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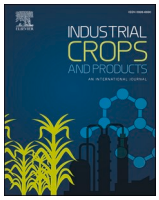

Ethanol production from *Agave tequilana* leaves powder by *Saccharomyces cerevisiae* yeast applying enzymatic saccharification without detoxification

Jorge Carlos Avila-Gaxiola [a,1], Evangelina Avila-Gaxiola [b,*,1]

[a] Facultad de Ciencias de la Tierra y el Espacio, Mexico
[b] Facultad de Ciencias Químico-Biológicas, Mexico

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ABSTRACT

The replacement of fossil resources with renewable biomass in a bioeconomy is seen as a major contribution to climate change mitigation. The transformation from a petrochemical-based economy to a bio-based economy necessitates the novel exploitation of cost-effective natural materials for both future biorefinery development and a range of value-added products of interest. The present investigation proposes the use of *Agave tequilana* Weber leaves, an agro-industrial residue with a huge potential to produce liquid biofuels. The objective of the present work is to evaluate the alcoholic fermentation by *S. cerevisiae* yeast in powdered *A. tequilana* leaves (dry-mill,100 °C, diameter ≤ 300 μm) pretreated with two enzymatic saccharification processes without detoxification and determine the highest yield bioconversion of sugars to ethanol. Alcoholic fermentation was evaluated using yeast at different times (0−42) h with an initial concentration of 34.06 ± 0.4 g/L reducing sugars. *S. cerevisiae* has the highest ethanol production 12.20 ± 0.3 g/L within 18 h obtained an ethanol yield of 0.41 g/g (81% of theoretical value), and volumetric ethanol productivity 0.68 ± 0.02 g/L/h. Yeast was able to consume the 86.4% reducing sugars and increase to 17.2-fold cell concentration in the presence of 80.30 ± 0.70 mg/L phenolic compounds. This biotransformation of waste has great potential and significant prospects for wider industrial and biotechnological applications, the results show the feasibility and efficiency to produce ethanol, is a clean source of energy and offers a solution for countries that produce agave or similar feedstocks. It is firmly believed by the author that, due to the large amounts of waste produced by the tequila industry, the best solution for this problem does not lie in this paper or implementation of a single treatment. On the contrary, a mix of some of the alternative treatments presented in other works would probably represent the most efficient option, from both an economic and environmental point of view.

1. Introduction

Fossil fuel reserves are showing a decrease and are strongly associated with negative environmental impacts. The demand for fossil fuels is constantly increasing, actually representing 80% of the primary energy consumed in the world (EIA, 2021). Therefore, it is important to research alternative materials that can replace fossil fuels and resolve the major issues of pollution. So, it is necessary to focus on the use of renewable, sustainable, efficient, and cost-effective energy resources with lesser emissions to make the world energy matrix sustainable (Ali et al., 2019; He et al., 2010; Singh et al., 2010). One source of renewable energy production is biomass, which can be converted into biofuels. Biofuels have emerged as one of the most strategically important sustainable fuel sources and are considered an important way of progress in reducing greenhouse gas emissions, improving air quality, and finding new energy resources. The main advantages of biofuels include their biodegradable and renewable properties; the generation of employment and technical development in rural areas; decentralized production from locally available domestic biomass; besides the combustion of biomass feedstock has been considered as carbon neutral or low-carbon fuel since the plant crops assimilate carbon dioxide from the atmosphere during the growth (Ali et al., 2019; Demirbas, 2009; Lal, 2005). The economical and societal transformation from fossil-based to the biomass-based economy (bioeconomy), will be implemented in many countries, this transformation considers sustainable bioeconomy in the development of flexible and integrated biorefineries to produce

* Corresponding author.
E-mail address: evangelina_gaxiola@uas.edu.mx (E. Avila-Gaxiola).
[1] Universidad Autónoma de Sinaloa, Av. de las Américas y Blvd. Universitarios, Cd. Universitaria, Culiacán, Sinaloa, México. 161154.

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biofuels and bioproducts from biomass sources. One of the relevant objectives is the development of technologies in biochemical conversion processes for the reuse, recycling, and restoration of biomass materials and products (Delzeit et al., 2021; Manzanares, 2020). Therefore, is necessary more research in biomass characterization and the development of technologies efficient and environmentally friendly for the conversion of biomass into bioproducts of industrial interest to fulfill the diverse needs of society.

