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Acetone–butanol–ethanol fermentation from sugarcane bagasse hydrolysates: Utilization of C5 and C6 sugars

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ABSTRACT

Background: Fuels and chemicals from renewable feedstocks have a growing demand, and acetone, butanol and ethanol (ABE) are some relevant examples. These molecules can be produced by the bacterial fermentation process using hydrolysates generated from lignocellulosic biomass as sugarcane bagasse, one of the most abundant sources of lignocellulosic biomass in Brazil. It originates as a residue in mills and distilleries in the production of sugar and ethanol.
Results: In the present work, two strategies to generate hydrolysates of sugarcane bagasse were adopted. The fermentation of the first hydrolysate by *Clostridium acetobutylicum* DSM 6228 resulted in final concentrations of butanol, acetone and ethanol of 6.4, 4.5 and 0.6 g/L, respectively. On the other hand, the second hydrolysate presented better results (averages of 9.1, 5.5 and 0.8 g/L, respectively), even without the need for nutrient supplementation, since key elements were already present in the medium. The productivity (Q_P) and yield ($Y_{P/S}$) of the solvents with second hydrolysate were 0.5 g/L·h^{-1} and 0.4 g/g, respectively.
Conclusions: The results described herein open new perspectives for the production of important molecules from residual lignocellulosic biomass for the fuel and chemical industries within the context of second-generation biorefinery.
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1. Introduction

The industrial demand for renewable and sustainable fuels and chemicals is already a reality today and is expected to grow significantly in the future [1]. Several companies are currently investing in development for the production of biobutanol, using the infrastructure of corn or sugarcane ethanol production. Efforts are being applied in genetic modification and in downstream stages, in addition to the use of other substrate sources [2]. In this context, lignocellulosic biomass represents a feedstock with massive potential for the production of biofuels and chemicals because it is the most abundant renewable material in the world and because in many cases, it is considered a residue that does not compete with food. In Brazil alone, the main agricultural sectors generate approximately 350 million tons of biomass annually, of which approximately 20% is available for use [3], and sugarcane bagasse is the major biomass. The 2019/2020 harvest season yielded a sugarcane production of 615.98 million tons, with an average amount of bagasse of 154 million tons [4].

In recent decades, there has been an increase in microbial production aimed at high-value molecules in the market and, at the same time, the search for inexpensive and environmentally friendly substrates that can make the biotechnology process viable. In this context, traditional fermentations such as the production of acetone, butanol and ethanol, named ABE fermentation, have returned to the market, adding new technologies of bioprocessing, genetic and metabolic engineering [5]. This bioprocess occurs in two steps: the first step is an acidogenic phase, where substrates (sugars) are consumed for the production of organic acids (butyric acid and acetic acid), resulting in a pH drop to 4–5; then, acids are converted to solvents (butanol, acetone and ethanol) in a second step, known as the solventogenic phase. Butanol and acetone are the main

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metabolites from ABE fermentation, produced by *Clostridia*, followed by ethanol (in a small amount).

The literature presents several fermentation studies based on wild or genetically modified microorganisms to obtain these chemicals. Al-Shorgani et al. [6] used batch and continuous fermentations of dilute acid-pretreated deoiled rice bran for butanol production. Pretreatment of biomass with dilute sulfuric acid (1%) resulted in the production of hydrolysate with 42.1 g/L total sugars (25.6 g/L glucose, 15.1 g/L xylose and 1.5 g/L cellobiose). The hydrolysate was used as the fermentation medium. The maximum concentration of solvents achieved was 11.2 g/L (4.4 g/L acetone, 5.9 g/L butanol and 0.9 g/L ethanol), which was attained by hydrolysate detoxification with activated charcoal to remove fermentation inhibitors such as furfural, 5-hydroxymethilfurfural (5-HMF), acetic acid, formic acid and levulinic acid.

Hydrolysates of corn fiber, cotton stalk, soybean hull and sugarcane bagasse obtained from dilute acid pretreatment and enzymatic hydrolysis were submitted to acetone–butanol–ethanol fermentation without a previous detoxification step. The maximum butanol concentration of 15.6 g/L was obtained with a corresponding yield of 0.31 g/g and productivity of 0.31 g/L·h^{-1} by the fermentation of cotton stalk hydrolysate. These values are the highest reported in the literature for a lignocellulosic feedstock obtained by immobilized genetically engineered *Clostridium tyrobutyricum* [7].

Liao et al. [8] compared the different performances of naturally occurring and genetically modified *Clostridium acetobutylicum* bacteria. The authors used soybean straw that was pretreated with dilute acid and enzymatically hydrolyzed. The initial glucose and xylose levels were 50 and 15 g/L, respectively. The butanol concentrations were 8 g/L with the genetically modified strain, reaching maximum volumetric productivity and butanol yield of 0.11 g/L·h^{-1} and 0.14 g/g, respectively. The hydrolysate was not detoxified, and the engineered strain presented twice the productivity when compared to that of the wild strain.

