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Using high pressure processing (HPP) to pretreat sugarcane bagasse

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ABSTRACT

High pressure processing (HPP) technology was used to modify the structural composition of sugarcane bagasse. The effect of pressure (0, 150 and 250 MPa), time (5 and 10 min) and temperature (25 and 50 °C) as well as the addition of phosphoric acid, sulfuric acid and NaOH during the HPP treatment were assessed in terms of compositional analysis of the lignocellulosic fraction, structural changes and crystallinity of the bagasse. The effect of HPP pretreatment on the bagasse structure was also evaluated on the efficiency of the enzymatic hydrolysis of bagasse. Results showed that 68.62 and 45.84% of the hemicellulose fraction was degraded by pretreating at 250 MPa with sulfuric and phosphoric acids, respectively. The removal of lignin (54.10%) was higher with the HPP-NaOH treatment. The compacted lignocellulosic structure of the raw bagasse was modified by the HPP treatments and showed few cracks, tiny holes and some fragments flaked off from the surface. Structural changes were higher at 250 MPa and 50 °C. The X ray diffraction (XRD) patterns of the raw bagasse showed a major diffraction peak of the cellulose crystallographic 2θ planes ranging between 22 and 23°. The distribution of the crystalline structure of cellulose was affected by increasing the pressure level. The HPP treatment combined with NaOH 2% led to the higher glucose yield (25 g/L) compared to the combination of HPP with water and acids (>5 g/L). Results from this work suggest that HPP technology may be used to pretreat sugarcane bagasse.

1. Introduction

Lignocellulosic materials (LCM) are the most abundant renewable organic component all over the world and can be used for bioethanol production because they have a high content of cellulose and hemicelluloses, coming from agricultural residues, wood, paper and yard waste in municipal solid waste, and dedicated energy crops (Brethauer & Wyman, 2010; Singh et al., 2010).

There have been research efforts in developing cost-effective process to produce biofuels from LCM which are commonly called second generation biofuels, or advanced technologies. However, few challenges still remain for the commercial applications of advanced technologies, such as the low accessibility of cellulose and hemicelluloses due the complex tight structure of the lignocelluloses, low activity of cellulase enzymes and the difficulty to ferment 5-C sugars from the hydrolysis of hemicelluloses to ethanol (Cheng & Timilsina, 2011).

One of the major lignocellulosic materials found in great amount in tropical countries is sugarcane bagasse (SCB), the fibrous agro industrial by-product of the sugar and alcohol industry, which is a renewable resource with short remove cycle (Pandey, Soccol, Nigam, & Soccol, 2000; Vargas & Pereira, 2010). SCB is commonly used in paper industry and as a fuel for sugar factory, but the remaining bagasse is still a menace to environment. Hence, there is a need to explore novel applications for SCB. This lignocellulosic material is mainly composed by cellulose (about 50%), hemicelluloses (about 25%) and lignin (about 25%), which are associated to each other by hydrogen bonds and some other covalent bonds (Cardona, Quintero, & Paz, 2010).

Conversion of lignocellulosic materials to ethanol is more difficult than conversion of sugar or starch materials to ethanol (Cheng & Timilsina, 2011) since it involves three steps: pretreatment, hydrolysis and fermentation. From these, pretreatment is the more expensive and least technologically mature and hence there is a need to get more insight into this step through research and development.

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Combining pretreatment strategies is generally more effective in enhancing biomass digestibility and often employed in designing leading pretreatment technologies to lignocellulosic residues (Mosier, Wyman, et al., 2005; Sun & Cheng, 2002). Acid and alkaline pretreatments have been used alone or in combination with physical methods (Chen, Tu, & Sheen, 2011; Velmurugan & Muthukumar, 2011). Dilute acid pretreatment using hydrochloric acid, nitric acid, phosphoric acid and sulfuric acid converts hemicelluloses from LCM into soluble sugars (xylose, glucose, mannose, galactose and arabinose) and facilitates the subsequent enzymatic hydrolysis of cellulose (Chen, Tu, & Sheen, 2010). Otherwise, alkaline pretreatments adding sodium and calcium hydroxide remove or solubilize a greater fraction of lignin and part of hemicelluloses besides decreasing cellulose cristallinity and the degree of sugar polymerization. The mechanism of alkaline pretreatment involves saponification of the intermolecular ester bonds cross-linking lignin to other components as hemicelluloses (Arni, Zilli, & Converti, 2007).

