred grams of bagasse was impregnated with either SO_2 (1.1 g per 100 g of dry matter (DM)) for 15 min or with H_2SO_4 (1% DM) overnight at room temperature. The impregnated material was steam-pretreated afterwards at 205°C for 10 min as described previously[12]. Steam explosion (STEX) without impregnation was also performed as reference. The steam-pretreated material was submitted to enzymatic hydrolysis without separating the streams.

Steam explosion with alkaline impregnation

Batches of 0.3-3 kg of sugarcane biomass were blended with water and hydrated lime at a load of 2-20 kg per 100 g DM, and allowed to soak either in covered containers at ambient temperature or in a temperature-controlled tank set to 50 or 90ÚC for one day to three weeks. The whole lime-impregnated and acidified (pH 5) biomass was transferred into a 60 L rotary reactor heated with steam through a jacket at 9 bar and 125-160°C for 5-45 min. After a predetermined period, the reactor was stopped in the up-right position and biomass released tangentially into a receiver. The steam-exploded biomass was then kept refrigerated in plastic bags until analysis.

Analysis of the solid fraction

The contents of cellulose, xylan and lignin in the raw and pretreated bagasse were determined by analytical acid hydrolysis followed by chromatographic determination of the sugars[21]. Sugars, acids and furans were analysed by HPLC using previously described procedures[9]. The total content of phenolic compounds was analysed by the Folin-Ciocalteu method[20].

Enzymatic hydrolysis

For the enzymatic convertibility of the material pretreated by H_2SO_4- or SO_2-assisted STEX, the slurry was diluted to a 5% DM content, adjusted to pH 4.8, mixed with commercial cellulases (Celluclast 2 L and Novozym 188, Novozymes A/S, Denmark) at a loading of 25 FPU/g DM and incubated at 40° for 96 h. The protocol used for the enzymatic saccharification of the material pretreated by lime-assisted STEX was based on NREL's procedures[2].

Results and discussion

Steam explosion with acidic impregnation

The yields of fibrous material, glucose, xylose, furan aldehydes and aliphatic acids after pretreatment are shown in Table 1. Glucose and xylose were formed from the hydrolysis of cellulose and xylan, respectively. The furan aldehydes furfural and 5-hydroxymethyl-2-furfural (HMF) were formed as result of the degradation of pentoses and hexoses, respectively. Acetic acid was derived from the deacetylation of hemicelluloses, and formic and levulinic acids resulted from the degradation of the furan aldehydes. A decrease of the yield of fibrous material was observed for all the pretreatments. That was due to the solubilisation of hemicelluloses and some of the cellulose, lignin and extractives. The lowest fibre yield was obtained in the H_2SO_4-impregnated pre-treatment, and that was a result of a major solubilisation of cellulose and hemicelluloses. Under H_2SO_4-assisted steam explosion cellulose was hydrolysed to a high extent as can be deduced from the high glucose yield. Roughly 50% of the glucose content in the raw material was released already in the pre-treatment step of the H_2SO_4-impregnated bagasse. In contrast, only a negligible part of the glucose was released during pretreatment after impregnation with sulphur dioxide or without any impregnation.

Table 1. Yields after pretreatment of bagasse pretreated by steam explosion assisted by impregnation with SO_2 or H_2SO_4[1], g/100 g dry bagasse (DB).

Impregnating agent	Fibre	Glucose	Xylose	Furan aldehydes[2]	Aliphatic acids[3]	Phenolics
None	72.1	1.0	6.1	2.3	5.0	4.5
SO_2	69.2	1.0	8.2	2.3	5.8	4.4
H_2SO_4	58.2	22.6	3.6	6.2	13.8	4.0

[1]Mean values from two replicates. [2]Sum of furfural and HMF. [3]Sum of acetic, formic and levulinic acids.

A high degree of hydrolysis of hemicelluloses was achieved in all the pretreatments. The resulting xylose was well preserved in the pretreatments with SO_2 impregnation and without impregnation, whereas in the H_2SO_4-impregnated pretreatment sugars were degraded yielding high formation of furan aldehydes and aliphatic acids (Table 1). Lignin solubilisation was not affected for any of the impregnation agents as indicated by the comparable yield of phenolic compounds observed in all the pretreatments. Since furan aldehydes, aliphatic acids and phenolic compounds are inhibitors of ethanolic fermentation, the fermentability of hydrolysates obtained by steam explosion of H_2SO_4-impregnated bagasse are poorly fermentable with *Saccharomyces cerevisiae*.

Table 2. Enzymatic convertibility of polysaccharides in the material pretreated by steam explosion assisted by impregnation with SO_2 or H_2SO_4, g/100 g dry bagasse (DB).