Magalhães et al. [2] used sugarcane bagasse hydrolysate to produce ABE by *C. saccharoperbutylacetonicum* DSM 14923. The authors reported the production of 4.5, 1.4 and 0.3 g/L of butanol, acetone and ethanol, respectively, with an ABE yield and volumetric productivity of 0.09 g/g and 0.30 g/L·h^{-1}, respectively. The hydrolysate was not detoxified, and the strain showed a consumption of 96.1% glucose and 53.1% xylose.

Liu et al. [9] used switchgrass hydrolysate for butanol production by naturally occurring *C. acetobutylicum*. Fermentability of the hydrolysate was only possible after the detoxification procedure with activated carbon, generating a concentration of 11 g/L butanol with a total solvent (ABE) concentration of 17 g/L. The corresponding response variables included a butanol yield of 0.20 g/g and a volumetric productivity of 0.15 g/L·h^{-1}.

In another study, sugarcane bagasse was pretreated with alkali and enzymatically hydrolyzed. The hydrolysates were used to produce butanol in fed-batch fermentation by *C. acetobutylicum*. At 60 h, 14.2 and 21.0 g/L of butanol and acetone–butanol–ethanol (ABE) were produced from 68.9 g/L of total sugars with yields of 0.22 and 0.33 g/g, respectively. In fed-batch fermentation, glucose was completely consumed in 56 h, but xylose was consumed more slowly; after 56 h, xylose utilization ceased, and 45% of the pentose remained in the fermented medium [10].

The main motivation of this work is to contribute to the development of a bioprocess within the context of the second-generation (2G) biorefinery, i.e., lignocellulosic biorefinery. This concept is of utmost importance, particularly for countries with agricultural activities as a natural vocation. The approach is based on exploiting the use of biomass for the production of fuels and chemicals in a more integrated production system. The most interesting aspect of the second-generation technologies is the use of abundant agricultural residues, positioning them as feedstocks. In this context, this work is aimed at investigating ABE fermentation, defining strategies for improving the production performance. Thus, dilute acid pretreatment and enzymatic hydrolysis were defined and executed producing hydrolysates rich in sugars, which are fermented promptly, with no detoxification step and nutrient addition prior to fermentation. Therefore, the present work aims to produce acetone, butanol and ethanol using non-detoxified sugarcane bagasse hydrolysates rich in xylose and glucose by *C. acetobutylicum* DSM 6228.

2. Materials and methods

2.1. Hydrolysates

Sugarcane bagasse was provided by a sugarcane mill in Goiás State, Brazil. The wet biomass (5 kg with 50% moisture) was submitted to pretreatment with 25% (*w*/*w*) bagasse in 1% (*v*/*v*) sulfuric acid at 121°C for 30 min in an 80 L pilot plant. The resulting wet solid was pressed, and then, the pH of the liquid fraction (named HC5) and solid fraction was adjusted to 5.0 using 30% (*v*/*v*) ammonium hydroxide. The solid fraction was subjected to enzymatic hydrolysis with two different liquid phases (water or HC5) in a mechanically stirred bioreactor with the commercial preparation CellicCTec® 3 (15 mg protein/g cellulose), which was kindly supplied by Novozymes® Latin America LTDA. The total solid content was 20% (*w*/*w*) suspended in water or HC5, and hydrolysis was carried out for 72 h at pH 5 and 50°C. The reaction mixture was centrifuged at 30,000 × g for 20 min at 20°C. The resulting liquid fraction using water was named sugarcane bagasse hydrolysate 1 (SBH-1), and the resulting liquid fraction using HC5 instead of water (under the same operating conditions described above) was named sugarcane bagasse hydrolysate 2 (SBH-2).

2.2. Microorganism, activation and propagation steps

The study was carried out with *C. acetobutylicum* DSM6228, purchased from the DSMZ (Deutsche Sammulung von Mikroorganismen und Zellkulturen) culture collection. The microorganism was kept in a stock solution of 30% (*v*/*v*) glycerol in 2 mL tubes at -80°C [11]. The activation step consisted of transferring one tube to each of the two 100 mL vials each containing 50 mL of RCM (reinforced clostridial medium) and resazurin at 1 mg/L (anaerobiosis indicator). The components of RCM are as follows: beef extract (10 g/L), peptone (10 g/L), sodium chloride (5 g/L), glucose (5 g/L), yeast extract (3 g/L), sodium acetate (3 g/L), starch (1 g/L), L-cysteine-HCl (0.5 g/L) and agar (0.5 g/L). The flasks were kept without stirring at 37°C for 24 h [12]. The microorganism propagation step was carried out in a 1.3 L bioreactor (BioFlo 110, New Brunswick Co.) at 37°C and 50 rpm for 8 h with 500 mL of medium containing: glucose (12 g/L), peptone (6 g/L) and yeast extract (6 g/L). This step used 100 mL of inoculum from the activation flask.