Otherwise, high pressure processing (HPP) is an emerging technology used to preserve taste and nutritional properties of foods (San Martin, Barvosa-Canovas, & Swanson, 2002; Torres & Velazquez, 2005), which has not been tested to pretreat lignocellulosic materials. HPP treatment is based on two fundamental principles: the Le Chatelier principle, which postulates that pressure favors all structural reactions and changes that involve a decrease in volume; and the isostatic principle, which assumes that the distribution of pressure is proportional in all parts of a foodstuff irrespective of its shape and size (Heremans, 2002; Valdez-Fragoso, Mu ′jica-Paz, Welti-Chanes, & Torres, 2011). Unlike thermal treatments, pressure treatment is not time/mass dependent, since pressure acts by disrupting mainly hydrogen bonds without affecting covalent bonds and thus reducing the processing time. Besides, HPP technology induces a number of changes in biological systems – morphological, biochemical and genetic – as well as changes in the membrane and wall of microbial cells. HPP effects are uniform and nearly instantaneous throughout the product. Industrial HPP installations typically operate discontinuously and can attain pressures of up to 800 MPa, although pressures exceeding 400 MPa are not normally used for foods because they can bring about a reversible or irreversible disruption of inter and intramolecular bonds (Heinz & Buckow, 2009; Knorr, Heinz, & Buckow, 2006). For a proper cost analysis of the whole process, other aspects need to be considered when the high-pressure system is first installed; for example, the cost of equipment, automation, installation, plan preparation, labor, utilities, product design and packaging. The main cost involved is the equipment and its installation, but since high-pressure equipments are currently available, the cost of products processed by this technology has dropped significantly in recent years, making more products, accessible to consumers. According to Rastogi, Raghavarao, Balasubramaniam, Niranjan, and Knorr (2007), the average cost of HPP is around US$0.05–0.5 per liter or kilogram depending on processing conditions, which is lower than thermal processing costs. However, according to Hernando-Sainz, Tárrago-Mingo, and Purroy-Balda (2008), the cost of high-pressure processing under some common processing conditions, for example, ready-to-eat meats (585 MPa, 3 min, filling vessel 50%), is really between US$0.08 and 0.22/kg, which makes HHP technology suitable for products of premium quality. Hence the main goal of this work was to evaluate the effect of high pressure processing assisted by chemical agents (sulfuric acid, phosphoric acid and sodium hydroxide) to elucidate if HPP can be used to improve enzymatic hydrolysis of sugarcane bagasse to produce ethanol and other chemical products of interest. Pretreatments were carried out at two different temperatures (25 and 50 °C) and its effect was evaluated in terms of composition and structural features of sugarcane bagasse.

2. Materials and methods

2.1. High hydrostatic press equipment

Pressure treatments were carried out by using a Cold Isostatic Press Model CIP42260 (Avure Autoclave Systems, Columbus, OH) with a pressuring chamber of 101.6 mm of inner diameter and 584.2 mm of length. The operating pressure capacity of the equipment was up to 30,000 psi. A mixture of 5:1 water with anticorrosive lubricant (Hydrolubric 120-B; EF Hounghton, Valley Forge, PA) was used as pressurizing fluid in order to keep temperature near to 25 and 50 °C during treatments. The time required to reach process conditions was less than 30 s and the release time was always less than 20 s.

2.2. Pretreatment of cane bagasse with HPP

Sugarcane (*Saccharum officinarum*) bagasse (SCB) was provided by the local sugar factory, The Higo, Veracruz. SCB was dried, milled and storage in bags. Treated SCB samples were placed in 120 mL bottles with water, phosphoric acid (2%, v/v), sulfuric acid (2%, v/v) and NaOH (2%, v/v) in the ratio of 9:1 (mL/g) and subjected to pressurization using a experimental design under the following conditions: pressure (0, 150 and 250 MPa), temperature (25 and 50 °C), and time (5 and 10 min). Experiments were carried out by duplicate using both, non-treated bagasse (NB) and prehydrolyzed bagasse (PHB) by using: 2% (v/v) sulfuric acid, 121 °C, 40 min and a liquid–solid ratio of 6:1 (mL/g).

