Journal of Energy Chemistry 27 (2018) 535–543

Contents lists available at ScienceDirect

Journal of Energy Chemistry

journal homepage: www.elsevier.com/locate/jechem

http://www.journals.elsevier.com/journal-of-energy-chemistry/

Effect of steam-pretreatment combined with hydrogen peroxide on lignocellulosic agricultural wastes for bioethanol production: Analysis of derived sugars and other by-products

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ARTICLE INFO

Article history:
Received 25 July 2017
Revised 30 October 2017
Accepted 9 November 2017
Available online 14 November 2017

Keywords:
Lignocellulose
Hydrogen peroxide
Steam explosion
Enzymatic hydrolysis
Bioethanol

ABSTRACT

The hydrogen peroxide, a green impregnating agent suitable for lignocellulosic biomass to bioethanol process, was used to pretreat sugarcane bagasse by steam explosion. Two different concentrations of hydrogen peroxide (0.2% and 1%) were investigated. Then, the biomass was hydrolyzed after pretreatment using cellulase. The amount released of: (i) cellobiose; (ii) monosaccharides, as glucose, xylose, arabinose and mannose and (iii) lignocellulose derived by-products, as furans and small organic acids (acetic, formic, and levulinic acid), was evaluated in the hydrolysate samples, previously pretreated both in the presence and absence of impregnating agent. By adding of hydrogen peroxide in steam-pretreatment, the average yield increase was 12% for glucose and as high as 34% for xylose, and cellobiose yield was decreased of about 30%. No significant increase has been observed in arabinose and mannose yield. Furthermore, the hydrogen peroxide seems not increased the formation of lignocellulose derived by-products during pretreatment process, with the exception of the levulinic acid.

1. Introduction

The development of renewable, clean and environmentally sustainable energy sources is the great challenge of our times. Biomass is a carbon neutral renewable energy source that can be used for sustainable production of biofuels [1,2], such as biogas/syngas [2–4], biohydrogen [1], and bioalcohols [5]. Bioethanol is one of the most important bioalcohols used as substitutes for petroleum-based fuels; the increasing replacement of oil-derived fuels by bio-ethanol could contribute to the environmental impact reduction [5]. Bioethanol can also be mixed with petrol to make a composite fuel and used in existing internal combustion engines with little or no modification [6]. Currently, Brazil is the world's largest ethanol producer from sugarcane as a source of biomass [7]. Sugarcane (*saccharum officinarum*) is a tall, perennial grass belonging to the family of Poaceae, to the *saccharum* genus and is now cultivated in many countries: Brazil, China, Thailand, Mexico, Colombia, Australia, Indonesia, and United States of America. The average annual production of sugarcane in Brazil is approximately 623 million tons, estimated in the years 2010/2016: the 2015/2016 sugarcane harvest in Brazil led to a sugar production of about 34 million tons, and a bio-ethanol production of approximately 30 billion liters [8].

Ethanol production from sugarcane is mainly based on the fermentation of sucrose stored in the stalk during ripening stage. The sucrose content in this plant is extracted by a milling process, generating a lignocellulosic residual known as sugarcane bagasse (SCB): per every ton of sugarcane processed, 140 kg of SCB are generated [9]. At the beginning of the National Fuel Alcohol Program (Proálcool project), SCB was considered a fluffy waste: in most case, it remained unused and left to natural degradation or burnt in the field, causing severe environmental aggression

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https://doi.org/10.1016/j.jechem.2017.11.007

[10,11]. Nowadays, SCB is recognized as one of the most promising feedstock for the production of advanced, or second generation, bioethanol obtained from lignocellulosic biomass, due to its high content of fermentable sugars [12]. SCB consists of long-chain cellulose, composed of β-1,4-linked D-glucose units and packed into microfibrils by hydrogen and van der Waals bonds; the microfibrils are closely embedded in a matrix of hemicellulose, pectins, glycosylated proteins and lignin [13]. Hemicellulose is a mixture of heterogeneous polysaccharides with different structures, such as xylan, glucuronoxylan, arabinoxylan, glucomannan, and xyloglucan [14]. These polymers include branches which interact with cellulose, guaranteeing stability and flexibility to the aggregate [15]. Lignin, a complex molecular structure, contains cross-linked phenolic polymer formed by three constituent monolignols (p-coumaryl, coniferyl and sinapyl alcohols), which are linked together by alkyl–aryl, alkyl–alkyl, and aryl–aryl ether bonds [16]. The conversion of lignocellulose to ethanol is favored by de-polymerization of cellulose and hemicellulose in fermentable sugars through the dilute and concentrated acid, or enzymatic hydrolysis; then, sugars fermentation in ethanol can be performed by different microorganisms: bacteria, yeasts and fungi [17]. However, the compact and rigid structure of lignocellulosic matrix acts as a physical barrier able to prevent cellulase-catalysed hydrolysis, fermentable sugars release, and subsequent ethanolic fermentation. This complex and heterogeneous architecture is the cause of biomass recalcitrance to microbial and enzymatic deconstruction [18]. Thus, a physical and chemical pretreatment process of SCB is required to reduce recalcitrance biomass and increase the reactive surface area [19]. The steam explosion is one of the most efficient pretreatment method that promotes the breakdown of the lignocellulosic matrix as a result of an explosive decompression of biomass [20]: biomass is treated to high-pressure (0.7–4.8 MPa) saturated steam, at elevated temperatures ($160-260$ °C) for few seconds (30 s) to several minutes (20 min), then pressure is swiftly reduced to atmospheric condition within (milli) seconds [21]. The steam explosion promotes: (i) the disruption of lignocellulose matrix in individual fibers (hemicelluloses, cellulose and lignin); (ii) the partial removal and/or redistribution of lignin; (iii) the partial degradation of hemicelluloses via a mechanism known as the "auto-hydrolysis" or "auto-cleave-steam pretreatment", in which the hydrolysis of acetyl groups, included in heterogeneous polysaccharides, in turn causes further hydrolysis of hemicelluloses; (iv) the breaking of linkages between hemicellulose and cellulose; (v) the increasing in cellulose accessibility, allowing the polysaccharide to be readily hydrolyzed into glucose units by a multi-step reaction catalyzed from enzymes, known as cellulase [22]. Initially, cellulose is hydrolyzed in soluble intermediate products, as short cellulo-oligosaccharides and cellobiose, via the synergistic action of endoglucanases (EG) and exo-glucanases/cellobiohydrolases (CHB). The conversion of these soluble intermediate products into glucose is carried out by the action of β-glucosidase (BG) [23]. The auto-hydrolysis process of hemicellulose led to production of xylose and other monomeric sugars, such as arabinose, mannose and glucose [24]. A side effect of steam explosion is the formation of lignocellulose-derived by-products, such as dehydrated sugar monomers (furans) and small organic acids, which are inhibitory to hydrolytic enzymes and fermenting microorganisms [25]. During pretreatment step, biomass is exposed to high temperatures, and it can lead to the dehydration of hexoses (glucose) and pentoses (xylose) into the corresponding furans, 5-(hydroxymethyl)furfural (HMF) and furfural, respectively [26]. These furaldehydes have a negative fallout on the rate of enzymatic hydrolysis, inhibiting the enzymes used to liberate the sugars from the (hemi-)cellulose fractions [27]. They also inhibit the yeast and bacterial growth, and consequently alcohol fermentation, in a dose-dependent manner [28,29]. Large amounts of organic acids, such as acetic, formic, and levulinic acid, are contained in pretreated lignocellulose. Acetic acid is derived from hydrolysis of acetyl groups in hemicellulose, while formic acid and levulinic acid arise a degradation products of furfural and HMF [30].

