Assessment of Different Saccharification and Fermentation Configurations for Ethanol Production from *Agave lechuguilla*

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Different strategies were assessed for the production of ethanol from *Agave lechuguilla* that was pretreated by autohydrolysis. Separate hydrolysis and fermentation (SHF) was compared against simultaneous processes including simultaneous saccharification and fermentation (SSF) and prehydrolysis and simultaneous saccharification and fermentation (PSSF) using different solids (15%, 20%, and 25% w/w) and enzyme loadings (15 FPU/g, 20 FPU/g, and 25 FPU/g glucan). The results showed that the maximum ethanol concentration (53.7 g/L) and productivity (1.49 g/L h^{-1}) was obtained at 36 h in the SHF configuration at the highest solids and enzyme loadings (25% w/v and 25 FPU/g glucan, respectively). The ethanol concentration and productivity obtained in the PSSF configuration at the same time were 45 g/L and 1.25 g/L h^{-1}, respectively. The SSF configuration exhibited the lowest ethanol concentration and productivity (10.4 g/L and 0.29 g/L h^{-1}, respectively) at 36 h. The enzyme used, Cellic CTec3, allowed for high glucose yields at the lower enzyme dosage assessed. The SHF configuration exhibited the best results. However, the PSSF configuration can be considered an attractive alternative because it eliminated the need for solid-liquid separation devices, which simplifies the industrial implementation of the process.

Keywords: Agave lechuguilla; Autohydrolysis; Different process configuration; Ethanol

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INTRODUCTION

Agave lechuguilla is a common plant found in northern Mexico and occupies the largest range of all agaves with almost 20 million hectares of the arid and semiarid lands of Mexico (Castillo *et al*. 2013). The species has traditionally been exploited for fiber extraction (Pando-Moreno *et al*. 2008) and has recently been reported as a feedstock for ethanol production (Ortíz-Méndez *et al*. 2017). *Agave lechuguilla* cogollos (heart or pulpy central stem with attached leaf bases) can be harvested several times without sacrificing the whole plant. The annual productivity is 4 tons per hectare, with an average rainfall of 427 mm (Escamilla-Treviño 2012).

The production of ethanol from lignocellulosics can be performed by three major steps, including the pretreatment of the raw material, hydrolysis of cellulose, and biological conversion of sugars to ethanol (Triwahyuni *et al*. 2015). The hydrolysis of cellulose can

be achieved *via* an acid or enzymatic process. However, enzymatic hydrolysis presents diverse advantages compared to acid hydrolysis because it requires less energy, mainly because the process is carried out at lower temperatures (approximately 50 °C for enzymatic hydrolysis *vs.* over 150 °C for acid hydrolysis), it does not produce inhibitory by-products, and it is an environmentally friendly process (López-Linares *et al*. 2014). However, it has been suggested that to make the lignocellulose conversion process more economically feasible, the enzymatic hydrolysis process must be carried out using high solids loadings. Theoretically, high concentrations of sugars will result in a higher ethanol production, which could reduce energy use and costs associated with the distillation process (Modenbach and Nokes 2013). Nevertheless, increasing the solids concentration in enzymatic hydrolysis leads to decreased yields, particularly due to the initial high viscosity of fibrous materials, resulting in poor mixing and impaired enzyme performance (Sant'Ana da Silva *et al*. 2016).

The hydrolysis and fermentation process can be achieved by several strategies, including separate hydrolysis and fermentation (SHF), simultaneous saccharification and fermentation (SSF), and prehydrolysis and simultaneous saccharification and fermentation (PSSF) (Paulova *et al*. 2015). To the knowledge of the authors, few reports on ethanol production from agave hydrolysates are available, and those that exist are mainly focused on the use of the SHF configuration with yeast (Hernández-Salas *et al*. 2009; Saucedo-Luna *et al*. 2011; Caspeta *et al*. 2014; Corbin *et al*. 2015; Mielenz *et al*. 2015; Rios-González *et al*. 2017) or on the SSF configuration with ethanologenic bacteria (Pérez-Pimienta *et al*. 2017).

