Biomass Conversion and Biorefinery
https://doi.org/10.1007/s13399-020-00619-y

ORIGINAL ARTICLE

Agave bagasse response to steam explosion and anaerobic treatment

Bernd Weber[1] · Andrea C. Sandoval-Moctezuma[1] · Andrea Estrada-Maya[1] · Iván G. Martínez-Cienfuegos[1] · Ma. Dolores Durán-García[1]

Received: 2 October 2019 / Revised: 20 December 2019 / Accepted: 21 January 2020

Abstract

Global aspirations for more sustainable development and governmental agreements at the international level have incentivized the tequila industry, an important producer of beverages in Mexico, to look for greener production schemes. The main by-product, agave bagasse, generated during production has not been handled properly up to now, even though it could be a low-cost resource to cover energy requirements in facility. In this context, this study aims to reduce this waste product in a process combination of steam explosion and anaerobic digestion to find a more sustainable solution for agave bagasse management. At the laboratory scale, it is demonstrated that the steam explosion pretreatment can accelerate hydrolysis of agave bagasse; however, the methane yield of 235 $mL_N\ g_{VS}^{-1}$ for the steam-exploded substrate was only 11% higher than that of the bioconversion of the untreated lignocellulosic material. The process combination proposed in this study is able to concentrate 49% of the original energy content in the biogas.

Keywords Thermochemical conversion · Lignocellulosic fiber · Biogas · Tequila industry

1 Introduction

Semisolid organic wastes are generated in huge quantities in common food production schemes. At a glance, they present a potential to produce added value products or to serve as an energy carrier available on-site. However, the latter is hard to access due to moisture associated. Thus, prior energy-consuming drying steps are needed to make semisolid wastes valuable in combustion systems [1, 2]. Obviously, a decision to implement treatment strategies for semisolid waste processing is mainly based on economic criteria. The simple alternative to give wastes generated away should be regulated more rigorously by the government to encourage on-site processing. The alternatives for on-site processing of semisolid organic wastes should be analyzed conscientiously because biomass firing systems are roughly 4 to 5 times more expensive than steam boilers fired with liquid of gaseous fuels [3, 4]. However, recent studies have shown that biomass firing systems represent an attractive return on investment, especially when biomass is available on-site free of cost [5, 6]. As a result of the biomass combustion process, biomass bed ash and fly ash have distinct chemical compositions. While the bed ash may be disposed of at low-cost because there are some applications for reuse [7–9], the fly ash is often enriched in heavy metals and contains toxic organic compounds like polycyclic aromatic hydrocarbons [10]. Additionally, dioxins may emerge from de novo synthesis when heavy metals are present [11]. Thus, depending on a nation's regulations, fly ash management may be a serious problem. To look for alternative processing of semisolid wastes, this study is focused on the objective of evaluating how much semisolid wastes from a tequila factory can be reduced in a process combination of

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s13399-020-00619-y) contains supplementary material, which is available to authorized users.

✉ Bernd Weber
bweber@uaemex.mx

Andrea C. Sandoval-Moctezuma
andrea.smu02@gmail.com

Andrea Estrada-Maya
aem_@outlook.es

Iván G. Martínez-Cienfuegos
igmartinezc@uaemex.mx

Ma. Dolores Durán-García
mddg_2210@hotmail.com

[1] Faculty of Engineering, Universidad Autónoma del Estado de México, Cerro de Coatepec s/n, Toluca, Mexico

Published online: 03 February 2020

steam explosion and anaerobic digestion with the benefit to obtain biogas.

The main solid wastes generated in tequila industry are the remaining crushed agave heads (lignocellulosic fibers) following juice extraction of inulin in diffusors or hydrolyzed inulin in roller mills. About 1.4 kg of dry agave bagasse is generated during the production of 1 L of tequila [12]. Recently, various investigations have dealt with alternative uses of agave bagasse, e.g., built composite materials or some uses in the agricultural sector [13], or its integration into a biorefinery scheme [12, 14]. The agave bagasse is mainly composed of fibers with a length of 0.5 to 12 cm and a thickness ranging from 0.2 to more than 0.85 μm [15]. The mineral content is between 3 and 7%. Besides the extractive content, which depends on the processes applied by the tequila industry, carbohydrates (hemicellulose and cellulose) and lignin are in the range of 10–22%, 31–43%, and 10–20%, respectively. Such a composition leads to a net calorific value of about 16.5 MJ kg^{-1} [16]. Similar to other fibers like sugarcane bagasse, the biodegradation of agave bagasse in an anaerobic digestion without a pretreatment is slow, resulting in a low methane yield (stated as milliliter methane per gram of volatile solids (VS)). In the literature, biochemical methane potential (BMP) for sugarcane bagasse is reported to be 85 mL_N CH_4 $g_{VS}{}^{-1}$ [17]. Gomez-Guerrero et al. [18] report a BMP for two different agave species of 194 and 324 mL_N CH_4 $g_{VS}{}^{-1}$ when inoculated with granular sludge. An important impact on BMP is the quantity of remaining sugars in the fibers, which depends on the technology used for sugar extraction in tequila industry [19]. In contrast, sugarcane bagasse is commonly Brix-free since sugar processing is realized in more profit-orientated large-scale facilities.

As such, investigations are searching for solutions to make hemicellulose and cellulose in lignocellulosic structure available for faster biological degradation with the aim to produce bioalcohols [20, 21], biomethane [14, 22], or biohydrogen [23, 24]. For these processes, the applied pretreatments are similar, e.g., some are aiming to remove lignin using different organic solvents [25], alkaline solutions, or by oxidative reactions with ozone or hydrogen peroxide. In some pretreatments with parallel or consecutive steps, hemicelluloses and cellulose are hydrolyzed. In the steam explosion treatment, the cell structure is disintegrated mechanically and modified chemically [26, 27]. Steam explosion takes place at 150–250 °C [28], with temperatures > 180 °C forcing hydrolysis of hemicellulose and solubilization of lignin compounds. Most of the biogas produced is converted into electricity on-site, which offers thermal excess heat at adequate temperature level to run a steam explosion pretreatment. Further, anaerobic digestion is capable of producing exocellular enzymes for the biodegradation of biomass. This investigation determines the degradation of agave bagasse in such a process combination.

2 Methodology

The proposed process combination of steam explosion treatment and anaerobic digestion is shown in Fig. 1. The agave bagasse was fed into a static pressure reactor, where solid and liquid phases are produced through steam explosion, and subsequently were converted into biogas in an anaerobic digester. Batch operation of the steam explosion process is mandatory at full-scale and in this laboratory experiment. In this study, the anaerobic digestion is simulated in a batch test; however, for full-scale applications, a continuous operation is preferable as shown in the process scheme. The carbon balance of biomass conversion steps, also shown in the figure, is discussed further below.