In addition, other limits of steam explosion concern the only partial destruction of xylan and the incomplete disruption of the lignin-carbohydrate matrix. One major drawback of steam explosion is the partial removal of lignin, in fact high residual lignin content is redistributed on the surface of cellulose, hampering the hydrolysis by cellulase enzymes [31,32]. The use of alkaline hydrogen peroxide (H_2O_2), in combination with steam explosion, has been successfully developed for pretreatment of lignocellulose biomass [31,33,34]. It is effective for hemicellulose and promotes the delignification of lignocellulosic biomass, due to its strong oxidizing ability that causes detachment and solubilization of lignin, loosening the lignocellulose matrix [34,35]. It is believed that delignification process is induced by products of H_2O_2 decomposition, such as hydroxyl radicals and superoxide anion radicals: they cause the oxidation of lignin structures, which leads to the introduction of hydrophilic (carboxyl) groups and cleavage of some interunit bonds [36]. Furthermore, alkaline hydrogen peroxide not leaves residues in the biomass, as it degrades into oxygen and water, the formation of secondary products is practically inexistent, and the costs of H_2O_2 are lower than other pretreatment chemicals [12,33]. Hydrogen peroxide ranging from 0.2% to 1% (v/v) presents low toxicity and corrosivity [31].

2. Experimental

2.1. Experimental design

SCB was pretreated by steam explosion in combination with H_2O_2, using two concentrations of impregnating agent: 1% and 0.2% by weight based on the bagasse water content. Three parallel conditions were investigated conditions were tested: (a) steam explosion without impregnating agent (WI); (b) steam explosion with 1% H_2O_2; (c) steam explosion with 0.2% H_2O_2. The choice of alkaline hydrogen peroxide concentration (%, w/w solution) was adapted from others' studies [31–33]. Solutions of hydrogen peroxide, ranging from 0.2% to 1% (v/v), as applied in our experimental study, are low toxic and corrosive [33].

The SCB pretreatment produced a slurry material in which it was possible to distinguish a solid (water insoluble solid, WIS) and a liquid fraction. The two fractions were separated by filter press; the 10 wt% WIS fraction was hydrolyzed by cellulases. The concentrations (g/L) of cellobiose, monosaccharides (glucose, xylose, arabinose and mannose), and other lignocellulose derived by-products (furfural, HMF, and acetic, levulinic, formic acid), were assessed after the hydrolysis reaction. It presents low toxicity and corrosivity [31].

2.2. Raw material

The SCB raw material was obtained from the sugarcane located in Seranna, São Paulo, Brazil, consisting of 70% carbohydrates and 24% lignin (% of dry weight, DW). The composition of raw material, shown in Table 1, was analyzed according to the standardized methods of the National Renewable Energy Laboratory (NREL) [37]. The dry matter (DM) of the raw material was estimated by drying the samples in an oven at 105 °C until constant weight [28]. The raw material, with an initial dry matter (DM) of 91.6%, was stored in plastic buckets at 5 °C.

