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ORIGINAL PAPER

Efficient chemical and enzymatic saccharification of the lignocellulosic residue from *Agave tequilana* bagasse to produce ethanol by *Pichia caribbica*

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Abstract Bagasse of *Agave tequilana* (BAT) is the residual lignocellulosic waste that remains from tequila production. In this study we characterized the chemical composition of BAT, which was further saccharified and fermented to produce ethanol. BAT was constituted by cellulose (42%), hemicellulose (20%), lignin (15%), and other (23%). Saccharification of BAT was carried out at 147°C with 2% sulfuric acid for 15 min, yielding 25.8 g/l of fermentable sugars, corresponding to 36.1% of saccharificable material (cellulose and hemicellulose contents, w/w). The remaining lignocellulosic material was further hydrolyzed by commercial enzymes, ~8.2% of BAT load was incubated for 72 h at 40°C rendering 41 g/l of fermentable sugars corresponding to 73.6% of the saccharificable material (w/w). Mathematic surface response analysis of the acid and enzymatic BAT hydrolysis was used for process optimization. The results showed a satisfactory correlation (R^2 = 0.90) between the obtained and predicted responses. The native yeast *Pichia caribbica* UM-5 was used to ferment sugar liquors from both acid and enzymatic hydrolysis to ethanol yielding 50 and 87%, respectively. The final optimized process generated 8.99 g ethanol/50 g of BAT, corresponding to an overall 56.75% of theoretical ethanol (w/w). Thus, BAT may be employed as a lignocellulosic raw material for bioethanol production and can contribute to BAT residue elimination from environment.

Keywords Lignocellulose residue · Acid hydrolysis · Enzymatic hydrolysis · Fermentation alcoholic · *Agave tequilana* · *Pichia caribbica*

Introduction

Countries around the world are interested in the potential use of ethanol as fuel in order to reduce greenhouse gas emissions. This has increased the demand for ethanol worldwide, resulting in a demand of fermentation facilities that comply and with regulations and need. Presently, ethanol is mainly obtained from food crops, which causes a rise of food prices in both developed and underdeveloped countries.

The use of lignocellulosic biomass as a source of fermentable sugars has received increased attention due to its low cost and abundance. Furthermore, the combination of pretreatment of the lignocellulosic material with an efficient and cost-effective enzyme blend is being examined to obtain fermentable sugars from food crops [1]. *Agave tequilana* Weber is a plant that is cultivated in Mexico and used as a raw material in the production of the alcoholic beverage tequila. Middle-aged Agave plant is harvested and the pinecone is subjected to cooking. The juice obtained is yeast fermented and distilled. The remaining lignocellulosic residue from the squeezed and cooked

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pinecone is called agave bagasse of the tequila manufacturing (BAT). The 2008 Mexican consumption of *Agave tequilana* Weber for tequila production has been estimated at 1.12×10^6 tons. Approximately 40% (in weight) corresponds to cooking bagasse residue (BAT) [Consejo Regulador del Tequila, CRT; 2].

BAT (var. Weber, from the tequila industry) is composed of 43% cellulose, 19% hemicellulose, and 15% lignin. This composition makes BAT of potential use for pulp and paper production [2]. Thus, we suggest that BAT is a suitable candidate for bioconversion to ethanol.

The utilization of agave residues from *Agave atrovirens* for ethanol conversion has recently been described [3]. This study examined the by-products from "pulque" manufacturing (fermented beverage where the pinecone or sap is not submitted to cooking). The bagasse from "pulque" manufacturing also is a lignocellulosic residue that following sap extraction generates cellulosic residues uncooked (metzal) and whole agave plant at end of its productive life (metzontete) are generated. This suggests that agave lignocellulosic residues are suitable to produce ethanol and other chemical compounds with biotechnological importance [3].