2.1 Characterization of agave bagasse and pretreated bagasse

Agave bagasse was obtained from a tequila factory located in the municipality of Amatitán, Jalisco (20° 50′ 09.7″ N 103° 43′ 52.7″ W) in December 2017. The agave bagasse was dried in a solar tunnel to reduce natural degradation during storage. The moisture and ash contents of the agave bagasse were determined according to NREL/TP-510-42621 and NREL/TP-510-42621, respectively. To determine the gross calorific value (GCV), the sample was pelletized as cylinders with a diameter of 10 mm and height of about 12 mm. Then the pellets were incinerated in a Parr 6200 calorimeter according to ASTM D240 using a metallic crucible. Benzoic acid was used as a reference material for the calorific value control. Chemical composition of the agave bagasse was described by determination of extractives, hemicellulose, cellulose, and lignin according to Van Soest and Wine [29]. CNS elemental composition was determined with a vario EL cube microcombustion analyzer from Elementar Analysensysteme, Langenselbold, Germany. Optical microscopy was realized with a fluorescent stereomicroscope MZ16F from Leica Microsystems, Wetzlar, Germany. Scanning electron microscopy (SEM) was realized with a JSM-IT100 from JEOL Ltd., Japan. Energy-dispersive spectroscopy (EDS) was realized with a Bruker system mounted on the SEM. To determine the color of untreated agave bagasse and steam-exploded agave bagasse, a grinded sample was formed into a pellet with a pressure of 130 N mm^{-2}. From the pellets, a picture was taken with a Leica V-Lux digital camera (Type 114) from Leica Camera AG, Wetzlar, Germany. The optical density of the pellets was measured with a densitometer X-Rite

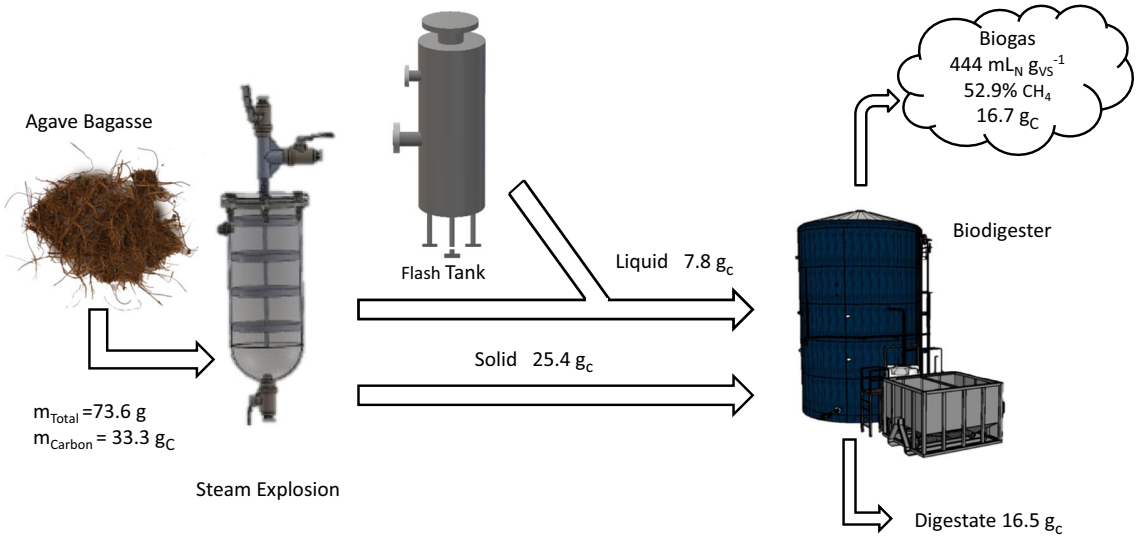

Fig. 1 Process combination of steam explosion and anaerobic digestion used for the biomethanation of agave bagasse. Carbon transfer of the different process streams is indicated for favorable operation conditions at 0.98 MPa

500 series from X-Rite company, Grand Rapids, Michigan, and the result was given as CIELAB color space (CIE L* a* b*).

2.2 Steam explosion treatment

The steam explosion of agave bagasse was realized in a stainless steel vessel with a total volume of 1.64 L. Before filling the vessel with biomass, the reactor walls where preheated for 10 min with steam. A cover of half-inch thick Armaflex® heat insulation foam reduced heat losses over the surface. The pressure reactor was then filled with 80 g (73.6 g dry mass) agave bagasse. The size distribution as obtained from the roller mill (fibers 0.5–8 cm long) was not modified for the experiments. Saturated steam was produced in a 3.5″ tube mounted in a parabolic solar concentrator with a pressure of at least 0.2 MPa higher than the chosen pressure for the experiment. The steam was injected into the pressure reactor at 0.38, 0.68, and 0.98 MPa absolute pressure (corresponding to 142, 164, and 179 °C), maintained manually by adjusting the valve. Pressure release was realized after 2 min 48 s, 6 min, or 22 min depending on the number of expansions chosen. The pressure release was done by opening a valve connected to a 25-L flash tank. After 2 min release time, one, three, or five pressure cycles were applied for a total of 24 min reaction time. Such reaction conditions correspond to severity factors ranged from 2.4 to 3.7 according Eq. (1), which is based on the algorithm proposed by Chum et al. [30].

$$\mathrm{SF} = \mathrm{Log}_{10}(R_0) = \mathrm{Log}_{10}\left(t \cdot e^{\left(\frac{T-100}{14.75}\right)}\right) \quad (1)$$

After the reaction, the pressure reactor was purged by opening a valve at the bottom of the pressure reactor. The extract was drained, centrifuged (1150×*g*, 10 min), and stored at − 20 °C for further analysis. The moisture of the pretreated biomass was further reduced from 75 to 60% by mechanical dewatering and then dried overnight in an oven at 60 °C to obtain the dry mass, a parameter necessary for mass and energy balances. This experimental design was realized in triplicate. After characterization of liquid and solid products of each treatment, the samples were merged for biological tests with a reduced number of samples.

2.3 Batch assay for determination of biological methane potential

The efficiency of bioconversion into biogas of the different hydrolysates was determined by batch tests according to VDI 4630 in standard 500-mL glass bottles. A local gaffer adapted an additional connection for septum, where the gas samples were taken during experiment. The assay was started with an inoculum: substrate ratio of 2:1 and incubated at 36 °C. Each biological test was done in triplicate. The dry matter concentration of substrate selected for the batch test was 5 g L^{-1}. The blank samples contained only inoculum. Cellulose filter paper was used as a positive control. Granular sludge from an anaerobic digester that treats brewery effluents was used as inoculum. The granular sludge, with a total solid concentration of 9%, was acclimatized for 3 days before starting the test. Before incubation, the pH was adjusted to a minimum alkalinity of 3 g L^{-1} by adding sodium carbonate. Temperature control and horizontal shaking at 90 rpm were performed in an LSI-3016R incubator (Lab-Tech Instruments, Korea). Biogas production was sampled daily using an eudiometer; ten milliliters of the biogas collected in the eudiometer was sampled with a glass syringe and injected into a gas cell for measurement in the FTIR spectrometer (Shimadzu IR Prestige). The

gas cell, with a total volume of 65 mL, was purged with a small vacuum pump for 1 min to make the cell ready for the next measurement. The equipment was calibrated using pure methane and pure carbon dioxide. The BMP is the maximum methane yield obtainable under specific conditions, which is reached in the case of solid substrates when the biogas production is lower than 0.5% of the accumulated biogas for three consecutive days. After this stop criterion, the flasks were opened for pH control and determination of remaining soluble chemical oxygen demand (COD) in the digestate.