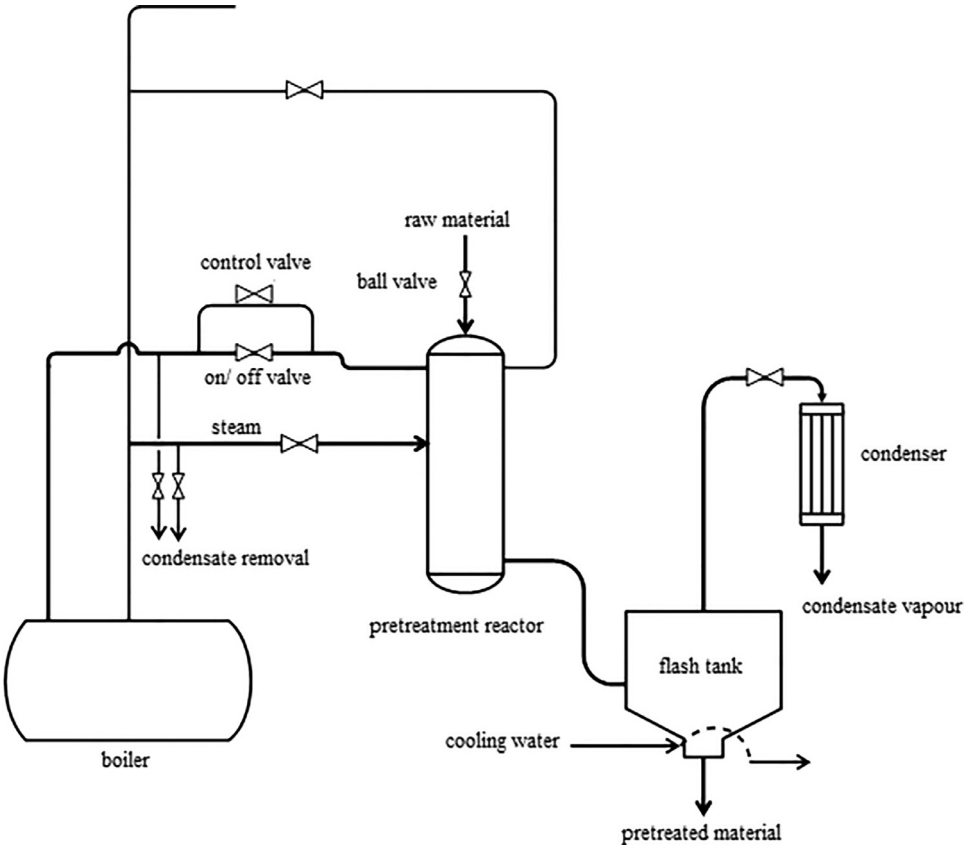

Fig. 1. The pretreatment unit.

Table 1. Composition of sugarcane bagasse as percentage of dry matter.

Compounds	Content (%)
Glucan	41.4
Xylan	22.5
Arabinan	1.3
Mannan	3.4
Lignin	23.6
Other compounds	1.9

2.3. Steam pretreatment

Two different concentrations of H_2O_2-gas, corresponding to 1% and 0.2% by weight based on the water content of SCB, were sprayed into raw material using a rotary system, which allows a better surface area contact between the substrate and peroxide solution [31]. Thus, the plastic bags containing impregnated materials were sealed and kept for 2 h at room temperature. The steam explosion was performed in 10 L reactor and the pretreated material was collected in a flash tank. The pretreatment unit is shown in Fig. 1. Steam was provided using a 110 kW electrical boiler (Pann-Partner, Stockholm, Sweden) able to provide steam up to a maximum pressure of 3 MPa, equivalent to a saturated steam temperature of 235 °C [38].

The reactor included a ball valve at the top for the feedstock input and an air-actuated ball valve positioned at the bottom outlet, allowing for the swift expulsion of the pretreated material. Two different air-actuated valves injected high-pressure steam into reactor, one of which (located near to the bottom) was necessary for rapid heat up of the material, and the other (located higher up) was required for temperature and pressure control. The temperature, pressure and hold-up time in the reactor were monitored and regulated by a computer, using the software Intouch (Wonderware, USA) [38].

The bagasse equivalent to 600 g of dry matter was loaded into reactor of steam explosion system and treated for 15 min with a steam pressure of 1.89 MPa, corresponding to temperatures of 210 °C, in according to literature data [31–33,39]. Before each experiment, the reactor was first pre-heated in order to achieve the required experimental temperature; time zero (for pretreatment) was taken when the pressure in the reactor reached 99% of the target pressure. The pretreated-SCB was collected in a flash tank and then stored at 5 °C in containers covered with plastic lids.

2.4. Enzymatic hydrolysis

The enzymatic hydrolysis of pretreated-fraction was performed using cellulase mixture Cellic® Ctec2 (193.7FPU/ml enzyme solution) by Novozymes A/S (Bagsvaerd, Denmark), an enzymatic complex containing a blend of aggressive cellulases, high level of β-glucosidases, and hemicellulases. The enzymatic hydrolysis was made using 10% (w/w) WIS fraction. This fraction was placed in a 0.5 L glass flask, diluted with 0.1 M sodium acetate buffer (pH = 4.8) and mixed with Cellic® Ctec2; buffer solution was added to a final weight of 500 g. The enzymatic loading corresponded to a cellulase activity of 10 FPU (filter paper units) per gram of WIS. The pretreated biomass underwent enzymatic hydrolysis according to the NREL standard procedure [30]. The following changes were made: sodium azide at a final concentration of 0.01 g/L was used instead of antibiotics, and the pH was adjusted to 5 (0.05 mol/L sodium citrate buffer after sample preparation). A pH of 4.8–5.0 corresponds at optimal condition of pH for Cellic® Ctec2, as referred by enzyme manufactures. The enzymatic hydrolysis was conducted for

96 h under mechanical stirring at 45–50 °C, corresponding at optimal conditions of temperature for Cellic® Ctec2, as referred by enzyme manufactures. The adjustment of the pH value was carried out during the enzymatic hydrolysis experimental activity to keep pH within the correct range. The reaction mixing was performed at 100/150 rpm by overhead stirrer equipped with propellers stirrer 3 blades PFTE-coated. The hydrolysis unit was equipped with a pH-controlling device and a water jacket to maintain constant temperature. Aliquots of 0.5 mL were taken at different time (0, 6, 12, 24, 48, 72, and 96 h), immediately chilled on ice, and centrifuged at 5000 rpm for 10 min. Analysis of sugars and lignocellulose by-products were carried out on the resultant supernatants. All the experiments were performed twice and the average values were used for data analysis.

