Vol. 16, No. 2 (2017) 467-476
Revista Mexicana de Ingeniería Química

BIOETHANOL PRODUCTION FROM *Agave lechuguilla* BIOMASS PRETREATED BY AUTOHYDROLYSIS

PRODUCCIÓN DE BIOETANOL A PARTIR DE BIOMASA DE *Agave lechuguilla* PRETRATADA POR AUTOHIDRÓLISIS

O.H. Ortíz-Méndez[1], T.K. Morales-Martínez[1], L.J. Rios-González[1]*,
J.A. Rodríguez-de la Garza[1], J. Quintero[2], G. Aroca[2]
[1]*Biotechnology Department, Chemistry Faculty, Universidad Autónoma de Coahuila, Blvd. V. Carranza, C.P. 25280, Saltillo, Coahuila, México.*
[2]*School of Biochemical Engineering, Pontificia Universidad Católica de Valparaíso, Av. Brasil 2085, Valparaíso, Chile*
Received January 21, 2017; Accepted April 25, 2017

Abstract
Agave lechuguilla cogollos (heart or pulpy central stem with attached leaf bases) were used as feedstock for production of second generation bioethanol following a scheme based on pretreatment by autohydrolysis, enzymatic saccharification and fermentation of hydrolysates. Autohydrolysis was carried out in a Parr reactor at different severity factors with a solid/liquid ratio of 1/6 (w/v) and 200 rpm. Solid fraction obtained was hydrolyzed using the commercial cellulase Accellerase 1500 (Genencor®). Hydrolysate was fermented with the *Saccharomyces cerevisiae* ATCC 4126 yeast. Using a severity factor of 4.127 (190 °C during 30 min) it was possible to preserve most of the glucans while increasing its enzymatic digestibility. Enzymatic hydrolysis of the solid fraction showed a maximum yield of 60.85%, with a final glucose concentration of 59 g/L. Ethanol concentration at the end of the fermentation was 25.4 g/L (91% of the theoretical ethanol yield).
Keywords: *Agave lechuguilla*, autohydrolysis pretreatment, bioethanol, cogollos, severity factors.

Resumen
Se evaluó el uso de los cogollos (tallo) de *Agave lechuguilla* como materia prima para la producción de bioetanol de segunda generación, siguiendo un protocolo basado en el pretratamiento por autohidrólisis, sacarificación enzimática y fermentación de hidrolizados. La autohidrólisis fue llevada a cabo en un reactor Parr a diferentes factores de severidad (SF), con una relación sólido/líquido de 1/6 (p/v) a 200 rpm. La fracción sólida obtenida fue hidrolizada utilizando el complejo comercial celulolítico Accellerase 1500 (Genencor®). El hidrolizado fue fermentado con la levadura *Saccharomyces cerevisiae* ATCC 4126. Los resultados obtenidos mostraron que a un factor de severidad de 4.127 (190 °C por 30 minutos) fue posible preservar la mayoría de los glucanos presentes, incrementándose la digestibilidad enzimática. La hidrólisis enzimática de la fracción sólida mostró un máximo rendimiento de 60.85%, con una concentración final de glucosa de 59 g/L. La concentración de etanol al final de la fermentación fue de 25.4 g/L (correspondiendo a un 91% de acuerdo al valor teórico).
Palabras clave: *Agave lechuguilla*, bioetanol, cogollos, factor de severidad, pretratamiento por autohidrólisis.

1 Introduction

Bioethanol is produced commercially at large scale from sugarcane and corn starch, in Brazil and USA respectively. However, the production of biofuels from feedstocks suitable for food or feed entails a number of undesirable consequences, particularly the shortage of supply and the concomitant increase in prices of basic foods (Fischer *et al*., 2010). Because of this, there is a growing interest in new sources of feedstocks for biofuels that can be cultivated without competing for key resources, such as land and water, with food crops. Some of the advantages of second generation bioethanol production from lignocellulosic materials (LCM) are: less greenhouse gas emissions compared to the first generation bioethanol; the possibility of using low-cost feedstocks; and geographical diversity of supply (Chuck-Hernández *et al*., 2011; Morales-

* *Corresponding author. E-mail*: leopoldo.rios@uadec.edu.mx
Tel. +52 (844) 4155752, Fax: +52 (844) 4159534.

Martínez *et al*., 2014; Sivamani and Baskar, 2015).