2.4 Mass and energy balances

The mass balance of the steam explosion treatment was based on the initial mass of agave bagasse with moisture content in equilibrium with ambient laboratory conditions. This initial mass is split into three phases through steam explosion treatment. The total solid content (TS) and content of volatile solids (VS) of the solid and liquid phases were determined with the same procedure as mentioned for the substrate. The relation between the TS found in liquid and solid phases to the initial mass is defined as the recovery rate. The recovery rate is calculated according Eq. (2) using the following parameters: initial mass filled into the pressure reactor (m_{initial}), moisture of the substrate (ζ_{H2O}), dry mass of the residual solid product (m_{residual}), density of the extract (δ), drained extract (Vdrained), squeezed extract (Vsqueezed), and the TS content of the extract.

$$\omega = \frac{m_{\text{residual}} + \text{TS} \cdot \delta \cdot \left(V_{\text{drained}} + V_{\text{Squeezed}}\right)}{m_{\text{initial}} \cdot \left(1 - \zeta_{\text{H}_2\text{O}}\right)} \tag{2}$$

The difference between quantified products to the initial mass is attributed to incomplete recollection of solids from the pressure reactor, incomplete recollection of the liquid phase from the expansion tank (note: 300 mL of liquid was found in a 25-L bottle), loss of organic matter as vapor from the expansion tank, dehydrating reactions, and formation of non-condensable gases.

2.5 Statistical analysis

Data provided in both the table and the text with an error range represent the mean value and standard deviation of the conversion and bio-digestion experiments, each of which were carried out in triplicate. ANOVA and non-parametric tests were used to identify the optimum operation conditions for the transfer of organic matter into the liquid phase as well as the potential for methanogenic fermentation. Experimental data were statistically analyzed at a 5% significance in a least significant difference (LSD) test.

3 Results and discussion

3.1 Mass balance of the steam explosion process

The transformation of organic matter during different steam explosion conditions is shown in Table 1. Water content in the substrate used for steam explosion treatments was the difference of mass used in the experiment (80 g) and the initial mass (73.6 g) mentioned in the table. Mineral content of the pretreated bagasse fibers did not change significantly in comparison with the initial mineral matter of 5.1%. Consequently, the yields of TS and VS in the liquid phase were similar. The VS/TS relation in the liquid phase ranged from 0.932 to 0.934. It seems that steam explosion does not solubilize much additional mineral matter as this is already achieved during the production stage when cooking agave heads at temperatures between 90 and 120 °C and at low pH [31]. For example, in Fig. 6b, a mineral incrustation in the agave fiber is visible. Contrary to the mineral matter, the organic matter experiences a significant mass reduction. The effect of pressure and temperature is stronger than that of the number of expansions. Statistical analysis applying multiple comparisons with LSD classifies the factor pressure into three subsets and the factor number of expansions into only two subsets. In addition, the severity factor (SF) describes the impact of applied conditions accordingly. When a pressure of 0.98 MPa was applied with five expansions, the highest mass reduction of the solid phase (> 30%) was observed. Previous investigations attribute this mass reduction to hydrolysis of extractives, hemicelluloses, but also lignin [28].

The mass recovery of data presented in Table 1 was 0.93 ± 0.037 without any correlation to the applied treatments. The occurrence of mass recovery in steam explosion experiments is not well-discussed in literature. Laser et al. [32] report an overall mass recovery of 90%, an observation similar to this investigation. The distribution of present mass loss to the mentioned factors is done by estimation. The literature does not report on mass loss in the steam explosion process due to the formation of non-condensable gases and is also scarce for other thermal hydrolysis treatments. However, patents [33] and technical applications, like the Cambi® process [34], consider the liberation of non-condensable gases to downstream anaerobic digesters in their plant design. It is assumed that the contribution of non-condensable gases mainly composed of CO_2 without a share to energy balance is 0.6%. Other mass losses are estimated to be 5% in solid phase, 1% in liquid phase, and 0.6% organic matter, which escapes from the flash tank with uncondensed vapor. These mass corrections are considered in balance of constituents, biogas potential of products, and further energy balances.

Table 1 Mass transfer of organic and mineral matter during different steam explosion treatments applied to agave bagasse

	Number of expansions	SF	Solid phase Y_{Ash} (g (%))	Solid phase Y_{VS} (g (%))	Liquid phase Y_{TS} (g (%))	Liquid phase Y_{TS} (g (%))
Initial	-	-	3.8 (5.1)	69.8 (94.9)	-	-
	1	2.58	4.0 (6.6 ± 0.2)	62.3 (84.7 ± 2.3)	4.9 (6.6 ± 2.2)	4.6 (6.2 ± 2.1)
0.38 MPa	3	2.50	3.8 (5.2 ± 0.3)	58.6 (79.7 ± 0.9)	6.2 (8.5 ± 2.1)	5.9 (7.9 ± 1.9)
	5	2.40	3.8 (5.1 ± 0.2)	57.9 (78.7 ± 0.6)	7.2 (9.8 ± 0.2)	6.8 (9.2 ± 0.1)
	1	3.23	3.5 (4.8 ± 0.2)	54.3 (73.7 ± 0.7)	5.8 (7.9 ± 1.4)	5.4 (7.4 ± 1.3)
0.68 MPa	3	3.14	3.6 (4.9 ± 0.2)	55.6 (75.6 ± 3.9)	7.2 (9.8 ± 1.2)	6.8 (9.1 ± 1.1)
	5	3.03	3.5 (4.7 ± 0.2)	53.4 (72.5 ± 3.8)	8.2 (11.1 ± 0.9)	7.7 (11.1 ± 0.9)
	1	3.67	3.3 (4.4 ± 0.2)	50.1 (68.1 ± 1.5)	11.3 (15.4 ± 0.3)	10.6 (14.3 ± 0.2)
0.98 MPa	3	3.58	3.3 (4.5 ± 0.2)	55.6 (69.7 ± 5.5)	10.6 (14.4 ± 0.2)	9.9 (13.5 ± 0.3)
	5	3.47	3.1 (4.2 ± 0.2)	47.9 (65.1 ± 1.4)	10.1 (13.7 ± 0.7)	9.4 (12.8 ± 0.7)

3.2 Balances of constituents in agave bagasse throughout the steam explosion process

The elemental composition of the agave bagasse was 45.2% carbon, 0.34% nitrogen, and 0.05% sulfur. Under the most severe conditions, the treatment solubilizes about 7.8 g of carbon, which is transferred mainly in the liquid phase (Fig. 1). Because the main constituents of the agave bagasse are carbohydrates, there was no noticeable change in the carbon concentration (45.7%) through hydrolysis of these constituents in the steam-exploded agave bagasse. The first extraction step in fiber analysis according to Van Soest and Wine [29] releases neutral detergent solubles (remaining sugars, proteins, lipids, and minerals). These components, called extractives, are easily metabolized in anaerobic digesters, with the exception of the inorganic fraction. In the substrate, the extractives have a mass of 20.3 g and a concentration of 27.6% (Fig. 2). Through the steam explosion process, mainly remaining sugars are separated from this fraction. However, proteins, which need more severe conditions for hydrolysis according to Weber et al. [35], did not solubilize. The obtained extract had a dark brown color, a pleasant smell, and a pH of 3.56 ± 0.2. The COD ranged from 16 to 32 g L^{-1} and the main components were sugars [19]. The COD yield (3–17 g_{COD} per 100 g of substrate) ranged even wider because at higher pressure, more liquid is obtained. The experimental setup of two factors at three levels was used to draw a response surface for the COD yield, which is presented in Fig. 7 (Supplementary material).