2.3. Enzymatic hydrolysis of cellulose

The enzymatic hydrolysis was performed in 250 mL flasks in a rotary shaker at 150 rpm and 50 °C previously added with the cellulase enzymes: Novozyme 188 and Celluclast (both enzymes from Sigma, Aldrich) in a concentration of 0.5 g enzyme/10 g biomass for each enzyme in 50 mM acetate buffer (pH 4.8). Samples (2.0 mL) were periodically removed and boiled for 5 min to quench the enzymatic reaction. After centrifugation of the boiled samples, glucose concentration was measured by HPLC. All reactions were carried out by duplicate.

2.4. Compositional analysis of cane bagasse

Neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL) were determined in treated and untreated cane bagasse using the Ankom Fiber Analyzer (Ankom Technology, Macedon, N.Y.). Differences between NDF and ADF provide an estimate of hemicelluloses and differences between the values of ADL and ADF provide the concentration of cellulose according to McIntosh and Vanconv (2011).

2.5. Cellulose crystallinity measurement

The Powder method was used to observe the crystalline structure of treated and non-treated SCB. Dry bagasse samples were ground into a fine powder and sieved through a 150-μm mesh. Powder samples were densely packed into an aluminum frame. The X-ray diffraction patterns of samples were recorded by using a diffractometer (Siemens D5000, Madison, WI) operating at 35 kV and 15 mA. A Cu Kα radiation wavelength of $\lambda = 1.5406$ Å, from 10° to 45° on a 2θ scale and a step size of 0.05° was used. Measurements were conducted at room temperature.

Table 1
Composition and origin of lignocellulosic fractions from different types of sugarcane bagasse.

Cellulose (%)	Hemicellulose (%)	Lignin (%)	Origin	Reference
43.6	27.7	17.7	Brazil	Teixeira et al., 1999
43.6	33.5	18.1	China	Sun, Xu, Sun, Sun, and Wu (2004)
40.5	19.7	26.4	Brazil	Rabelo, Amezquita-Fonseca, Andrade, Maciel-Filho, and Costa (2011)
44	34.2	16.8	Oaxaca	Gutierrez (2011)
52.45	25.97	12.72	Taiwan	Chen et al. (2011)
34	27	18	India	Binod et al. (2012)
44.6	26.3	18.4	Veracruz	This work

2.6. Analytical methods

Sugars and inhibitors were quantified by using a Waters Alliance HPLC (Model 2695, Water Corporation, Milford, MA) supplied with a refractive index detector (Waters 2414) and with a Shodex SH1011 column operating at 50 and 55 °C, respectively. The mobile phase of 0.05 N H_2SO_4 was run at an elution flow rate of 0.600 mL/min. The concentration of sugars was obtained based on calibration with sugar standards.

Morphological changes on SCB were observed under a scanning electron microscope (SEM) (JEOL JSM-6060 LV, Tokyo, Japan). The bagasse samples were fixed with an electro conductive carbon ribbon duplex brass sample holder. Previous to the analysis a gold pleating was conducted with an acceleration voltage of 20 kV.

3. Results and discussion

3.1. Characterization of the lignocellulosic fraction of sugarcane bagasse

Results from the detergent fiber technique showed that the lignocellulosic fraction of sugarcane bagasse before pretreatment was made of tree basic constituents: cellulose (44.60%), hemicelluloses (26.30%) and lignin (18.40%). This composition is similar to that found by Teixeira, Linden, and Herbert (1999) in a sugarcane bagasse from Brazil (Table 1) but is quite different from the composition reported for a sugarcane bagasse from a different Mexican region (Gutierrez, 2011) and other countries. Differences can be attributed to the type of plant from which the biomass was obtained, crop age and harvesting method (Classen et al., 1999). Constituents of lignocellulosic materials are associated with each other in a hetero-matrix to different degrees and the relative composition depends on type, species and even source of biomass (Chandra et al., 2007). This heterogeneity and high degree of complexity, inherent to the nature and composition of the constituents of SCB and other lignocellulosic materials is a limiting factor and makes a difficult task to establish a pretreatment applicable to all lignocellulosic biomass in order to produce compounds of interest such as ethanol (Sanchez and Cardona, 2008). In this context, it is interesting to study the effect of high pressure processing on the structure of cellulose or as pretreatment to remove hemicelluloses and lignin from the lignocellulosic fraction of SCB, since no reports have been found on this application.