2.5. Analysis methodologies

2.5.1. Determination of FPU activity in Cellic® Ctec2

The cellulase activity was determined in terms of "filter paper units" (FPU) per milliliter of original (undiluted) enzyme solution, using IUPAC procedure [40]. Then, FPU is the measure unit of activity enzyme (FPU = international unit (I.U)/mL; I.U = μmol sugars produced/min). At first, 1 mL of Na-citrate buffer (0.05 M), pH 4.8 and 50 mg Whatman No. 1 filter paper strip (1 × 6 cm) were added to a test tube. Then, 0.5 mL of diluted cellulase was added to the tube. Two dilutions must be made of each cellulase sample: one dilution should release slightly more than 2.0 mg of glucose (absolute amount) and the other one slightly less than 2.0 mg of glucose. The tubes were incubated at 50 °C for 60 min. At the end of the incubation period, each tube was removed from the 50 °C bath and the enzyme reaction was stopped by adding 3.0 mL of DNS reagent and mixing. All tubes were boiled in a vigorously boiling water bath for 5.0 min. The colored solution was diluted with 20 mL of H_2O, and the absorbance was measured at 540 nm.

The cellulase concentration, which would have exactly 2.0 mg of glucose released by means of a plot of glucose liberated against the logarithm of enzyme concentration, was estimated. Two data points very close to 2.0 mg were taken, and a straight line was drawn between them. This line was used to interpolate between the two points to find the enzyme dilution that would produce exactly 2.0 mg glucose equivalents of reducing sugar. The dilutions were express as follows:

Cellulase concentration releasing 2.0 mg glucose = 1/Dilution

FPU was calculated as:

$$\text{FPU} = \frac{0.37}{\text{Cellulase concentration releasing 2.0 mg glucose} \times \text{units/mL}}$$

2.5.2. Determination of sugars and lignocellulose by-products concentrations

Sugars and lignocellulose by-products analysis was performed by HPLC (high performance liquid chromatography), as described by Sluiter et al. [41]. The instrument used (Shimadzu LC-10AD, Tokyo, Japan) was equipped with a Refractive Index detector (Shimadzu). All samples were diluted and filtered through a 0.20 µm filter prior to HPLC analysis, and acidic samples were neutralized by the addition of $CaCO_3$. The concentrations of sugars, as cellobiose, glucose, xylose, galactose, mannose and arabinose, in the liquid collected after pretreatment and in the samples from enzymatic hydrolysis were separated using an Aminex HPX-87P column (Bio-Rad laboratories, Hercules, CA, USA), operating at 85 °C with deionized water as the mobile phase, at a flow rate of 0.6 mL/min. The concentrations of lignocellulose derived by-products, as furfural, HMF, and acetic, levulinic, formic acid, were analyzed with an Aminex HPX-87H (Bio-Rad laboratories, Hercules, CA, USA), operating at 65 °C, with 5 mM H_2SO_4 as mobile phase at a flow rate of 0.6 mL/min. The obtained data were subjected to analysis of variance (ANOVA) for the model of sugars and lignocellulose by-products yields after hydrolysis for pretreatment of SCB by steam and H_2O_2, in order to determine if any significant differences ($p < .05$) occurred between factors. It can be seen that the model presents a high correlation coefficient and can be considered statistically significant with 90% of confidence according to the F test.

3. Results and discussion

3.1. Sugars concentration in hydrolysate samples

The concentrations (g/L) of cellobiose and monosaccharides (glucose, xylose, arabinose and mannose) obtained in hydrolysate samples, pretreated with steam and H_2O_2 1% and 0.2%, were compared with those obtained in hydrolysate samples, pretreated with steam in absence of impregnating agent (Figs. 2 and 3).

3.1.1. Cellobiose

Cellobiose is a homoglucan disaccharide, composed of two molecules of β-glucose linked by a β-(1→4) bond. Cellobiose is a stronger inhibitor for cellulase [23] and can be derived from enzymatic hydrolysis of cellulose: it is released synergistically by endo-β-glucanase (EC 3.2.1.4) and exo-β-glucanase (EC 3.2.1.91), and then hydrolyzed to glucose by β-glucosidase (EC 3.2.1.21). Cellobiose concentrations were monitored over a time course of 96 h during the hydrolysis reaction. In absence of H_2O_2 in steam-pretreatment, cellobiose concentration increases with increasing hydrolysis time. On the contrary, in hydrolysate samples pretreated in combination with H_2O_2, cellobiose concentration reaches a maximum between 24 and 48 h of hydrolysis and it seems to decrease with the prolonged reaction time (Fig. 2a).

3.1.2. Glucose and xylose

The glucose is the most abundant monosaccharide contained into hydrolysate samples; its concentration increases with increasing hydrolysis time. Both in absence and presence of 0.2% H_2O_2 in steam-pretreatment, similar concentrations of glucose are maintained throughout the period of hydrolysis reaction, reaching the plateau state after 78 h. The highest glucose concentrations are to be found in hydrolysate samples pretreated in combination with 1% H_2O_2; in the latter case, glucose conversion shows a gradual achievement of the plateau state after about 90 h of hydrolysis reaction (Fig. 2b).

The xylose concentration is lower compared to that of glucose; it increases slightly with increasing hydrolysis time, reaching a plateau state after 48 h reaction under each experimental condition. The highest xylose concentrations are determined in hydrolysate samples pretreated in combination with H_2O_2, in particular by adding of 1% impregnating agent (Fig. 2c).

3.1.3. Arabinose and mannose

The monomeric sugars, as arabinose and mannose, account for a very small quantity in the SCB extract. The highest concentrations of arabinose are attained in samples pretreated in combination with 0.2% and 1% H_2O_2, after 6 h hydrolysis time. However, low arabinose levels are detected in samples pretreated in absence of H_2O_2 only at zero time (Fig. 3a). On the contrary, the highest concentrations of mannose are measured in samples pretreated in absence of impregnating agent. In samples pretreated in combination with 1% and 0.2% H_2O_2, the mannose concentration has a value equal to zero after 12 and 78 h hydrolysis time, respectively (Fig. 3b). Then, concentrations of two monomeric sugars decrease with the prolonged reaction time. It can be postulated that arabinose and mannose degrade with time.