With an increase in pressure of the steam explosion treatment, the mass of extractives in the solid phase reduces from 21.0 ± 1.2 g for the treatment with 0.38 MPa down to 18.5 ± 2.1 g for the treatment with 0.98 MPa. This reduction is visible in Fig. 2 but is not significant. The second step of fiber analysis quantifies acid detergent fiber (ADF), which are mainly the hydrolyzed hemicelluloses. Before treatment, the hemicellulose is 17.3 g (23.5%), and for the most severe treatment (0.98 MPa with one expansion), the hemicellulose is reduced to 10.0 ± 2.9 g. Thus, about 41% of the hemicellulose is removed by steam explosion. It seems the more labile hemicelluloses, like arabinan, are converted and more stable hemicelluloses, like galactans and xylans, hydrolyze at a lower rate, as reported in other investigations [36, 37]. Statistical analysis of the hemicellulose fraction showed that the treatments with 0.98 MPa are significantly different from treatments only applying 0.38 or 0.68 MPa. The cellulose fraction is removed

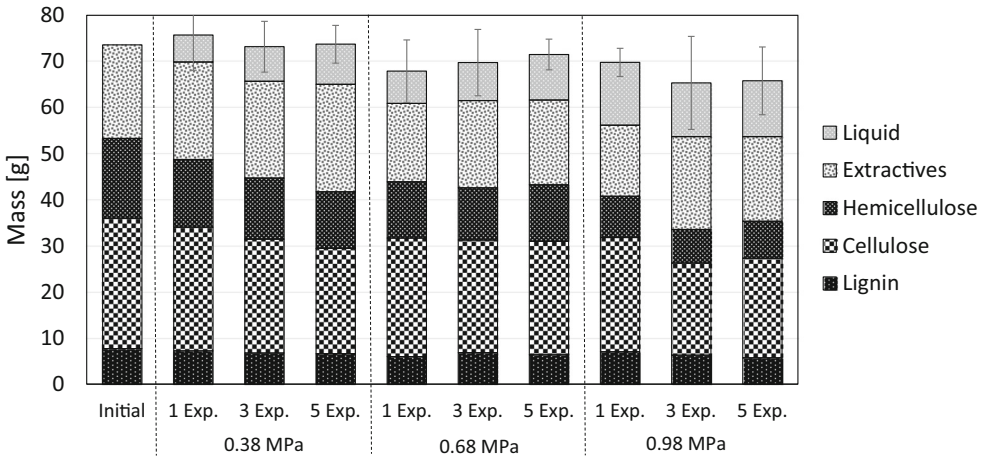

Fig. 2 Composition of solid phase (according to Van Soest and Wine analysis) and the liquid phase formed through steam explosion for substrate (untreated agave bagasse) and treatments with one expansion applied

only slightly, from 28.3 g of substrate down to 23.2 ± 3.7 g. Additionally, lignin is removed through steam explosion only slightly, from 7.7 to 5.8 ± 0.5 g. The literature reports that lignin dissolution in hydrothermal treatments under a neutral pH initiates at 180 °C [38]. Apparently, under the conditions in the present study, with a maximum temperature of 180 °C, the lignin degradation still is not perceptible. In summary, the steam explosion treatment applied to agave bagasse shifts mainly extractives and hemicellulose to a liquid phase, rich in organic matter. In the next chapter, the biodegradability in an anaerobic environment is investigated.

3.3 Biodegradation of agave bagasse and products obtained through steam explosion in batch assays

Biogas/methane production from the extracts is reported in a previous study [19]. There, it was demonstrated that in the batch assay for the determination of BMP, the specific methane yield reached 290 mL_N CH_4 per g_{COD} added and the average methane concentration was 53.9%. The COD removal in the batch assay was 89%. Thus, it is possible to treat such a stream in a high-rate anaerobic digester. However, an alternative is the anaerobic digestion of the liquid and solid phases in a suspended matter digester (e.g., a continuous-stirred tank reactor), a strategy studied here. In the biological batch assay, a control (only granular sludge) and a positive control (cellulose) were run parallel to the treatments. The specific biogas production of the cellulose was 687 mL_N biogas g_{VS}^{-1}, quite close to the theoretical yield of 745 mL (VDI 4630), confirming good practice of the conducted experiment. In Fig. 3a, cumulative biogas formation of the three analyzed treatments is shown. This graph also includes the contribution of organic matter converted to biogas from the previous experiment, where the liquid phase was the exclusive point of interest [19]. It is worth mentioning that about 28% of that liquid substrate is still impregnated in the solid phase after mechanical dehydration, an argument to justify the combination of data from two experiments. The final biogas production (444 ± 43 mL_N g_{VS}^{-1}) from pretreated agave bagasse under 0.98 MPa and three expansions is 21% higher than the biogas release from the untreated agave bagasse (364 mL_N g_{VS}^{-1}). The average methane concentration for each variation in the batch assay were 70.8% for the blank sample (endogenous respiration of protein-rich biomass); 52.5% for cellulose; 56.6% for agave bagasse without pretreatment; 51.0% for 0.38 MPa with three expansions; and 52.7% for 0.98 MPa with three expansions. Thus, the methane yields for the three substrates were 206 ± 16 mL_N g_{VS}^{-1} (untreated agave bagasse), 216 ± 36 mL_N g_{VS}^{-1} (0.38 MPa with three expansions), and 235 ± 23 mL_N g_{VS}^{-1} (0.98 MPa with three expansions). In the literature, the methane yield of agave bagasse is only reported for co-digestion with granular sludge as 323 mL_N g_{VS}^{-1} for *Agave tequilana* var. azul and

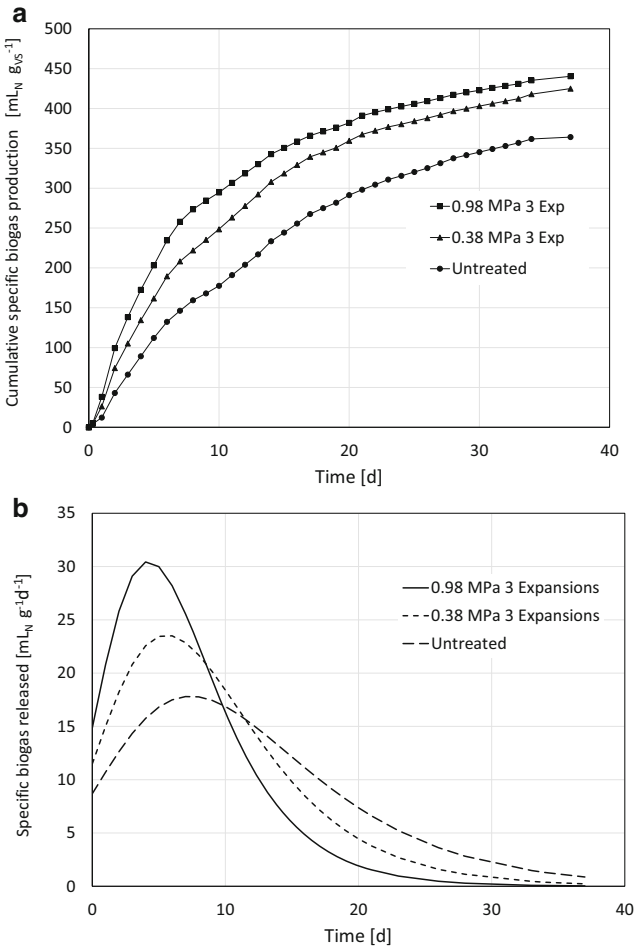

Fig. 3 **a** Specific accumulated biogas production obtained in liquid and solid phase batch assays realized with untreated agave bagasse and the steam explosion treatments (0.38 MPa and 0.98 MPa both with three expansions realized). **b** Derivative of Gompertz function (velocity of biogas release) obtained from modeling with cumulative production data from Fig. 3a