The integration of two or more process steps is important for simplifying the process and reducing the production cost (Wang *et al*. 2013; Narra *et al*. 2015). The SHF configuration is performed in two separate steps: first the enzymatic hydrolysis of pretreated cellulose and then the fermentation of sugars to ethanol; and each step can be carried out at its optimal process condition (de Barros *et al*. 2017). In the SSF configuration, the enzyme and microbe are synergically performing. This configuration is also advantageous because both processes happen in a single step. However, enzymatic hydrolysis has a low performance because the optimal temperature for yeasts is lower than that for enzymatic hydrolysis (Neves *et al*. 2016). In the PSSF configuration, the pretreated material is prehydrolyzed at the optimal temperature of the enzyme complex and the temperature is then lowered for further inoculation with no other additional step. The main advantage of PSSF over SHF is that it simplifies the process by eliminating the need to separate the slurry before fermentation. Regarding the advantage of the PSSF configuration compared to the SSF configuration, the rate of enzymatic hydrolysis is not reduced by the suboptimal temperature and the ethanol production rate is not limited by the low concentration of carbon source (Paulova *et al*. 2015).

The aim of this work is to assess and compare the SHF, SSF, and PSSF configurations at different solids and enzyme loadings for ethanol production from *A. lechuguilla* biomass pretreated by autohydrolysis.

EXPERIMENTAL

Materials

Agave lechuguilla cogollos were collected from the municipality of Ramos Arizpe, Coahuila, Mexico. The cogollos were dried in a tray dehydrator (model KL10, Koleff S.A.

de C.V., Queretaro, Mexico) at 45 °C until the moisture content was less than 10% of the total weight. Subsequently, the dried cogollos were milled and sieved in a Retsch SM100 cutting mill (Retsch SM100, Retsch, Haan, Germany) to 2-mm particle size prior to compositional analysis and autohydrolysis pretreatment. The material was mixed to obtain a homogeneous sample and stored at room temperature in hermetic containers.

Feedstock composition and autohydrolysis pretreatment

The moisture content was determined with a moisture analyzer (Moisture Analyzer OHAUS, Ohaus Co., Parsippany, NJ, USA). The extractives and ash content were determined using the National Renewable Energy Laboratory (NREL) analytical methods NREL/TP-510-42619 (Sluiter *et al*. 2005) and NREL/TP-510-42622 (Sluiter *et al*. 2008), respectively. The Laboratory Analytical Procedure (LAP) from the NREL (NREL/TP-510-42618) was modified for the determination of cellulose (glucan), hemicellulose (xylan), and lignin according to Mussatto *et al*. (2011). The material (500 mg) was hydrolyzed with 72% (w/w) sulfuric acid (H_2SO_4) for 7 min at 50 °C. The obtained hydrolysate was subsequently diluted to 4% (w/w) H_2SO_4 by adding distilled water. A second hydrolysis was performed by autoclaving the reaction mixture at 121 °C for 1 h. The autoclaved solution filtered through 0.2-µm filters for High Performance Liquid Chromatography (HPLC) analysis, and the solid residues that remained after filtration were used to determine the acid insoluble lignin (Klason lignin). The proteins were determined by the Kjeldahl method (Ortíz-Méndez *et al*. 2017).

Autohydrolysis pretreatment of *A. lechuguilla* was performed in a 5-gallon high-pressure stainless steel reactor (Parr Instruments Co., Moline, IL, USA). The dried and milled material (2.192 kg of *A. lechuguilla*) was suspended in 13.15 L of distilled water (resulting in a 1:6 w/v solid/liquid ratio) at 190 °C, 200 rpm for 30 min; these conditions were established previously by Ortíz-Méndez *et al*. (2017).

The reactor was rapidly cooled down once the reaction time was reached. The pretreated material was then separated by filtration. The liquid fraction was analyzed by HPLC, injecting a 20 µL sample to determine the concentration of glucose, xylose, other sugars (mannose, arabinose, and galactose), and a 10 µL sample to determine inhibitors, such as, formic acid, acetic acid, furfural, and hydroxymethylfurfural (HMF). The solid fraction was washed with water (30 times the volume of the material) and stored at 4 °C until further use in the SHF, SSF, and PSSF experiments. The glucan, xylan, and lignin in the solid fraction were determined as described above.