Different species of agaves has been traditionally used for fiber and alcoholic beverage production in the American continent. It has high drought resistance and water-use efficiency and can be grown on marginal lands in arid conditions (Borland *et al*., 2009; Somerville *et al*., 2010). There are at least 200 species worldwide; more than 150 can be found in Mexico (García-Mendoza, 2007). *Agave lechuguilla* (lechuguilla) is a common plant in the Chihuahuan desert, covering large areas (200,000 km^2) of the arid and semiarid lands of northern Mexico (Nobel and Quero, 1986; Marquez *et al*., 1996). Lechuguilla fiber is used in metal polishing brushes, furniture and car seat filling, carpets and cleaning brushes, as construction material in combination with thermoplastic resins and has recently been suggested as a concrete reinforcement (Pando-Moreno *et al*., 2008). To our knowledge *A. lechuguilla* has not yet been considered for bioethanol production; however their carbohydrates could be used as feedstock for biofuel production (Vieira *et al*., 2002). The main advantage of *A. lechuguilla* compared to other agave species, such as, *A. tequilana*, *A. salmiana*, *A. americana*, *A. atrovirens*, is it can be harvested many time with no need of sacrificing the whole plant due to only the heart or pulpy central stem with attached leaf bases of the plant is harvested (known as "cogollo"). Harvesting the cogollo also promotes a longer life span of the plant, up to 15-20 years (Sheldon, 1980) compared with 4 to 6 years of life when no harvesting is accomplished. The regeneration of the cogollo (at a height of 25 cm) takes from 14 to 25 month in natural conditions, however according to the National Institute for Forestry, Agriculture and Fisheries Research of Mexico (INIFAP), in the cases of commercially cultivated plantations of *A. lechuguilla*, total regeneration of the cogollo can be achieved in only 8 months (Narcia *et al*., 2012).

Bioethanol production from LCM involves three major steps: (a) pretreatment of the raw material to increase its susceptibility to further processing, with the eventual generation of valuable byproducts; (b) enzymatic hydrolysis of cellulose and hemicellulose to obtain sugars, and (c) biological conversion of sugars to ethanol. A wide number of technologies have been proposed for pretreatment of lignocellulosic materials in aqueous alkali and acid media, or thermal and mechanical pretreatments to increase enzymatic digestibility (Saucedo-Luna *et al*., 2010; Das *et al*., 2014; Molina *et al*., 2014; Buratti *et al*., 2015; Wang *et al*., 2015).

Hydrothermal treatment, also known as autohydrolysis or hot compressed liquid water treatment has the advantage of avoiding the use of chemicals for catalyzing the physicochemical transformation of the material during the pretreatment. In autohydrolysis, some of the acetyl esters present in the raw material are cleaved to produce acetic acid (Garrote and Parajo, 2002; Lee *et al*., 2010). In addition, hydronium ions produced from water autoionization of acidic species (i.e. formic acid) further catalyze a series of autohydrolysis reactions (Palmqvist and Hahn-Hagerdal, 2000) in which hemicellulose is depolymerized and converted into soluble oligomers (the major reaction products), and mono-sugars. Simultaneously, a variety of side-processes take place, including removal of extractives, partial dissolution of lignin (acid-soluble lignin) and ashes, solubilization of proteins and generation of byproducts from sugars (Lee *et al*., 2009).

The aim of the present work was to establish the best conditions of *A. lechuguilla* cogollos pretreatment by autohydrolysis for increasing the enzymatic digestibility and to assess the fermentation of the hydrolysate using *S. cerevisiae* ATCC 4126.

2 Materials and methods

2.1 Feedstock

A. lechuguilla cogollos of 25 cm in height were collected from the Ejido Independencia in the municipality of Jaumave, Tamaulipas, México. The material was dried in a Koleff tray dehydrator (model KL10, Querétaro, Mexico) at 45 °C during 24 h, and subsequently milled and sieved using a 2 mm mesh in a Retsch cutting mill (model SM100, Haan, Germany). Milled material was stored at room temperature.

2.2 Feedstock characterization

Cellulose (glucan), hemicellulose (xylan) and lignin composition were determined according to the analytical methods of the National Renewable Energy Laboratory (NREL) (Sluiter *et al*., 2011). Three hundred milligrams of material were hydrolyzed with 72% (w/w) H_2SO_4 during 1 h at 30 °C. Subsequently, acid present in liquid hydrolysate was diluted to 4% by adding distilled water. A second hydrolysis was carried out by autoclaving the mixture at 121 °C during 1 h. Filtration of the autoclaved solution was carried out using 0.2 μm filters for HPLC analysis and the solid residue obtained after

filtration was used to determine the acid insoluble lignin (Klason lignin). Sugars quantification was carried out by HPLC. Extractives and ashes were determined using the National Renewable Energy Laboratory (NREL) analytical methods: NREL/TP-510-42619 (Sluiter *et al*., 2005) and NREL/TP-510-42622 respectively (Sluiter *et al*., 2008). Moisture content was determined with a MB45 Moisture Analyzer (OHAUS; Parsippany, NJ). Proteins were quantified by Kjeldahl method, while pectins were determined according to procedure described by Contreras *et al*. (2006).

2.3 Autohydrolysis pretreatment

Autohydrolysis of the material was carried out in a Parr pressure reactor (model 4551, IL, USA) with a total volume of 3.75 L. The conditions for autohydrolysis were set up depending on the Severity Factor (SF) which relates reaction time and temperature according to equation proposed by Fan and Ragauska (2012):

$$SF = \log\left\{t \exp\left[\frac{T_H - T_R}{14.75}\right]\right\} \quad (1)$$

where t is reaction time in minutes, T_H is the reaction temperature in °C, T_R is the reference temperature, most often and in this case 100 °C. The value 14.75 is an empirical parameter related to activation energy and temperature.