Impregnating agent	Cellulose	Xylan
None	66.0	45.6
SO_2	70.4	56.8
H_2SO_4	71.8	20.7
No pretreatment	5.4	4.7

Mean values from three replicates.

All steam pretreatment conditions were efficient for enhancing the enzymatic hydrolysis of cellulose and xylan. H_2SO_4 impregnation was the most effective method for enhancing the enzymatic convertibility of cellulose, but it was outstandingly ineffective for xylan hydrolysis due to the high degradation of xylose that occurred during pretreatment (Table 2). On the other hand, the pretreatments with SO_2 impregnation and without impregnation yielded materials, whose cellulose and xylan were successfully hydrolysed with the enzyme preparation used.

Steam explosion with alkaline impregnation

Based on previous experience with lime and steam pretreatment of bagasse[4,7,16], this work was focused on the process employ of only such unit operations that have a reasonable chance of acceptance by the sugarcane industry; by avoiding chemical recovery, specialized equipment to handle high pressures or very corrosive acidic media, and reduction of biomass particle size beyond the one that is readily achieved with present-day sugarcane factory equipment.

It was found that the enzymatic conversion was improved when prior to the steam treatment, the lime-treated biomass was acidified to pH close to 5. Acidification prior to the hydrolysis is unavoidable in alkaline pretreatment in order to satisfy the pH requirements of the cellulases. Therefore, this does not increase the chemical requirements of the overall process. Since this pH is higher than in acid or autohydrolytic processes the production of furans was minimal.

Increasing the hydrated lime dose or reducing solids loading during impregnation improves the yield while the temperature effect appears to be only secondary by preventing any microbial action at 90ÚC that at lower temperatures sets in readily at lime doses below 10%. Low solids load during impregnation appears to lead to higher sugar yield but from the standpoint of industrial design, 10% solids is considered as minimum for a viable process. At this level biomass is fully saturated with little free liquor, yet sufficiently fluid so it can still be handled relatively easily.

In agreement with literature reports[7], lime consumption during treatment fitted well with the acetyl content in sugarcane bagasse, which is known to be 4-5% (w/w). The 4% lime dose is sufficient to neutralize acetic acid that is quickly released from the biomass under alkaline conditions (Figure 1-A) with the pH dropping as lime is converted to calcium acetate and, afterwards as infection sets in (Figure 2-A). Even the 10% lime level pH is not always high enough to maintain pH above 10 long enough and 20% hydrated lime appears needed for safe, infection-free impregnation. Formate levels in solution are negligible as long as the microbial action is kept to minimum at pH above 11, but increase rapidly if the pH drops because of insufficient lime (Figure 1-B).

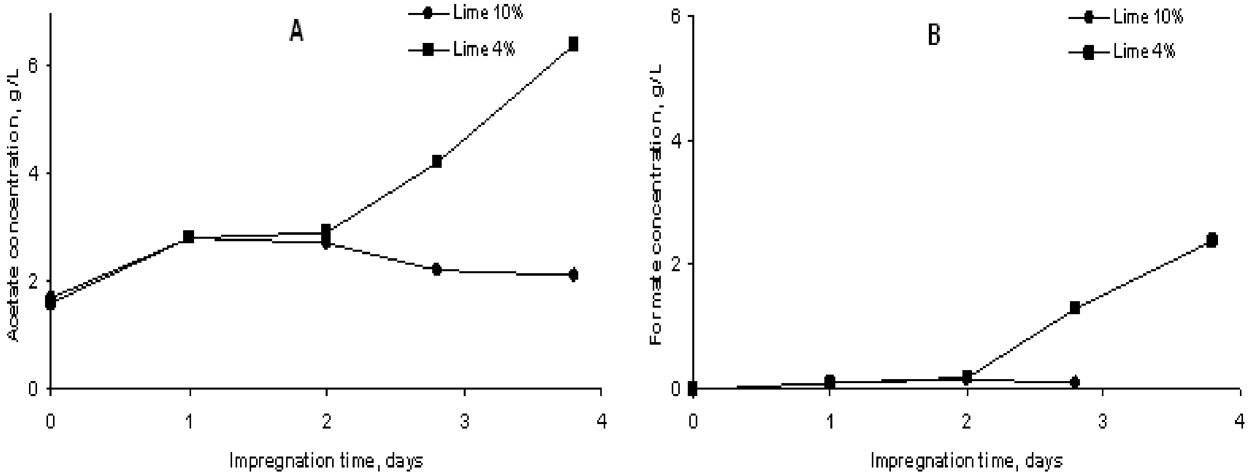

Figure 1. Concentrations of acetate (A) and formate (B) ions in the liquor during impregnation in two tests of lime impregnation.