3.2. Effect of high pressure treatments on the lignocellulosic fraction

The composition of the lignocellulosic fraction of sugarcane bagasse after being exposed to different levels of pressure (0, 150 and 250 MPa) at 50 °C during 10 min is shown in Fig. 1. It can be seen that the three basic components of SCB (cellulose, hemicelluloses and lignin) remained almost unaltered in dry bagasse (DB) and bagasse with 44% water, after exposition to the different pressure levels tested. A slight reduction (≈10%) on the cellulose content was observed when combining HPP with phosphoric acid, while the combined treatment of HPP with sulfuric acid rather affected the hemicellulose fraction. Otherwise, an important removal (≈50%) of lignin was observed when HPP treatments were carried out with 2% NaOH and low temperature (50 °C). This result suggests that this treatment could be used as delignification instead of alkaline pretreatments with potassium, calcium and ammonia hydroxides, which cause swelling of biomass and decrease the polymerization degree of cellulose by destruction of links between lignin, making the cellulose fraction more accessible (Arni et al., 2007; McIntosh & Vanconv, 2011; Mosier, Hendrickson, et al., 2005). A lignin reduction of 95% with 10% NaOH for 1 h was observed in SCB by Varga, Scengyel, and Recaey (2002).

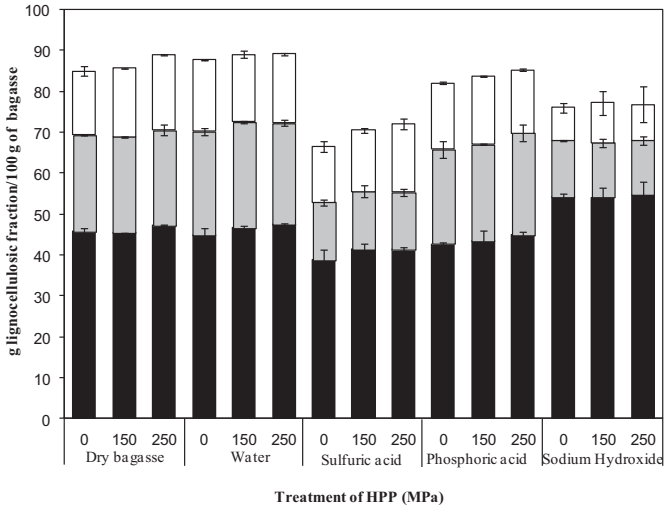

Fig. 1. Effect of the high pressure processing and chemicals used during pretreatment on the lignocellulosic fraction of raw sugarcane bagasse. Cellulose (black), hemicelluloses (gray), and lignin (white).

An effective pretreatment should alter not only the macroscopic and microscopic structure of LCM but also should modify the chemical composition and the structure of the carbohydrate fractions into simple sugars, quickly and with higher yields (Agbor, Cicek, Saprling, Berlin, & Levin, 2011; Mosier, Wyman, et al., 2005). Results from this work showed that high pressure technology importantly reduced the lignocellulosic fraction but it did not affect the hemicellulose content of SCB. These results agree with Yang, Jiang, Wang, Zhao, and Sun (2009), who pointed out that hemicellulose was less susceptible to degradation than other polysaccharides and showed