To establish the SF that can yield the highest glucan content, initially four SF were selected, temperature and time were in the range of 160-200 °C and 15 to 60 min respectively (Table 1), with a minimum SF of 3.465 and a maximum of 4.723. In a second stage six SF values were selected and assessed (4.12, 4.127, 4.133, 4.303, 4.428 and 4.597).

The reactor was loaded with 200 g (dry weight base) of material and 1.2 L of distilled water to obtain a solid/liquid ratio of 1:6 (w/v). Water impregnation was allowed by keeping the material at room temperature during 30 min. All runs were carried out by duplicate at 200 rpm.

The reactor was cooled down immediately after the reaction time was reached. The pretreated material was then separated by filtration. The liquid fraction was analyzed by HPLC to determine the concentration of glucos, xylose, arabinose, formic acid, acetic acid, furfural, hydroxymethylfurfural (HMF), furfuryl alcohol and syringaldehyde. The solid fraction was homogenized in water and disintegrated in a blender during 5 min, and subsequently washed with water (30 times the volume of the material) and stored at 4 °C for enzymatic hydrolysis. Cellulose, hemicellulose and lignin content in the solid fraction was determined according to Sluiter *et al*. (2011).

Glucan content in the solid fraction (g per 100 g of dry weight base) was taken as the response variable of the experimental procedure.

2.4 Enzymatic digestion test

Solid fractions obtained were hydrolized using Acellerase 1500 (Genencor®, USA) to assess the enzymatic digestibility. All tests were carried out at a solid load of 5% (w/w), corresponding to 2.5 g of solid pretreated material (dry weight base) and 46 g of 0.05 M citrate buffer at pH 4.8, using an enzyme load of 10 FPU per gram of glucan, in 125 mL erlenmeyer flasks. All test were carried out by duplicate in an orbital shaker at 50 °C and 200 rpm during 72 h.

Table 1. Experimental conditions of autohydrolysis pretreatment at various severity factors.

Experiment No.	Temperature (°C)	Time (min)	SF (Severity factor)
Exploratory conditions			
1	160	50	3.465
2	180	15	3.532
3	190	30	4.127
4	200	60	4.723
SF (selected range)			
5	180	60	4.133
6	190	30	4.127
7	190	45	4.303
8	190	60	4.428
9	200	15	4.12
10	200	45	4.597

Glucose yield was calculated as the ratio between the amount of glucose released in the enzymatic hydrolysis and the initial amount of glucans in the material after pretreatment, multiplied by a factor of 0.9 to include the hydration of the glucose.

2.5 Enzymatic hydrolysis and fermentation

Enzymatic hydrolysis of pretreated material was carried out in 1L Erlenmeyer flasks at a solid load of 20% (w/w) adding 0.05 M citrate buffer (pH 4.8) and an enzyme dosage of 25 FPU per gram of glucan. The flasks were incubated in orbital shaker at 50 °C and 200 rpm during 120 h. After hydroysis, glucose released was quantified by HPLC.

Saccharomyces cerevisiae ATCC 4126 was used to ferment the hydrolysates obtained from the enzymatic hydrolysis of the pretreated material. The yeast was maintained at 4 °C in Petri dishes containing YPD-agar medium with the following composition in g/L: glucose 20, peptone 20, yeast extract 10 and agar 20. Yeast growth was carried out in 250 mL Erlenmeyer flasks containing 100 mL of YPD liquid medium, incubated in an orbital shaker at 30 °C and 100 rpm during 24 h. After this time, the obtained culture was used as inoculum in fermentation runs.

Fermentation was carried out by duplicate in 125 mL Erlenmeyer flasks containing 70 mL of hydrolysate obtained in enzymatic hydrolysis, supplemented with (g/L): yeast extract 10, KH_2PO_4 1.17, $CaCl_2$ 0.09, $MgSO_4{\cdot}7H_2O$ 0.36, $(NH_4)_2SO_4$ 4.14, and 15 mL/L of a salts solution containing in g/L: NaCl 1.26, $CuSO_4{\cdot}5H_2O$ 0.26, $FeSO_4{\cdot}5H_2O$ 0.22, $MnCl_2{\cdot}4H_2O$ 0.12 and $ZnCl_2{\cdot}7H_2O$ 0.32, adjusted to pH 5.5 with NaOH. Enriched hydrolysates were inoculated with 10% (v/v) of culture grown in YPD medium and incubated in an orbital shaker at 30 °C and 100 rpm during 9.5 h. Samples were taken every 2 h and centrifuged at 10,000 rpm during 10 min. Subsequently, samples were filtered with PVDF membranes, pore size 0.22 μm, for further quantification of glucose and ethanol.

2.6 Analytical methods

Glucose, xylose, arabinose, formic acid, acetic acid and ethanol were determined and quantified by Agilent HPLC (model 1260 Infinity, CA, USA) equipped with a refractive index detector, using a BioRad Aminex HPX-87H column (7.8 x 300 mm; Bio-Rad Chemical Division, CA, USA) and 5 mM H_2SO_4 as mobile phase at a flow rate of 0.6 mL/min and 45 °C. Furfural, hydroxymethylfurfural (HMF), furfuryl alcohol and syringaldehyde were determined and quantified using the same equipment and column describe above with a UV detector at 220 nm and 65 °C, with an acid (5 mM H_2SO_4) to solvent (acetonitrile) ratio of 9:1 as eluent and a flow rate of 0.6 mL/min.