The low severity treatment after impregnation is aimed to sterilize the biomass and increase the enzyme saccharification rate. The severity factor[15] that was employed here (log R_0 ~ 2.9 - 3.0) is lower than was used in the acidic pretreatment (log R_0 3.8 - 4.4). The low severity is expected to be favourable for the design and choice of construction materials for the steam treatment reactor

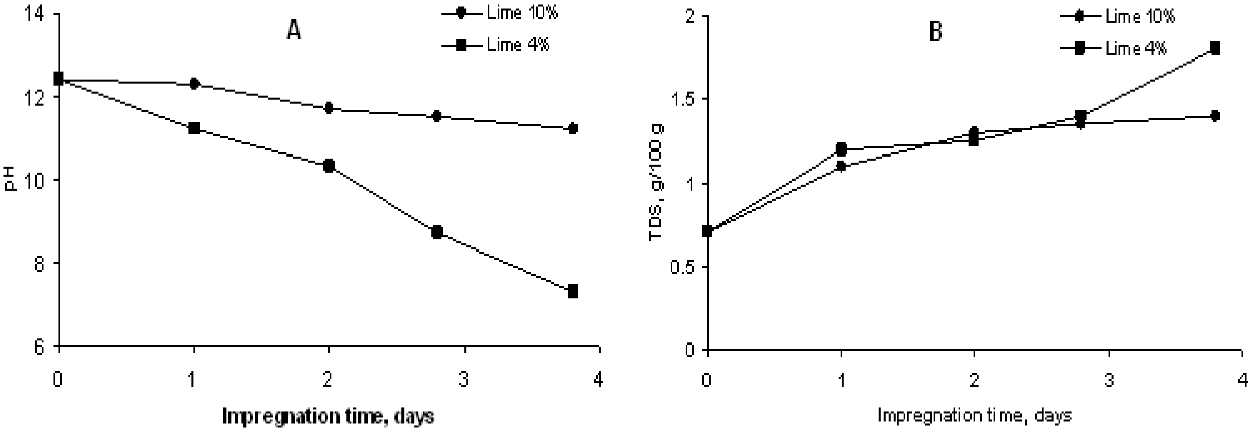

Figure 2. pH (A) and total dissolved solids (B) in two tests of lime impregnation.

The combined 72-hour enzymatic conversion of polysaccharides in lime-impregnated steam-pretreated cane leaves and bagasse pith corresponded approximately to 80% glucan, and 50% xylan (Fig. 3). The 24-hour conversion and yield are close to 90% of the final 72-hour values. The 40% yield of total sugars was lower than the 47 and 53% yields after acid impregnation followed by steam explosion at log R_0 = 4.1, but that was apparently due to the higher glucan content in the bagasse sample from a Cuban sugar mill[9] than in the Louisiana samples.

Major difference between acidic and alkaline pretreatments is the level of furans that are known to inhibit ethanol fermentation. A range of 100 – 5 000 mg furan/kg total dissolved solids (TDS) is common in acid hydrolysates from even low severity treatments[5,12], and 10 000 – 40 000 mg/kg TDS furan levels were reported in hydrolysates from higher severity autohydrolysis or dilute sulfuric acid processes[13]. In the alkaline tests, no measurable quantities of either furfural or HMF were detected in any of the pretreatment liquors. These results are in agreement with the low formation of furan aldehydes reported during alkaline wet oxidation of bagasse[10]. Whether the absence of furans in alkali-impreganted steam-exploded biomass hydrolysates translates to improved fermentability as expected will be tested in future simultaneous saccharification and fermentation (SSF) trials.

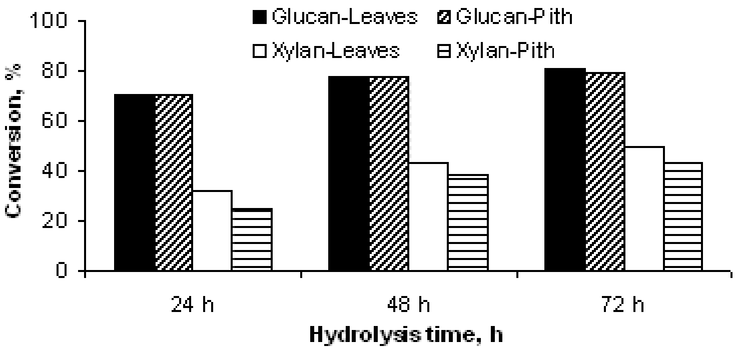

Figure 3. Glucan and xylan conversion in lime-impregnated steam-exploded cane trash and bagasse pith.