193 mL_N g_{VS}^{-1} for *Agave angustifolia* [18]. Comparatively, Croce et al. [39] report methane yields of 138 mL_N g_{VS}^{-1} for rice straw, 225 mL_N g_{VS}^{-1} for corn stover, and 330 mL_N g_{Vs}^{-1} for wheat straw. The methane yield of Brix-free sugarcane bagasse is reported as 70–90 mL_N g_{VS}^{-1} [17, 40] and 139 mL_N g_{VS}^{-1} for Miscanthus [41]. In most cases, a pretreatment of the mentioned substrates gives higher methane yields than feeding digesters directly with untreated biomass. The data extracted from Schroyen et al. [41] and Croce et al. [39] to analyze how much pretreatments increase methane yields can be summarized as application of steam explosion pretreatment results in an average increase of 26.7% with a standard deviation of ± 26%. For other pretreatment technologies applied, including steam explosion, the increase is 28 ± 33%. Thus, in some cases a pretreatment is not beneficial for higher methane yield.

Pretreatment of agave bagasse for subsequent anaerobic digestion may be advantageous because of the accelerated

conversion of organic matter into biogas [39]. A parameter used by other authors to describe degradation velocity is the relation of specific biogas yield at the 7th day of batch test to the final yield [42]. This parameter increased from 40% in the untreated agave bagasse to 49% and 58% for the pretreatments with 0.38 MPa and 0.98 MPa, respectively. The kinetic model for methanization based on the Gompertz equation showed maximum biogas release for the three treatments at days 7.5, 6, and 4 (Fig. 3b). Maximum biogas release for the treatment 0.98 MPa with three expansions is 1.7 times more than the maximum release obtained during bio-digestion of agave bagasse without a pretreatment.

It must be mentioned that the wide C:N relation, far over 40, is a reason for reduced digestibility in continuous operation of the digester. In the batch assay realized with an inoculum:substrate rate of 2:1, such a growth inhibition due to nutrient absence cannot be revealed. Likewise, in the tequila industry, the co-digestion of agave bagasse to shift the C:N relation with protein-rich vinasses is always a given.

3.4 Mass balance of anaerobic digestion and energy recovery in biogas

The biogas yield of cellulose used as the positive control is almost twice than that in the different bagasse samples. Consequently, some organic matter (dissolved or suspended) must remain at the end in the batch assay using agave derivatives as substrate. One of these remaining components must be lignin, which is degraded very slowly under anaerobic conditions [43, 44]. Given that mainly the extractives hemicellulose and cellulose are hydrolyzed and transformed into biogas with a methane concentration of about 50%, the residual mass estimated by applying stoichiometric balances under the most favorable conditions (0.98 MPa with three expansions) has to be 35.4 (48.0% of input). Assuming that the lignin fraction is not degraded, the 5.5 g of lignin present before anaerobic digestion remain, making up 15.6% of the residual digestate. Additionally, 3.1 g (8.8%) of the material is attributable to the mineral content of the agave bagasse. Thus, the residual mass is 26.8 g (75.8%) organic matter, which must be mainly cellulose. The same calculation for digestion of untreated agave bagasse results in 45.3 g (62% of input) of residual organic matter. In addition, it was visually observed that the amount of residual fibers at the end of the batch experiment was greatly reduced for the steam-exploded treatments in comparison with the untreated agave bagasse.

In the 0.98 MPa treatment with three expansions, carbon transfer occurred as 16.7 g of carbon was released with the biogas, and consequently, the difference of 16.5 g forms part of the digestate (Fig. 1). Thus, carbon makes up 47% of digestate.

The soluble COD in the digestate after the batch assay was 402 ± 79 mg L^{-1} (about 0.38 g_{VS} L^{-1}), which is almost 8% of the organic matter added to the batch assay. Such circumstances show that the limiting factor in the anaerobic degradation process is the hydrolysis of suspended organic matter, in this case cellulose. However, this cellulose in agave bagasse is not degraded as fast as the cellulose used for the positive trail. The reason for the slow degradation of cellulose in agave bagasse is found in the different crystallinity and/or that fibers still are not disintegrated through steam explosion treatment to make them susceptible to enzymatic attack.

The anaerobic digestion of energy crops, manures, and other wastes in continuous-stirred tank reactors is common practice in Europe, especially in Germany. This kind of technology requires hydraulic retention times of about 100 days for the mentioned substrates [45]. In comparison, the batch test was realized with only a duration of 37 days; therefore, a higher methane yield under continuous digestion is realistic. However, the steam explosion process could also be improved, at least to solubilize the total of the hemicellulose fraction and increase disintegration of hollow cells.

The GCV of a biogas with a methane concentration of 52.7% is 20.9 MJ m^{-3}. Taking into account mass balance for the most favorable pretreatment, an energy recovery of 49% is balanced. With the corresponding GCV of the untreated biomass, the energy recovery is 45%, slightly lower. Comparing these results with biomass combustion systems, the overall efficiency is lower. However, biogas has to be considered as a high-end fuel with the potential to realize combined heat and power generation on-site. It is worth mentioning that the GCV of the digestate is enriched due to a higher lignin concentration, which still represents a product with an energy value and is easier to handle than original agave bagasse.

3.5 Microscopic analysis of agave bagasse, steam-exploded agave bagasse, and digestate

After the batch assay, it was possible to separate some remaining agave fibers from the granular sludge. Thus, it was possible to analyze the agave fibers by optical and scanning electron microscopy at three different process stages: untreated agave bagasse, steam-exploded agave bagasse, and digestate of the treatments. First, photographs were taken from the untreated agave bagasse, agave bagasse after 0.38 MPa with three expansions, and the more severe treatment at a pressure of 0.98 MPa with three expansions (Fig. 4a–c). According to the literature, agave fibers exhibit a median diameter of about 0.7 mm [15]. In Fig. 4a–c, the bundle structure of hollow fibers is visible. These hollow fibers are composed of various shells of different composition. The external layer of lignin protects the internal layers composed of hemicellulose and cellulose, mostly in the formation as microfibrils [46]. The untreated agave bagasse has a more glossy surface giving way to a more harsh surface with increasing