Cellular growth (biomass) was determined by measuring optical density of cells using a UV/Vis spectrometer at 660 nm and correlated with dry weight. Ethanol yield and productivity, and the conversion efficiency, considering a maximum theoretical ethanol yield of 0.51 g ethanol/g glucose were determined to assess the viability of fermentation of the enzymatic hydrolysates obtained from *A. lechuguilla* cogollos pretreated by autohydrolysis. An analysis of variance (ANOVA) was carried out, along with Fisher's F test with a p value of <0.05.

3 Results and discussion

3.1 Composition of A. lechuguilla cogollos

The composition of *A. lechuguilla* cogollos is shown in Table 2. The carbohydrates (glucan and xylan) are the main component; 27.49%, w/w, followed by the extractives; 25.69%. Vieira *et al*. (2002) reported that *A. lechuguilla* fiber has almost 80% of cellulose and 3-6% of hemicellulose, however that material was subjected to a purification process, moreover fiber in *A. lechuguilla* represents only a small fraction (14%) of the whole cogollo fresh weight (Reyes *et al*., 2000). In spite of the low glucan and xylan content of *A. lechuguilla* cogollos, this agave has agroecological advantages over other agaves species as mentioned above.

Table 2. Chemical composition of *A. lechuguilla* cogollos.

Component	% (w/w) dry weight base
Glucan	14.65
Xylan	12.84
Lignin	9.07
Extractives	25.69
Ash	12
Protein	5.5
Pectin	16.46
Total	**96.21**
Others	3.79

Table 3. Solid fraction composition and digestion test after autohydrolysis pretreatment of *A. lechuguilla* cogollos.

Experiment No.	Solids composition (g per 100 gDWB)		Cellulose conversion	
	Glcn	Xyln	Glucose released (g/L)	Hydrolysis Yield (%)
1	29	14.98	2.17	13.11
2	31	13.1	3.18	18.03
3	41	9.66	7.28	31.39
4	27	0	7.97	51.75

DWB (dry-weight base). Cellulose conversion (Percentage of the conversion of cellulose to glucose). Solid composition refers to solids recovered after the pretreatment: Glcn: glucan; Xyln: xylan. Values are the mean of two runs.

Table 4. Solid and liquid fraction composition after autohydrolysis pretreatment and digestion test of *A. lechuguilla* cogollos (second stage experiments).

Experiment No.	Solids composition (g per 100 gDWB)			Liquor composition (g/L)									Cellulose conversion	
	KL	Glcn	Xyln	Glc	Xyl	Ara	FA	AA	Fur	HMF	Fa	Si	Glucose released (g/L)	Hydrolysis Yield (%)
5	31.43	36.2	9.55	0.43	4.79	0.08	1.92	5.05	0.99	0.62	0.38	0.01	7.27	35.34
6	38.53	41	9.66	0.26	6.43	0	1.95	4.66	0.84	0.57	0.59	0.01	10.15	39.88
7	36.48	38.7	9.2	0.2	5.07	0	1.81	6.07	1.26	0.76	0.49	0.01	8.33	37.92
8	35.32	30.5	0	0.47	4.79	0.03	2.02	6.39	1.19	0.83	0.54	0.1	8.43	48.51
9	31.95	32.8	9.25	0.2	5.77	0	2.07	6.11	0.33	0.35	0.31	0.01	8.6	46.17
10	37.57	29.2	0	0.12	4.41	0.14	1.91	6.25	0.83	0.81	0.6	0.03	8.99	54.02

DWB (grams per 100 g of dry-weight base). Cellulose conversion (Percentage of the conversion of cellulose to glucose). Solid composition refers to solids recovered after the pretreatment: KL: Klason lignin; Glcn: glucan; Xyln: xylan. Liquor composition refers to components released from pretreatment: Glc: glucose; Xyl: xylose; Ara; arabinose; FA: formic acid; AA: acetic acid; Fur: furfural; HMF: hydroxyl-methyl-furfural; Fa: Furfuryl alcohol; Si: Syringaldehyde. Values are the mean of two runs.

3.2 Pretreatment and digestion test

The results of the enzymatic hydrolysis of the solid fraction after pretreatment by autohydrolysis for the initial four operation conditions are shown in Table 3. Taking in account that xylan is more susceptible (low crystallization degree) to solubilization than glucan, it was expected that xylan solubilization increased as severity factor increased as shown in Table 3. The highest yield of enzymatic hydrolysis, 51.75%, with a final glucose concentration of 7.97 g/L, was obtained for the highest SF tested i.e.: SF 4.723, at 200 °C and 60 min. This can be due to the complete solubilization of the xylan and partial glucan degradation (mainly amorphous cellulose), however the initial glucan content was the lowest as a consequence of the high severity and therefore the loss of glucan during the pretreatment. In comparison at SF 4.127, a similar glucose concentration was achieved 7.28 g/L, with a lower hydrolysis yield of 31.39%, but obtaining the highest glucan composition (41%) due to the lower severity of the pretreatment.