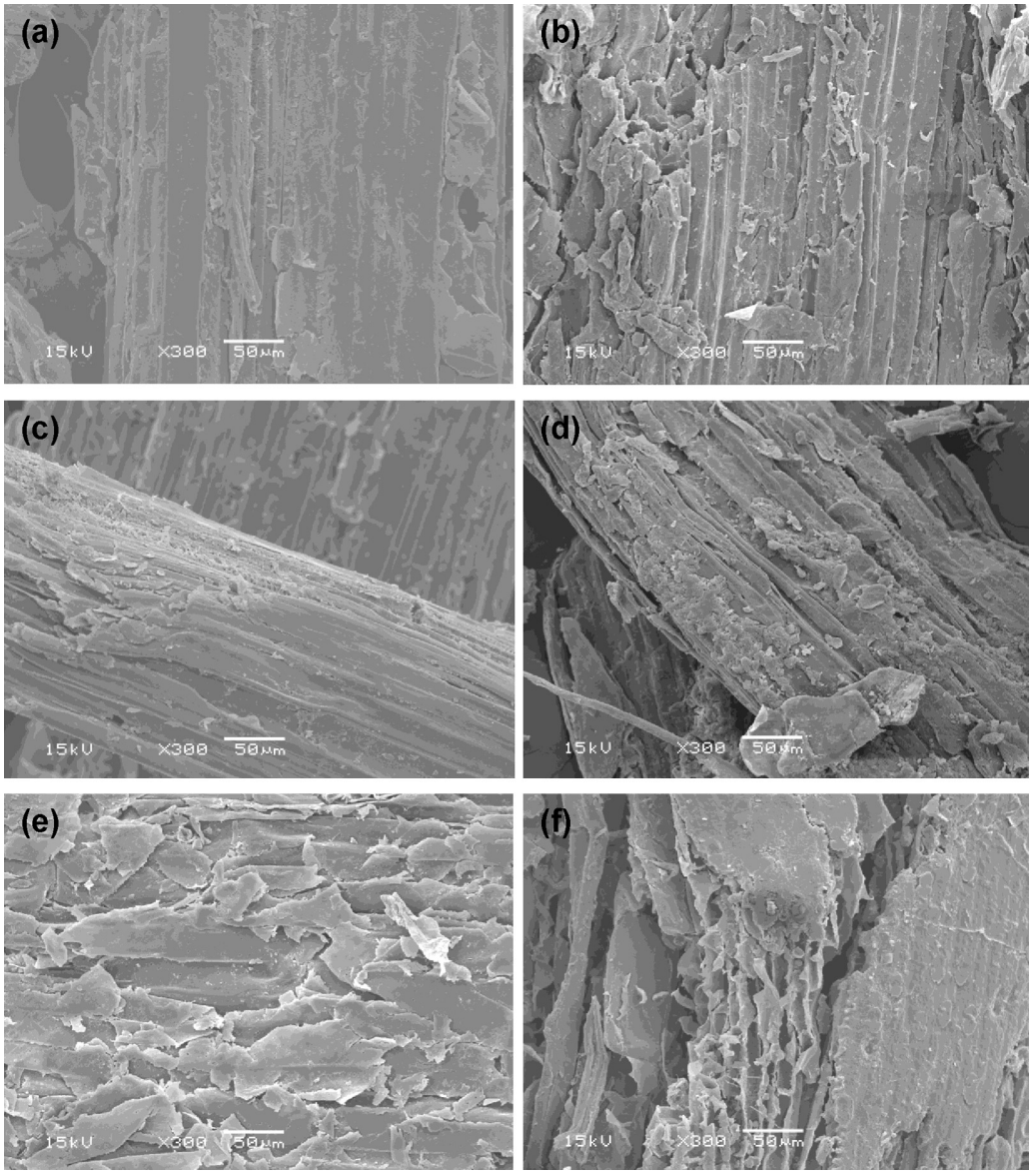

Fig. 2. SEM images of raw and pressurized sugarcane bagasse at 250 MPA and suspended in different media. (a) Raw bagase, (b) pre-hydrolyzed bagasse, (c) SCB with water, (d) SCB with sulfuric acid, (e) SCB with sodium hydroxide and (f) SCB with phosphoric acid.

that the covalent bond of this macromolecule was not modified by ultra high pressure treatments.

The main reduction of hemicellulose fraction was observed by combining acids with HPP. A removal of hemicelluloses of 68.62 and 45.84% were obtained with HPP and, sulfuric or phosphoric acids, respectively. However, no significant ($P > 0.05$) difference on the hemicellulose concentration was found between the HPP treated and the non HPP treated samples, though the cellulose fraction increased as the level of pressure rised. Treatments with dilute sulfuric acid (between 0.5 and 4%) removed 80–90% hemicellulose and low lignin was dissolved or suffered degradation, increasing the susceptibility to enzymatic attack (Yang & Wyman, 2008). Although the hemicellulose remotion was lower in treatments with sodium hydroxide acids, this solubilization of hemicellullose released oligomers and monomeric sugars and affected the reactivity of the biomass carbohydrate polymers and hence increased the accessibility of cellulose.