Enzyme

Cellic® CTec3 was kindly provided by Novozymes® (Kalundborg, Denmark). The cellulase activity (with a value of 217) of the enzyme complex was determined as described by Ghose (1987) in Filter Paper Units per mL (FPU/mL).

Inoculum and medium

Saccharomyces cerevisiae ATCC 4126 was used for the ethanol production. The inoculum was grown in 125-mL Erlenmeyer flasks with 50 mL of the following medium: yeast extract (10 g/L), monopotassium phosphate (1.17 g/L), calcium chloride (0.09 g/L), magnesium sulfate (0.36 g/L), and ammonium sulphate (4.14 g/L). The medium was supplemented with 15 mL/L of a salts solution containing: sodium chloride (1.26 g/L), cupric sulfate (0.26 g/L), ferrous sulphate (0.22 g/L), manganese chloride (0.12 g/L), zinc chloride (0.32 g/L), and glucose (100 g/L). The pH medium was adjusted to 5.5 with 2M

NaOH before inoculation. The flasks were incubated in an orbital shaker (New Brunswick™ 124/24R, New Brunswick Scientific Co., Inc., Hauppauge, NY, USA) at 100 rpm and 35 °C for 24 h. Five g/L of cells (10 % v/v) were used as inoculum in all of the experiments.

Methods

Process configurations- SHF

The enzymatic hydrolysis was conducted in 125-mL Erlenmeyer flasks using different enzyme (15 FPU/g, 20 FPU/g, and 25 FPU/g glucan) and solids loadings (15%, 20%, and 25% w/w dry matter) in a sodium citrate buffer at 0.05 M (pH 4.8). The solids loading was 15% (w/w) for experiments 1, 2, and 3, 20% (w/w) for experiments 4, 5, and 6, and 25% (w/w) for experiments 7, 8, and 9. Each solids loading was assessed at 15 FPU/g, 20 FPU/g, and 25 FPU/g glucan. The experiments were conducted in an orbital shaker at 50 °C and 200 rpm for 24 h. At the end of the hydrolysis reaction, the glucose concentration was measured by HPLC.

The hydrolysates were centrifuged at 5,500 rpm for 15 min in a Thermo Scientific centrifuge (Haraeus™ Megafuge™ 16 R, Rockford IL, USA). The supernatants were fermented (10% v/v inoculum) in a 125-mL Erlenmeyer flask with 15 mL of hydrolysates (supplemented with the nutrients described above; pH 5.5) and were incubated in an orbital shaker at 35 °C and 150 rpm for 24 h. Samples were taken at 6 h, 12 h, 18 h, and 24 h for ethanol and glucose quantification by HPLC.

The enzymatic hydrolysis yield was expressed as the relationship between the amount of glucose released during saccharification and the initial amount of glucan present in the pretreated material. The ethanol yield was reported as a percentage of the theoretical yield assuming all the potential glucose present can be fermented, with a maximum theoretical ethanol yield of 0.51 g ethanol/g glucose.

SSF

The SSF assays were conducted in 125-mL Erlenmeyer flasks at the same conditions described for the SHF configuration. The SSF assays were performed for 72 h at 35 °C, adding simultaneously the enzyme and the inoculum (10% v/v) at the beginning of the process. Samples were taken at 12 h, 24 h, 48 h, and 72 h and centrifuged at 5,500 rpm for 15 min in a micro-centrifuge (Heraeus™ Biofuge® Pico, Thermo Fisher Scientific, Waltham, MA, USA) for ethanol and glucose quantification by HPLC.

Prehydrolysis and simultaneous saccharification and fermentation (PSSF)

The pre-hydrolysis was performed under the same conditions described for the SHF configuration for 24 h; after this time and without separating the slurry from the flasks, the temperature was readjusted to 35 °C, inoculated (10% v/v), and incubated in an orbital shaker at 150 rpm for 72 h. The samples were removed and centrifuged for analysis at 12 h, 24 h, 36 h, 48 h, 72 h, and 96 h to determine the ethanol and glucose concentrations by HPLC.