Bioethanol is one of the most important biofuels due to its positive impact on the environment, the easy adaptability of this fuel to existing engines with a higher octane rating than gasoline (Antunes et al., 2018; Grad, 2006; Wheals et al., 1999). One of the most promising processes for producing ethanol is by microbial fermentation of lignocellulosic biomass. For commercial production of ethanol, the yeast *Saccharomyces cerevisiae* is one of the most used due to its ability to ferment sugars (Mohd Azhar et al., 2017; Nanda et al., 2018).

Lignocellulosic materials are among the most important resources for biorefineries to produce fuels, chemicals, and materials in such a way to substitute in part the role of petrochemistry in modern society. Lignocellulose is a complex mixture main composed of cellulose, hemicellulose, and lignin that needs an efficient pretreatment to make accessible pathways to enzymes for the production of fermentable sugars and avoiding the formation of phenolic and furans compounds related to an inhibitory effect in enzymatic activity and the fermentative process of ethanol. Furans as the 2-furaldehyde (furfural) and 5-hydroxymethylfurfural (HMF) are considered the most representative and inhibitory toxic compounds for the fermentative capacities of yeasts. Furfural significantly reduces cell proliferation, ethanol production and inhibits several enzymes (Palmqvist and Hahn-Hägerdal, 2000; Taherzadeh and Karimi, 2011; Ximenes et al., 2010; Zha et al., 2012). Pretreatment technologies are constantly being developed to improve the technical and economic utilization of lignocellulose in biorefineries for ethanol production. Pretreatment technologies differ in their mode of action and their effects on different lignocellulosic materials (Silveira et al., 2015).

This study proposes the use of leaves of *Agave tequilana* Weber variety Blue, an agro-industrial residue, as a viable attractive alternative for a renewable feedstock to liquid biofuels production and other compounds of industrial interest. Actually, the tequila industry demands 1.4 million tonnes of head *Agave tequilana* Weber blue, producing a similar amount of leaves that can be used as a source of energy (CRT, 2021). This agro-industrial residue represents 38% of total plant weight, is a rich source of polysaccharides such as fructans, cellulose, hemicellulose, and monosaccharides (Arrizon et al., 2010; Avila-Gaxiola et al., 2017; Iñiguez-Covarrubias et al., 2001). Fructans and monosaccharides present in agave leaves represent an advantage as feedstock compared with other agro-industrial residues (e.g., corn (*Zea mays L.*) husk, rice (*Oryza sativa L.*) husk, wheat (*Triticum aestivum L.*) straw, among others), that does not contain these carbohydrates. In a previous study realized in agave leaves (Avila-Gaxiola et al., 2017, 2018) were used treatment of dry-mill (dried at 100 °C, diameter $\leq$ 300 μm) for subsequent enzymatic hydrolysis were effective in the conversion of polysaccharides to reducing sugar and minimized formation of inhibitory compounds. Most of the studies for bioethanol production by microbial fermentation are realized in the head agave because it represents the main raw material of the tequila industry. In the case of the agave leaves few works have been realized for conversion to ethanol by microbial fermentation. Recent studies used the juice from the agave leaves subject to thermal acid and enzymatic hydrolysis to obtain ethanol using the *S. cerevisiae* yeast, it has been found that yeast did not grow in the hydrolysates from *A. fourcroydes* (Villegas-Silva et al., 2014). However, in other reports the *S. cerevisiae* fermented *A. tequilana* leaf juice with a 66% of theoretical yield (Corbin et al., 2015) and for *A. tequilana* leaf bagasse and juice, using acid pretreatment and enzymatic hydrolysis with a theoretical ethanol yield of 68% and 61% respectively (Rijal et al., 2016). It is worth mentioning that the agave leaves, there is still a lack of a systematic study for the production of ethanol by alcoholic fermentation using microorganisms. The objective of this study was to evaluate the alcoholic fermentation of sugar obtained of *A. tequilana* leaves treated with dry-mill and subsequent enzymatic saccharification without detoxification process using *S. cerevisiae* yeast to determine the highest yield of bioconversion of sugars to ethanol.

2. Materials and methods

2.1. Material

Leaves of *Agave tequilana* Weber plants were collected eight years after planting in crops from Culiacan, Sinaloa, and México. The samples were cleaned, the impurities from the environment in the agricultural field were removed using 1% chlorine solution, and the spines were cut. They were stored at a temperature of 12 °C ± 2 °C for a minimum of 24 h before drying treatment.

