Biomass Conversion and Biorefinery
https://doi.org/10.1007/s13399-020-00776-0

ORIGINAL ARTICLE

Alkali-peroxide treatment of sugar cane bagasse. Effect of chemical charges on the efficiency of xylan isolation and susceptibility of bagasse to saccharification

Yamil Nahún Solier[1] · Paulina Mocchiutti[1] · María Noel Cabrera[2] · Mario Carlos Nazareno Saparrat[3,4] · Miguel Ángel Zanuttini[1] · María Cristina Inalbon[1]

Received: 20 February 2020 / Revised: 17 April 2020 / Accepted: 15 May 2020

Abstract

Sugar cane is a promising raw material for the extraction of hemicelluloses. An alkali treatment of sugar cane bagasse under proper conditions followed by a precipitation using a reasonable quantity of ethanol can be an effective method to isolate these polysaccharides. In this work, bagasse is treated to obtain two products: (a) polymeric hemicellulose and (b) an enzymatic hydrolysate from the treated bagasse after hemicellulose extraction. The effects of charges of sodium hydroxide (10, 20, 40% w/w) and hydrogen peroxide (0, 10, 20% w/w) in the alkali treatment were evaluated. A 3^2 experimental design was considered under control of metal ions and inert atmosphere during alkaline treatment. An acceptable proportion of xylose plus arabinose could be extracted from bagasse (up to 18.4 g/100 g of bagasse). Both the alkali and peroxide showed strong effects on the yield of the extraction, as well as on the yield of the precipitation in ethanol-water solution. Besides, the susceptibility of bagasse for cellulolytic enzymatic hydrolysis is improved. The highest hemicellulose precipitation yield was 85% which corresponded to the treatment with the highest alkali charge without peroxide. The highest yield of enzymatic hydrolysis was obtained for the highest alkali and peroxide charges.

Keywords Hemicellulose extraction · Alkali consumption · Peroxide effect · Enzymatic hydrolysis

1 Introduction

In recent years, the substitution of petroleum-based materials for those obtained from renewable resources has gained great interest. Optimizing the use of main components of biomass in an integrated industrial process is important to achieve this objective.

Hemicelluloses are the second most abundant polysaccharide in nature after cellulose and they can be used for hydrogels, packaging films, or biomedical materials, among other purposes [1]. Nevertheless, they are not yet industrially exploited on a large scale. When paper pulps are produced, a high proportion of the original hemicelluloses are burned together with the lignin in the recovery boiler, although it is known that they have half of the calorific value of lignin [2, 3].

Among agricultural crop residues, sugar cane bagasse is the lignocellulosic by-product generated in the sugar and ethanol industry. Sugar cane is cultivated in tropical and subtropical countries throughout the world. The main producers are Brazil (721 million tons), India (347 million tons), China (123 million tons), and Thailand (96 million tons) [4]. Considering that approximately 250–280 kg of bagasse is generated by 1 ton of processed sugar cane, it represents an attractive raw material source for hemicellulose extraction [5]. An efficient well-known procedure of wet storage of this biomass in piles located close to the sugar mill allows its use during the whole year

✉ Yamil Nahún Solier
ysolier@fiq.unl.edu.ar

[1] Instituto de Tecnología Celulósica, Facultad de Ingeniería Química, Universidad Nacional del Litoral-CONICET, Santiago del Estero 2654, S3000AOJ Santa Fe, Santa Fe, Argentina

[2] Instituto de Ingeniería Química, Facultad de Ingeniería, Universidad de la República, J. Herrera y Reissig 565, 5to piso, 11300 Montevideo, Uruguay

[3] Instituto de Fisiología Vegetal, Universidad Nacional de La Plata-CONICET, Diag. 113 y 61 #495, La Plata, Argentina

[4] Instituto de Botánica Spegazzini, Facultad de Ciencias Naturales y Museo, Universidad Nacional de La Plata, 53 #477, La Plata, Argentina

Published online: 23 May 2020

as a raw material for paper production. Bagasse can be used for other industry processes to produce fuels and chemicals that offer economic, environmental, and strategic advantages, according to biorefinery [6]. Particularly, in Argentina, 18.4 million tons of sugar cane were processed annually [7]. The major hemicelluloses in sugar cane bagasse, as in other grasses, are L-arabino-(4-O-methyl-D-glucurono)-D-xylans (or just xylan) [8–11].

Different treatments can be applied for obtaining hemicelluloses, but alkaline extractions are the most effective ones for extracting high molecular weight hemicelluloses [9, 12–14].

Bagasse can be alkali treated under mild conditions (up to 6% NaOH on bagasse and temperature lower than 100 °C) and then refining in a disk mill to obtain a chemi-mechanical pulp [15]. Using higher alkali charges (between 8 and 11% on bagasse) and temperature up to 140 °C, a pulp suitable for packaging papers can be obtained [16]. A charge of 16% NaOH on bagasse can be enough for obtaining a highly delignificated chemical pulp.

On the other hand, if the extraction of hemicellulose from bagasse is desired, higher alkali charge and lower temperature than those used for pulping are necessary. Under these conditions, hydroxide ions cause high swelling of the cell wall, which facilitates hemicellulose removal. Besides, the α-ether bonds between lignin and hemicelluloses as well as the ester bonds between lignin, and/or hemicelluloses and hydroxycinnamic acids, such as p-coumaric and ferulic acids, are broken [12]. Thus, it can be expected that the hemicelluloses obtained through alkaline extraction have a smaller amount of bonded lignin.

It is known that, in alkaline conditions, carbohydrates suffer undesirable reactions: hydrolysis, which breaks the polymer chains randomly, and the "peeling" reaction in which the monomeric units are progressively eliminated from the reducing end groups. These reactions are favored at high temperature. Besides that, radicals, i.e., reactive species of oxygen, can also degrade hemicelluloses. In this regard, low temperature, an inert atmosphere in the reactor, and a control of the presence of metal ions could be useful to preserve the molar mass of the extracted hemicelluloses.

Despite the economic impact of chemical consumption of an alkaline extraction from bagasse, this aspect of the process is rarely reported in the literature.