The enzymatic degradation rate of lignocellulosic materials is less common due to the resistance of the cellulose crystalline structure and the physical barrier formed by the lignin surrounding the cellulose polymer [4]. Therefore, it is essential to pretreat the material to disrupt the lignin bonds and thereby enhancing susceptibility of lignocellulosic materials to enzyme activity. An efficient pretreatment of lignocellulosic residues includes to reduction of lignin content, decrease of crystallinity, and increase surface area for enzymatic reactions [4, 5]. Chemical and mechanical methods (e.g., acid [3, 6], steam [7], alkali [3], and ammonium freeze explosion (AFEX) [8]) for the pretreatment of sugar cane and agave bagasses have been described. Pretreatment of the lignocellulosic substrates should consider endoglucanase, cellobiohydrolase, β-glucosidase and different hemicellulolytic activities which play important roles in the efficiency of hydrolysis [9]. Due to the fact that the commercial cellulase from the *Trichoderma reesei* displays a limiting activity of β-glucosidase [10, 11], the enzymatic mixture is supplemented with β-glucosidase [12].

The efficiency in hydrolysis of the lignocellulosic material depends on catalyst concentration, substrate concentration, reaction time, enzyme concentration, and hemicellulolytic activities [13]. These factors are interrelated, thus the analysis of multiple variables or factors is required. This obstacle can be overcome by the use of response surface methodology that analyzes the effect of multiple variables, independently or in combination for a given process with minimal number of experiments while keeping a high degree of statistical significance [14]. This approach can be used to optimize pretreatment conditions and enzymatic saccharification of the lignocellulosic materials.

Although fermentation of the hydrolysates obtained from both chemical and enzymatic treatments have been studied, the efficiency of ethanol production needs to be increased in order to achieve an economical benefit. Glucose, mainly from cellulose and xylose of hemicelluloses may be obtained from lignocellulosic residues [15]. While recombinant yeasts have been proposed to ferment xylose-containing hydrolysates [16], *Saccharomyces cerevisiae* is recommended for the fermentation of glucose. Other studies describe the utilization of microbial consortia (yeast and/or bacteria) to ferment both glucose and xylose [17]. Furthermore, the current trend to obtain ethanol is to use genetically modified microorganisms able to carry out coupled processes of saccharification and fermentation of lignocellulosic residues [18]. Another possibility is the selection of native microorganisms possessing the ability to use hydrolysates from specific raw material. Previously, 200 yeast and bacterial isolates were obtained from putrid BAT. These isolates were submitted to screening for ethanologenic properties (ability to use glucose and xylose to produce ethanol by fermentation). From that screening, the UM-5 strain was selected and identified by 26S rRNA sequencing as *Pichia caribbica*.

Here, we find optimal conditions for BAT saccharification and ethanol production. We achieved this by using acid and enzymatic hydrolysis followed by a fermentation step using a native microorganism (*Pichia caribbica* UM-5).

Materials and methods

Raw material

Agave bagasse from *A. tequilana* Weber was collected from tequila distilleries (El Consuelo, El Quiote, and El Viejito) located in Jalisco, Mexico. BAT samples were washed with distilled water causing removal of remaining sugars. BAT was dried in an oven at 40°C to obtain constant weight, milled, and sieved to 0.5-mm-diameter particles. The material was mixed to obtain a homogeneous sample and stored at room temperature in polyethylene bags.

Compositional analysis

Analysis of the main polymeric fractions was carried out by quantitative inorganic acid hydrolysis as described [19]. Concentrations of glucose, xylose, arabinose, and ethanol were determined by HPLC with a Metacarb 87C column 300 × 7.8 mm (Varian). The HPLC system was equipped with a Prostar 350 differential refractometer, HPLC pump,

and an external heater module Metatherm (Varian). The temperature of the column was maintained at 70°C and the products eluted with water at a flow rate of 0.6 ml/min. Compound quantification was carried out using calibration plots ($R^2 = 0.99$) for glucose, xylose, arabinose, and ethanol (Sigma or Baker). Furfural and methyl furfural were spectrophotometrically analyzed at 280 nm (UV). The concentration of sugars from acid and enzymatic hydrolysis were calculated as described previously [14]:

$$\text{Hydrolysis yield}\,(\%) = [\text{total sugars}\,(\text{g})][0.9][100] / [\text{cellulose}\,(\text{g}) + \text{hemicellulose}\,(\text{g})] \quad (1)$$

Acid hydrolysis of BAT

Acid hydrolysis was carried out using a Multiple Reactor System (Parr Instruments, Model 5000) provided with six independently controlled reactors of 75 ml each. Reactors were loaded with 50 g of material (5 g of BAT and 45 g of 1–3% sulfuric acid, w/w; corresponding to 1:10 solid:liquid proportion) and incubated at temperatures ranging from 125 to 175°C. Samples were taken at different times according to the experimental design. Samples from the reactors were filtered on a 0.45-μm membrane (Millipore), and the retained solids were weighed. The concentrations of carbohydrates, furfural, and methyl furfural of the filtrate were determined as described above.