Table 4 shows the composition of solid fraction obtained after autohydrolysis and enzymatic digestion test for each SF tested in the second stage of experiments and its respective operational conditions. The glucan content in solids fraction was higher than 29% w/w of total solids in all the conditions. The best results were obtained at SF 4.127 corresponding to 190 °C and 30 min of time reaction, with the highest glucan content in solid fraction (41%).

At SF values higher than 4.428 a complete solubilization of xylan is achieved, it has been also reported for other materials (Moniz *et al*., 2013) in which a complete or nearly complete solubilization of xylan is reached at high SF values. These results are relevant because xylan is a physical barrier to cellulase, and its presence would decrease the hydrolysis yield. This behavior is in agreement with results shown also in Table 4, a higher hydrolysis of glucan was achieved as a result of the complete solubilization of xylan (48.51% and 54.02% at SF of 4.428 and 4.597 respectively). When xylose will not

be able to be fermented, the solubilization of xylan does not affect the overall ethanol yield. Due to that pretreatment method used in this work had a relatively low pH, lignin was not significantly removed at the SF tested.

Table 4 also shows the composition of the liquid fraction after pretreatment, it can be observed that the main sugar present is xylose (6.43 g/L at SF 4.127). Results obtained at SF; 4.428, showed the highest loss of xylan and glucan in the solid fraction, and rendered in the highest concentration of acetic acid: 6.39 g/L. This is attributed to a coupled process of byproduct formation and sugar degradation, where acetic acid is generated from hydrolysis of the acetyl groups of the hemicellulose, and furfural is produced from further degradation of pentose. These compounds are the dominant byproducts in the liquid fraction after pretreatment, other byproducts are present also, such as, formic acid and HMF (Mills *et al*., 2009). The lowest loss of glucan was obtained at SF 4.127, in this case a lower concentration of acetic acid, formic acid, furfural and HMF were also produced.

3.3 Enzymatic hydrolysis

Figure 1 shows the kinetic of glucose release from the pretreated *A. lechuguilla* cogollos during enzymatic hydrolysis. A glucose concentration of 41 g/L was reached after 4 h of incubation, obtaining the highest concentration after 48 h: 59 g/L, with a yield of 60.85%, due to the high enzyme dosage used, most of the glucan was hydrolyzed during the first hours.

Compared with other reports of enzymatic hydrolysis using different pretreated material, e.g. alcoholic beverage production residues, fibers, etc., the yields obtained in the present study are higher, except by those reported by Caspeta *et al*. (2014). Hernández-Salas *et al*. (2009), reported a 56% yield from *Agave atrovirens* pretreated by NaOH, Medina-Morales *et al*. (2011), mentioned a maximum yield of 42% from *Agave salmiana* leaves fibers and Saucedo-Luna *et al*. (2011), obtained a 73.62% yield from *Agave tequilana* bagasse, however in this report the glucose release was lower (41 g/L). Yang and Pan (2012) reported a 68.9% yield from *Agave americana* biomass pretreated with NaOH and Caspeta *et al*. (2014) obtained a higher yield (91% with a glucose release of 225 g/L), however this result was achieved at a solid load of 30% from pretreated agave residues by ethanosolv with a higher carbohydrates content (69%, 24% and 6% of glucan, xylan and arabinan respectively), than *A. lechuguilla* cogollos.

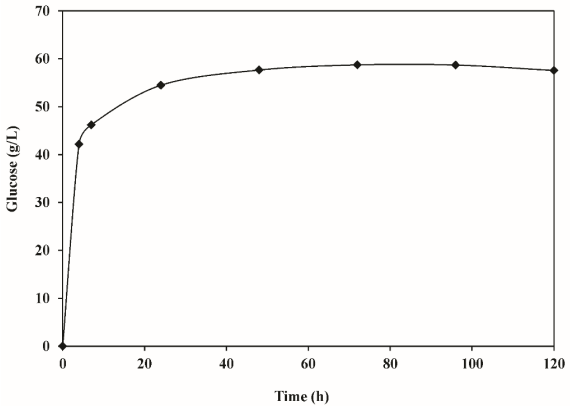

Fig. 1. Glucose released from pretreated *A. lechuguilla* cogollos during enzymatic hydrolysis (commercial enzyme Acellerase 1500 from Genencor®).

In addition, all raw materials used in the other studies reported a higher content of glucan and xylan, above 35%.

3.4 Fermentation of enzymatic hydrolysates

Figure 2 shows glucose consumption and ethanol production in the fermentation of the hydrolysate obtained after enzymatic saccharification using *S. cerevisiae* ATCC 4126 containing 54.41 g/L of initial glucose concentration. Results were compared with a control culture containing 88.64 g/L of glucose. Glucose consumption in the control was 86% after 9.5 h, producing 35.41 g/L of ethanol at a yield ($Y_{E/S}$) of 0.46 $g_{ethanol}/g_{glucose}$, which corresponds to a 91% of the theoretical value and a productivity of 3.72 g/L h^{-1}. In the case of the fermentation of hydrolysates, a complete consumption of glucose was achieved after 7.5 h, reaching an ethanol concentration of 25.4 g/L, at a yield of 0.46 $g_{ethanol}/g_{glucose}$ corresponding to a 91% of the theoretical value, with a productivity of 3.38 g/L h^{-1}. Fig. 2 also shows a short lag phase of 2 h, achieving a biomass yield of 6.32×10^{-2} $g_{biomass}/g_{glucose}$, (Table 5).