It is known that the addition of hydrogen peroxide in an alkaline medium leads to an improvement in yield of a hemicellulose extraction which can be ascribed to a delignification effect. Doner and Hicks [17] showed that the presence of hydrogen peroxide in a corn fiber extraction operation increased the amount of hemicelluloses extracted by approximately one-third. In that case, a lighter color of hemicelluloses was also obtained. Alkaline hydrogen peroxide has been used to pretreat different types of biomass to improve the efficiency of enzymatic hydrolysis [18]. One of the limitations for the use of peroxide is the requirement of an equipment material that can withstand this reactant. Besides, the degradation of the hemicelluloses should be minimized by controlling the presence of metal ions in the medium. These metal ions promote the decomposition of hydrogen peroxide reducing its delignification performance [9]. Peroxide induces changes in the chemical characteristics of the hemicelluloses that should be considered.

For isolation, xylan can be precipitated by the addition of ethanol to the extraction liquor. For a potential industrial operation, a low ethanol:liquor ratio should be considered in order to reduce the energy requirement for the ethanol recovery process.

On the other hand, sugar cane bagasse after hemicellulose extraction can be used for cellulosic ethanol production. A successful alkali treatment removes lignin and hemicelluloses, and makes the cellulose more accessible to enzymatic attack considering a biological process for bioethanol production [19–21].

In this paper, the extraction and isolation of hemicelluloses from sugar cane bagasse are analyzed in detail considering the following: (a) metal ion control, (b) inert atmosphere in the reactor, and (c) low ethanol:liquor ratio for xylan precipitation. Charges between 10 and 40% of NaOH and between 0 and 20% of H_2O_2 on bagasse were considered. The chemical consumption, the yield of the hemicellulose extraction, the yield of their precipitation in alcoholic medium, and the contents of lignin are analyzed. The enzymatic digestibility of the solid treated bagasse as a function of the alkaline treatment conditions is also studied. To facilitate a technical-economic analysis, all results are referred to the original bagasse mass.

2 Materials and methods

2.1 Materials

Sugar cane bagasse (fiber and pith) was provided by the Tacuarendí Experimental Center, Santa Fe, Argentina.

2.1.1 Raw bagasse characterization

The size of the raw bagasse was reduced by two passes through a 300-mm disk mill using clearances of 2.5 and 1.0 mm, respectively. After that, bagasse was air dried (final moisture content of 10%).

The content of the main carbohydrates (glucose, xylose, and arabinose), and acid-soluble and acid-insoluble lignin was determined according to Sluiter et al. [22] in the extractive-free fresh bagasse. For that, milled raw material (40 mesh) was subjected to a two-step of Soxhlet extraction using water first and then ethanol (96%). By duplicate, 300 mg of sample was hydrolyzed with 72% (w/w) H_2SO_4

during 60 min at 30 °C. Hydrolyzates were then diluted to 4% (w/w) H_2SO_4 and treated at 121 °C for 1 h in an autoclave. The material was filtered through 0.5-μm glass filter and the mass retained was quantified as acid-insoluble lignin plus ash. The ash content was determined according to TAPPI T 211, at 525 °C. Acid-soluble lignin was quantified, in the filtrate, by UV-Visible spectrophotometer, determining the absorbance at 240 nm and considering an absorptivity of 25 l (g cm)$^{-1}$. For carbohydrate analysis, a Shimadzu Lab Solution HPLC system was used, which was equipped with refractive index (RID-10A) and UV-VIS (SPD-20A) configured at 210-nm detectors. Column and guard column BioradAminex HPX-87H, sulfuric acid solution (0.005 M) as mobile phase, a flow rate of 0.6 ml min^{-1}, and a column temperature of 35 °C were also employed. Standard sugars were supplied by Sigma-Aldrich. The acetyl content in bagasse was also determined by this method using acetic acid from Sigma-Aldrich as standard.

2.2 Alkali-peroxide treatment

Different charges of sodium hydroxide and hydrogen peroxide were considered in a 3^2 experimental design, shown in Table 1.

Prior to alkali treatment, in order to control the presence of metal ion, air dry milled bagasse was treated during 30 min at 50 °C in water containing a chelating agent (DTPA, diethylenetriaminepentaacetate), 0.2 g/100 g of bagasse. This reactant was also added, in the same charge, to the alkali treatment.

In a 3-l glass reactor and with a moderate stirring (120 rpm) by a PTFE paddle stirrer, 60 g of bagasse was treated during 180 min at 50 °C in a bagasse:liquor ratio 1:25. An inert atmosphere of nitrogen was established in order to prevent degradation of carbohydrates by alkali reactive oxygen species. When hydrogen peroxide was used, a solution of this compound was added in aliquot portions every 10 min during the first 2 h of the extraction time. This procedure minimized foam formation.

Once the period (180 min) was reached, the bagasse was separated from the liquor (L_e) by filtration through a cloth bag and the liquor was removed by squeezing the bag in hot condition. The amount of liquor collected was 1420–1300 ml. The liquor at room temperature was centrifuged during 10 min at 1800*g* to separate the small debris of bagasse. Later, the pH of the liquor was quantitatively adjusted to 7.0 using a 3 M solution of hydrochloric acid. Then, the liquor was centrifuged again during 15 min at 1800*g* in order to separate all the material insoluble at this pH. The low amount of particles precipitated at this point was discharged. The treated bagasse (S_e) was washed with 1 l of distilled water at room temperature. Later it was dewatered, weighed, and stored at −15 °C. The washing liquid (L_w) was preserved. The susceptibility of the treated bagasse for enzymatic saccharification was assessed after washing. A complete scheme of the process is shown in Fig. 1.

The hydrogen peroxide consumption during the extraction was determined by titration with sodium thiosulfate (iodine method). Alkali consumption was determined by titration with hydrochloric acid solution. All determinations were made in duplicate.

Table 1 Identification code of alkali and alkali-peroxide treatments of bagasse (50 °C, 180 min, under inert atmosphere and metal ion control). Chemical charges followed a 3^2 experimental design

H_2O_2 charge (% w/w)	NaOH charge (% w/w)		
	10	20	40
0	$A_{10}P_0$	$A_{20}P_0$	$A_{40}P_0$
10	$A_{10}P_{10}$	$A_{20}P_{10}$	$A_{40}P_{10}$
20	$A_{10}P_{20}$	$A_{20}P_{20}$	$A_{40}P_{20}$

2.2.1 Precipitation process

Extracted hemicelluloses were precipitated mixing a volume of the neutralized liquor with 95% ethanol in 1:1 v/v ratio. The mixture was left overnight at 4 °C and precipitated hemicelluloses were separated by centrifugation at 1800*g* for 15 min. The supernatant mixture liquor-ethanol (L_p) was reserved to quantify the lignin content. The precipitate was washed with 50 ml of ethanol and centrifuged again under the same conditions. Hemicelluloses were dried (S_p) at low temperature (40 °C) and reserved in plastic-sealed tubes until use.