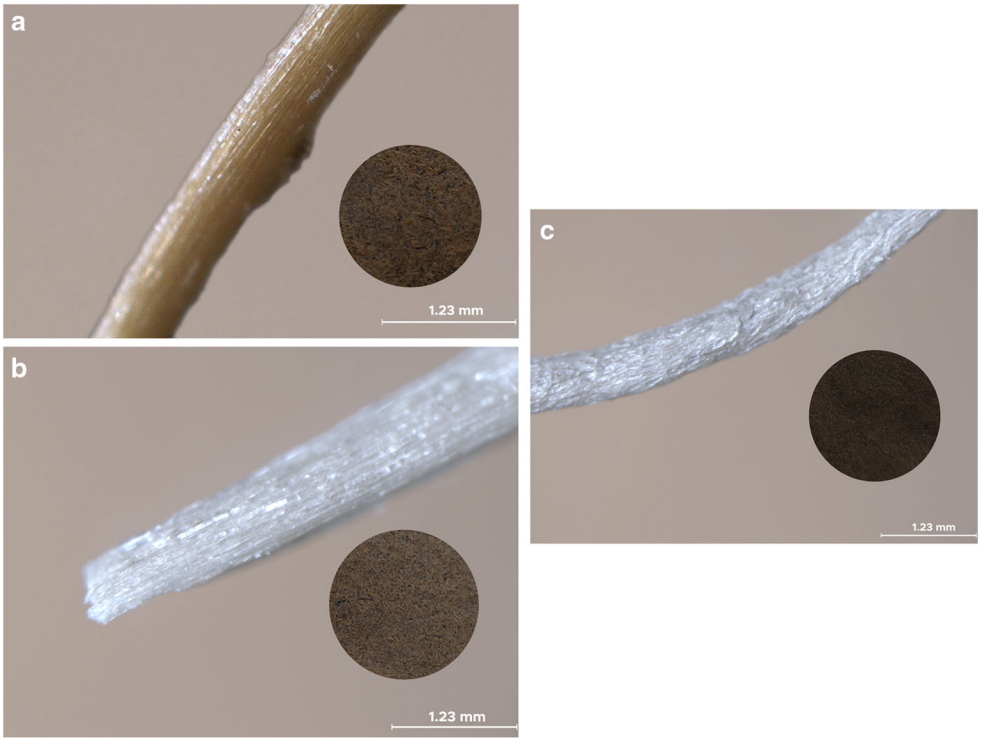

Fig. 4 Optic microscopy with a magnification × 63 of untreated agave bagasse and two treatments (0.38 MPa and 0.98 MPa both with three expansions), from left to right (**a**, **b**, **c**). The disc shows how a pellet of these fibers appears under daylight

severity of the treatment. The color of fibers (shown as a disc in the figure) changes noticeably to darker brown for the treatment applying 0.98 MPa in comparison with both the milder treatment at 0.38 MPa as well as the untreated agave bagasse. Color determination with the densitometer, reported as CIE L [L* a* b*], reveals the optical density of the untreated sample, the 0.38 MPa treatment, and the 0.98 MPa treatment to be [59.0, 7.0, 18.9], [55.3, 6.5, 16.8], and [45.6, 5.9, 13.7], respectively. The root squared difference between the optical density of the fibers from the first two samples was 4, while the 0.98 MPa treatment fiber optical density had a root squared difference of 14 and 10 with that of the fibers from the untreated and first treatment, respectively. Thus, the optical density of the fibers resulting from the untreated and the first treatment were more similar, while that of the second treatment was more dissimilar. In addition, it is observed that for the 0.98 MPa treatment, some of the hollow fibers are moved from their original aligned position.

Figure 5a shows the fibers after anaerobic digestion in batch test after a duration of 37 days. The surface of the agave bagasse without a thermal pretreatment (fiber on the left) resisted more strongly to conditions of anaerobic digestion than the steam-exploded fiber (0.98 MPa with three expansions). In Fig. 5b, the same fibers are shown under monochromatic illumination. The left fiber shows a stronger fluorescence effect than the right fiber. This effect may be promoted by a higher lignin content still present on the fiber without a thermal pretreatment. As consequence, lignin was hydrolyzed by process combination more effectively. It is supposed that solubilized lignin is still dissolved in aqueous media and contributes to the mentioned residual COD due to the slow biodegradation of lignin under anaerobic conditions [43]. The presence of such lignin-derived compounds with low biodegradability in forest industry wastewaters is also mentioned by Speece [47].

Figure 6a–c shows SEM micrographics of agave bagasse at different process stages. The agave fiber on the left was treated with a pressure of 0.38 MPa and one expansion. The small rods are aligned as a bundle, discovered by optical microscopy. Kestur et al. [15] showed that these rods are hollow cells with irregular lumens. The hollow cells have many black points on their surface, which are unidentifiable at this resolution. However, micrographs with a higher resolution from Kestur et al. identify them as small holes [15]. The main observation for the 0.38 MPa treatment with one expansion is that any rupture of the material in the figure cannot be detected. The agave fiber in the middle, which was treated with a pressure of 0.98 MPa and one expansion, has lost the bundle structure on the right side. The surface on the right side seems to be molten, while farther to the left, some crystals appear. EDS analysis of the surface of crystals detects only the elements calcium, carbon, and oxygen. It is assumed that these crystals are calcium oxalates as described more in detail by Perez-Pimienta et al. [48]. Figure 5c shows the fiber processed with 0.98 MPa and

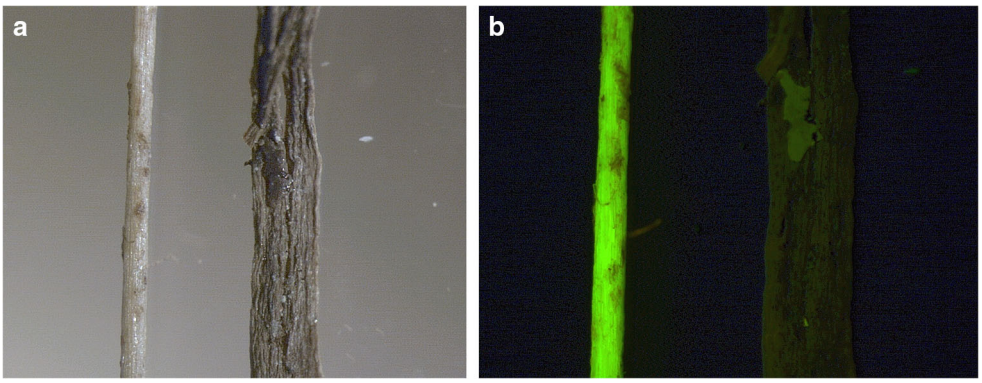

Fig. 5 Optic microscopy with a magnification × 80 of fibers after anaerobic digestion for untreated agave bagasse (fiber on the left) and for the 0.98 MPa treatment with three expansions (fiber on the right) (**a**, **b**). The right photograph shows the same fibers under fluorescence

three expansions and subsequent anaerobic digestion. The activity of microbial consortium formed a hole in the bundle, and some individual hollow cells can be seen inside the hole. The shell of the fiber now has some small holes instead of the black points. There are still crystals present, but they now appear deteriorated.