2.3. Fermentation of lignocellulosic hydrolysates

After 8 h of propagation in the bioreactor, 500 mL of substrate was added to the bioreactor, and the fermentation was carried out for the next 80–84 h under the same conditions used for propagation. Assays were first carried out with chemically defined media (glucose and xylose solutions, separately, according to the work of Sun and Liu [13] and Jiang et al. [14]), then with SBH-1 and finally with SBH-2. During all fermentations, the pH was measured but not controlled. The supplementation of nutrients was performed based on the medium composition reported by Sun and Liu [13], composed of (in g/L in the final solution) yeast extract (1.0), $MnSO_4$ (0.01), $MgSO_4$ (0.2), KH_2PO_4 (0.5), K_2HPO_4 (0.5), sodium acetate (0.01), and $FeSO_4 \cdot 7H_2O$ (0.01). SBH-2 was the chosen substrate to carry out fermentations with and without nutrient addition. The yield of product per consumed substrate ($Y_{P/S}$) was determined for each fermentation through the

ratio between the total solvent concentration (butanol, acetone and ethanol, in g/L) and the total consumed substrate (glucose and xylose, also in g/L). Productivity (Q_P) was calculated using the ratio between solvent concentration (g/L) and time (h).

2.4. Analytical methods

The concentrations of glucose, xylose, acetone, butanol, ethanol, butyric acid and acetic acid (or acetate) were determined using an HPLC (Thermo Scientific) according to the method developed for this work. The analyses were conducted with 0.005 M H_2SO_4 (mobile phase) at 0.5 mL/min at 80°C and a Vertex Eurokat® H column. External standards of each chemical with a minimum purity of 99% were used for the identification and quantification. The total polyphenols (TP) in lignocellulosic hydrolysates were quantified according to the Folin–Ciocalteu method [15] using a HACH DR6000 spectrophotometer ($\lambda = 510$ nm), and tannic acid was used as a standard. The cell concentration on a dry basis was quantified by turbidity in the same spectrophotometer at a wavelength of 600 nm [16,17,18]. Quantification of the sulfate and phosphate content in the hydrolysates was carried out by ion chromatography with an 861 Advanced Compact IC system (Metrohm) with a Metrosep A Supp4 250/4.0 column, eluent containing 1.8×10^{-3} mol/L Na_2CO_3 and 1.7×10^{-3} mol/L $NaHCO_3$, flow at 1 mL/min, and injection volume of 20 µL at room temperature.

Metal analysis (Mn^{2+}, Mg^{2+}, K^+, Na^+ and Fe^{2+}) was performed by inductively coupled plasma optical emission spectrometry (ICP-OES), with a Perkin Elmer Sciex Optima 3300 DV system, according to the methodology of ASTM D1976 96 [19]. Ammoniacal nitrogen and total nitrogen were measured according to the methodologies of 4500-NH_3 nitrogen (ammonia) [20] and ASTM D5176–08 [21], respectively.

3. Results and discussion

3.1. Fermentation of sugarcane bagasse hydrolysates

The fermentation tests carried out with chemically defined media using glucose and xylose as the carbon sources and with the addition of nutrients are presented in Fig. 1 and Fig. 2. For the assays with glucose and xylose as substrates, the yield ($Y_{P/S}$) values obtained, based on the solvents as the products, were 0.32 and 0.47 g/g, respectively, and the productivity (Q_P) values were 0.36 and 0.32 g/L·h^{-1}. The glucose fermentation assay was carried out for 80 h with almost total consumption of the substrate in 32 h, i.e., 35.4 g/L,

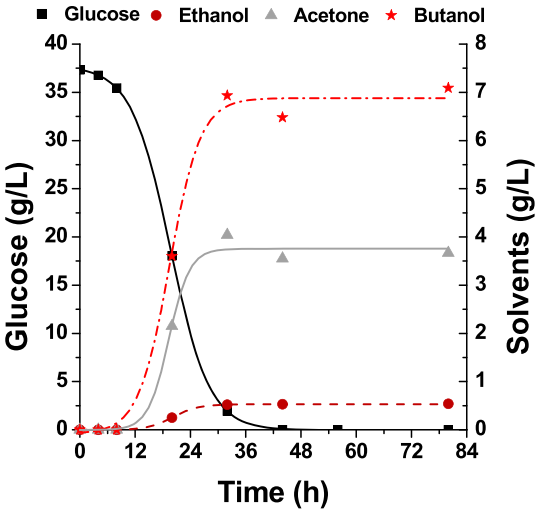

Fig. 1. Time course of ABE fermentation with glucose as substrate. Temperature: 37°C and 50 rpm in 1 L bioreactor.