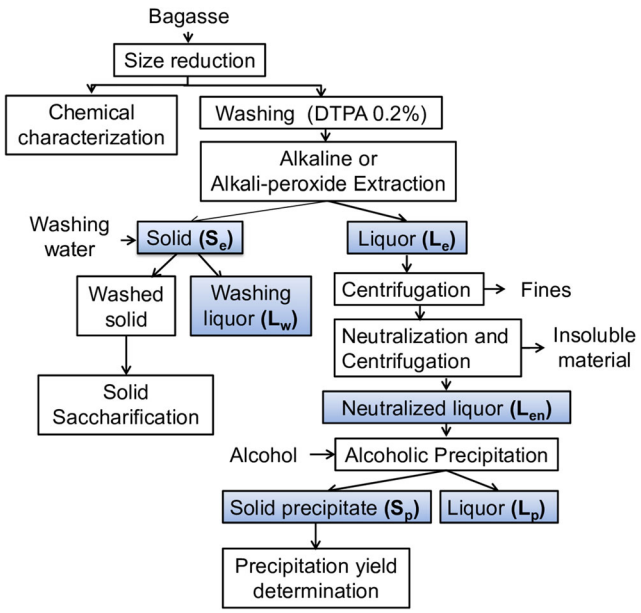

Fig. 1 Experimental process scheme. Hemicellulose extraction and isolation, and saccharification of treated bagasse are indicated

2.3 Determination of hemicellulose and lignin in the extraction liquor and in the precipitated solid

The concentrations of carbohydrate and lignin in the liquor (L_{en}) were determined according to the technique proposed by Sluiter et al. [23]. By duplicate, a sample of 20 ml of the neutralized liquor, after addition of 0.7 ml of sulfuric acid (72% w/w), was treated in an autoclave for 1 h at 121 °C. The hydrolyzed liquor was filtered through 0.5-μm glass filter. The mass retained was quantified as acid-insoluble lignin. Acid-soluble lignin was quantified in the filtrate, by UV-Visible spectrophotometer, determining the absorbance at 240 nm using an absorptivity of 25 l $(g\ cm)^{-1}$. Carbohydrates and acetyl content were determined by the same HPLC system with the mobile phase under the same conditions described above.

The xylose and arabinose concentrations in the neutralized extraction liquor (L_{en}) and washing liquor (L_w) were evaluated by the phenol/sulfuric acid method proposed by Hodge and Hofreiter [24]. This is a fast method that mainly allows determining the 5-carbon sugar concentration in a solution by a spectrophotometric measurement when a 480-nm wavelength is considered. In this case, xylose was used as standard. Supernatant solution (1 ml) was shaken in a glass tube with 1 ml of phenol at 5% (m/v) and 5 ml of concentrated sulfuric acid (95.5–96.5%). After 10 min, the tube was shaken again and placed in a water bath at 25–30 °C for 20 min. Absorbance was spectrophotometrically measured at 480 nm. Determinations were made in triplicate.

A quantified mass of the precipitate (S_p) was dissolved in 10^{-4} M sodium hydroxide solution in order to obtain a xylan concentration of 2 g xylose l^{-1}, approximately. The carbohydrates and lignin contents were determined, using the technique proposed by Sluiter et al. [23] explained above.

2.4 Enzymatic saccharification of bagasse after extraction

Bagasse treated under different extraction conditions and untreated bagasse as control, were submitted to saccharification with a cellulase from *Trichoderma longibrachiatum* (C9748 Sigma-Aldrich) according to NRELLAP standards [25], with a few modifications. Hydrolysis was carried out in triplicate in 50-ml tubes using acetate buffer (0.05 M, pH 5.0) with a solid loading of 2% (on dry basis) at 150 rpm and 50 °C for 96 h. The enzyme dosage used was 0.3% w/w (g enzyme/g bagasse). Azide (0.02%) was also added to avoid microbial growth during enzymatic treatment. Control enzymatic treatment (without cellulase) for each pretreated material as well as for untreated bagasse was done in parallel. After incubation, tubes were immersed in a boiling water bath for 10 min to inactivate the enzymes and conclude the reaction. Glucose quantification was done, immediately after saccharification, using a glucose oxidase kit (Wiener Laboratorios SA, Rosario, Argentina). Later, samples were filtered through 0.22-μm filter and glucose, xylose, and arabinose were quantified by HPLC, as explained above.

3 Results and discussion

3.1 Bagasse chemical composition

The chemical composition of the extractive-free sugar cane bagasse (in % w/w) was as follows: cellulose 42.2 ± 0.02, hemicellulose 33.1 ± 1.5 (xylose and arabinose in anhydrous form; 25.3 and 4.1, respectively; acetyl groups; 3.7), lignin 24.2 ± 0.8 (acid soluble lignin; 3.3, acid insoluble lignin; 20.9), and ash 1.2 ± 0.02. Results are in agreement with those reported by others [9, 10, 26, 27].

3.2 Chemical consumption during alkali treatment

Hydrogen peroxide was totally consumed according to iodometric titration for all the extraction conditions.

Alkali consumption is shown in Fig. 2, expressed as sodium hydroxide per 100 g of bagasse. The percentage of sodium hydroxide consumed referred to the alkali charge is shown in brackets.