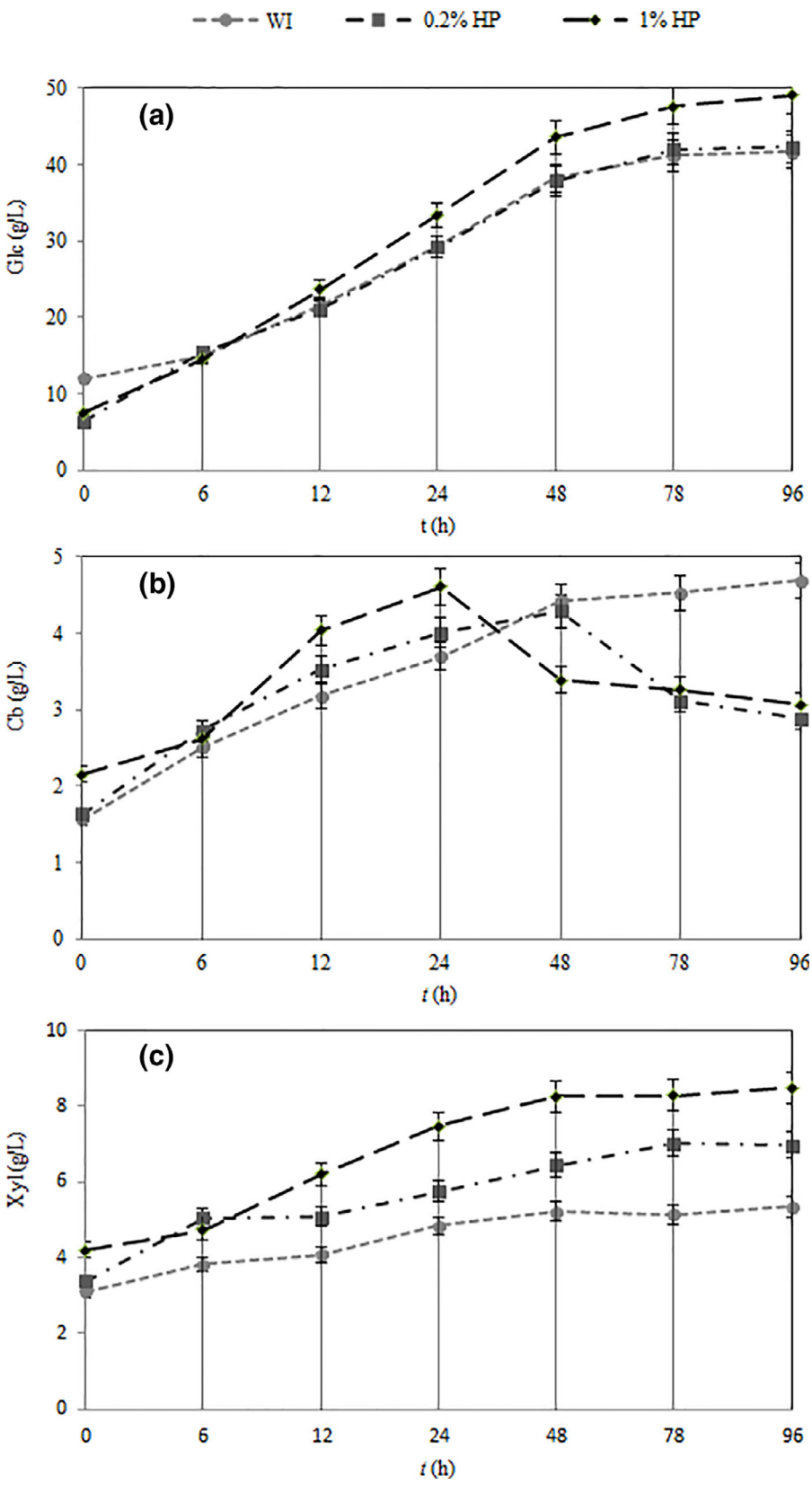

Fig. 2. Time evolution of main fermentable sugars concentration without impregnating agent (WI) and at different amounts of H_2O_2 as impregnating agent (H_2O_2 at 0.2 %, H_2O_2 at 1%). (a) Cellobiose, (b) glucose and (c) xylose; cb = cellobiose, glc = lucose, xyl = xylose; HP = hydrogen peroxide, H_2O_2.