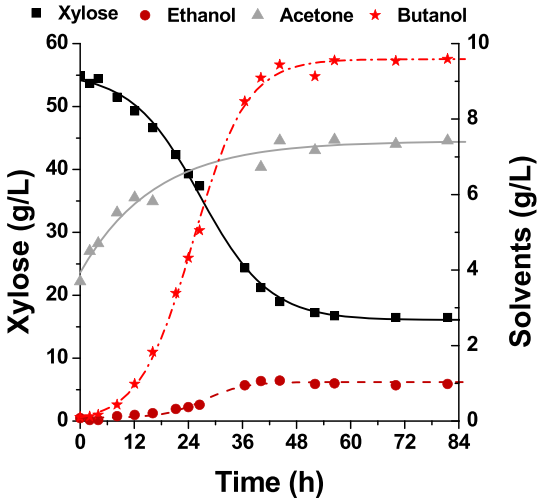

Fig. 2. Time course of ABE fermentation with xylose as substrate. Temperature: 37°C and 50 rpm in 1 L bioreactor.

with a butanol, acetone and ethanol production of 6.9, 4.0 and 0.5 g/L, respectively. The time required for xylose fermentation was 56 h, consuming 38 g/L of carbohydrate and leaving a residual of 16.7 g/L. In this test, the production of butanol, acetone and ethanol was 9.6, 7.5 and 1.0 g/L, respectively. Although xylose remained, the values obtained were even higher than those of the glucose assay. It is also seen that the amount of substrate used by the microorganism was between 30 and 40 g/L. The most important finding of these tests is the ability of the strain to metabolize both glucose and xylose to produce the solvents, with butanol as the major product. Jiang et al. [14] used glucose as a substrate for the production of butanol by *Clostridium beijerinckii* IB4, obtaining butanol, acetone and ethanol concentrations of 11, 3 and 0.2 g/L, respectively, after 40 h of fermentation. The glucose consumption in this period was 41 g/L, leaving a residual of 18 g/L. The yield and productivity of the solvents obtained were 0.34 g/g and 0.35 g/L·h^{-1}, respectively. Kheyrandish et al. [22] used glucose as a substrate for the production of butanol, acetone and ethanol at initial substrate concentrations of 20, 40, 60 and 80 g/L. The authors obtained an increase of 80% in butanol production when they increased the glucose concentration from 20 to 40 g/L but did not observe a significant increase in the product with higher initial concentrations of substrate. The authors attributed the results to catabolic repression caused by glucose at substrate amounts above 40 g/L. The butanol, acetone and ethanol concentrations achieved were 9.3, 3.4 and 0 g/L, respectively, after 48 h of fermentation and starting with 40 g/L of glucose. Sun and Liu [13] used xylose as a substrate for butanol production by *C. acetobutylicum* ATCC 824, obtaining butanol, acetone and ethanol concentrations of 7.9, 2.0 and 1.5 g/L after 96 h of fermentation. The consumption of xylose in this period was 47 g/L, leaving a residual of 13 g/L. The solvent yield and productivity were 0.24 g/g and 0.12 g/L·h^{-1}, respectively. After fermentation with chemically defined media,

Table 1
Composition of sugarcane bagasse enzymatic hydrolysates (in g/L) for 2G ABE fermentation.

Component	SBH-1	SBH-2
Glucose	51.30 ± 0.97	69.83 ± 0.05
Xylose	17.80 ± 0.35	52.06 ± 1.54
Acetic acid	5.42 ± 0.02	11.58 ± 0.52
5-HMF	0.02 ± 0.00	0.07 ± 0.02
Furfural	0.36 ± 0.01	0.62 ± 0.10
Total polyphenol	1.83 ± 0.02	3.21 ± 0.04

sugarcane bagasse hydrolysates (SBH-1 and SBH-2) were used separately as substrates for ABE production in the instrumented bioreactor. Table 1 presents the composition of the hydrolysates used in this work.

The processes for deconstructing the biomass fibers (pretreatment and enzymatic hydrolysis) were applied to use the main lignocellulosic sugars, glucose and xylose, which together account for more than 70% of the composition of sugarcane bagasse on a dry basis. SBH-1 contained a total carbohydrate concentration of approximately 70 g/L, with glucose being the predominant sugar, and hydrolysate SBH-2 presenting a total sugar concentration of approximately 122 g/L. The concentrations of furfural and total polyphenols obtained were higher in SBH-2 than in SBH-1 because of the C5 liquid stream that was gather together with the pretreated solids to generate SBH-2. The dilute acid pretreatment is intended to disrupt the lignocellulosic material and chemically hydrolyze most of the hemicellulose, with xylose as the major sugar in this fraction, which continues to react with the acid catalyst in the medium, generating furfural. A small portion of lignin is also solubilized under acid pretreatment conditions, generating polyphenols. For the preparation of SBH-1, the pretreatment liquid fraction was mostly removed by filter press. The residual solid was mixed with water and enzyme to undergo enzymatic hydrolysis and generate the hydrolysate SBH-1, thereby diluting furfural and polyphenols in the medium.