Figure 2 shows that the specific alkali consumption increased when alkali and peroxide charges were raised. Nevertheless, when the alkali charge was increased from 20 to 40% w/w, a reduction in the slope is observed, and particularly, without peroxide, the specific alkali consumption

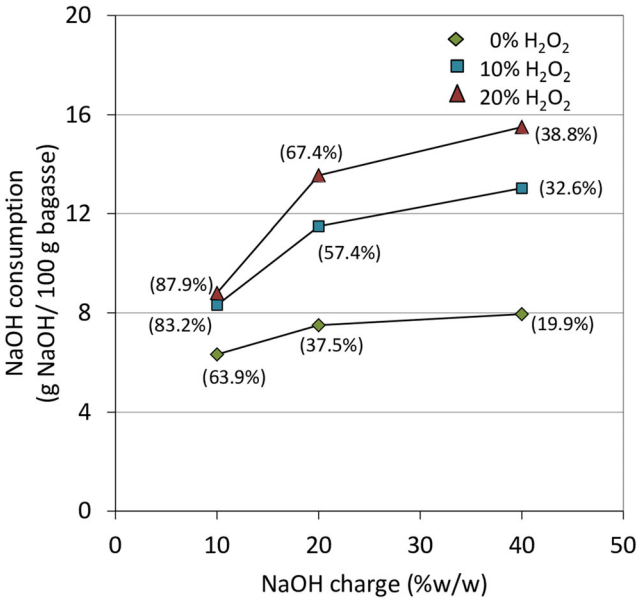

Fig. 2 Specific NaOH consumption of the different extractions expressed as sodium hydroxide per 100 g of bagasse. The percentage of NaOH consumed relative to the initial alkali charge is shown in brackets

remained almost constant. The highest specific alkali consumption, 15.5 g NaOH/100 g of bagasse, is observed for the $A_{40}P_{20}$ condition (the highest alkali and peroxide charges).

The higher alkali consumption brought about by peroxide addition can be better seen considering the relative consumption. It was 63, 37 and 19% of alkali charge for 10, 20 and 40 g NaOH/100-g bagasse, respectively. These values were increased to 88, 67, and 37%, respectively, when 20% of peroxide was added.

Considering the consumption of the reagents, at this point, it is clear that only a higher isolation yield or a better quality of xylans can justify the hydrogen peroxide addition in the alkaline extraction.

3.3 Hemicellulose and lignin extraction and precipitation yields

3.3.1 Hemicellulose extraction and precipitation yields

Figure 3 shows the yields of hemicellulose (xylose and arabinose) determined by HPLC in the liquor collected (L_{en}) and in the precipitated (S_p). They are expressed as a percentage on original dry bagasse (% w/w). The hemicellulose extraction yield was increased with the alkali charge. Regardless of the alkali charge, the extraction yield was doubled when hydrogen peroxide was added, but there was no difference between the use of 10 or 20% of peroxide charge.

For the maximum alkali charge (40%), the improvement in the hemicellulose extraction was increased only 6% when the peroxide charge was increased from 10 to 20%, even though the NaOH consumption was increased 19% (as was shown in Fig. 2). This result indicates that a 20% peroxide charge involves an important consumption of alkali that does not entail hemicellulose extraction yield improvement. Misailidis et al. [28] studied the feasibility of commercial arabinoxylan production in the context of a wheat biorefinery. They concluded that the cost of hydrogen peroxide is more significant than the cost of raw material for arabinoxylan production. In the present work it is shown that it is worthless to increase the amount of peroxide charge.

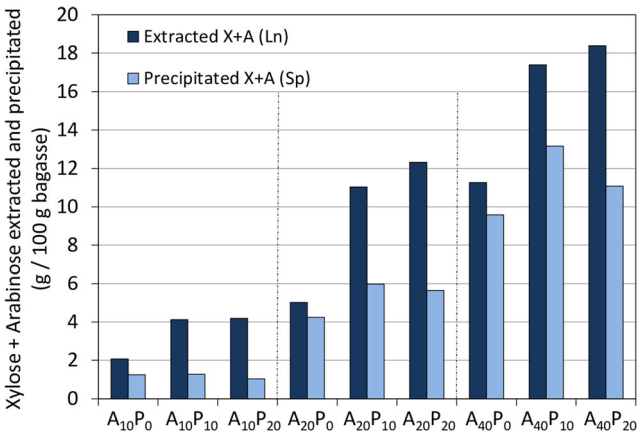

Fig. 3 Extraction and precipitation yield (xylose + arabinose) determined by HPLC

Peng et al. [27] reported yield values of 10.9% and 9.4% of hemicellulose (% on dry matter) applying successive NaOH treatments of 25 and 75 g NaOH/100 g of bagasse, respectively, at 50 °C and 180 min after a water treatment. The conditions of the first alkaline treatment applied in that work were similar to the $A_{20}P_0$ condition in the present work, but the water pretreatment and the lower size of raw material used (0.8-mm size screened) might have allowed them to obtain a double amount of hemicelluloses. On the other hand, in the present work, the addition of peroxide ($A_{20}P_{10}$ and $A_{20}P_{20}$ conditions) produces a greater yield than that reported by Peng et al. [27]. Campbell et al. [29] reported an arabinoxylan extraction yield of 66% from sugarcane bagasse (which corresponds to 12.9% w/w of the initial arabinoxylan in bagasse). The extraction was made using 2% H_2O_2 solution at pH of 11.5, and 1:45 bagasse:liquor ratio (which corresponds to 90% w/w H_2O_2 on bagasse). They concluded that the sugarcane is more amenable to arabinoxylan extraction compared with wheat bran.

The maximum amount of xylose plus arabinose was extracted using the highest charge of both reactants ($A_{40}P_{20}$), reaching 57% of these sugars existing in the original sugar cane bagasse. A similar amount of hemicelluloses was obtained by Carvalho et al. [20], who extracted 52.5% of the hemicelluloses under the optimal condition of their work (33 °C, 60 min, and a charge of 110 g of NaOH/100 g of bagasse).

Figure 3 also shows that for the lowest alkali charge, the amount of hemicelluloses extracted in treatments that included peroxide was twice the amount extracted without peroxide. Nevertheless, precipitated quantities were similar.

If we consider the amount of xylose and arabinose extracted, it can be noticed that the precipitation yield was decreased when the peroxide charge was increased (for all alkali charges).

It was previously found that hemicelluloses extracted and precipitated under these conditions show a narrow range of molecular weight (polydispersity ranged from 1.03 to 1.30) [11] which was ascribed to the relatively low ratio of liquor:ethanol used (lower than the 1:4 v/v ratio adopted by other authors [10, 30]).

This indicates that the peroxide addition not only reduces the alkalinity of the medium but can also cause depolymerization of the hemicellulose, hindering its precipitation. The metal-catalyzed decomposition of hydrogen peroxide is undesirable since it generates more active radicals, such as hydroxyl radicals, participating in degradation reactions of lignin and carbohydrates [31]. Although a washing step of the raw bagasse and DTPA

was applied in the present work to remove metal ions, probably they were not completely eliminated.