Analytical methods

The glucose, xylose, galactose, arabinose, mannose, formic acid, acetic acid, and ethanol were determined by a HPLC unit (Agilent 1260 Infinity, Santa Clara, CA, USA) equipped with a refractive index detector at 45 °C, using an Agilent Hi-Plex H column at 35 °C (7.7 × 300 mm, Santa Clara, CA, USA) and 5 mM H_2SO_4 as the mobile phase at a

flow rate of 0.5 mL/min. Furfural and hydroxymethylfurfural (HMF) were measured using the same equipment and column (at 55 °C) described above using a UV detector at 220 nm with a mixture of 5 mM H_2SO_4 and acetonitrile at a ratio of 9:1 as the eluent and a flow rate of 0.4 mL/min. The cellular growth of the inoculum was determined by correlating the optical density of cells using a UV/vis spectrometer (Varian, Palo Alto, CA, USA) at 660 nm with the dry weight. All experiments were performed in triplicate and the average values are reported. An analysis of variance (ANOVA) was conducted along with Fisher's F test with a p value of < 0.05 (Minitab® version 17, Minitab Inc., State College, PA, USA).

RESULTS AND DISCUSSION

Composition of *A. lechuguilla* and Autohydrolysis Pretreatment

The composition of *A. lechuguilla* cogollos on a dry basis was: extractives 37%, glucan 22.2%, xylan 7.86%, lignin 18.3%, ash 7%, protein 5.5%, and other non-quantified compounds 2.14%. The composition of *A. lechuguilla* pretreated by autohydrolysis is summarized in Table 1. The recovered sample after treatment was enriched in glucan and the total polymerized sugar content was higher compared to the untreated biomass (increasing from 22.2% to 41.0%). From the initial glucan content present in the untreated material, 71% remained in the solid fraction. Autohydrolysis pretreatment mainly affected the hemicellulosic components, and under these conditions 92% of the original xylan content was solubilized. This is in agreement with the results of Amiri and Karimi (2015) and Zhuang *et al*. (2016), who reported that most of the xylan was hydrolyzed during pretreatment while the glucan and insoluble lignin were retained in the solid fraction. The solid recovered from pretreatment was 39.5% of the original raw material; this loss was attributed to the removal of extractives and xylan during the process. The lignin was not significantly solubilized during the pretreatment.

Table 1. Composition of Solid and Liquid Fractions after Autohydrolysis Pretreatment of *A. lechuguilla*

Pretreated Solids (% w/w)		Liquid Fraction (g/L)	
Solids recovery	39.50 ± 2.12	**Sugars**	
		Glucose	0.26 ± 0.04
Glucan	40.98 ± 1.16	Xylose	6.43 ± 0.19
		Other sugars [a]	ND
Xylan	1.52 ± 0.47	**Inhibitors**	
		Acetic acid	4.66 ± 0.09
Lignin	44.25 ± 0.71	Formic acid	1.95 ± 0.03
		Furfural	0.84 ± 0.05
Ash	2.94 ± 0.48	HMF	0.57 ± 0.01
ND: Not detected; [a] Galactose, arabinose, and mannose			

During pretreatment, the main byproducts were acetic acid, formic acid, furfural, and HMF with concentrations in g/L of 4.66, 1.95, 0.84, and 0.57, respectively. The acetic acid formation during the pretreatment process promotes the xylan dissolution as a result of the pH decrease. This behavior was previously described by Rios-González *et al*. (2017) using agave bagasse pretreated by autohydrolysis; the authors reported an acetic acid concentration in the range of 5.33 g/L to 10 g/L at 190 °C with operation times ranging from 15 min to 60 min.

Process Configurations Assessment

Table 2 shows the effect of solids and enzyme loading on final glucose concentration and hydrolysis yield. It can be observed that in the three configurations (SHF, SSF, and PSSF) assessed, the glucose concentration increased as the solids loading increased within the experiments performed with a maximum of 108.8 g/L, 66.9 g/L, and 107.2 g/L for SHF, SSF, and PSSF, respectively, under the same conditions (solids and enzyme loadings of 25% and 25 FPU/g glucan, respectively). In the case of the SSF configuration, the glucose concentration decreased 38% and 37% (experiment 9) when compared with the SHF and PSSF configurations, respectively.