4 Conclusions

This study revealed that the steam explosion treatment can accelerate the biodegradation of agave bagasse; however, the methane yield of such a process combination only increases slightly. The relation of energy output to energy input of the two process combinations reaches 49%, which has the potential to reorient tequila industries towards more sustainable production when implementing the proposed process combination. However, the target to reduce derived solid wastes to a content equal to lignin and mineral matter was not achieved. Microscopic analysis showed still intact agave fibers after both steam explosion and anaerobic digestion, which explains the results related to mass and energy recovery. Hence, further investigation about more effective pretreatment as well as anaerobic digestion of agave bagasse should be realized.

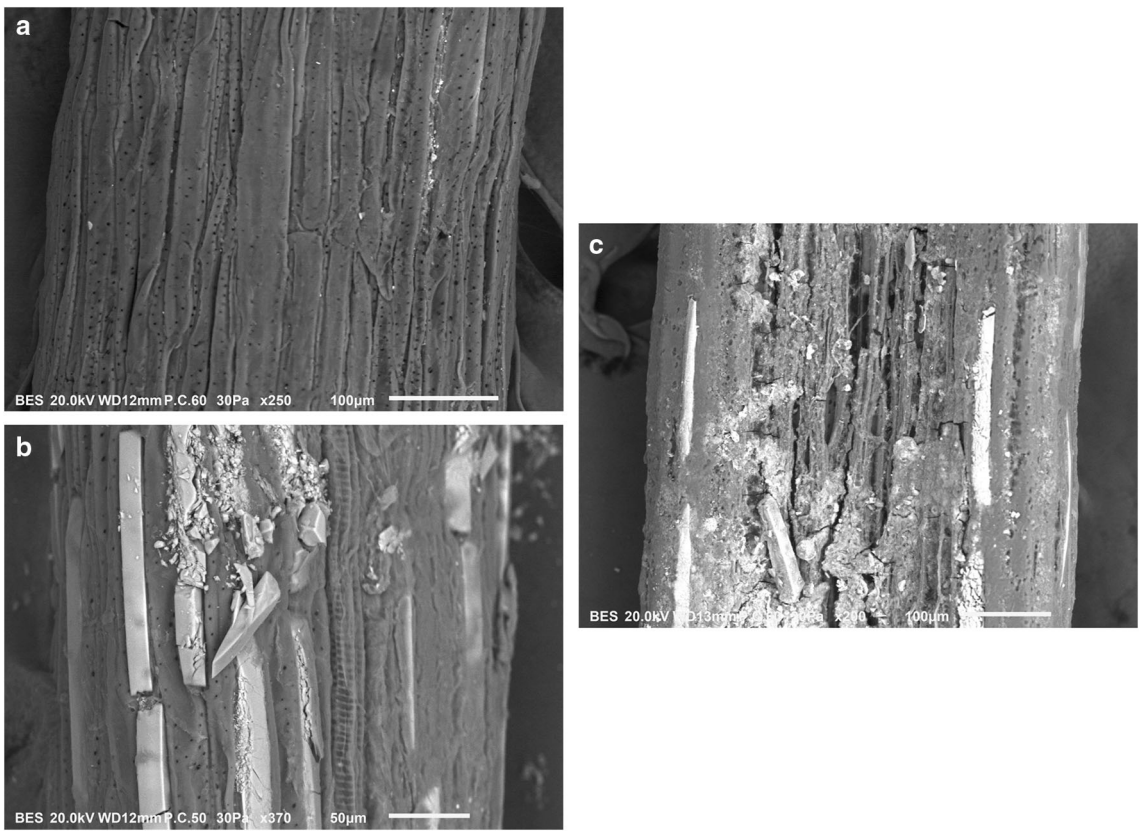

Fig. 6 SEM photographs of pretreated agave bagasse (0.38 MPa 1 expansion (**a**) and 0.98 MPa 3 Expansions (**b**)) and pretreated agave bagasse with 0.98 MPa 3 expansions after anaerobic digestion (**c**)

Acknowledgments The authors sincerely thank Dr. Jafargholi Imani for the pictures taken with the optical microscope.

Funding information This research was conducted with the support of Fondo Sectorial CONACYT-Sener, Cemie-Bio Project: 247006 "Biocombustibles Gaseosos."