Enzymatic hydrolysis of residues from BAT acid hydrolysis

BAT residues from acid hydrolysis (pretreated BAT) were filtered and buffered to undergo enzymatic hydrolysis using a commercial enzyme mixture (celluclast 1.5L from *Trichoderma reesei* (cellulase) and β-glucosidase preparation Novozyme 188 from *Aspergillus niger* (cellobiase) (Novozymes). The lignocellulosic material was resuspended in 50 mM sodium acetate pH 5. Cellulase (100 units/1 g of total solid (TS) and cellobiase (80 units/1 g of TS) were added to 10 ml of the recommended buffer solution. Pretreated BAT remaining was suspended in 50 mM sodium acetate, pH 5, and the reaction was carried out in a flask incubated at 40°C and 100 rpm. After incubation, the reactions were boiled for 10 min. Each sample was centrifuged and the supernatant was collected, filtered through a 0.2-μm membrane (Millipore) and subjected to HPLC analysis.

Fermentation of hydrolysates

The obtained hydrolysates were fermented with *Pichia caribbica* UM-5, a microorganism isolated from rotten BAT. The hydrolysates were adjusted to pH 5.0 with $Ca(OH)_2$ and filtered on Whatman No.1 paper. All fermentation experiments were carried out with 8 ml of hydrolysate inoculated with 2 ml of the overnight grown microorganism that was grown in YPD medium (1×10^8 cells/ml), at 30°C and 100 rpm. Ethanol and sugars quantification was carried out by HPLC as described above.

Statistical analysis and mathematical modeling

The statistical analysis was determined by an analysis of variance (ANOVA), along with Fisher's F test with a p value of 0.05. The three-dimensional surface plots generated by the polynomial model were obtained using the software Statgraphics Centurion XV (Statpoint Technologies, Inc. USA).

Results and discussion

Compositional analysis of agave bagasse (BAT)

The potential usage of BAT originated in the tequila fabrication process for ethanol production was evaluated. BAT from factories is processed to extract sugars by milling the cooking agave pinecone, which contains remaining free sugars accounting to 3–6% of dry weight. The sugars contained in the BAT were extracted by washing the residue as described above. The incorporation of these sugars in the acid or enzymatic hydrolysis may increase the overall yield of the bioconversion process; however, they were not used for this study.

The chemical components for BAT were analyzed before and after acid pretreatment (Table 1). Residual BAT from tequila production contains free sugars including fructose (3–6%, w/w), cellulose (42 ± 2%, w/w), hemicellulose (20 ± 1%, w/w), lignin (15 ± 1%, w/w), and other solids (20 ± 3%, w/w).

Following acid hydrolysis, the pretreated BAT contains the followed proportions: cellulose (60 ± 1%, w/w), hemicelluloses (not detected), lignin (25 ± 1%, w/w), and remaining solids (15 ± 1%, w/w). Thus, cellulose and

Table 1 Agave bagasse (BAT) composition before and after acid hydrolysis treatment

Component	Dry matter (%, w/w)[a]	
	Before	After
Cellulose	42 ± 2	60 ± 1
Hemicellulose	20 ± 1	No detected
Lignin	15 ± 1	25 ± 1
Other solids	20 ± 3	15 ± 3

[a] Analysis of the main polymeric fractions was carried out by quantitative inorganic acid hydrolysis as described [19]

lignin contained in BAT increased to 47.5 and 44.3%, respectively. In contrast, all hemicellulose was completely hydrolyzed with the acid treatment. These results indicate that the BAT residue from the production of tequila is a suitable cellulosic material for saccharification processes. This composition is similar to the proportions found in sugarcane bagasse (cellulose 38–50%, hemicellulose 25–27%, lignin 20–25%, and others 12% [3, 20]).