2.2. Drying and milling treatment

The leaves were sliced in a thickness of 1.0 mm using a slicer machine (Hobart, 1612E, USA), the thickness was verified with a vernier caliper (Uchida, M0–1, Japan). The slices were drying at 100ºC for 30 min ± 1 min in a convective oven, to later be reduced size with a blade mill (Pulvex, México, D.F., México). The powder was placed on the sieve number 50 to achieve a particle diameter $\leq$ 300 μm (Avila-Gaxiola et al., 2017). This dry-mill treatment increases the reaction surface of the material, increasing the extraction of sugars, improving the hydrolysis of polysaccharides as fructans and holocellulose in the material. This treatment minimizes the contamination of the material, by reducing the microbiological activity and the deterioration of the raw material.

2.3. Characterization agave leaves powder

The chemical composition in the samples was determined by proximal analysis according to AOAC methods (AOAC, 2012); ash (923.05), moisture (925.09), crude fiber (962.09), protein (979.09), and carbohydrates were figured by taking the difference of the other compounds. Hydrogen potential (pH) in the powder was obtained following the methodology AOAC 943.02 using a potentiometer (Hanna, HI 2211, México). Water activity (a_w) in the sample was measured with a hygrometer (AquaLab CX-2, Decagon, USA). The quantification of lignin content in the powder was performed following the Klason method. The holocellulose, cellulose, and hemicellulose content were determined following the methodology proposed by Wise et al. (1946). The details of the methods with minimal changes, as described (Avila-Gaxiola et al., 2018). Analyzes were realized in triplicate.

2.4. Quantification of fructans, reducing sugars and sucrose content

Fructans and sugars obtained from agave leaves powder used an extraction with distilled water according to Avila-Gaxiola et al. (2017) for afterward analysis.

Fructans were quantified in the aqueous extract of agave leaves powder with the enzymatic method (Avila-Gaxiola et al., 2018). The reducing sugars liberated of hydrolyzed fructans by commercial enzyme Inulinase from *Aspergillus niger* or Fructozyme L (Novozymes, Bagsvaerd, Denmark) were determined by the HPLC method.

The quantification of reducing sugars and sucrose content in the aqueous extraction of agave leaves powder were performed by HPLC equipment (Agilent Technologies, 1220 infinity LC, USA) with a refractive index detector and a 300 mm × 7.8 mm Aminex HPX-87 C (Biorad, Hercules, CA, USA) column at 50 °C. Samples were filtered using nylon membranes with a porosity of 0.45 μm (Millipore, SLHN033NK Millex, México) and the volume injected was 20 μL. Glucose (purity $\geq$ 99%), fructose (purity $\geq$ 99%), sucrose(purity $\geq$

99%), and arabinose (purity ≥ 99%), was used as standards and purchased from Sigma-Aldrich (St. Louis, MO, USA). Samples were realized in triplicate.

2.5. Enzymatic treatment

The enzymatic treatments and the conditions for the hydrolysis applied to the agave leaves powder to obtain sugars of fructans and lignocellulosic material were selected based on previous studies (Avila-Gaxiola et al., 2017, 2018) considering the conversion yield in reducing sugars. The aqueous extract was prepared with agave leaves powder and distilled water to obtain a 10% (w/v) concentration and stirred (Eppendorf, thermomixer comfort, Hamburg, Germany) at 60 °C, 37 rad s^{-1} for 30 min. Enzymatic hydrolysis of fructans was realized with the commercial enzyme Inulinase or Fructozyme L (Novozymes, Bagsvaerd, Denmark) using an enzyme dose of 0.02% (v/v) with respect to aqueous extract of agave leaves powder. The enzymatic reaction was carried using a stirrer (Eppendorf, Thermomixer comfort, Hamburg, Germany) at 50 °C, 37 rad s^{-1} for 24 h. The solid residue from agave leaves powder obtained after aqueous extraction was hydrolyzed using commercial enzyme Cellic CTec-2 (Novozymes, Bagsvaerd, Denmark) with an enzyme dose of 1% (v/w), the reaction was realized on a stirrer (Eppendorf, Thermomixer comfort, Hamburg, Germany) at 50 °C, 37 rad s^{-1} for 18 h. Sugars were identified and quantified by high-performance liquid chromatography (HPLC). Samples were realized in triplicate. The extracted agave leaves obtained from all treatments were used for evaluating the alcoholic fermentation using *S. cerevisiae* yeast.