The highest percentage of xylose plus arabinose precipitated relative to the extracted quantity was 85%, using $A_{40}P_0$ condition.

An interesting alternative is a charge of 40% w/w, without peroxide, since the excess of alkali can be recovered. One option is the ultra-filtration of the extraction liquor before neutralization to obtain two fractions, one of them mostly composed of high molecular weight hemicelluloses, and the other, of low molecular weight compounds and a high proportion of the sodium hydroxide. For alkali hemicellulose extraction from birch wood, Testova et al. [32] reported that a high level of recovering can be obtained since only 2% of the residual sodium hydroxide remained in the concentrated fraction after sequential nano-and diafiltration. Schild et al. [33] also reported an efficient separation of xylan from strong alkaline solutions through ultrafiltration in bench-scale experiments. Campbell et al. [29] successfully used polyethersulfone membranes with a molecular weight cut-off of 10 kDa, to reduce the initial extraction volume to one-fifth. They suggested that ultrafiltration would be useful in a commercial process to reduce the cost of the ethanol needed for the subsequent precipitation.

Sugar concentration that was determined by the phenol/sulfuric (PS) acid method resulted in acceptable agreement with those obtained by HPLC. If concentration is expressed in g l^{-1}, the correlation found was as follows:

$$(\text{xylose} + \text{arabinose})_{\text{HPLC}} = 1.0858\ (\text{xylose} + \text{arabinose})_{\text{PS}} + 0.0407; R^2 = 0.977$$

In a previous work [1] for liquor of hydrothermal treatment of eucalyptus wood, it was shown that the phenol/sulfuric acid method was also acceptable to estimate the 5-carbon sugars. This method presents the great advantage of being a rapid and trustworthy technique.

3.3.2 Acetyl content

The acetic acid content in the extraction liquors ranged from 3.05 to 3.41 g of acetyl/100 g of bagasse. These values correspond to 82 and 92% of the original acetyl present in the original bagasse. Deacetylation is the main reaction that takes place, at low temperature, during the first stage of any alkaline pulping process [34, 35]. The specific amount of acetic acid quantified in the extraction liquor indicates, as expected, that hemicelluloses were almost deacetylated before being extracted or later.

3.3.3 Lignin content

Figure 4 shows soluble, insoluble, and total lignin content in the neutralized extraction liquor (L_{en}) and total lignin precipitated (S_p), relative to the original dry bagasse (% w/w). For the lowest alkali charge, the total lignin extracted decreases when the peroxide charge is increased. This result suggests that for the delignification process, some alkalinity is needed in the medium, and the peroxide presence slightly decreases it (as shown in Fig. 2). When 20 and 40% of alkali were used, the alkalinity reached was high enough, so the peroxide helped for delignification, and the total lignin extracted did not show the effect of peroxide increment. The highest value for total lignin extracted, obtained by $A_{40}P_{10}$ and $A_{40}P_{20}$ conditions, corresponds to 48.3% of the total lignin in the original bagasse. In the optimal conditions found by Carvalho et al. [20] the total lignin extracted was 37%. As these authors suggested, higher lignin solubility in alkaline solutions compared with other lignocellulosic raw materials (such as eucalyptus wood) can be explained by the amount of free phenolic groups and ester bonds, which are usually higher in grass lignins. It is remarkable how even though working with less than half the NaOH charge used by Carvalho et al. [20] (110%), but adding peroxide, a greater amount of lignin could be extracted in the present work.

The amount of acid-insoluble lignin extracted was notably higher than the amount of acid-soluble lignin in all cases and had the same tendency as the total lignin. The high amount of acid-soluble lignin obtained by $A_{20}P_{20}$ and $A_{40}P_{20}$, was 66.6% of the acid-soluble lignin reported for the original bagasse.

The percentage of lignin precipitated relative to the extracted was increased as both chemicals were raised. The peroxide charge had an unfavorable effect in the content of lignin in the precipitate, because the percentage of hemicelluloses was decreased and the percentage of lignin was increased.

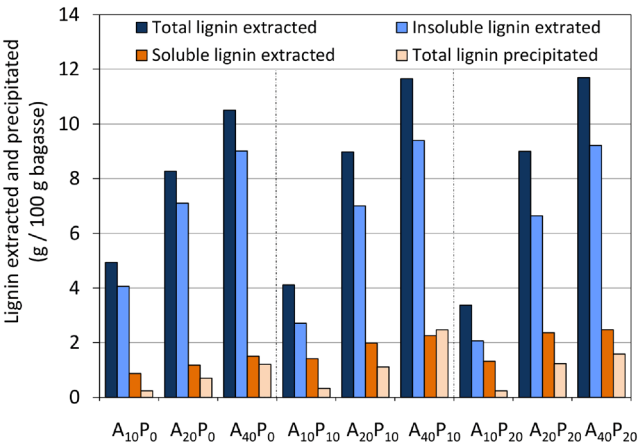

Fig. 4 Soluble, insoluble, and total lignin determined in the neutralized extraction liquor (L_{en}) and total lignin in the precipitated (S_n)

3.4 Hemicellulose recovery by washing

Table 2 shows the content of xylose plus arabinose in the washing liquor (L_w). It was quantified by the phenol/sulfuric acid method and is expressed in relation to the original mass of bagasse. This fraction of xylose plus arabinose that was not possible to collect in the liquor by the squeezing stage is also reported a proportion of the total extracted mass.

It was found that the washing step was able to recover most of the hemicelluloses remaining in bagasse at the end of the extraction. As alkali and peroxide charges were increased, the liquor not collected was lower and this is the main reason why the amount of hemicelluloses in washing water was decreased (from 16 to 5.7%).

In an industrial process, this washing streaming could be added to the main one, in case of being economically convenient.

3.5 Alkali consumption and alcohol demand related to xylose and arabinose content in the precipitate

For an economic analysis of the process, it is necessary to know the specific alkali consumption and the specific alcohol demand, i.e., values related to the obtained quantity of xylose plus arabinose.