Besides deacetylation, the exact mechanism of alkaline action on sugarcane biomass is not known. Thermogravimetric analysis of the three main components of the biomass reveals very different TGA patterns, with xylan decomposing very rapidly at about 320ÚC. Cellulose decomposes later, at about 380ÚC, and lignin breaks down over a wide range 300 to 500ÚC (Fig. 4-A). Untreated sugarcane pith (Fig. 4-B) displays both xylan and cellulose peaks, shifted higher by 20ÚC. After pretreatment with hydrated lime, pure cellulose behavior remains. Curiously, after treatment with magnesium hydroxide the TGA pattern is shifted lower matching the xylan behavior. Whether this is related to the different effects of the two alkalis on the biomass structure of whether the residual salts catalyze thermal degradation is under investigation.

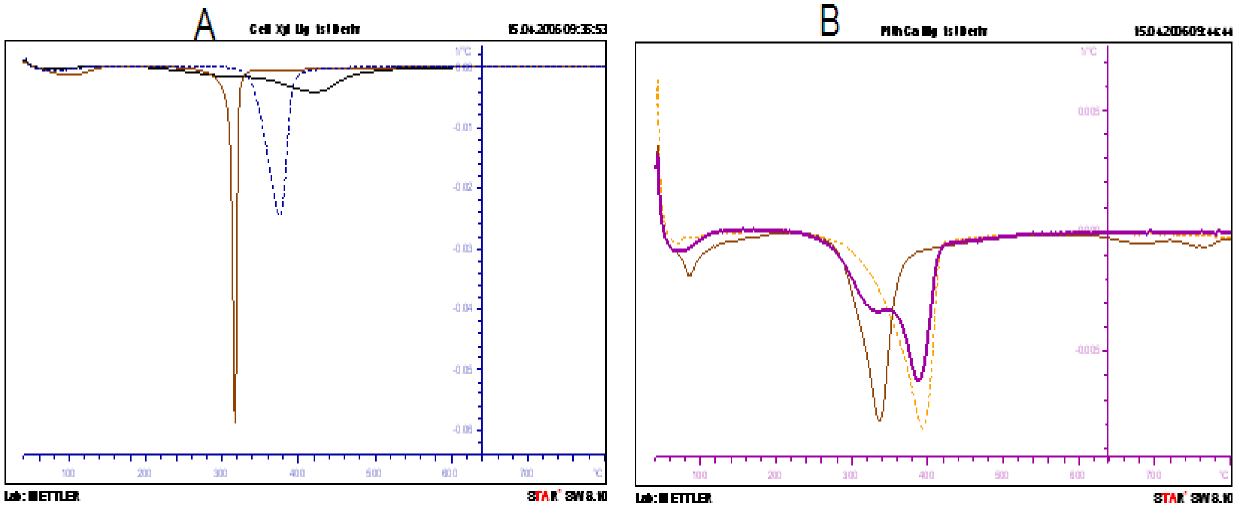

Figure 4. First derivative TGA curves of pure biomass macromolecules (A) and bagasse pith (B). A: Xylan (narrow-peaked solid line), cellulose (dotted line), and lignin (wide distribution solid line). B: raw bagasse pith (thick solid line), pith after impregnation with hydrated lime (dotted line) and pith after impregnation with magnesium hydroxide (thin solid line).

At current prices, it is estimated that the costs of the chemicals of the present unoptimized process are some $0.10 per kg sugar produced, of which $0.04 is for hydrated lime, $0.03 for the neutralization acid and $0.03 for the industrial enzymes. The acid requirements can be reduced by pressing the alkali-pretreated fiber prior to acidification. Unlike for bagasse, no equivalent-fuel value or any collection cost need to be assigned to cane trash, only the expense of transportation from the field to the mill. At present this averages $3-4 per ton of cane in Louisiana, adding only $0.01/kg fermentable sugar produced. Further improvements in the economics of the process are expected from potential co-products that are under investigation.

Conclusions

The two-step pre-treatment combining low-temperature acidic or alkaline impregnation followed by low-severity steam explosion treatment was found effective for pretreatment of sugarcane biomass. In the alkaline treatment, the sugar yield of 40 g/100 g BDS was achieved with the standard enzyme loading, at an estimated chemical cost of $0.10 /kg sugar. The process is the reverse of the standard dilute acid-detoxification by overliming process - but avoids the corrosive high-temperature acidic environment and the associated high-cost construction materials, sugar degradation and generation of fermentation toxins.

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