3.3. *Effects of high pressure processing on macro and microstructure of SCB*

The SCB when submitted to different treatments suffered changes in appearance and coloration depending on the type of suspension media or chemical added before being pressurized. It can be seen that the greatest discoloration and degradation of the fiber occurred with sodium hydroxide. With acids, the structure of SCB became more brittle and color was less intense than regular bagasse (data not shown). Similar changes have been observed in other lignocellulosic materials, such as polar species. Foston and Ragauskas (2010) attributed these changes to the breakdown of carbohydrates and to the extraction and release of phenols, plus residual lignin. Darkening became more evident as increased the residence time of the SCB on acid.

Sugarcane bagasse samples obtained before and after high pressure treatments were analyzed by scanning electron microscopy in order to assess structural and morphological changes. Micrographs

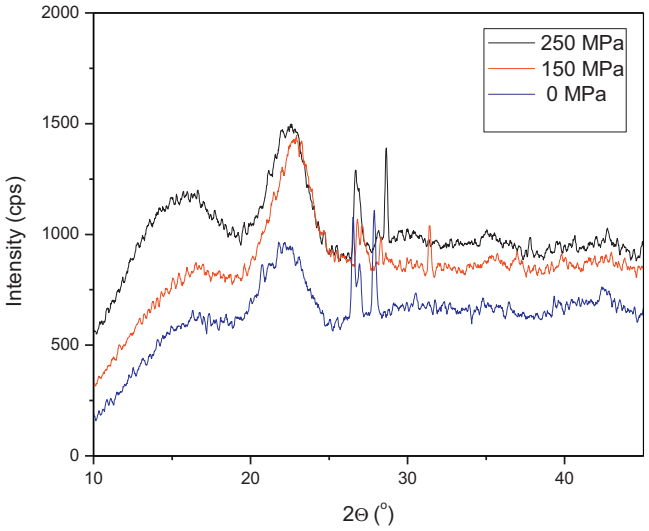

Fig. 3. Effect of pressure level used during high pressure processing on the crystalline structure of sugarcane bagasse.

(Fig. 2) suggest that changes occurred in the fibrous structure of pretreated samples, where visible breaks in the structure left exposed zones or hollow areas while raw bagasse showed a complete and compact structure (Fig. 2a). Structural changes varied according to pressure level and the chemical added. At 250 MPa and 50 °C, the lignocellulosic structure was more severely damaged showing some cracks and fragments on the surface, which were more evident in treatments with NaOH (Fig. 2e). HPP treatments combined with acids provoked holes and the structure of bagasse was destroyed in a significant way (Fig. 2d and f). These changes could be associated to the partial disruption of the fiber and the removal of lignin and hemicellulose. Similar structural changes were reported by Chen et al. (2011).

3.4. Effect of high-pressure treatments on the crystallinity of sugarcane bagasse

Changes on the crystallinity of sugarcane bagasse submitted to HPP can be observed in the XDR patterns of the raw bagasse shown in Fig. 3. A major diffraction peak was observed at 2θ ranging between 22 and 23°. The crystalline structure was clearly observed in the bagasse control (0 MPa). However, according to the distributions the SCB treated with 150 and 250 MPa has an *s* trend which indicates a modified crystalline structure of cellulose. In addition, it can be observed that the amorphous domain of SCB was more susceptible to changes than the crystalline cellulose. A reduction of the amorphous structure was more evident in the treatment of HPP combined with sodium hydroxide. Given that the crystalline structure of cellulose itself represents an extra obstacle to hydrolysis, the extent of reduction on the crystalline structure is an indicative of the effectiveness of pretreatments, since the amorphous regions are more easily converted to glucose (Cardona et al., 2010). The crystalline structure is sensitive to heat and it disappears at about 190 °C in the presence of dilute acid (Chen et al., 2011) while the amorphous domain of SCB is more susceptible to acid hydrolysis (Velmurugan & Muthukumar, 2011). Experimental results showed a similar behavior with increments of pressure level in pretreatments combined with sulfuric acid (Fig. 3).