Table 6 shows reports of the fermentation of hydrolysates obtained from different material, as mentioned above (alcoholic beverage production residues, fibers), with the operational conditions at which fermentation were carried out. It can be observed that ethanol concentration and yield are lower than the obtained in this work, with the exception of the report by Caspeta *et al*. (2014), in which the glucose concentration in the hydrolysate was considerably higher compared with those concentrations obtained in other reports.

Table 5. Fermentation kinetic parameters of enzymatic hydrolysates of *A. lechuguilla* cogollos using *Saccharomyces cerevisiae* ATCC 4126.

Kinetic Parameters	Control	Enzymatic hydrolysate
$Y_{E/S}$ ($g_{ethanol}\ g^{-1}_{glucose}$)	0.46	0.46
Conversion efficiency (%)	91	91
EP ($g_{ethanol}\ L^{-1}\ h^{-1}$)	3.72	3.38
$Y_{X/S}$ ($g_{biomass}\ g^{-1}_{glucose}$)	0.0632	-

YE/S: Ethanol yield. EP: Ethanol productivity. YX/S: Biomass yield.

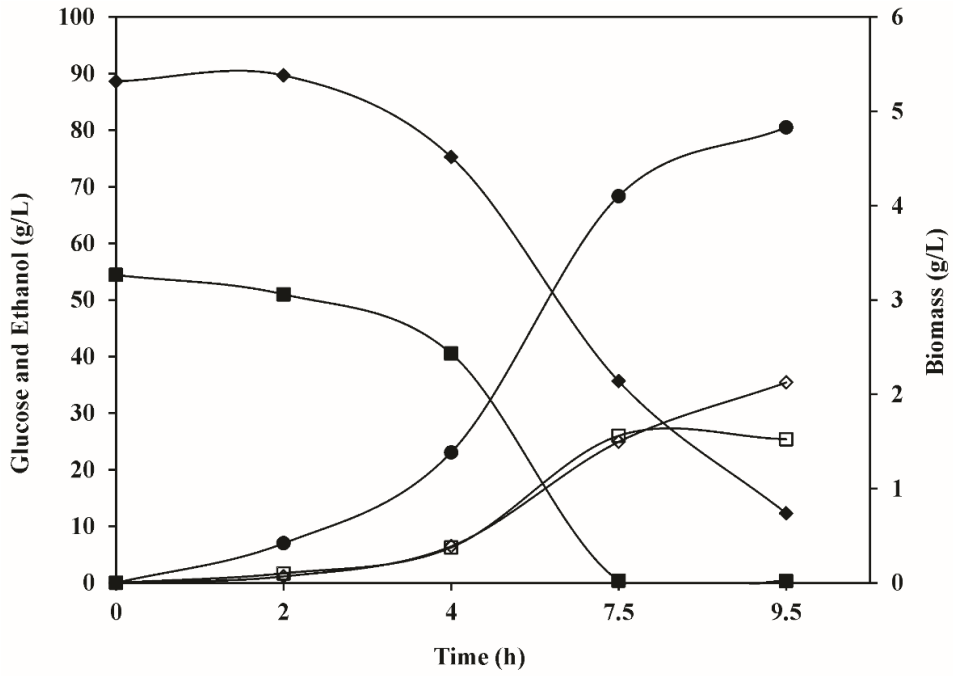

Fig. 2. Fermentation of enzymatic hydrolysates obtained from pretreated *A. lechuguilla* cogollos using the *Saccharomyces cerevisiae* ATCC 4126. Glucose consumption (Filled markers); ethanol production (unfilled markers) in control (♦) and enzymatic hydrolysates (■); and *Saccharomyces cerevisiae* ATCC 4126 biomass growth in control (●).

Conclusions

Acetic acid released during the pretreatment of *A. lechuguilla* cogollos catalyzed the hydrolysis of xylan and in some extend of glucan at certain SF. The higher the SF the higher the xylan and glucan hydrolysis, however the loss of glucan is not preferred when pretreatment liquor is not further fermented. In spite of the presence of some inhibitory compounds, the fermentation process of the hydrolysates by *S. cerevisiae* ATCC 4126 obtained a 25.4 g/L of ethanol and a yield of 0.46 $g_{ethanol}/g_{glucose}$ corresponding to a 91% of the theoretical value. Finally, it is worthy to mention that *A. lechuguilla* can tolerate desertic and semidesertic climates giving a special advantage over other energy crops for 2G bioethanol production in Mexico.

Acknowledgments

The authors are grateful to the Secretariat of Agriculture, Livestock, Rural Development, Fisheries and Food of Mexico (SAGARPA), The National Council of Research and Technology of Mexico (CONACYT) and National Commission of Research and Technology of Chile (CONICYT) for financial support.

Table 6. Hydrolysis and fermentation of different agave materials.