2.6. Fermentation

2.6.1. Microorganism

The alcoholic fermentation of the sugar obtained from agave leaves treated without detoxification process was realized using commercial *Saccharomyces cerevisiae* strain Ethanol Red (Fermentis, France). Yeast was grown in yeast peptone dextrose (YPD) agar (Sigma-Aldrich, St. Louis, MO, USA) at 30 °C and 250 rpm. This yeast was stored at − 80 °C in a YPD medium with 20% (v/v) glycerol until they were required.

2.6.2. Inoculum preparation

Inoculums were transferred to each 250 mL Erlenmeyer flasks containing 50 mL of sterile YPD agar (Sigma-Aldrich, St. Louis, MO, USA), and pH was adjusted to 4.5. The cultures were incubated for 18 h in an orbital shaker (250 rpm, 30 °C). After incubation, the concentration of yeast was determined by cell count using a Neubauer chamber.

2.6.3. Fermentation process

Fermentation was performed in an Erlenmeyer flask of 500 mL with a working volume of 85 mL of sterile extract agave leaves as a culture medium. Culture media were inoculated aseptically with *S. cerevisiae* yeast to an initial concentration of 11.5×10^6 cells per mL with initial pH of 4.5. The flasks were covered with cotton caps and incubated for 42 h in an orbital shaker (100 rpm, 30 °C). Samples were taken at (0, 3, 6, 12, 18, 24, 30, 36, and 42) h of alcoholic fermentation. During fermentation were determined sugars consumption, ethanol production, and yeast growth.

2.6.4. Fermentation parameters

The reducing sugars conversion (%) was calculated as a ratio of reducing sugars consumed to the initial reducing sugars concentration. The yield of ethanol to reducing sugars consumed (g/g) was defined as the ratio of ethanol concentration to the reducing sugars consumed. The efficiency of reducing sugars conversion to ethanol (%) has been estimated by the ratio of ethanol yield to the theoretical value of ethanol yield (0.511 g/g). The ethanol volumetric productivity (g/L/h) was calculated as the ratio of ethanol concentration (g/L) at the end of the run to the fermentation time (h) at the highest ethanol concentration.

2.7. Quantification of phenolic and furan content

The determination of phenolic and furan compounds in the aqueous extract of agave leaves powder for the alcoholic fermentation process was carried out by high-performance liquid chromatography (HPLC; Agilent Technologies, 1220 infinity LC, USA) equipped with an Agilent ZORBAX Eclipse Plus C18 column (250 mm × 4.6 mm×5 μm). Samples were filtered using nylon membranes with a porosity of 0.45 μm (Millipore, SLHN033NK Millex, México) and the volume injected was 20 μL. Furfural (purity ≥ 99%), hydroxymethylfurfural (purity ≥ 99%), 2-furoic acid (purity ≥ 98%), hydroquinone (purity ≥ 99%), 4-hydroxybenzaldehyde (purity ≥ 98%), pyrocatechol (purity ≥ 99%), phenol (purity ≥ 99%), 4-hydroxybenzoic acid (purity ≥ 99%), vanillic acid (purity ≥ 97%), syringic acid (purity ≥ 95%), 4-hydroxyacetophenone (purity ≥ 99%), vanillin (purity ≥ 99%), acetovanillone (purity ≥ 98%), acetosyringone (purity ≥ 97%), and coniferyl aldehyde (purity ≥ 98%), were used as standards and purchased from Sigma-Aldrich (St. Louis, MO, USA). Samples were realized in triplicate.

2.8. Gas chromatography ethanol quantification

The quantification of ethanol content in the extract of agave leaves powder was performed by gas chromatography (GC; Hewlett Packard; Agilent Technologies, HP 6890, USA) coupled to a Hewlett Packard (Agilent Technologies, HP 7694E, USA) equipped with Agilent HP-INNOWax capillary column (60 m × 0.25 mm × 0.25 μm). The sample volume injected was 2 mL. Ethanol (Sigma-Aldrich, St. Louis, MO, USA, purity ≥ 99%) was used as standard. Samples were analyzed in triplicate.

2.9. Statistical analysis

For the statistical examination of data for each response variable was employed analysis of variances ANOVA for one factor. The means were compared using the least significant difference test LSD with $p < 0.05$.