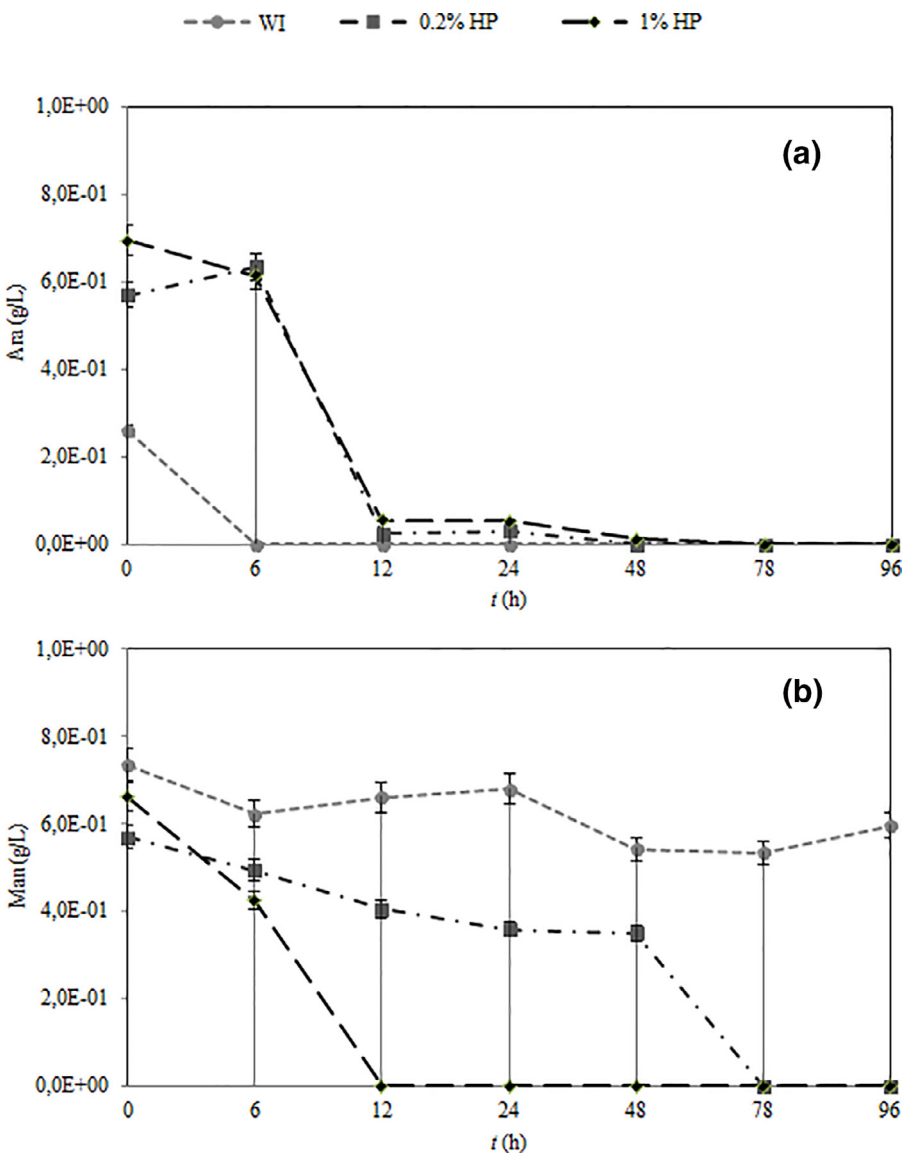

Fig. 3. Time evolution of minor fermentable sugars concentration without impregnating agent (WI) and at different amounts of H_2O_2 as impregnating agent (H_2O_2 at 0.2 %, H_2O_2 at 1%). (a) Arabinose and (b) mannose; ara = arabinose, man = mannose, HP = hydrogen peroxide, H_2O_2.

3.2. Yield of cellobiose and monomeric sugars

The fermentable sugars yields have been calculated as:

$$Y_i = \frac{m_i^a}{m_i^{exp}} \times 100\% \quad (1)$$

where Y is the percent yield of interest compound; i (cellobiose, glucose, xylose, arabinose, or mannose); m_i^a is the actual mass (g/L) of interest compound that was compared with the expected mass (g/L) of interest compound (m_i^{exp}).

Then, percent yield of cellobiose (cb), glucose (glc), xylose (xyl), arabinose (ara) and mannose (man) were evaluated as reported in the equations included in Table 2. The obtained percent yield of fermentable sugars is summarized in Fig. 4. After 96 h hydrolysis time, the highest cellobiose yield was achieved in hydrolysate samples pretreated in absence of impregnating agent. When this

Table 2. The percent yield of cellobiose (cb), glucose (glc), xylose (xyl), arabinose (ara) and mannose (man).

Equations	Yield (%)	Actual mass (g/L)	Expexted mass (g/L)
$Y_{cb} = \frac{m^a_{cb}}{m^{exp}_{cb}}$	Y_{cb}=percent yield of cb	m^a_{cb}=actual mass yield of cb	m^{exp}_{cb}=expected amount of cb, as glucan percent per dry fiber mass
$Y_{glc} = \frac{m^a_{glc}}{m^{exp}_{glc}}$	Y_{glc}=percent yield of glc	m^a_{glc}=actual mass yield of glc	m^{exp}_{glc}=expected amount of glc, as glucan percent per dry fiber mass
$Y_{xyl} = \frac{m^a_{xyl}}{m^{exp}_{xyl}}$	Y_{xyl}=percent yield of xyl	m^a_{xyl}=actual mass yield of xyl	m^a_{xyl}=expected amount of xyl, as xylan percent per dry fiber mass
$Y_{ara} = \frac{m^a_{ara}}{m^{exp}_{ara}}$	Y_{ara}=percent yield of ara	m^a_{ara}=actual mass yield of ara	m^{exp}_{ara}=expected amount of ara, as arabinan percent
$Y_{man} = \frac{m^a_{man}}{m^{exp}_{man}}$	Y_{man}=percent yield of man	m^a_{man}=actual mass yield of man	m^a_{man}=expected amount of man, as mannan percent per dry fiber mass