Process stage		Feedstock					
		Agave tequilana bagasse	*Agave tequilana* bagasse	*Agave tequilana* bagasse	*Agave atrovirens* residues	*Agave atrovirens* residues	*Agave lechuguilla* cogollos
	Pretreatment	Diluted acid	Diluted acid	Ethanosolv	NaOH	NaOH	Autohydrolysis
Hydrolysis	Catalyst	Acid	Enzymes	Enzymes	Enzymes	Enzymes	Enzymes
	Yield (%)	36.1	73.6	91	56	28	60.8
Fermentation	Microorganism	Pichia caribbica UM-5	Pichia caribbica UM-5	*S. cerevisiae* SuperStart	*S. cerevisiae* SAFMEX	*S. cerevisiae* SAFMEX	*S. cerevisiae* ATCC 4126
	Sugars conc. (g/L)	24.9	41	225	56.37	28.44	54.41
	Temperature (°C)	30	30	37	30	30	30
	pH	5	5	N.R	5	5	5.5
	RPM	100	100	N.R	N.R.	N.R.	100
	Time (h)	48	48	9	48	48	9.5
	Ethanol conc. (g/L)	6.37	18.31	64	6.6	6.3	25.4
	Ethanol yield (%)	50	87	85	11.71	22.23	91
Reference		Saucedo-Luna *et al.* (2011)	Saucedo-Luna *et al.* (2011)	Caspeta *et al.* (2014)	Hernández-Salas *et al.* (2009)	Hernández-Salas *et al.* (2009)	This work

N.R. Not reported.

References

Borland, A.M., Griffiths, H., Hartwell, J. and Smith, J.A.C. (2009). Exploiting the potential of plants with crassulacean acid metabolism for bioenergy production on marginal lands. *Journal of Experimental Botany 60*, 2879-2896.

Buratti, C., Barbanera, M. and Lascaro, E. (2015). Ethanol Production from vineyard pruning residues with steam explosion pretreatment. *Environmental Progress & Sustainable Energy 34*, 802-809.

Caspeta, L., Caro-Bermudez, M.A., Ponce-Noyola, T. and Martínez, A. (2014). Enzymatic hydrolysis at high-solids loadings for the conversion of agave bagasse to fuel ethanol. *Applied Energy 113*, 277-286.

Chuck-Hernández, C., Pérez-Carrillo, E., Heredia-Olea, E. and Serna-Saldívar, S.O. (2011). Sorghum as a multifunctional crop for bioethanol production in Mexico: technologies, advances and improvement opportunities. *Revista Mexicana de Ingeniería Química 10*, 529-549.

Contreras-Esquivel, J.C., Espinoza-Pérez, J.D., Montanez-Saez, J.C., Charles-Rodriguez, A.V., Renovato, J., Aguilar, C.N., Rodríguez-Herrera, R. and Wicker, L. (2006). Extraction and characterization of pectin from novel sources. *Advances in Biopolymers. ACS Symposium Series 935*, 215-229.

Das, S.P., Ravindran, R., Ghosh, A., Deka, D., Das, D., Jawed, M., Fontes, C.M.G.A. and Goyal, A. (2014). Efficient pretreatment for bioethanol production from water hyacinth (*Eichhornia crassipes*) involving naturally isolated and recombinant enzymes and its recovery. *Environmental Progress & Sustainable Energy 33*, 1397-1404.

Fan, H. and Ragauska, A. (2012). Pretreatment and lignocellulosic chemistry. *Bioenergy Resources 5*, 1043-1066.

Fischer, G., Prieler, S., van Velthuizen, H., Berndes, G., Faaij, A., Londo, M. and de Wit, M. (2010). Biofuel production potentials in Europe: sustainable use of cultivated land and pastures, Part II: land use scenarios. *Biomass and Bioenergy 34*, 173-187.

García-Mendoza, A.J. (2007). Los agaves de México. *Ciencias 87*, 14-23.

Garrote, G. and Parajo, J.C. (2002). Non-isothermal autohydrolysis of *Eucalyptus* wood. *Wood Science and Technology 36*, 111-123.

Hernández-Salas, J.M., Villa-Ramírez, M.S., Veloz-Rendón, J.S., Rivera-Hernández, K.N. and González-César, R.A., Plascencia-Espinosa, M.A., Trejo-Estrada, S.R. (2009). Comparative hydrolysis and fermentation of sugarcane and agave bagasse. *Bioresource Technology 100*, 1238-1245.

Lee, J.M., Jameel, H. and Venditti, R.A. (2010). One and two stage autohydrolysis pretreatments for enzyme hydrolysis of Coastal bermuda grass to produce fermentable sugars. *BioResources 5*, 1496-1508.

Lee, J.M., Shi, J., Venditti, R.A. and Jameel, H. (2009). Autohydrolysis pretreatment of Coastal bermuda grass for increased enzyme hydrolysis. *Bioresource Technology 100*, 6434-6441.

Márquez, A., Casaruang, N., González, I. and Colunga-GarcíaMarín, P. (1996). Cellulose extraction from *Agave lechuguilla* fibers. *Economic Botany 50*, 465-468.

Medina-Morales, M.A., Contreras-Esquivel, J.C., De la Garza-Toledo, H., Rodríguez, R. and Aguilar, C.N. (2011). Enzymatic bioconversion of Agave leaves fiber hydrolysis using Plackett-Burman design. *American Journal of Agricultural and Biological Sciences 6*, 480-485.