In spite of the glucose concentration increment, when increasing the solids loading, the hydrolysis yield in the cases of the SHF and PSSF configurations decreased (Table 2). As reported by López-Linares *et al*. (2014), using acid pretreated rapeseed straw in a SHF configuration, the glucose concentration increased roughly linearly with the increase of solids loading; however the hydrolysis yield diminished. It has been suggested (López-Linares *et al*. 2014) that this phenomenon is caused by diffusional limitations in the medium containing a high proportion of solids rather than to a loss of enzymatic activity due to end-product inhibition. Xue *et al*. (2012) mentioned that high enzyme loadings can improve enzymatic hydrolysis yield at high solids loading. However, in this work, the enzyme complex Cellic® CTec3 showed that a smaller enzyme loading of 15 FPU/g glucan can be used to obtain similar hydrolysis yield (maximum difference of 7.4%) compared with the maximum enzyme loading assessed of 25 FPU/g glucan.

The increase in enzyme loading from 15 FPU/g to 20 FPU/g of glucan and 20 FPU/g to 25 FPU/g of glucan increased the hydrolysis yield between 1% to 4.4%, regardless of the solids loading; it is therefore not recommended to increase the enzyme loading because it will not result in a noticeably higher hydrolysis yield. According to Olofsson *et al*. (2008), an increase of 50% in enzyme loading should be justified if an increase in the hydrolysis yield is greater than 6%. Therefore, the enzyme loading can be optimized to provide the maximum glucose concentration at the lowest unit cost (Wang *et al*. 2012).

Table 2. Glucose Released and Hydrolysis Yield in Different Configurations (SHF, SSF, and PSSF) at 24 h of Enzymatic Hydrolysis

Exp. No.	Final Glucose (g/L)					Hydrolysis Yield (%)[c]		
	Process Configurations							
	SL[a]	EL[b]	SHF	SSF	PSSF	SHF	SSF	PSSF
1		15	65.0 ± 0.14	22.1 ± 1.27	63.7 ± 0.52	96.1	32.6	94.2
2		20	66.3 ± 0.57	23.7± 1.32	65.5 ± 0.47	98.0	35.0	96.8
3		25	67.0 ± 0.34	25.2 ± 1.19	66.3 ± 0.38	99.0	37.2	98.0
4		15	81.9 ± 0.82	42.0 ± 1.41	82.2 ± 1.06	90.8	46.5	91.2
5		20	84.5 ± 0.67	44.5 ± 0.71	84.7 ± 0.75	93.7	49.3	94.0
6		25	87.9 ± 1.34	47.0 ± 1.13	88.7 ± 1.21	97.5	52.1	98.4
7		15	101.1 ± 0.57	58.5 ± 0.99	100.5 ± 0.71	89.7	51.9	89.1
8		20	105.5 ± 0.71	62.0 ± 1.7	104.5 ± 0.7	93.6	55.0	92.7
9		25	108.8 ± 0.49	66.9 ± 1.55	107.2 ± 0.35	96.5	59.3	95.1

[a] SL: Solids loading (%); [b] EL: Enzyme loading (FPU/g glucan)
[c] Enzymatic hydrolysis yield expressed as the relationship between the amount of glucose released and the initial amount of glucan present in the pretreated material.

The highest hydrolysis yield (99%) was obtained in the SHF configuration at 15% solids loading and 25 FPU/g glucan enzyme loading. A similar hydrolysis yield (98.4%) was obtained in the PSSF configuration using 20% solids loading and 25 FPU/g glucan enzyme loading. In contrast, glucan conversion in the SSF configuration was lower (59.4%; the highest value for this configuration) compared to the SHF and PSSF configurations at 25% solids loadings and 25 FPU/g glucan enzyme loading. The hydrolysis yields obtained in the SSF configuration were not considered an absolute magnitude, but rather an apparent magnitude because the glucose released from the cellulose in the enzymatic reaction was consumed by yeasts during the fermentation process. In addition, the optimal temperature in SSF was different for saccharification and fermentation. Paulová *et al*. (2014) reported a plunge in the hydrolysis yield due to discrepancies in the optimal temperatures for both processes. In the present study, when using a temperature of 50 °C in the SSF configuration, no ethanol production was detected at 72 h (data not shown).