Mathematical model for BAT saccharification

Factorial experiment design 3^3 for acid hydrolysis and 3^2 for enzymatic hydrolysis was carried out. A three-level factorial experiment design was used to investigate the parameters affecting the sugars yielded from the acid and enzymatic hydrolysis of BAT. For acid hydrolysis, H_2SO_4 (1–3% w/w), temperature (125–175°C), and hydrolysis time (10–30 min) were variable input parameters and fermentable sugars were an output parameter. For enzymatic hydrolysis, acid pretreated BAT was loaded (6–14% w/w) and hydrolyzed for 24–72 h; the yield of hydrolysis (%) was an output parameter. Coded values are described in Table 2, and their relation with the actual values is described with the dimensionless equation:

$$X_i = \left(X_i - X_j^0\right) \Big/ (\Delta X_i) \quad (2)$$

where

X_i is the coded value of the ith independent variable;

X_i is the actual value of the ith independent variable;

X_i^0 is the actual value of the ith independent variable at center point;

and ΔX_i is the step change value.

The relationship between independent variables is described by the general second degree quadratic polynomial equation:

$$Y = \beta_0 + \Sigma\beta_i X_i + \Sigma\beta_{ii} X_i^2 + \Sigma\Sigma\beta_{ij} X_i X_j \quad (3)$$

Table 2 Coded values of the variables for the experiment designs in mathematical model for agave bagasse (BAT) saccharification

Variable	Coded symbol	Values of coded levels		
		−1	0	1
Acid hydrolysis				
H_2SO_4 loading (%, w/w)	X1	1	2	3
Hydrolysis time (min)	X2	10	20	30
Temperature (°C)	X3	125	150	175
Enzymatic hydrolysis				
Agave bagasse (BAT) loading (%, w/w)	X1	6	10	14
Hydrolysis time (h)	X2	24	48	72

where for acid hydrolysis:

Y, fermentable sugars (g/l); X_i, when $i = 1$, H_2SO_4 (% w/w);
$i = 2$, hydrolysis time; and
$i = 3$, temperature

For enzymatic hydrolysis:

Y, hydrolysis yield (%);
X_i, when $i = 1$, BAT loading (% w/w);
$i = 2$, hydrolysis time;
β_0 is the offset term;
β_i, are linear coefficients;
β_{ij}, are cross-product coefficients'
and β_{ij}, when $i = j$, are quadratic coefficients.

The reliability of the polynomial model was evaluated by its correlation coefficient R^2.

Acid hydrolysis of BAT

The acid hydrolysis was optimized with response surface method using a 3^3 full factorial design as described above (Eq. 3), and yielding an empirical second-order polynomial equation (Eq. 4).

$$\begin{aligned}\text{Fermentable sugars} = {} & 25.0526 + 1.81611 \\ & \times X1 - 0.261667 \times X2 - 0.173333 \times X3 - 4.47944 \\ & \times X1^2 - 3.025 \times X1 \times X2 + 0.158333 \times X1 \\ & \times X3 - 2.53278 \times X2^2 - 0.115833 \times X2 \\ & \times X3 - 0.567778 \times X3^2 \end{aligned} \quad (4)$$

The quality of fit for the second-order polynomial equation was checked using the coefficient of determination (R^2) of 0.90, indicating a satisfactory correlation between the observed and predicted responses. The p values obtained for the different coefficients implied that X1 (H_2SO_4%, w/w) and X2 (hydrolysis time) have significant effect on the release of fermentable sugars from the fiber (supplemental material, Table S1). Thus, according with the predicted model, the optimal values for obtaining fermentable sugars during acid hydrolysis of BAT were 147°C, 2.3% H_2SO_4, and 15 min of hydrolysis time (Fig. 1). The predicted concentration of fermentable sugars under these conditions was 25.8 g/l, which corresponds to 37.4% of total hydrolysis. Additionally, liquors obtained after acid hydrolysis gave concentrations lower than 1 g/l of furfural and methyl-furfural (data not shown), which was non-toxic for yeast fermentation. The reliability of the model for acid hydrolysis of 10% (w/w) BAT was measured at 147°C, 2.0% (w/w) H_2SO_4, and 15 min of hydrolysis time (Table 3). A total of 24.9 g/l of fermentable sugars was obtained, which corresponds to a 36.14% hydrolysis yield with respect to