Mills, T.Y., Sandoval, N.R. and Gill, R.T. (2009). Cellulosic hydrolysate toxicity and tolerance mechanisms in *Escherichia coli. Biotechnology for Biofuels 2*, 1-11.

Molina, C., Sánchez, A., Serafín-Muñoz, A. and Folch-Mallol, J. (2014). Optimization of enzymatic saccharification of wheat straw in a micro-scale system by response surface methodology. *Revista Mexicana de Ingeniería Química 13*, 765-778.

Moniz, P., Pereira, H., Quilhó, T. and Carvalheiro, F. (2013). Characterization and hydrothermal processing of corn straw towards the selective fraction of hemicelluloses. *Industrial Crops and Products 50*, 145-153.

Morales-Martínez, T.K., Rios-González, L.J., Aroca-Arcaya, G., Rodríguez-de la Garza, J.A. (2014). Ethanol production by *Zymomonas mobilis* NRRL B-806 from enzymatic hydrolysates of *Eucalyptus globulus. Revista Mexicana de Ingeniería Química 13*, 779-785

Narcia, V.M., Castillo Q.D., Vázquez, R.J.A. and Berlanga, R.C.A. (2012). Turno técnico de la lechuguilla (*Agave lechuguilla* Torr.) en el noreste de México. *Revista Mexicana de Ciencias Forestales 3*, 81-88.

Nobel, P.S. and Quero, E. (1986). Environmental productivity indices for a Chihuahuan Desert CAM plant *Agave lechuguilla. Ecology 67*, 1-11.

Palmqvist, E. and Hahn-Hagerdal, B. (2000). Fermentation of lignocellulosic hydrolysates. II. Inhibitors and mechanisms of inhibition. *Bioresource Technology 74*, 25-33.

Pando-Moreno, M., Pulido, R., Castillo, D., Jurado, E. and Jiménez, J. (2008). Estimating fiber for lechuguilla (*Agave lechuguilla* Torr., Agavaceae), a traditional non-timber forest product in Mexico. *Forest Ecology and Management 255*, 3686-3690.

Reyes, A.J.A., Aguirre, R.J.R. and Peña V.C.B. (2000). Biología y aprovechamiento de *Agave lechuguilla* Torrey. *Boletín de la Sociedad Botánica de México 67*, 75-88.

Saucedo-Luna, J., Castro-Montoya, A.J, Rico, J.L. and Campos-García J. (2010). Optimization of acid hydrolysis of bagasse from *Agave tequilana* weber. *Revista Mexicana de Ingeniería Química 9*, 91-97.

Saucedo-Luna, J., Castro-Montoya, A.J., Martinez-Pacheco, M.M., Sosa-Aguirre, C.R. and Campos-Garcia, J. (2011). Efficient chemical and enzymatic saccharification of the lignocellulosic residue from *Agave tequilana* bagasse to produce ethanol by *Pichia caribbica. Journal of Industrial Microbiology and Biotechnology 38*, 725-732.

Sheldon, S. (1980). Ethnobotany of *Agave lechuguilla* and *Yucca carnerosana* in Mexico's Zona Ixtlera. *Economic Botany 34*, 376-379.

Sivamani, S. and Baskar, R. (2015). Optimization of bioethanol production from Cassava peel using statistical experimental design. *Environmental Progress & Sustainable Energy 34*, 567-574.

Sluiter, A., Hames, B., Ruiz, R., Scarlata, C., Sluiter, J. and Templeton, D. (2008). Determination of ash in biomass. Golden, Colorado: National Renewable Energy Laboratory; Jan, Report No. TP-510-42622.

Sluiter, A., Hames, B., Ruiz, R., Scralata, C., Sluiter, J. and Templeton, D. (2011). Determination of structural carbohydrates and lignin in biomass, NREL/TP-51042618. Laboratory Analytical Procedure (LAP), National Renewable Energy Laboratory.

Sluiter, A., Ruiz, R., Scarlata, C., Sluiter, J. and Templeton, D. (2005). Determination of extractives in biomass. Golden, Colorado: Jan, Report No. TP-510-42619.

Somerville, C., Youngs, H., Taylor, C., Davis, S.C. and Long, S.P. (2010). Feedstocks for lignocellulosic biofuels. *Science 329*, 790-792.

Vieira, M.C., Heinze, T., Antonio-Cruz, R. and Mendoza-Martinez, A.M. (2002). Cellulose derivatives from cellulosic material isolated from *Agave lechuguilla* and *fourcroydes. Cellulose 9*, 203-212.

Wang, G., Tan, L., Sun, Z-Y., Gou, Z-X., Tang, Y-Q. and Kida, K. (2015). Production of bioethanol from rice straw by simultaneous saccharification and fermentation of whole pretreated slurry using *Saccharomyces cerevisiae* KF-7. *Environmental Progress & Sustainable Energy 34*, 583-588.

Yang, Q. and Pan, X. (2012). Pretreatment of *Agave Americana* stalk for enzymatic saccharification. *Bioresource Technology 126*, 336-340.