The maximum ethanol concentration was obtained when using the hydrolysate with the highest glucose concentration: 53.7 g/L in SHF, 25.9 g/L in SSF, and 50.3 g/L in PSSF, which corresponded to ethanol yields of 96.8%, 75.9%, and 91.9%, respectively (Table 3). The lowest ethanol concentration obtained in the SSF configuration was attributed to the low glucose concentration. Long exposure at non-optimum temperatures contributes to enzyme deactivation (Kristensen *et al*. 2009), which was confirmed by the low glucose concentration in the media available for ethanol production.

Table 3. Ethanol Concentration, Hydrolysis, and Ethanol Yield in Different Configurations: SHF (at 24 h fermentation), SSF (at 72 h fermentation), and PSSF (at 24 h fermentation)

Exp. No.	Final Ethanol (g/L)					Ethanol Yield ($g_{ethanol}/g_{glucose}$) - (%)[c]		
	Process Configurations							
	SL[a]	*EL*[b]	*SHF*	*SSF*	*PSSF*	*SHF*	*SSF*	*PSSF*
1		15	31.7 ± 0.42	8.5 ± 0.64	31.2 ± 0.54	0.49 (95.7)	0.39 (75.8)	0.49 (96.1)
2		20	32.0 ± 0.57	9.2 ± 0.31	31.7 ± 0.99	0.48 (94.6)	0.39 (76.5)	0.48 (94.9)
3		25	32.6 ± 0.21	9.5 ± 0.64	32.1 ± 0.92	0.49 (95.5)	0.38 (74.3)	0.48 (95.0)
4		15	40.4 ± 0.68	16.7 ± 0.28	39.2 ± 0.81	0.49 (96.7)	0.40 (77.9)	0.48 (93.5)
5		20	41.2 ± 0.39	17.3 ± 0.49	40.5 ± 0.77	0.49 (95.7)	0.39 (76.4)	0.48 (93.7)
6		25	43.7 ± 1.18	18.0 ± 0.71	42.7 ± 0.3	0.50 (97.5)	0.38 (75.0)	0.48 (94.4)
7		15	49.2 ± 0.35	22.9 ± 1.28	46.2 ± 0.35	0.49 (95.5)	0.39 (76.7)	0.46 (90.2)
8		20	50.6 ± 0.92	23.7 ± 1.13	48.5 ± 0.71	0.48 (94.1)	0.38 (75.1)	0.46 (91.0)
9		25	53.7 ± 0.35	25.9 ± 1.34	50.3 ± 0.42	0.49 (96.8)	0.39 (75.9)	0.47 (91.9)

[a] SL: Solids loading (%); [b] EL: Enzyme loading (FPU/g glucan); [c] (%): Percentage of the theoretical yield (0.51 $g_{ethanol}/g_{glucose}$)

Comparing the three process configurations, the ethanol concentrations were higher in the SHF and PSSF configurations, which was attributed to the better fermentation

performance in the separate process, because in the SSF configuration the difference between the optimal temperatures of both processes (hydrolysis and fermentation) affected the cellulose hydrolysis rate and caused carbon limitation in fermentation at lower temperatures. In contrast, it could affect the activity of cellulolytic enzymes, thus slowing down the metabolism of the microbial strain at higher temperatures. Both approaches resulted in a reduction of productivity and a lower or non-existent ethanol production.

Figure 1 shows the glucose consumption and the ethanol production kinetics during the fermentation stage in the SHF configuration at different solids loadings (15%, 20%, and 25% and 25 FPU/g glucan). The glucose was consumed before 10 h with solids loadings of 15% and 20%. However, at a 25% solids loading, total glucose consumption occurred at 12 h. The maximum ethanol production obtained was 53.7 g/L in experiments conducted with a 25% solids loading and 24 h of incubation (without noticeable increase after 12 h). Other studies reported ethanol production using different agave species residues as feedstock, such as Hernández-Salas *et al*. (2009), Saucedo-Luna *et al*. (2011), Caspeta *et al*. (2014), and Rios-González *et al*. (2017), in which final ethanol concentrations of 6.6 g/L, 24.68 g/L, 64 g/L, and 65.2 g/L, respectively, were reported when pretreating with NaOH, diluted H_2SO_4, organosolv and ionic liquid, and autohydrolysis, respectively. The difference in ethanol production was attributed to the cellulose content of the agave species and type of pretreatment method.