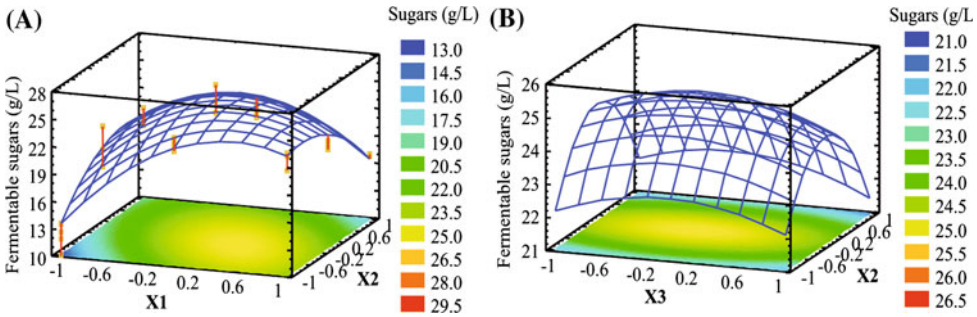

Fig. 1 Response surface plots of acid saccharification of agave bagasse (BAT). **a** Catalyst (X1) and time (X2) effect. **b** Temperature (X3) and time (X2) effect. *Bars* represent experimental points obtained in the confirmation of conditions predicted (147°C, 2.0% of H_2SO_4, and 15 min), $n = 3$

Table 3 Confirmation assays of agave bagasse (BAT) under acid and enzymatic hydrolysis treatment

Conditions			Fermentable sugars (g l^{-1})			
H_2SO_4 (%, w/w)	Hydrolysis time (min)	Temperature (°C)	Actual[a]	Predicted[a]	Residual	Error (%)
Acid hydrolysis						
1.0	20	150	18.5 (26.8)	18.12 (26.3)	0.40	2.20
2.0	15	147	24.9 (36.1)	25.80 (37.4)	–0.90	3.51
2.0	20	150	24.9 (36.1)	25.24 (36.6)	–0.33	1.34
3.0	20	150	21.0 (30.4)	21.70 (31.4)	–0.70	3.22

Conditions		Fermentable sugars (g l^{-1})			
BAT (%, w/w)	Hydrolysis time (h)	Actual[a]	Predicted[a]	Residual	Error
Enzymatic hydrolysis					
6	48	17.49 (43.7)	18.12 (45.3)	–1.57	3.47
8.2	72	37.08 (67.8)	36.71 (67.1)	0.67	0.99
10	72	41.02 (61.5)	40.89 (61.3)	0.17	0.34

[a] In parenthesis, percentage of hydrolysis yield are shown. Experimental conditions are described in "Materials and methods"

cellulose and hemicellulose contents present in the saccharificable material of BAT. This result is considering the stoichiometric parameters shown in Eq. (1). The result deviated from the predicted value by 3.51%, suggesting that this model fits adequately the experimental data.

Saccharification of agave bagasse derived from *Agave atrovirens* has been described recently [3]. In this study of "pulque" production, the lignocellulosic by-products (metzal from pinecone and metzontete from leaves agave plants) remaining were not submitted to cooking as occurs in the tequila manufacturing process. By acid hydrolysis (HCl), reducing sugars yielded 10 and 5% [3]. In our case, the first step (hydrolysis using H_2SO_4), we obtained 36.14% of fermentable sugars with respect to cellulose and hemicellulose contents present in the saccharificable material of BAT. This shows an increased efficiency of threefold. The difference in the hydrolysis yield could be the result of the different temperature and acid employed (121°C, HCl). In addition, the nature of BAT, which suffers a cooking process prior to sugar extraction from the agave pinecone used for the tequila manufacturing, may play an important role in opening the lignocellulosic matrix, thus facilitating the action of the catalyst during the acid hydrolysis. Scanning electronic microscopy (SEM) indicates that relaxing the fiber structure after the cooking process occurs in the BAT material (data unpublished).

Enzymatic hydrolysis of BAT

Enzymatic hydrolysis of BAT residue from acid treatment was carried out using commercial cellulases-glucosidase mix as described in above. This remaining lignocellulosic material contained mainly cellulose and lignin (Table 1). The cellulases-glucosidase enzyme mixture used yielded 61.5% using 10% w/w of total solids and a sugar concentration of 41.02 g/l (Table 3) was obtained. This result coincides with previous reports using other fibers; however, if the stoichiometry factor of the hydrolysis yield equation is disregarded, the value will increase to ~11%, which is one of the highest hydrolysis yields reported [21–23].