3. Results and discussion

3.1. Characterization of agave leaves powder

Agave leaves are mainly composed of carbohydrates 52.2 ± 1.1% and crude fiber 33.5 ± 1.4% on a dry basis (Table 1), the values were according to the literature (Avila-Gaxiola et al., 2017, 2018) make it an agro-industrial residue potential, renewable and low-cost material as a good source of carbon for the production of biofuel. The 3.1 ± 0.3% moisture in the agave leaves powder was low obtained several advantages; kept microbiologically stable during storage, and reduced the cost of transport due to reduction of moisture content. The pH of agave leaves powder has a value of 4.8 ± 0.1 is within the optimum pH range of *S. cerevisiae* 4.0–5.0 for survival and growth, representing an adequate

Table 1
Chemical composition, pH, and water activity (a_w) of agave leaves powder.

Compounds	Content
Moisture (%)	3.1 ± 0.3
Carbohydrates (%)	52.2 ± 1.1
Crude Fiber (%)	33.5 ± 1.4
Ash (%)	6.6 ± 0.4
Protein (%)	3.4 ± 0.2
Lipids (%)	1.4 ± 0.2
pH	4.8 ± 0.1
a_w	0.32 ± 0.03

The value represents mean ± standard deviation.

medium for the fermentation process (Lin et al., 2012). The a_w in agave leaves powder was 0.32 ± 0.03, this value is below of critical level reported ($a_w < 0.6$) that will reduce enzymatic and microbiological activity avoiding the contamination of material, physical and chemical changes are minor under this condition (Rahman and Labuza, 1999), therefore the material is stable for storage.

Polysaccharides as fructans, holocellulose, and reducing sugars in dry basic (Table 2) are carbohydrates detected in agave leaves powder with 69.8 ± 1.0% and lignin with a 15.9 ± 0.6%. The non-structural carbohydrates represent 52.2 ± 1.0% of total carbohydrates while the rest 17.6 ± 0.5% correspond to structural carbohydrates. Fructans are the main carbohydrates with 37.1 ± 1.1% in material, this polysaccharide makes agave leaves a good prospect as a source of carbon for biofuel production. Fructans and reducing sugars in agave leaves make it an attractive material with respect to other agro-industrial residues (e.g., corn (*Zea mays L.*) husk, rice (*Oryza sativa L.*) husk, wheat (*Triticum aestivum L.*) straw, among others), that do not contain these carbohydrates. Polysaccharides present in agave leaves can be bioconvert into reducing sugars and fermented by microorganisms to produce ethanol. The major constraint in bioconversion of agave leave to ethanol is to apply an adequate treatment for lignocellulose and fructans hydrolysis for producing reducing sugars and avoiding the formation of inhibitory compounds in enzymatic activity and yeast for the fermentation process.

The concentration of reducing sugars from agave leaves powder in aqueous extract untreated, enzymatic hydrolysis, and the solid residue treated with enzyme are shown in Table 3. The powder agave leaves treated with enzyme for hydrolyzed fructans in the aqueous show a higher concentration of sugars ($p < 0.05$) in 2.4-fold with respect to sample untreated. This result shows the enzymes were compatible with material obtaining a high conversion yield of 97% to reducing sugars between the hydrolysis of fructans and lignocellulose of solid residue. Comparable to previous studies that found 62% of the efficiency of enzymatic hydrolysis for *A. tequilana* juice (Ávila-Fernández et al., 2009), another report maximum yield of 69% of enzymatic saccharification of *A. tequilana* leaves (Rijal et al., 2016). The good yield in this study of enzymatic saccharification of polysaccharides was obtained because the agave leaves previously treated with drying and milled, show various benefits for releasing reducing sugars, making accessible pathways to enzymes hydrolysis, during this process the presence of furfural and hydroxymethylfurfural compounds were not detected (Table 4), indicating the that did not produce degradation of reducing sugars. Phenolic compounds detected in the extract of agave leaves powder for alcoholic fermentation were 4-hydroxybenzoic acid (61.8 ± 2.5) mg L^{-1}, vanillin (14.5 ± 0.3) mg L^{-1}, 2-furoic acid (1.8 ± 0.4) mg L^{-1}, acetovanillone (1.2 ± 0.3) mg L^{-1}, pyrocatechol (1.0 ± 0.2) mg L^{-1}, respectively. These compounds are related to a possible alteration of the physical structure and chemical composition of lignin, this could facilitate the enzymatic hydrolysis of polysaccharides. The dry-mill and enzymatic treatment used in the agave leaves were workable, efficient, clean processes, avoided degradation of reducing sugars and did not produce the formation of inhibitory compounds for yeast fermentation.

Table 2
Carbohydrates content (non-structural and structural) and lignin in agave leaves powder.