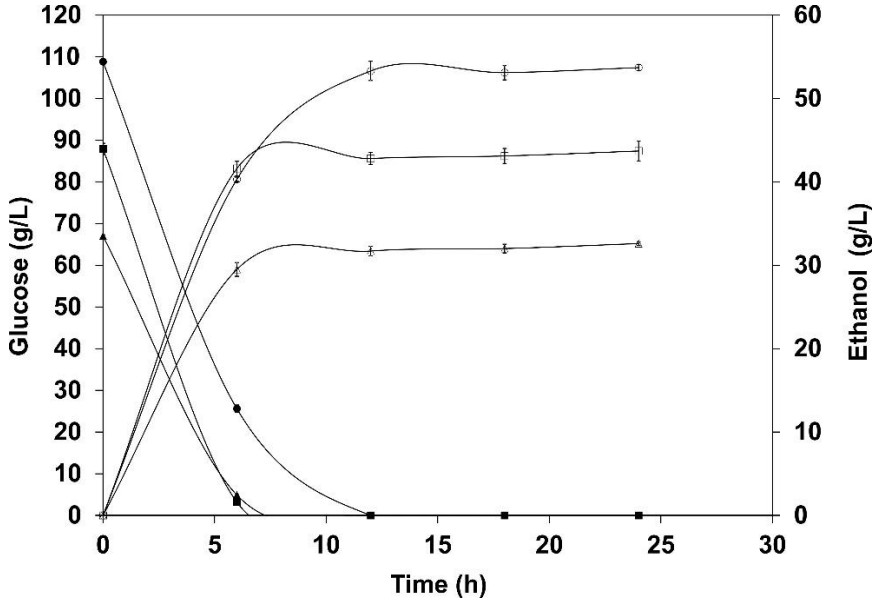

Fig. 1. Kinetics of glucose consumption (filled markers) and ethanol production (unfilled markers) during the fermentation stage of the SHF configuration at different solids loadings (w/w): 15% (▲), 20% (■), and 25% (●), and 25 FPU/g glucan

Figure 2 shows the kinetics of the SSF configuration at different solids loadings and 25 FPU/g glucan. The ethanol concentrations obtained at 72 h were 9.5 g/L, 18 g/L, and 25.9 g/L at 15%, 20%, and 25% of solids loading, respectively. The results indicated that increased solids loading led to higher glucose concentrations, reaching the maximum glucose concentrations after 24 h for the different solids loading assessed. Glucose was not detected in the simultaneous process at 72 h for the solids loadings assessed. A 12 h lag was observed in the ethanol production, which can be attributed to yeast adaptation and

propagation as reported by Neves *et al*. (2016). The SSF configuration usually achieves an ethanol yield in the range of 60% to 85% according to different reports, regardless of the feedstock, enzyme complex, or pretreatment method used (García-Aparicio *et al*. 2011; López-Linares *et al*. 2014; Pérez-Pimienta *et al*. 2017). The results obtained in the present study were in agreement with the aforementioned reports.

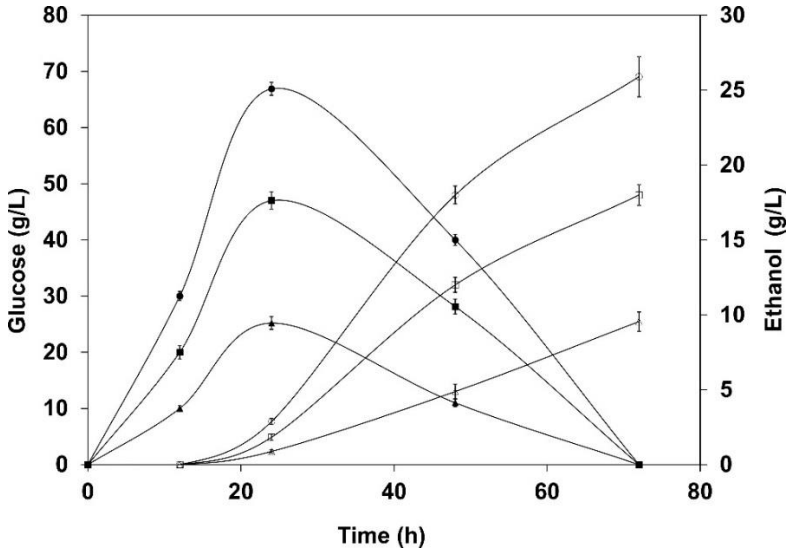

Fig. 2. Kinetics of glucose production and consumption (filled markers) and ethanol production (unfilled markers) in the SSF configuration at different solids loadings (w/w): 15% (▲), 20% (■), and 25% (●), and 25 FPU/g glucan