Figure 5 shows that the specific NaOH consumption and ethanol demand are both clearly beneficed by a high alkali charge. For 40% of NaOH, the alcohol demand was reduced by the addition of 10% of hydrogen peroxide, but the alkali consumption was not changed.

Nevertheless, to find the most favorable conditions, an economic analysis is necessary taking into account the quality of the hemicelluloses (according to their use), hydroxide, and peroxide cost, as well as the energy to recover the ethanol involved in the precipitation step and the requirement of special reactor material resulting from peroxide addition. As was shown before, when peroxide is used in the extraction, it was completely consumed. Misailidis et al. [28] concluded that ethanol and H_2O_2 requirements represent the main portion of arabinoxylan production costs. They also affirm that maximizing yield is the most effective approach for reducing production costs of arabinoxylan. A priori, conditions of highest alkali charge seem to be the best, considering the possibility of recycling the excess of alkali. In the case of bioethanol production, arabinoxylans appear as a potential co-product that would give process integration opportunities [28].

Table 2 Xylose + arabinose (X + A) content in washing liquor

Extraction condition	(X + A) in washing liquor	
	g/100-g bagasse	Total mass extracted (%)
$A_{10}P_0$	0.32	16
$A_{20}P_0$	0.69	12
$A_{40}P_0$	0.89	8.3
$A_{10}P_{10}$	0.46	13
$A_{20}P_{10}$	0.67	6.8
$A_{40}P_{10}$	1.00	6.2
$A_{10}P_{20}$	0.43	13
$A_{20}P_{20}$	0.93	9.3
$A_{40}P_{20}$	0.91	5.7

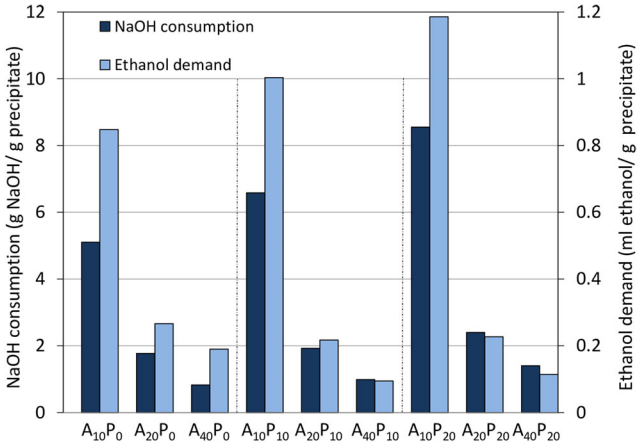

Fig. 5 NaOH consumption and ethanol demand considering the quantity of xylose plus arabinose in the precipitate

3.6 Bagasse enzymatic digestibility

Following the biorefinery concept, it is worth studying alternatives for the integration of process. The susceptibility of treated bagasse to saccharification as a potential previous step for ethanol production was investigated. Figure 6a shows the amount of glucose, xylose, and arabinose generated during saccharification of untreated bagasse (Ref) and alkali or alkali-peroxide-treated bagasse. The glucose quantity determined using the enzymatic kit (data not shown here) was similar to those obtained by HPLC.

It is observed that the released glucose is raised as the alkali charge and also as the peroxide is increased. There is no difference between the mass of the glucose generated from $A_{10}P_0$ (lowest alkali charge and no peroxide addition) and the reference. The mass of released xylose was increased when alkali was raised from 10 to 20%, but remained constant when alkali was increased to 40%. On the other hand, in all cases, there was no difference with the reference in the quantity of arabinose released.

Figure 6b shows that the total of sugars generated during saccharification is clearly related to the total lignin removed during hemicellulose extraction stage. It can

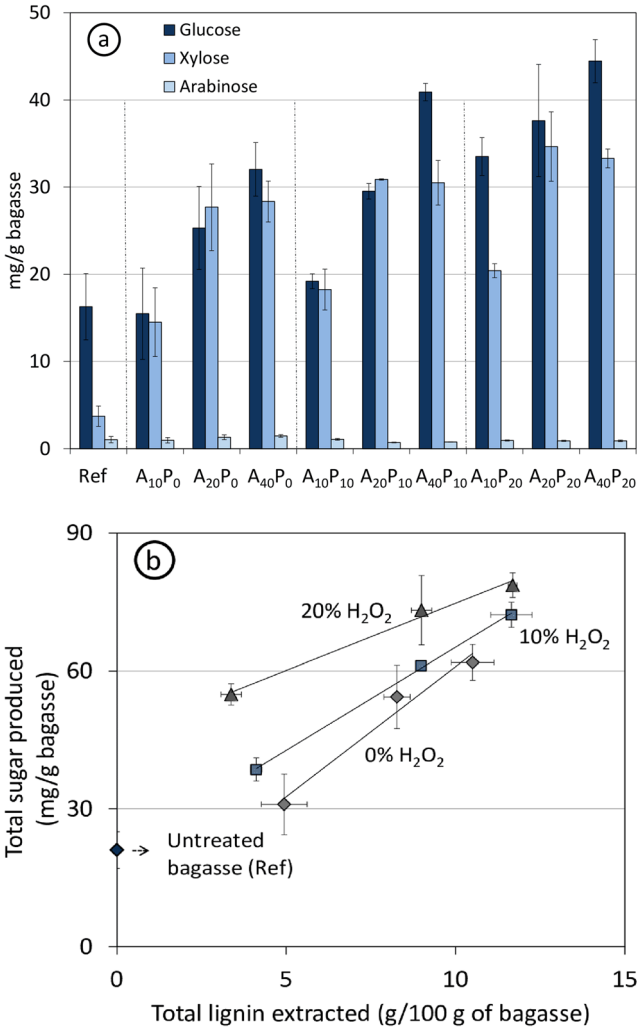

Fig. 6 Glucose, xylose, and arabinose generated (**a**) and relationship between total lignin extracted from bagasse and total sugar generated by saccharification (**b**)

be observed that the alkali had a favorable effect in the enzymatic hydrolysis. Besides that, the peroxide improved the enzymatic accessibility during saccharification beyond the favorable effect of this reactant on the delignification of bagasse.

The effect of lignin as an inhibitory biopolymer for the enzymatic hydrolysis of lignocellulosic biomass can be partially ascribed to differences in lignin structure. For instance, Rahikainen et al. [36], by quantifying the enzyme adsorption onto lignin and the inhibition that lignin layer produces, showed that difference in lignin chemistry can change its role in the non-productive adsorption of enzyme.