SBH-1 and SBH-2 were subjected to ABE fermentation with *C. acetobutylicum* DSM 6228, and the results are presented in Fig. 3 and Fig. 4, respectively. It can be observed from the time course of Fig. 3 that the acidogenic phase occurred in the first 24 h of fermentation. After this period, butanol, acetone and ethanol (solventogenic phase) were produced, achieving concentrations of 6.4, 4.5 and 0.6 g/L, respectively, after 48 h, corresponding to a total concentration of solvents of 11.5 g/L. The volumetric productivity (Q_P) was 0.25 g/L·h^{-1}. Concerning the consumption of substrates, the bacterium used 25 g/L of glucose and 8.3 g/L of xylose during the process. These results showed that it was possible to harness the SBH-1 hydrolysate without the need for detoxification to remove potential inhibitors, such as 5-HMF, furfural and polyphenols.

Regarding SBH-2, the acidogenic phase was shorter (8 h), followed by a partial consumption of organic acids and the production of solvents (Fig. 4). The substrate consumption was 28.4 g/L for glucose and 9.4 g/L for xylose. Glucose was preferably consumed by the strain, and at the end of the process, 55% of xylose remained unconsumed. The total use of glucose and part of xylose was due to the higher initial concentration of sugars in this stream (approximately 55 g/L), which inhibited xylose uptake due to catabolic repression caused by glucose, as reported by Kheyrandish et al. [22]. It is worth noting that in contrast to SBH-2, in the SBH-1 strategy, both sugars were totally consumed. The reason behind this result is that the initial sugar concentration was approximately 35 g/L lower in SBH-1 than in SBH-2. *C. acetobutylicum* consumes sugars in a range of 30–40 g/L. The maximum butanol, acetone and ethanol production was 9.1, 5.5 and 0.9 g/L, respectively. The values of Q_P and yield ($Y_{P/S}$) were 0.5 g/L·h^{-1} and 0.4 g/g, respectively. The possibility of eliminating inputs of nutrients in the fermentation medium was evaluated by assessing the fermentability of SBH-2, without supplementation, to verify whether such additions were truly essential. Fig. 4 shows the fermentation profiles of the media with and without nutrient addition. No significant differences were observed in either medium. Table 2 shows the overall results of the 2G ABE fermentation processes reported in the literature.

The maximum solvent concentration in this work of approximately 15 g/L was obtained after only 30 h of fermentation, reflecting the highest productivity values of the fermentation process (approximately 0.5 g/L·h^{-1}). Another interesting feature of the process developed herein is that by using the combined hydrolysate SBH-2, there was no need for nutrient supplementation to achieve high butanol, acetone, and ABE yield and productivity values. At the beginning of this work, the liquid fraction HC5, arisen from dilute acid pretreatment, presented toxicity to fermentation media. Since enzymatic hydrolysis presents the advantage of non-generation of fermentation inhibitors, two strategies were planned: SBH-1 (enzymatic hydrolysis of pretreated solids in water) and SBH-2 (enzymatic hydrolysis of pretreated solids gather together with the HC5). Nonetheless, the SBH-2 displayed the best results in terms of yield and productivity. In other word, the hemicellulosic hydrolysate did not impair fermentation performance in this case.