To assess the PSSF configuration, a prehydrolysis was performed after 24 h, followed by the SSF operation at 15%, 20%, and 25% solids loadings and 25 FPU/g glucan. Figure 3 shows that after 24 h of prehydrolysis, the maximum glucose concentrations were 66.3 g/L, 88.7 g/L, and 107.2 g/L at 15%, 20%, and 25% solids loadings, respectively.

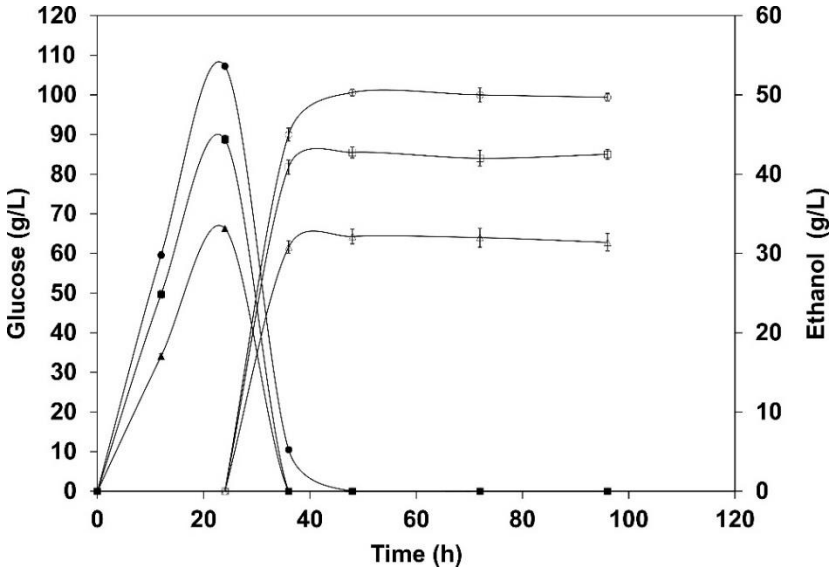

Fig. 3. Kinetics of glucose production and consumption (filled markers) and ethanol production (unfilled markers) in the PSSF configuration at different solids loadings (w/w): 15% (▲), 20% (■), and 25%, (●) and 25 FPU/g glucan

It was observed in all experiments that after inoculation, the glucose was rapidly consumed and the ethanol concentration increased, which achieved the highest ethanol concentrations of 32.1 g/L, 42.7 g/L, and 50.3 g/L at 15%, 20%, and 25% solids loadings, respectively. No additional increment in ethanol concentration was detected after 24 h. However, the maximum ethanol production and total glucose consumption after inoculation was achieved after more time when compared to the SHF configuration. This can be attributed to the fact that yeast was subjected to stress conditions due to high solids loading (López-Linares *et al*. 2014).

CONCLUSIONS

1. Comparing the SHF and PSSF configurations, the ethanol concentrations and productivity achieved at 36 h (saccharification plus fermentation time) were 53.7 g/L and 1.49 g/L h^{-1} and 45 g/L and 1.25 g/L h^{-1}, respectively.
2. In contrast, the SSF configuration exhibited the lowest achieved ethanol concentration and productivity at the same time (10.4 g/L and 0.29 g/L h^{-1}, respectively).
3. The results obtained in the present work show that the SHF configuration can be considered the best alternative using the Cellic® CTec3 enzyme complex.
4. This enzyme complex allowed for a high hydrolysis yield with the lower enzyme dosage assessed in this study (15 FPU/g glucan).
5. A lower enzyme requirement is a relevant factor for operation cost reduction when scaling-up the ethanol production process for *A. lechuguilla*. The PSSF configuration can be an attractive alternative if the initial investment and reduction in the number of required stages of the process are taken into account.

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