A surface response analysis of the enzymatic hydrolysis of pretreated BAT depicted a direct dependence on the incubation time (72 h), quadratic behavior against the BAT

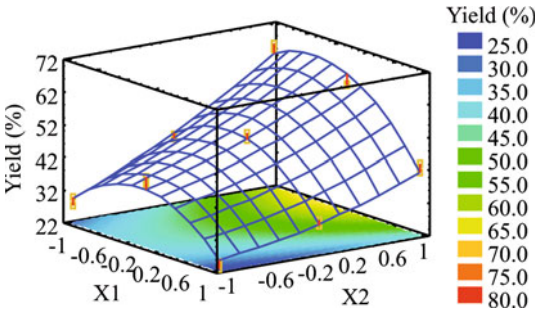

Fig. 2 Response surface plot of enzymatic saccharification of agave bagasse (BAT). Effect of BAT loaded (X1) over time (X2) effect. *Bars* represent experimental points obtained in the confirmation of conditions predicted (40°C, 8.2% of BAT loaded, and 72 h), $n = 2$

loading (8.2%, w/w) (Fig. 2). The mathematical model of the process is shown in Eq. (5).

$$\text{Yield (enzymatic hydrolysis)} = 49.605 - 6.50833 \times X1 + 12.4125 \times X2 - 12.11 \times X1^2 - 4.3225 \times X1 \times X2 + 2.4025 \times X2^2 \quad (5)$$

The p values obtained for the different coefficients implied that X1 (BAT loading %) and X2 (hydrolysis time) have a significant effect on the released of fermentable sugars from the fiber (supplemental material, Table S2). The reliability of the model for enzymatic hydrolysis of BAT was measured at reaction conditions of 8.2% (w/w) BAT load and 72 h. Under these conditions, 37.8 g/l of fermentable sugars were obtained, which corresponded to 67.8% hydrolysis yield. This result deviates from the predicted value by 0.99%, suggesting that the model fits the experimental data adequately.

The optimal combined hydrolysis yields for the acid and enzymatic hydrolysis of BAT are summarized as follows: for acid hydrolysis as 1st batch stage, 10% (w/w) of BAT in sulfuric acid (2%, w/w) at 147°C for 15 min reaction; for a 2nd batch step, enzymatic hydrolysis was carried out with 8.2% (w/w) of pretreated BAT load from 1st acid treatment, pH 5 and incubation for 72 h at 40°C rendering 37.08 g/l of fermentable sugars (Table 3). These BAT saccharifications rendered 25.36 g of fermentable sugars for 50 g de BAT treated, corresponding to a hydrolysis yield of 73.62% with respect to saccharificable material.

Our data describes a process where the acid pretreatment is very efficient at breaking down the hemicelluloses. This process was able to fully unfold the hemicellulose fraction present in BAT. Further, the enzymatic preparation catalyzed the breakdown of the remaining cellulose fraction, rendering a yield of approximately 69%. Interestingly, lignocellulosic residue of agave plant submitted to alkaline delignification using NaOH solution (2% w/v), autoclaving at 121°C for 4 h and enzymatic saccharification yielded 12–58% of saccharification, and given a total yield of 56% using a combined alkaline-enzymatic treatment [3]. The process of saccharification followed in our work shows that the efficiency was increased significantly to 73.62%. Thus, BAT residue is a suitable material to obtain sugar liquors for ethanol production.

Fermentation of hydrolysates

Fermentation of liquors obtained from both chemical and enzymatic saccharification processes were carried out using the yeast UM-5 strain isolated from putrid BAT and selected due to its ethanologenic properties (data unpublished). We sequenced a 550-bp fragment of 26S rRNA from UM-5 that allowed us to identify it as *Pichia caribbica*. The sequenced fragment was 100% identical with these species (data not shown) suggesting that this yeast probably corresponds to this lineage.