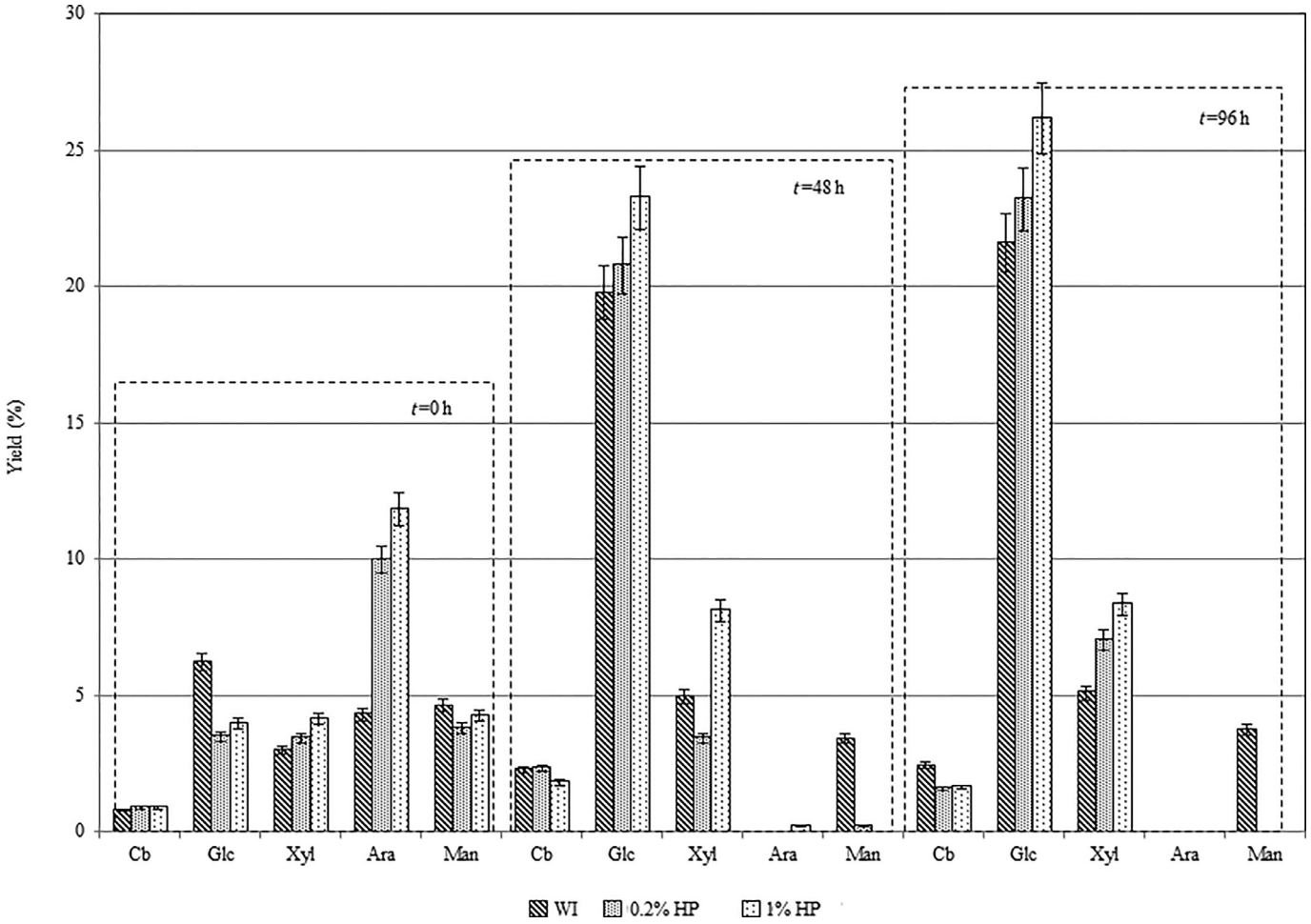

Fig. 4. Final yields of fermentable sugars at different time during hydrolysis reaction. Abbreviations: Cb = cellobiose, Glc = glucose, Xyl = xylose, Ara = arabinose, Man = mannose, HP = hydrogen peroxide, WI = without impregnating agent; $t = 0$ h at begin, $t = 48$ h and $t = 96$ h after 48 and 96 h of hydrolysis, respectively.

yield was compared with those obtained from peroxide impregnated samples, pretreated and hydrolyzed at the same conditions, one can observe that the peroxide impregnation resulted in a decrease in the cellobiose yield of about 30%. The highest final glucose yields obtained in the hydrolysate samples pretreated in combination with 0.2 and 1% H_2O_2, show an average increase of 7 and 17%, respectively, compared to the glucose yields obtained using hydrolysate samples steam-pretreated without H_2O_2.

Following an assessment of the results for cellobiose and glucose yields, it can be observed that hydrogen peroxide impregnation resulted in an increase in the total cellulose conversion. The results obtained show the highest glucose yield when the material was pretreated at 210 °C, 15 min and 1% H_2O_2. Similar glucose yields were observed of cellulose conversion in Verardi et al. using unwashed material (slurry), hydrolyzed at the same experimental conditions and obtained from SCB pretreated at 200 °C, 5 min and 2% SO_2 (as impregnating agent) [12]. A higher cellulose conversion was obtained when the enzymatic hydrolysis of slurry material was performed in a bioreactor at 300 rpm [24].

Although the hemicellulose is the main constituent extract in steam pretreatment, the hemicellulose conversion was lower than conversion of cellulose. It is probably due to a higher rate of hemicelluloses degradation; it is known that the steam explosion pretreatment degrades hemicelluloses-derived sugars [42]. In addition, a higher hemicellulose conversion was obtained using other impregnating agents in steam pretreatment, such as SO_2 [12,41]; the impregnation of the material with hydrogen peroxide causes, on the contrary, a slightly larger degradation of hemicelluloses-derived sugars, increasing, consequently, the formation of inhibitors [31]. However, a higher yield of xylose was obtained in the material impregnated with hydrogen peroxide compared to a pretreatment in absence of impregnating agent: in hydrolysate samples steam-pretreated in combination with 0.2 and 1% H_2O_2 was achieved a substantial increase of 28% and 39% xylose yields, respectively, compared to that reached in samples steam-pretreated without H_2O_2.

The total content of arabinose was lost after 96 h hydrolysis reaction in each experimental condition, while the minimum yield, resulting from hydrolysis reaction of the samples pretreated in absence of H_2O_2, was lost in the other experimental conditions.

3.3. Analysis of lignocellulose derived-by-products

The steam-explosion in combination with H_2O_2 is able to selectively break bonds within lignin by oxidation mechanism, increasing the lignin degradation to an appreciable extent. If, on the one hand, the greater lignin degradation allows a higher accessibility of hydrolytic enzyme to hemicellulose and cellulose surface, on the other hand the release of lignocellulose derived-by-products, includes furans that are degradation products of hemicellulose (such as furfural and HMF), small organic acids (mainly acetic acid), and phenolic compounds from lignin. Some of these lignocellulose derived-by-products, formed during hemicellulose and lignin degradation, are released into the liquor of steam-explosion process, whereas others are incorporated in the biomass and released during successive bioconversion [43]. The lignocellulose derived-