Compounds	Content (% dry matter)
Non-structural carbohydrates	
Reducing sugars	15.1 ± 1.0
Fructans	37.1 ± 1.1
Structural carbohydrates	
Holocellulose	17.6 ± 0.5
Lignin	15.9 ± 0.6

The value represents mean ± standard deviation.

Table 3
Reducing sugars concentration of aqueous extract untreated, enzymatic hydrolyzed and solid residue of agave leaves powder enzymatic hydrolyzed.

Treatment	Reducing sugars (% dry matter)
Aqueous extract	
Untreated	15.1 ± 1.0[a]
Enzymatic hydrolysis Fructozyme	36.2 ± 1.2[b]
Solid residue	
Enzymatic hydrolysis Cellic CTec-2	16.8 ± 1.0[a]

The value represents mean ± standard deviation, different superscripts letters within the column indicate significant differences ($p < 0.05$) by LSD test.

Table 4
Phenolic and furan compounds of the extract obtained of agave leaves powder for the alcoholic fermentation process.

Compounds	Concentration (mg/L of extract)
4-Hydroxybenzoic acid	61.8 ± 2.5
Vanillin	14.5 ± 0.3
2-Furoic acid	1.8 ± 0.4
Acetovanillone	1.2 ± 0.3
Pyrocatechol	1.0 ± 0.2
Hydroxymethylfurfural	ND
Furfural	ND
Hydroquinone	ND
4-Hydroxybenzaldehyde	ND
Phenol	ND
Vanillic acid	ND
Syringic acid	ND
4′-Hydroxyacetophenone	ND
Acetosyringone	ND
Coniferyl aldehyde	ND

The value represents mean ± standard deviation. ND: Not detected.

3.2. Cell growth and reducing sugars consumption

The kinetic cell growth and reducing sugars consumed during alcoholic fermentation by *Saccharomyces cerevisiae* yeast in the extract obtained of agave leaves powder enzymatic hydrolyzed without detoxification process is reported in Fig. 1. The yeast was capable of growth and consuming reducing sugars in the extract containing 34.06 ± 0.4 g/L reducing sugars and 80.30 ± 0.70 mg/L phenolic compounds (Table 4), this shows the extract agave obtained of treatment was adequate and without inhibitory effect for *S. cerevisiae*. This result agrees with that reported for cause inhibition in the yeast for alcoholic fermentation, are requires at least 4-hydroxybenzoic acid (1.0 g/L), vanillin (0.5 g/L), 2-furoic acid (4.5 g/L), of phenolic compounds

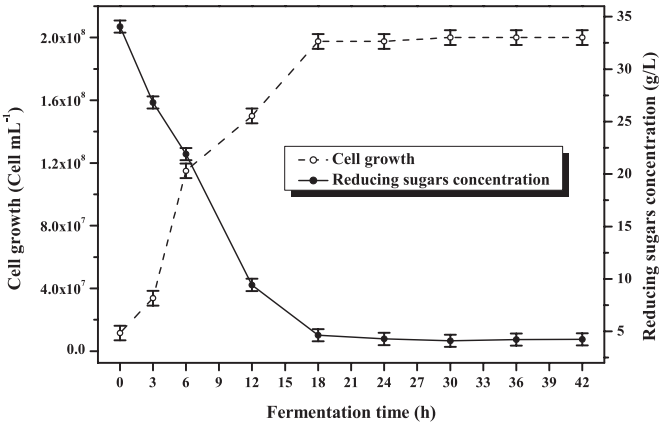

Fig. 1. Kinetic cell growth and reducing sugars consumed during alcoholic fermentation with *Saccharomyces cerevisiae* yeast in the extract obtained of agave leaves powder enzymatically saccharified. The point indicates the mean and vertical bars represent the significant difference ($p < 0.05$) by the LSD test.

respectively (Palmqvist and Hahn-Hägerdal, 2000; Taherzadeh and Karimi, 2011; Ximenes et al., 2010; Zha et al., 2012). The highest cell growth of yeast was during the first 18 h fermentation process, obtained cell concentration of (19.8 ± 0.4) × 10^7 cells per mL, representing a significant increase ($p < 0.05$) of 17.2-fold with respect to the cell concentration initial of process. The results show that the exponential growth phase of the yeast was during the first 18 h, it was observed that the highest consumption of reducing sugars ($p < 0.05$) reach up to 86%, for after initial with the stationary growth phase. *S. cerevisiae* had a preference for consuming glucose during the first 3 h of the fermentation process (Fig. 2). After the yeast co-ferment, the consumption of sugars increased significantly ($p < 0.05$) for glucose and fructose with 91% and 80%, respectively.