The liquor obtained after acid pretreatment (containing 24.9 g/l of fermentable sugars) (Table 3), was adjusted to pH 5 and inoculated with the UM-5 strain. After 48 h of fermentation, a concentration of 6.37 g/l ethanol was obtained, giving a 50.16% yield (Fig. 3). Interestingly, glucose was completely exhausted, while xylose was only consumed up to 45% of its initial concentration. On the other hand, the liquor obtained after enzymatic hydrolysis had 41.02 g/l of fermentable sugars, all in the form of glucose (Table 3). This liquor was fermented with the UM-5 strain, giving a final ethanol concentration of 18.31 g/l after 48 h (Fig. 4). Under these conditions, all the glucose was consumed, giving a yield of 87.05%. Thus, the combined yield of both acid and enzymatic treatments obtained liquors that were 8.99 g of ethanol for 50 g of BAT. This corresponds to a yield of 56.77%, which is based on maximal theoretical ethanol yield on available hexoses and pentoses into the saccharificable BAT material. The yields of fermentation (12–22%), obtained by the commercial strain of *S. cerevisiae* with hydrolysates from the agave plant remaining of "pulque" beverage production [3], are lower than our combined process and fermentation.

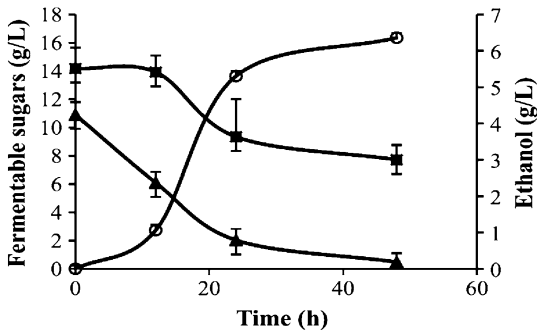

Fig. 3 Batch fermentation of acid hydrolysates obtained from agave bagasse (BAT) using the UM-5 yeast strain. *Filled triangle* glucose, *filled square* xylose, *open circle* ethanol. SD are shown as *bars*, $n = 3$

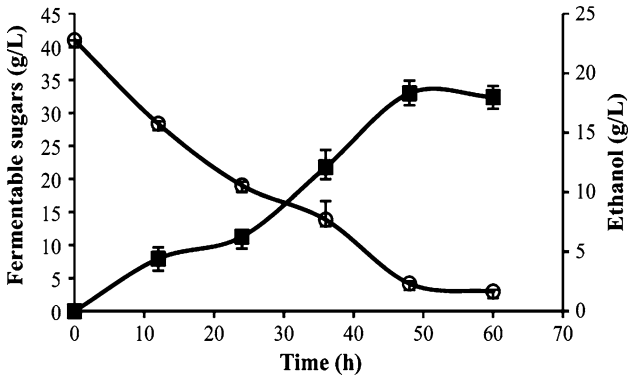

Fig. 4 Batch fermentation of enzymatic hydrolysates obtained from agave bagasse (BAT) using the UM-5 yeast strain. *Open circle* glucose, and *filled square* ethanol. SD are shown as *bars*, $n = 3$

Furthermore, the fermentation of the hydrolyzed liquors by the UM-5 strain was very promising since the strain was able to ferment both glucose and xylose with yields of 87 and 45%, respectively. This yield coincides with the data reported for other lignocellulosic sources when fermentation was done with the genetically engineered yeast *Saccharomyces cerevisiae* TMB3400 [22, 24]. Interestingly, the UM-5 strain had the ability to metabolize xylose to ethanol. Even though the causes for not consuming the totality of xylose during fermentation are unknown, it is possible that oxygen plays an important role as described for *Pachysolen tannophilus* [25] and *Mucor indicus* [26]. Further studies are needed to study factors that affect xylose consumption by the UM-5 strain.

Conclusions

This work indicates that BAT from *A. tequilana* residue remaining from the tequila manufacturing process may be considered as a suitable lignocellulosic material to obtain saccharification liquors that may be used efficiently to produce ethanol. The process presented involves acid pretreatment of BAT from *A. tequilana*, enzymatic hydrolysis of the residue and fermentation of its hydrolysates using the yeast *Pichia caribbica* UM-5. This process could be suggested as an efficient procedure for ethanol production. This process also may contribute to solve environment concerns of waste (1.12×10^6 annual tons), which is generated during the tequila production. Finally, the utilization of BAT from *A. tequilana* is as important as the use of sugarcane bagasse and other agricultural lignocellulosic residues.

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