The highest yields of glucose (44.45 mg/g bagasse) and xylose (33.30 mg/g bagasse) were obtained for the highest alkali and peroxide charges ($A_{40}P_{20}$), which increased 170% and 800% the yield of the reference in glucose and xylose, respectively.

4 Conclusions

Under metal ion control and inert atmosphere, in an alkaline-peroxide treatment of bagasse, alkali charge showed a strong beneficial effect on hemicellulose extraction yield. The increment in the NaOH charge from 20 to 40% on bagasse led to a moderate increase in alkali consumption but the hemicellulose extraction yield was clearly raised. The maximum amount of hemicellulose extracted corresponded to 56% of the original content in bagasse. The specific consumption of alkali and the specific alcohol demand were favorably reduced with the increment of the alkali charge. The enzymatic digestibility of the treated bagasse was improved by the increase in extraction chemical charges. For each level of hydrogen peroxide charge, total sugar generated during saccharification was clearly related to the total lignin removed during hemicellulose extraction stage.

The hydrogen peroxide addition showed advantages and disadvantages. The hemicellulose extraction yield was notably increased and, on the other hand, the sugar release during saccharification of the treated bagasse was increased. Nevertheless, there was a reduction in the precipitation yield of the hemicellulose in the alcoholic medium and there was an increment in the susceptibility of lignin to precipitate together with hemicellulose.

Considering that cost is increased when hydrogen peroxide is used in the hemicellulose extraction, it would not be convenient to raise this charge from 10 to 20% on bagasse. On the other hand, since alkali maximizes the xylan extraction yield, 40% NaOH charge could be the most convenient, particularly if alkali can be recovered.

Code availability Not applicable

Authors' contributions All authors contributed to the study conception and design. Material preparation, data collection, and analysis were performed by Yamil Nahún Solier, Paulina Mocchiutti, María Noel Cabrera, Mario Carlos Nazareno Saparrat, María Cristina Inalbon, and Miguel Ángel Zanuttini. The first draft of the manuscript was written by Yamil Nahún Solier and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Funding information The authors wish to acknowledge the financial support received from the Universidad Nacional del Litoral-CAI+D 2016-UNL No. 50420150100079LI and the Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET)-PIP 2013–No. 11220120100672. Thanks are given to the Tacuarendí Experimental Center, Santa Fe, Argentina, for providing the sugar cane bagasse, and to Dr. D. Lauff from the Instituto de Fisiología Veghetal (INFIVE) (CONICET-Universidad Nacional de La Plata) for her technical assistance for performing the glucose quantification. This research was partially supported by the Agencia Nacional de Promoción Científica y Tecnológica PICT 2015-1620 and the Universidad Nacional de La Plata (A11/307, N11/897).