3.3. Ethanol production

The ethanol production and reducing sugars consumed were monitored during 42 h of alcoholic fermentation using *S. cerevisiae* yeast in the extract obtained of agave leaves powder enzymatic hydrolyzed without detoxification process shown in Fig. 3. Ethanol concentration and the reducing sugar consumption show a significant increase ($p < 0.05$) until at 18 h of the alcoholic fermentation process, after this period it remained unchanged indicating the stationary growth phase for yeast. The highest ethanol production was at the 18 h of fermentation process with 12.20 ± 0.3 g/L ethanol concentration. These results show that the agave extract was appropriate for *S. cerevisiae* yeast because it was able to grow, consume reducing sugars, and produce ethanol without requiring a detoxification process. The phenolic compounds detected at a concentration of 80.30 ± 0.70 mg/L phenolic compounds (Table 4) in the extract of agave leaves did not show an inhibitory effect for yeast.

3.4. Fermentation parameters

Table 5 shows the parameters obtained for the optimal fermentation time of 18 h using *S. cerevisiae* in the extract obtained of agave leaves powder and enzymatic hydrolyzed. The yeast was capable of consuming the 86.40 ± 0.20% of reducing sugars for convert to ethanol with a rate of 1.64 ± 0.01 g/L/h and increase the yeast growth to (19.80 ± 0.40) × 10^7 cells per mL. The result show the yeast was efficient for converting sugars into ethanol with a yield of 0.41 g/g corresponding to 81.12 ± 2.00% with respect to the theoretical ethanol yield and the

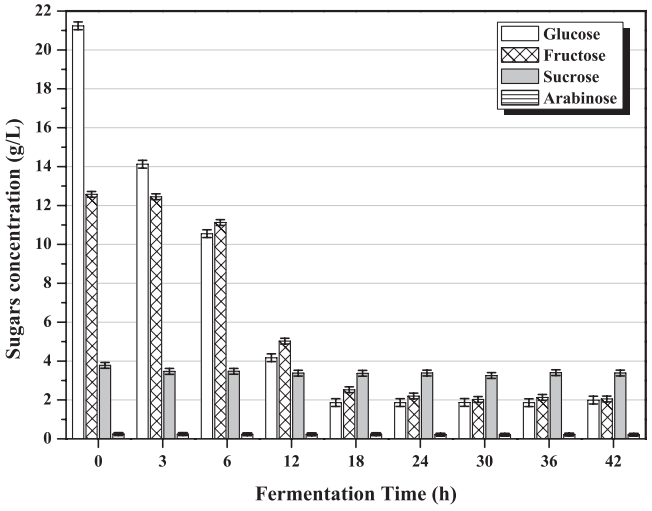

Fig. 2. Sugars consumed during alcoholic fermentation with *Saccharomyces cerevisiae* yeast in the extract obtained of agave leaves powder enzymatically saccharified. The columns indicate the mean and vertical bars represent the significant difference ($p < 0.05$) by the LSD test.

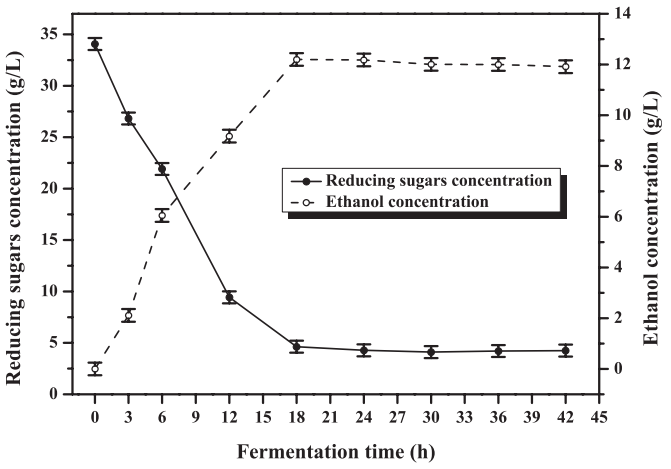

Fig. 3. Kinetic ethanol production and reducing sugars consumed during alcoholic fermentation with *Saccharomyces cerevisiae* yeast in the extract obtained of agave leaves powder enzymatically saccharified. The point indicates the mean and vertical bars represent a significant difference ($p < 0.05$) by the LSD test.