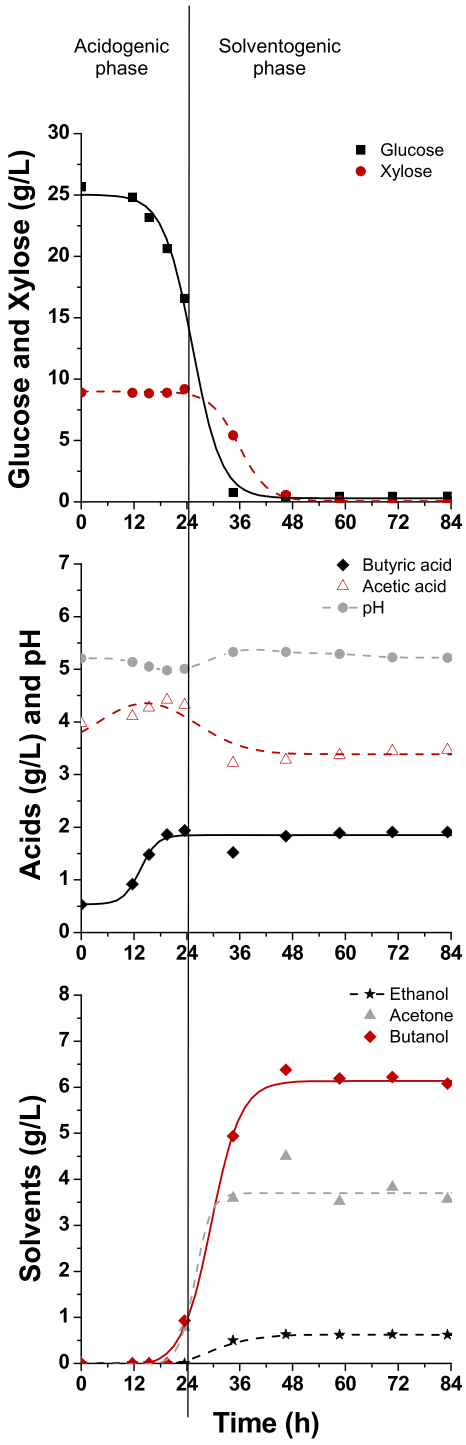

Fig. 3. Time course of 2G ABE fermentation by *C. acetobutylicum* DSM 6228 using SBH-1. (Temperature: 37°C, volume: 1 L, agitation: 50 rpm and addition of supplementation).

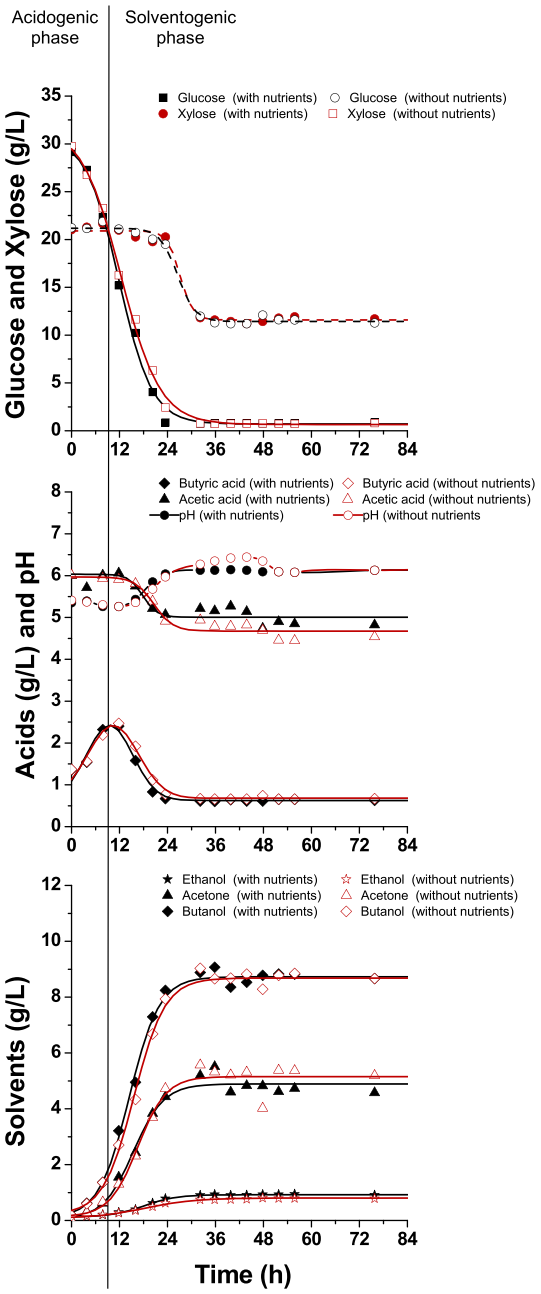

Fig. 4. Time course of 2G ABE fermentation by *C. acetobutylicum* DSM 6228 using SBH-2 with and without nutrient addition. (Temperature: 37°C, Volume: 1 L, Agitation: 50 rpm).

The results achieved in this work are in accordance with those reported in the literature, as can be observed in Table 2. Pang et al. [10] reported butanol and ABE production of 14.2 and 21.1 g/L, respectively, using sugarcane bagasse hydrolysate that contained 69 g/L of sugars. However, the reported values for the yield ($Y_{P/S}$) and volumetric productivity (Q_P) were slightly lower than those obtained in the present study, which used less sugar.

Zheng et al. [23] obtained an ABE concentration of 13.1 g/L using eucalyptus hydrolysate without nutrient supplementation. However, this result was obtained after 120 h of fermentation, resulting in a low value of productivity (0.11 g/L·h^{-1}). In this work, the achieved productivity value was 0.41 g/L·h^{-1}, even without the consumption of all xylose available, with a solvent production of 14.7 g/L.