3.5. Influence of HPP on the enzymatic saccharification of sugarcane bagasse

Changes on the composition of the SCB solid phase after high-pressure treatments at optimal conditions were evaluated by cellulose susceptibility to the enzymatic saccharification to obtain glucose. Fig. 4 shows the final concentration of glucose after 144 h of saccharification of both, milled bagasse (NB) and prehydrolysated bagasse (PHB) at 250 MPa, as well as their respective controls for HPP treatments with water, sulfuric acid (2%), phosphoric acid (2%) and sodium hydroxide (2%).

It can be seen from Fig. 4 that hydrolysis of cellulose appeared with increments on the incubation time. Currently this process is carried out using microbial enzymes and the best results have been found in fermentations with glucose produced by this method since the formation of inhibitors is avoided (Berlin, Maximenko, Gilkes, & Saddler, 2007). However the process is slow, as is shown in Fig. 4a–d, where glucose production was gradually occurring throughout the enzymatic process. The higher final concentrations of glucose were obtained with pressurized bagasse suspended in water and sodium hydroxide: 5–25 g/L, respectively (Fig. 4a and b). Otherwise, glucose concentrations obtained with normal bagasse were lower (3 and 20 g/L respectively). The observed differences suggest that pressure and prehydrolysis favored enzymatic hydrolysis because the accessibility of the enzymes to the fibers is enhanced by the removal of hemicellulose.

On the other hand, small differences were observed between the non-pressurized NB and PHB, and both HPP-acid treated bagasses. It can be seen from Fig. 4c that the kinetic behavior of bagasses treated with H_2SO_4 was similar and produced only between 3.0 and 3.5 g/L glucose at the end of the enzymatic process (144 h) while those treated with H_3PO_4 (Fig. 4d) achieved 6.5 g/L glucose. Differences between pressurized bagasse and unpressurized samples were more important at the end of the process, although glucose concentrations were quite low and the efficiency of hydrolysis was no more than 50%, indicating a low digestibility of BC. This low degree of digestibility may be associated to a possible denaturation or inactivation of cellulases.

The HPP treatment combined with NaOH 2% led to the higher glucose yield (25 g/L) compared to the combination of HPP with water and acids (>5 g/L). Results from this work suggest that HPP technology may be used to pretreat sugarcane bagasse.

Experimental results point out that the maximal concentration of glucose was obtained from the enzymatic hydrolysis of BPH pretreated with HPP and 2% NaOH. The increase on enzymatic digestibility may be associated to the low content of lignin and hemicelluloses and the high cellulose concentration remaining in the solid material, as suggested by Taherzadeh and Karimi (2007). It has been reported that enzymatic hydrolysis is affected by several factors such as substrate concentration, cellulose crystallinity, degree of polymerization, surface area, lignin and hemicelluloses, porosity and particle size (Alvira, Tomas-Pejo, Ballesteros, & Negro, 2010). From these, the lignin content is the main obstacle to enzyme attack of cellulose because it prevents the accessibility of cellulases and enzymes might be irreversibly adsorbed to it (Chandra et al., 2007; Zhu et al., 2009). Indeed, the results obtained with pressurized-NaOH bagasse confirm that there is a strong correlation between the fraction of hydrolyzed cellulose and the lignin fraction removed since there was a complete degradation of the structure as pressure level increased. Some researchers have observed that there is not need to remove most of the lignin to achieve higher than 90% cellulose degradability and suggested that residual lignin plays a role in binding the enzymes, and affecting hydrolysis rate (Adsul et al., 2005; Lee, Doherty, Linhardt, & Dordick,