Table 5
Parameters obtained of the alcoholic fermentation process using *Saccharomyces cerevisiae* yeast in the extract obtained of agave leaves powder enzymatic hydrolyzed.

Parameters	*S. cerevisiae*
Optimal fermentation time (h)	18
Consumption reducing sugars (g/L)	29.43 ± 0.40
Residual reducing sugars (g/L)	4.63 ± 0.40
Reducing sugars conversion (%)[1]	86.40 ± 0.20
Reducing sugars consumption rate (g/L/h)	1.64 ± 0.01
Cell growth (Cell/mL)	(19.80 ± 0.40) × 10^7
Ethanol (g/L)	12.20 ± 0.30
Ethanol yield[2]	0.41 ± 0.01
Conversion efficiency with respect to maximum theoretical ethanol yield (%)[3]	81.12 ± 2.00
Volumetric ethanol productivity (g/L/h)[4]	0.68 ± 0.02

The value represents mean ± standard deviation.
[1] g reducing sugars consumed / g reducing sugars initial.
[2] g ethanol production maximum / g reducing sugars consumed.
[3] g ethanol production maximum × 100 / g reducing sugars consumed × 0.511.
[4] g ethanol production maximum/optimal fermentation time.

volumetric ethanol productivity of 0.68 ± 0.02 g/L/h. The alcoholic fermentation efficiency obtained in this study was higher than those reported for hydrolysates from *A. fourcroydes* leaf juice, subject to thermal acid and enzymatic hydrolysis, in which the *S. cerevisiae* yeast is not able to grow (Villegas-Silva et al., 2014). In other work the *S. cerevisiae* fermented *A. tequilana* leaf juice with 66% of the theoretical yield (Corbin et al., 2015), and for *A. tequilana* leaf bagasse and juice, subject to acid pretreatment and enzyme saccharification obtained a conversion to ethanol of 68% and 61% respectively (Rijal et al., 2016), our result is also the higher yield of conversion to ethanol. These results show that the dry-mill treatment and subsequent enzyme saccharification were effective for *A. tequilana* leaves obtaining a higher ethanol yield by fermentation using *S. cerevisiae* yeast and therefore improvement of the production of sustainable liquid biofuel.

4. Conclusions

Lignocellulosic biorefinery is of global commercial interest, a lot of studies have been realized in different types of biomass sources to evaluate the biological production of biofuels or other biochemical

products. Overall wood and agricultural residues seem to be competitive from an economic and environmental point of view. The agave leaves are a promising feedstock for biofuel and biochemical production in arid regions, agave can be grown in unfavorable conditions which do not support food crop production. Agave leaves are an agro-industrial residue potential, renewable, not competitive with food and feed crops, and low-cost material as a good source of the production of sustainable ethanol by alcoholic fermentation using *Saccharomyces cerevisiae* yeast.

Highlighting the proposal of novel pretreatments, combined saccharification, and fermentation processes to improve productivity and reduce costs for the entire process. The main finding in this study is the high ethanol yield of 0.41 g/g after two enzymatic saccharification processes. In this study, the results show that the dry-mill treatment and subsequent enzyme hydrolysis were effectively for bioconversion of carbohydrates of agave leaves to ethanol by fermentation using *Saccharomyces cerevisiae* yeast, with an 81% with respect to theoretical value and volumetric ethanol productivity of 0.68 g/L/h. These treatments were sustainable environments and not required of detoxification step for alcoholic fermentation with yeast.

One of the essential and costly stages of the pretreatment process is the enzymatic saccharification, the study implies that the results can serve to direct further research efforts and investment towards the most promising pretreatments that can be developed and scale up these pretreatments to a full-scale process.

Ethanol obtained represent one alternative and renewable energy source for a viable solution that reduce dependence on fossil fuels and mitigate climate change by reducing greenhouse gas emission. Therefore, agave leaves, treatments, and alcoholic fermentation by *Saccharomyces cerevisiae* yeast used in this work for conversion to ethanol could be considered as a potential prospect to produce sustainable biofuels, taking into consideration other lignocellulosic residues to develop regional industries of diverse raw materials.

CRediT authorship contribution statement

Jorge Avila-Gaxiola: Conceptualization, Methodology, Software, Validation, Formal analysis, Investigation, Data curation, Writing – original draft, Writing – review & editing, Supervision. **Evangelina Avila-Gaxiola**: Conceptualization, Methodology, Software, Validation, Formal analysis, Investigation, Resources, Data curation, Writing – original draft, Writing – review & editing, Visualization, Supervision, Project administration, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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