Using a genetically modified *C. acetobutylicum* strain, Liao et al. [8] achieved a butanol and ABE production of 8 and 14.8 g/L, respectively, with soybean straw hydrolysate. The consumption of sugars was 58 g/L, and the authors achieved a yield ($Y_{P/S}$) and productivity (Q_P) of 0.25 g/L and 0.21 g/L·h^{-1}, respectively. These values are higher than those using the wild strain and lower than those found in this paper.

Al-Shorgani et al. [6] obtained results with detoxified deoiled rice bran hydrolysate that were similar to those achieved in this work when SBH-1 was used. However, the results with SBH-2 were superior, without the need for detoxification.

Table 3 shows the concentrations of cations and anions in the hydrolysates used in ABE fermentation and the required cell demand for some trace nutrients, according to Sun and Liu [13] and Monot et al. [24]. The ions Mn^{2+}, Mg^{2+}, K^+, Na^+, Fe^{2+} and SO_4^{2-} are at higher concentrations in SBH-2 than in SBH-1. Phosphate were not fully supplied by the hydrolysates and probably it was provided by the propagation step, but this was not analyzed in the present work. It is well known that some ions are important in the metabolism of microorganisms, as they represent cofactors of enzymes that catalyze bioreactions such as glycolysis, redox reactions, and electron transport and that are essential for maintaining cell viability. According to Monot et al. [24] the presence of ions Mg^{2+} and K^+ in the recommended concentration ranges plays an important role in the formation of acetone by bacterial enzymes. According to Zabihi et al. [25] the increasing of NaCl concentration from 0.01 to 20 g/L prolonged the lag phase of *C. acetobutylicum* from 30 to 93 h. The salt has a destructive effect on cell growth and ABE production, as well as inhibiting the shift between acidogenic and solventogenic phases. High salinity causes dehydration of the bacterial cytoplasm and cell lysis. According to Maiti et al. [26] NaCl is reported in a list of inhibitory compounds present in biomass hydrolysates. This salt inhibits cell growth at a concentration of 2 g/L, which corresponds to 786 mg/L of Na^+, and increases cell membrane permeability. The reduction in solvent production occurs at a concentration of 5 g/L, corroborating the previous study [25]. In the present work, the concentrations of Na^+ in SBH-1 and SBH-2 are 34 and 125 mg/L, respectively, which are lower than the inhibitory concentration. The enzyme phosphofrutokinase (PFK) catalyzes a single step in glycolysis, known as fructose-6-phosphate phosphorylation, using ATP and phosphate to form fructose-1,6-diphosphate and ADP. The PKF needs Mg^{2+} associated with NH_4^+ or only K^+. Magnesium cation is important in medium because it forms a complex with ATP that is probably the true substrate for PKF. NH_4^+ has been suggested to stimulate glycolysis through the action of PFK to increase synthesis rate [27]. One of the important steps in the *Clostridium* Embden-Meyerhof-Parnas (EMP) metabolic pathway is the cleavage of pyruvate, obtained from glycolysis, by the action of the pyruvate ferredoxin oxidoreductase (PFOR) enzyme in the presence of coenzyme-A (Co-A) to the formation of acetyl CoA and CO_2 with concomitant conversion of oxidized ferredoxin and its reduced form. The enzyme PFOR contains sulfur and iron chromophore in its composition that carries electrons from pyruvate to ferredoxin. Compositional analysis of the enzyme confirmed the presence of thiamine pyrophosphate, iron and sulfur [27].

The addition of iron ions in the culture medium influences *Clostridium* metabolism to alter the normal flow of carbon and electrons during xylose fermentation, thereby increasing both solventogenesis and xylose utilization in ABE-producing organisms. Both of these are desirable outcomes for eventual use in reactors with wild-type organisms in biofuel production [28]. Concerning the nitrogen source (yeast extract), the concentration required for ABE fermentation is 1.0 g/L, according to Sun and Liu [13]. However, the total nitrogen in SBH-1 and SBH-2 was determined to be 2.7 and 8.5 g/L, respectively, with ammoniacal nitrogen concentrations of 1.9 and 6.5 g/L, which is due to NH_4OH addition for pH adjustment during enzymatic hydrolysis of the pretreated bagasse. Based on the results shown in Fig. 4, these amounts were probably enough for the nitrogen supply since no significant differences were observed in $Y_{P/S}$ and Q_P in

Table 2
Comparison of literature data with the results obtained in the present work for ABE fermentation.