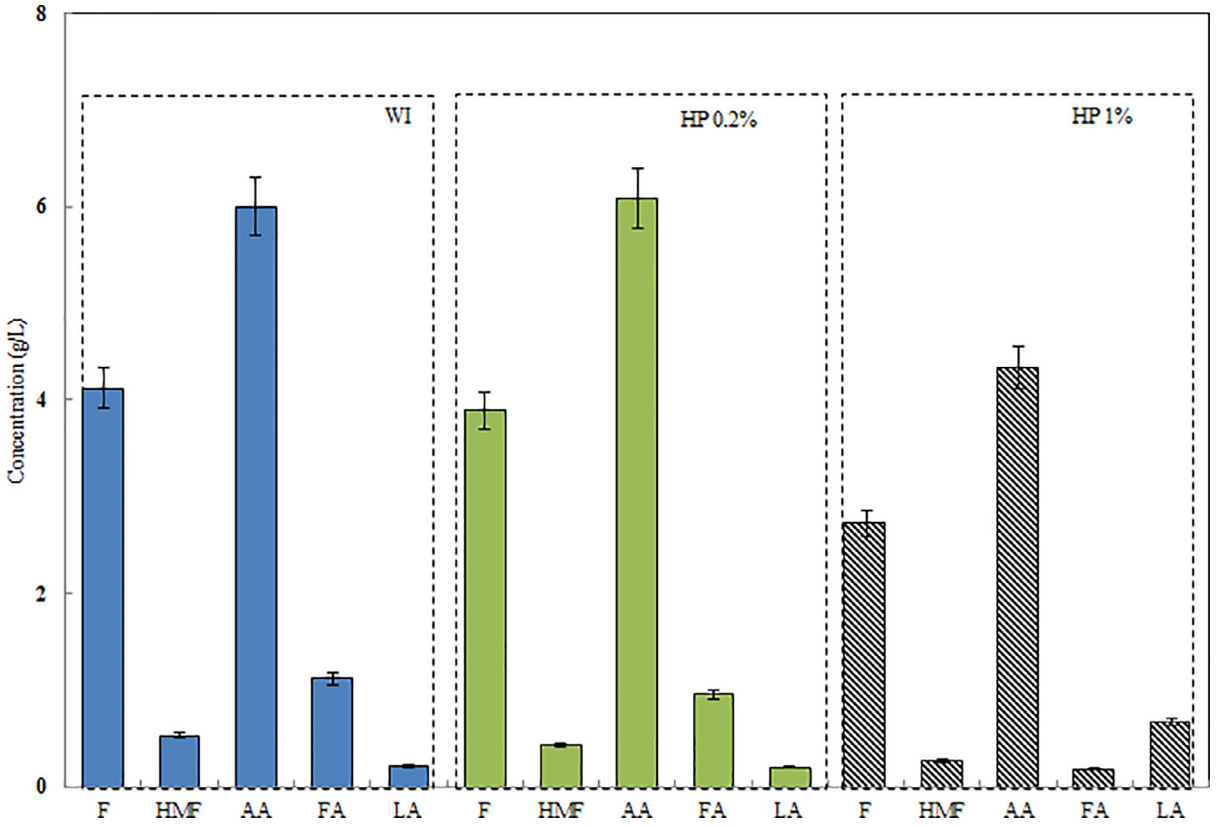

Fig. 5. Concentration of lignocellulose derived by-products. Abbreviations: F = furfural, HMF = 5-(hydroxymethyl)furfural, AA = acetic acid, FA = formic acid, LA = levulinic acid, HP = hydrogen peroxide, WI = without impregnating agent.

by-products are able to inhibit microbial and enzymatic biocatalysts; then, the concentrations of furfural, HMF, acetic acid, formic acid, and levulinic acid, resulting from SCB-pretreatment by steam explosion, both in the presence and absence of H_2O_2, were evaluated in this study (Fig. 5). The hemicelluloses losses in pretreatment are partially caused from degradation products during steam explosion pretreatment; however, hemicellulose and other compounds are also lost through volatilization of degradation products and recondensation reactions [44].

The concentrations of furans and organic acids, obtained from SCB-pretreatment by steam explosion, without or in combination with 0.2% H_2O_2, seem to be very similar. By addition of 1% H_2O_2, the furans concentrations decrease of about 32% for furfural, and 43% for HMF, compared to furans concentrations obtained by steam explosion with 0.2% H_2O_2 or without impregnating agent. Furthermore, when using 1% H_2O_2, the concentrations of acetic acid and formic acid show an average decrease of 28% and 82%, respectively, compared to those obtained by steam explosion with 0.2% H_2O_2 or without impregnating agent. The concentration of furans (furfural and HMF) and weak acids, mainly acetic acid, was higher in unwashed material (slurry), obtained from SCB pretreated at 200 °C, 5 min and 2% SO_2 as impregnating agent [12]. These results suggest that use of hydrogen peroxide does not seem to increase the formation of furans, acetic and formic acid released during SCB-pretreatment by steam explosion.

On the contrary, the levulinic acid concentration, in samples steam-pretreated by addition of 1% H_2O_2, is higher of about 70% than that achieved in other experimental conditions. In addition, the concentration of levulinic acid achieved in slurry, derived from steam explosion pretreatment with H_2O_2, is greater than those obtained using SO_2 as impregnating agent during steam explosion pretreatment [12]. It is probably caused by higher degradation of HMF to levulinic acids under pretreatment conditions with higher H_2O_2 concentrations.

4. Conclusions

This study has been focused on the use of hydrogen peroxide to optimize the efficiency of steam explosion, based on the increase of fermentable sugars concentrations after enzymatic saccharification. The use of hydrogen peroxide as impregnating agent has brought clear advantages with respect to: (i) the yield of the main polysaccharides hydrolysis, with an increase of 12% for glucose and 34% for xylose; (ii) the impact of lignocellulose-derived by-products that have negative effects on the rate of enzymatic hydrolysis and on alcohol fermentation. In the latter case, the use of H_2O_2 does not seem to increase the formation of the inhibitor compounds released during pretreatment by steam explosion, with the sole exception of the levulinic acid concentration.

In addition to the overall improvement identified, alkaline hydrogen peroxide not leaves residues in the biomass and its costs are lower than other pretreatment chemicals.

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