Data availability Not applicable

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

References

1. Inalbon MC, Solier YN, Zanuttini MÁ (2017) Hydrothermal treatment of eucalyptus wood: effects on ion permeability and material removing. Ind Crop Prod 104:195–200. https://doi.org/10.1016/j.indcrop.2017.04.042
2. Yoon SH, Van Heiningen A (2008) Kraft pulping and papermaking properties of hot-water pre-extracted loblolly pine in an integrated forest products biorefinery. TAPPI J 7:22–27
3. Mendes CVT, Carvalho MGVS, Baptista CMSG, Rocha JMS, Soares BIG, Sousa GDA (2009) Valorisation of hardwood hemicelluloses in the kraft pulping process by using an integrated biorefinery concept. Food Bioproduct Proc 87:197–207. https://doi.org/10.1016/j.fbp.2009.06.004
4. Brienzo M, Carvalho AFA, de Figueiredo FC, de Oliva NP (2016) Sugarcane bagasse hemicellulose properties, extraction technologies and xylooligosaccharides production. Food waste: practices, management and challenges:155–188
5. Rodrigues RCLB, Felipe MGA,e Sil JBA, M. Vitolo (2003) Response surface methodology for xylitol production from sugarcane bagasse hemicellulosic hydrolyzate using controlled vacuum evaporation process variables. Process Biochem 38:1231–1237. https://doi.org/10.1016/S0032-9592(02)00290-X
6. Rabelo SC, Carrere H, Maciel Filho R, Costa AC (2011) Production of bioethanol, methane and heat from sugarcane bagasse in a biorefinery concept. Bioresour Technol 102:7887–7895. https://doi.org/10.1016/j.biortech.2011.05.081
7. http://centroazucarero.com.ar/oldsite/zafras/zafra2016.html. December 18, 2018
8. Banerjee PN, Pranovich A, Dax D, Willför S (2014) Non-cellulosic heteropolysaccharides from sugarcane bagasse - sequential extraction with pressurized hot water and alkaline peroxide at different temperatures. Bioresour Technol 155:446–450. https://doi.org/10.1016/j.biortech.2014.01.020
9. Brienzo M, Siqueira AF, Milagres AMF (2009) Search for optimum conditions of sugarcane bagasse hemicellulose extraction. Biochem Eng J 46:199–204. https://doi.org/10.1016/j.bej.2009.05.012
10. Sun JX, Sun XF, Sun RC, Su YQ (2004) Fractional extraction and structural characterization of sugarcane bagasse hemicelluloses. Carbohydr Polym 56:195–204. https://doi.org/10.1016/j.carbpol.2004.02.002
11. Solier YN, Schnell CN, Cabrera MN, Zanuttini MA, Inalbon MC (2020) Alkali-peroxide extraction of xylan from sugar cane bagasse. Characteristics and film forming capacity. Ind Crops Prod 145:112056. https://doi.org/10.1016/j.indcrop.2019.112056
12. Egüés I, Sanchez C, Mondragon I, Labidi J (2012) Effect of alkaline and autohydrolysis processes on the purity of obtained hemicelluloses from corn stalks. Bioresour Technol 103:239–248. https://doi.org/10.1016/j.biortech.2011.09.139
13. Júnior DL, Ayoub A, Venditti RA, Jameel H, Colodette JL, Chang HM (2013) Ethanol precipitation of hetero-polysaccharide material from hardwood by alkaline extraction prior to the kraft cooking process. Bioresources 8:5319–5332
14. Svärd A, Brännvall E, Edlund U (2017) Rapeseed straw polymeric hemicelluloses obtained by extraction methods based on severity factor. Ind Crop Prod 95:305–315. https://doi.org/10.1016/j.indcrop.2016.10.038
15. Zanuttini MAM (1995) Alkaline treatment of sugar cane bagasse. Swelling and properties of chemimechanical pulps. Cell Chem Technol 29:751–759
16. Zanuttini MAM, Citroni MA, Martínez MJ, Marzocchi VA (1997) Soda semichemical bagasse pulp for corrugating medium: chemical and physical characteristics. Part 2: shives content and handsheet properties. Cell Chem Technol 31:77–85
17. Doner LW, Hicks KB (1997) Isolation of hemicellulose from corn fiber by alkaline hydrogen peroxide extraction. Cereal Chem 74:76–181. https://doi.org/10.1094/CCHEM.1997.74.2.176
18. Dutra ED, Santos FA, Alencar BRA, Reis ALS, de Souza RDFR, da Silva Aquino KA, Morais MAJr, Menezes RSC (2018) Alkaline hydrogen peroxide pretreatment of lignocellulosic biomass: status and perspectives. Biomass Convers Biorefinery 8:225–234. https://doi.org/10.1007/s13399-017-0277-3
19. Batalha LAR, Han Q, Jameel H, Chang HM, Colodette JL, Borges Gomes FJ (2015) Production of fermentable sugars from sugarcane bagasse by enzymatic hydrolysis after autohydrolysis and mechanical refining. Bioresour Technol 180:97–105. https://doi.org/10.1016/j.biortech.2014.12.060
20. Carvalho DM, Sevastyanova O, Queiroz JH, Colodette JL (2016) Cold alkaline extraction as a pretreatment for bioethanol production from eucalyptus, sugarcane bagasse and sugarcane straw. Energy Convers Manag 124:315–324. https://doi.org/10.1016/j.enconman.2016.07.029
21. Meléndez-Hernández PA, Hernández-Beltrán JU, Hernández-Guzmán A, Morales-Rodríguez R, Torres-Guzmán JC, Hernández-Escoto H (2019) Comparative of alkaline hydrogen peroxide pretreatment using NaOH and $Ca(OH)_2$ and their effects on enzymatic hydrolysis and fermentation steps. Biomass Convers Biorefinery:1–11. https://doi.org/10.1007/s13399-019-00574-3
22. Sluiter A, Hames B, Ruiz R, Scarlata C, Sluiter J, Templeton D, Crocker D (2008) Determination of structural carbohydrates and lignin in biomass. Technical Report: NREL/ TP-510-42618
23. Sluiter A, Hames B, Ruiz R, Scarlata C, Sluiter J, Templeton D (2008) Determination of sugars, byproducts, and degradation products in liquid fraction process samples. Technical Report: NREL/TP-510-42623
24. Hodge JE, Hofreiter BT (1962) Determination of reducing sugars and carbohydrates. In: Whistler RL, Wolfrom ML (eds) Method in carbohydrate chemistry. Academic Press, New York, San Francisco
25. Resch MG, Baker JO, Decker SR (2015) Low solids enzymatic saccharification of lignocellulosic biomass. Technical Report: NREL/TP-5100-63351
26. Xu F, Sun RC, Sun JX, Liu CF, He BH, Fan JS (2005) Determination of cell wall ferulic and p-coumaric acids in sugarcane bagasse. Anal Chim Acta 552:207–217. https://doi.org/10.1016/j.aca.2005.07.037
27. Peng F, Ren JL, Xu F, Bian J, Peng P, Sun RC (2009) Comparative study of hemicelluloses obtained by graded ethanol precipitation from sugarcane bagasse. J Agric Food Chem 57:6305–6317. https://doi.org/10.1021/jf900986b
28. Misailidis N, Campbell GM, Du C, Sadhukhan J, Mustafa M, Mateos-Salvador F, Weightman RM (2009) Evaluating the feasibility of commercial arabinoxylan production in the context of a wheat biorefinery principally producing ethanol: part 2. Process simulation and economic analysis. Chem Eng Res Des 87:1239–1250. https://doi.org/10.1016/j.cherd.2008.12.028
29. Campbell GM, Mustač NČ, Alyassin M, Gomez LD, Simister R, Flint J, Philips DJ, Gronnow MJ, Westwood NJ (2019) Integrated processing of sugarcane bagasse: arabinoxylan extraction integrated with ethanol production. Biochem Eng J 146:31–40. https://doi.org/10.1016/j.bej.2019.03.001
30. Li Z, Qin M, Xu C, Chen X (2013) Hot water extraction of hemicelluloses from aspen wood chips of different sizes. Bioresources 8:5690–5700

31. Pan GX, Bolton JL, Leary GJ (1998) Determination of ferulic and p -coumaric acids in wheat straw and the amounts released by mild acid and alkaline peroxide treatment. J Agric Food Chem 46:5283–5288. https://doi.org/10.1021/jf980608f
32. Testova L, Roselli A, Costabel L, Kovasin K, Tenkanen M, Sixta H (2014) Combined production of polymeric birch xylan and paper pulp by alkaline pre-extraction followed by alkaline cooking. Ind Eng Chem Res 53:8302–8310. https://doi.org/10.1021/ie500694c
33. Schild G, Sixta H, Testova L (2010) Multifunctional alkaline pulping, delignification and hemicellulose extraction. Cellul Chem Technol 44:35–45
34. Zanuttini M, Marzocchi V, Mocchiutti P, Inalbon M (2005) Deacetylation consequences in pulping processes. Holz Als Roh-Und Werkstoff 63:149–153. https://doi.org/10.1007/s00107-004-0557-z
35. Inalbon MC, Mocchiutti P, Zanuttini M (2009) The deacetylation reaction in eucalyptus wood: kinetics and effects on the effective diffusion. Bioresour Technol 100:2254–2258. https://doi.org/10.1016/j.biortech.2008.10.054
36. Rahikainen JL, Martin-Sampedro R, Heikkinen H, Rovio S, Marjamaa K, Tamminen T, Rojas OJ, Kruus K (2013) Inhibitory effect of lignin during cellulose bioconversion: the effect of lignin chemistry on non-productive enzyme adsorption. Bioresour Technol 133:270–278. https://doi.org/10.1016/j.biortech.2013.01.075