Feedstock	Microrganism	Glucose consumed (g/L)	Xylose consumed (g/L)	Butanol (g/L)	Acetone (g/L)	ABE (g/L)	Q_P (g/L·h^{-1}) ABE	$Y_{P/S}$ (g/g) ABE	Reference
De-oiled rice bran hydrolysate	*C. acetobutylicum*	25.6	15.1	5.9	4.4	11.2	0.22	0.34	[6]
Soybean straw hydrolysate	*C. acetobutylicum*	24.2	8.4	4.0	3.1	7.1	0.10	0.22	[8]
	C. acetobutylicum (engineered strain)	47.4	11.0	8.0	6.0	14.8	0.21	0.25	
Sugarcane bagasse hydrolysate	*C. acetobutylicum*	12.0	5.0	4.5	1.4	6.2	0.08	0.36	[9]
Sugarcane bagasse hydrolysate	*C. acetobutylicum*	57.0	12	14.2	5.9	21.1	0.36	0.33	[11]
Eucalyptus hydrolysate	*C. saccharoperbutylacetonicum*	29.9	–	8.2	4.3	13.1	0.11	0.41	[23]
Rice straw hydrolysate	*C. acetobutylicum*	7–8	2–3	2.0	0.8	2.8	0.04	0.20	[31]
Willow *Salix schwerinii* hydrolysate	*C. acetobutylicum*	Not cited	–	8.1	3.7	12.4	0.10	0.33	[32]
Palm kernel cake hydrolysate	*C. acetobutylicum*	Not cited	–	3.6	2.0	5.7	0.08	0.24	[33]
Brown seaweed hydrolysate	*C. beijerinckii*	7.6	–	7.2	0.9	8.2	0.08	0.27	[34]
SBH-1	*C. acetobutylicum*	25.0	8.3	6.4	4.5	11.5	0.23	0.34	This study
SBH-2 with nutrients*	*C. acetobutylicum*	28.4	9.4	9.1	5.5	15.5	0.43	0.41	This study
SBH-2 without nutrients*	*C. acetobutylicum*	29.0	9.9	8.7	5.3	14.7	0.41	0.38	This study

* Nutrients (in g/L): yeast extract (1.0); $MnSO_4$ (0.01); $MgSO_4$ (0.2); KH_2PO_4 (0.5); K_2HPO_4 (0.5); sodium acetate (0.01) and $FeSO_4.7H_2O$ (0.01).

the assays with and without added nutrients. In addition, the acidogenic phase with SBH-2 was shorter than that with SBH-1, i.e., the fermentation with SBH-2 showed higher productivity. Li et al. [29] used several nitrogen sources, including yeast extract, peptone, tryptone, urea and ammonium acetate, to improve ABE fermentation. The authors concluded that ammonium acetate used as the sole source of nitrogen at a concentration of 3 g/L increased the cell growth by at least 1.26 times, and that this component contributed by improving cell growth and solvent production and by shortening the fermentation time. It has also been reported that ammonium ions affect the relative expression of six genes involved in the butanol metabolic pathway, i.e., *ackA* (acetate kinase), *buk* (butyrate kinase), *adc* (acetoacetate decarboxylase), *ctfAB* (acetoacetyl-CoA/acyl-CoA transferase), *adhE2* (bifunctional acetaldehyde-CoA/alcohol dehydrogenase) and *bdhB* (NADH-dependent butanol dehydrogenase). The acetic acid (or acetate) concentration is higher in SBH-2 than in SBH-1 (Table 1). Therefore, the presence of higher ammonium acetate in SBH-2 possibly increased the productivity in this assay.

One of the advantages of the process developed in this work was the effectiveness of the pretreatment procedures and also the promptly fermentation of the generated hydrolysates without the need for detoxification, which includes some steps using chemical additives, enzymatic treatment, heating and vaporization, liquid–liquid extraction, liquid–solid extraction or microbial treatment [30]. Therefore, these results reduce the costs and increase the viability of the acetone–butanol–ethanol (ABE) fermentation process.

Table 3
Demand of nutrients in 2G ABE fermentation according to Sun and Liu [14] and Monot [24] and composition of trace nutrients in SBH-1 and SBH-2 (in mg/L).

Ions required	Range of concentration (mg/L)	Concentration in SBH-1 (mg/L)	Concentration in SBH-2 (mg/L)
Mn^{2+}	0–7.3	2	13
Mg^{2+}	10–40	16	75
K^+	314–4188	90	335
Na^+	0–1966	34	140
Fe^{2+}	0.4–18.4	14	550
PO_4^{3-}	700–1400	14	125
SO_4^{2-}	0.6–160	1750	6500

4. Conclusions

This study showed that fermentation of sugarcane bagasse hydrolysates achieved results that were similar to or even better than those reported in recent literature, achieving yield ($Y_{P/S}$) and volumetric productivity (Q_P) values of 0.4 g/g and 0.4 g/L·h^{-1}, respectively, when using SBH-2 as a source of sugars (glucose and xylose) and nutrients, without the need for detoxification to remove inhibitors. The use of lignocellulosic sugars by this strain highlighted the potential for future development of the ABE fermentation scale up using lignocellulosic biomass.

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Conflict of interest

PETROBRAS has intellectual property in this area. However, on behalf of all authors, the corresponding author states that there is no conflict of interest.

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