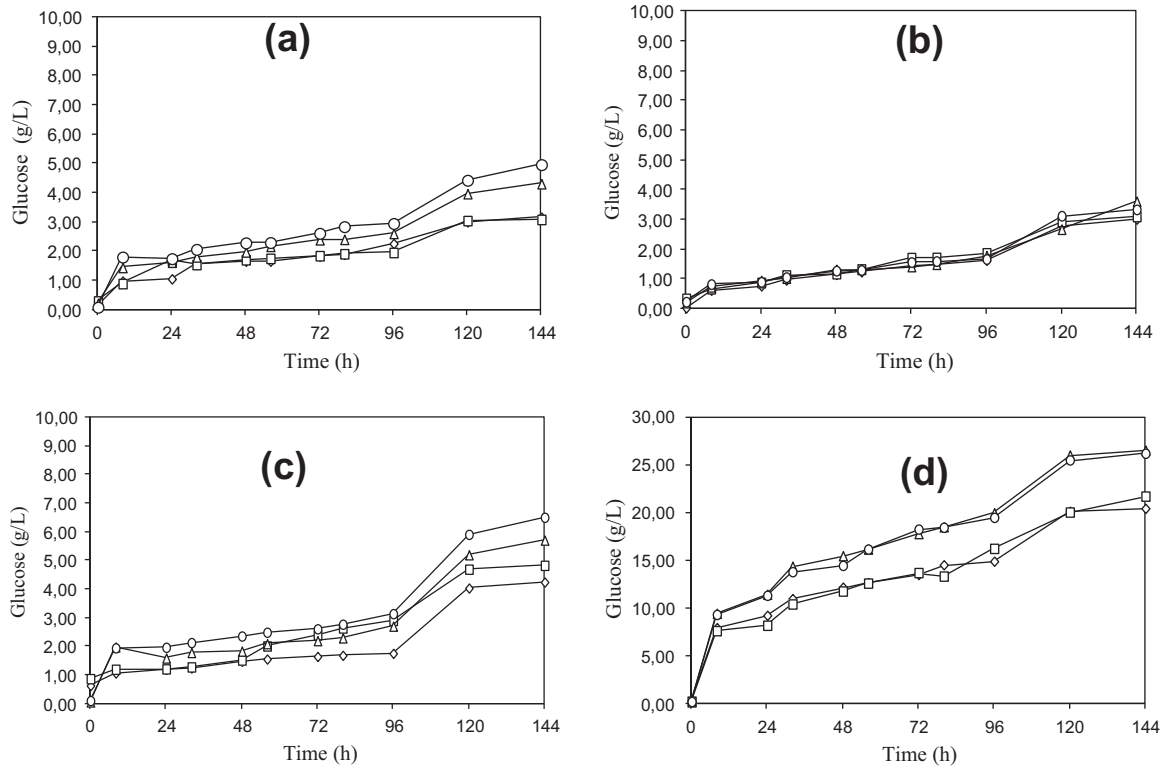

Fig. 4. Kinetics of enzymatic hydrolysis of the lignocellulosic fraction of sugarcane bagasse treated under different pressure conditions and suspended media. Normal bagasse – 0 MPa (◊), normal bagasse – 250 MPa (□), prehydrolyzed bagasse – 0 MPa (△), and prehydrolyzed bagasse – 250 MPa (○). Water (a), sulfuric acid (b), phosphoric acid (c), and sodium hydroxide (d).

2009). Results from these work show experimental evidence of the effectiveness of the combined pretreatment HPP-NaOH on the lignocelluloses fraction of SCB and on the enzymatic hydrolysis of cellulose.

4. Conclusions

The effect of high pressure processing on the lignocelluloses fraction of sugarcane bagasse was a function not only of pressure level and temperature, but also on the chemical used to pretreat SCB. Results indicated that even though HPP, in the pressure range tested, induced changes on microstructure and crystalline structure of SCB, it did not enhance susceptibility of pretreated SCB to the enzymatic hydrolysis and neither increased glucose yields. Such results evidenced the complexity and recalcitrant structure of the cellulose fraction of SCB. However, results from this work suggest that increasing high pressure processing (HPP) up to 400–800 MPa and using different concentrations of chemical compounds might increase the susceptibility of pretreated SCB to enzymatic hydrolysis and rise glucose concentrations. It should be noted that this work is the first to use HPP to pretreat lignocellulose biomass and to point out that this technology has potential to increase glucose yield and bioethanol production from it. Although the effectiveness of HPP technology has been known for some time at the food industry, it is time now to start developing and applying this technology for treating lignocellulosic biomass; hence it could be applied on a large scale, if the process is optimized.

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