References

1. Salunke VV, Deshmukh KB, Garud RP, Patil AS, Kulkarni SC (2017) Improvement of boiler efficiency using bagasse dryer. Int Res J Eng Technol 4:1286–1289
2. Sosa-Arnao JH, Nebra SA (2011) First and second law to analyze the performance of bagasse boilers. Int J Thermodyn 14:51–58
3. Chau J, Sowlati T, Sokhansanj S, Preto F, Melin S, Bi X (2009) Techno-economic analysis of wood biomass boilers for the greenhouse industry. Appl Energy 86:364–371. https://doi.org/10.1016/j.apenergy.2008.05.010
4. Gudmundsson O, Thorsen JE, Zhang L (2013) Cost analysis of district heating compared to its competing technologies. In: Brebbia CA, Marinov AM, Mammoli AA, Safta CA (eds) WIT transactions on ecology and the environment. Wessex, Bucharest, pp 10–118
5. Saygin D, Gielen DJ, Draeck M, Worrell E, Patel MK (2014) Assessment of the technical and economic potentials of biomass use for the production of steam, chemicals and polymers. Renew Sust Energ Rev 40:1153–1167. https://doi.org/10.1016/j.rser.2014.07.114
6. Akhtari S, Sowlati T, Day D (2014) Economic feasibility of utilizing forest biomass in district energy systems – a review. Renew Sustain Energy Rev 33:117–127. https://doi.org/10.1016/j.rser.2014.01.058
7. Knapp BA, Insam H (2011) Recycling of biomass ashes: current technologies and future research needs. In: Insam H, Knapp B (eds) Recycling of biomass ashes. Springer, Berlin, Heidelberg, pp 1–16
8. Tan Z, Lagerkvist A (2011) Phosphorus recovery from the biomass ash. Renew Sust Energ Rev 15:3588–3602. https://doi.org/10.1016/j.rser.2011.05.016
9. Cabrera M, Galvin AP, Agrela F, Carvajal MD, Ayuso J (2014) Characterisation and technical feasibility of using biomass bottom ash for civil infrastructures. Constr Build Mater 58:233–244. https://doi.org/10.1016/j.conbuildmat.2014.01.087
10. Mastral AM, Callén MS (2000) A review on polycyclic aromatic hydrocarbon (PAH) emissions from energy generation. Environ Sci Technol 34:3051–3057. https://doi.org/10.1021/es001028d
11. Dickson LC, Lenoir D, Hutzinger O (1992) Quantitative comparison of de novo and precursor formation of polychlorinated dibenzo-p-dioxins under simulated municipal solid waste incinerator postcombustion conditions. Environ Sci Technol 26:1822–1828. https://doi.org/10.1021/es00033a017
12. Alemán-Nava GS, Gatti LA, Parra-Saldivar R, Dallemand JF, Rittmann B, Iqbal HMN (2018) Biotechnological revalorization of Tequila waste and by-product streams for cleaner production – a review from bio-refinery perspective. J Clean Prod 20:3713–3720. https://doi.org/10.1016/j.jclepro.2017.07.134
13. Íñiguez-Covarrubias G, Bernal CJJ, Ramírez MW, Villalvazo NJ (2014) Recycling agave bagasse of the tequila industry. Adv Chem Eng Sci 4:135–142. https://doi.org/10.4236/aces.2014.42016
14. Palomo-Briones R, López-Gutiérrez I, Islas-Lugo F, Galindo-Hernández KL, Munguía-Aguilar D, Rincón-Pérez A, Cortés-Carmona MA, Alatriste-Mondragón F, Razo-Flores E (2018) Agave bagasse biorefinery: processing and perspectives. Clean Technol Environ Pol:1423–1441. https://doi.org/10.1007/s10098-017-1421-2
15. Kestur S, Flores-Sahagun THS, Pereira Dos Santos L, Dos Santos J, Mazzaro I, Mikowski A (2013) Characterization of blue agave bagasse fibers of Mexico. Composities: Part A 45:153–161
16. Liñán-Montes A, Parra-Arciniega SM, Garza-González MT, García-Reyes RB, Soto-Regalado E, Cerino-Córdova FJ (2014) Characterization and thermal analysis of agave bagasse and malt spent grain. J Therm Anal Calorim 115:751–758. https://doi.org/10.1007/s10973-013-3321-y
17. Inyang M, Gao B, Pullammanappallil P, Ding W, Zimmerman AR (2010) Biochar from anaerobically digested sugarcane bagasse. Bioresour Technol 101:8868–8872. https://doi.org/10.1016/j.biortech.2010.06.088
18. Gómez AV, Valdez-Vazquez I, Caballero-Caballero M, Chiñas-Castillo F, Alavéz-Ramírez R, Montes-Bernabé JL (2019) Co-digestion of *Agave angustifolia* Haw bagasse and viansses for biogas. Rev Mex Ing Quim 18:1073–1083. https://doi.org/10.24275/uam/izt/dcbi/revmexingquim/2019v18n3/Gomez
19. Weber B, Estrada-Maya A, Sandoval-Moctezuma AC, Martínez-Cienfuegos IG (2019) Anaerobic digestion of extracts from steam exploded Agave tequilana bagasse. J Environ Manag 245:489–495. https://doi.org/10.1016/j.jenvman.2019.05.093
20. Ye S, Cheng J (2002) Hydrolysis of lignocellulosic materials for ethanol production. Bioresour Technol 83:1–11. https://doi.org/10.1016/S0960-8524(01)00212-7
21. Delfín-Ruíz ME, Calderón-Santoyo M, Ragazzo-Sánchez JA et al (2019) Acid pretreatment optimization for xylose production from *Agave tequilana* Weber var. *azul*, *Agave americana* var. *oaxacensis*, *Agave karwinskii*, and *Agave potatorum* bagasses using a Box-Behnken design. Biomass Conv Bioref:1–10. https://doi.org/10.1007/s13399-019-00497-z
22. Santosh Y, Sreekrishnan TR, Kohli S, Rana V (2004) Enhancement of biogas production from solid substrates using different techniques—a review. Bioresour Technol 95:1–10. https://doi.org/10.1016/j.biortech.2004.02.010
23. Nissilä ME, Lay CH, Puhakka JA (2014) Dark fermentative hydrogen production from lignocellulosic hydrolyzates – a review. Biomass Bioenergy 67:145–159. https://doi.org/10.1016/j.biombioe.2014.04.035
24. Contreras-Dávila CA, Méndez-Acosta HO, Arellano-García L, Alatriste-Mondragón F, Razo-Flores E (2017) Continuous hydrogen production from enzymatic hydrolysate of Agave tequilana bagasse: effect of the organic loading rate and reactor configuration. Chem Eng J 313:671–679. https://doi.org/10.1016/j.cej.2016.12.084
25. Taherzadeh MJ, Karimi K (2008) Pretreatment of lignocellulosic wastes to improve ethanol and biogas production: a review. Int J Mol Sci 9:1621–1651. https://doi.org/10.3390/ijms9091621
26. Negro MJ, Manzanares P, Oliva JM, Ballesteros I, Ballesteros M (2003) Changes in various physical/chemical parameters of Pinus pinaster wood after steam explosion treatment. Biomass Bioenergy 25:301–308. https://doi.org/10.1016/S0961-9534(03)00017-5
27. Chen HZ, Liu LY (2007) Unpolluted fractionation of wheat straw by steam explosion and ethanol extraction. Bioresour Technol 98:666–676. https://doi.org/10.1016/j.biortech.2006.02.029
28. Hendriks ATWM, Zeeman G (2009) Pretreatments to enhance the digestibility of lignocellulosic biomass. Bioresour Technol 100:10–18. https://doi.org/10.1016/j.biortech.2008.05.027
29. Van Soest PJ, Wine RH (1967) Use of detergents in the analysis of fibrous feeds. IV Determination of plant cell-wall constituents. J Assoc Off Anal Chem 50:50–55
30. Chum HL, Johnson DK, Black SK, Overend RP (1990) Pretreatment-catalyst effects and the combined severity parameter.

Appl Biochern Biotechnol 24(25):1–14. https://doi.org/10.1007/BF02920229
31. Gschaedler-Mathis AC, Acevedo F, Aroca G (2017) Tequila and Pisco. In: Pandey A, Sanromán MA, Du G, Soccol CR, Dussap CG (eds) Current developments in biotechnology and bioengineering: Food and beverages industry. Elsevier, Amsterdam, The Netherlands, pp 469–486
32. Laser M, Schulman D, Allen SG, Lichwa J, Antal MJ, Lynd LR (2002) A comparison of liquid hot water and steam pretreatments of sugar cane bagasse for bioconversion to ethanol. Bioresour Technol 81:33–44. https://doi.org/10.1016/S0960-8524(01)00103-1
33. Ampulski RS, Turner JT Simmons WW (2015) Pretreatment of biomass using steam explosion methods. US-Patent: US 8961628
34. Fernandez-Polanco F, Velazquez R, Perez-Elvira SI, Casas C, del Barrio D, Cantero FJ, Fernandez-Polanco M, Rodriguez P, Panizo L, Serrat J (2008) Continuous thermal hydrolysis and energy integration in sludge anaerobic digestion plants. Wat Sc Technol 57: 1221–1226. https://doi.org/10.2166/wst.2008.072
35. Weber B, Stadlbauer EA (2017) Sustainable paths for managing solid and liquid waste from distilleries and breweries. J Cleaner Production 149:38–48. https://doi.org/10.1016/j.jclepro.2017.02.054
36. Ballesteros I, Oliva LM, Negro MJ, Manzanares P, Ballesteros M (2002) Enzymic hydrolysis of steam exploded herbaceous agricultural waste (*Brassica carinata*) at different particule sizes. Process Biochem 38:187–192. https://doi.org/10.1016/S0032-9592(02)00070-5
37. Menardo S, Bauer A, Theuretzbacher F, Piringer G, Nilsen PJ, Balsari P, Pavliska O, Amon T (2013) Biogas production from steam-exploded miscanthus and utilization of biogas energy and CO_2 in greenhouses. Bioenerg Res 6:620–630. https://doi.org/10.1007/s12155-012-9280-5
38. Bobleter O (1994) Hydrothermal degradation of polymers derived from plants. Prog Polym Sci 19:797–841
39. Croce S, Wei Q, D'Imporzano G, Dong R, Adani F (2016) Anaerobic digestion of straw and corn stover: the effect of biological process optimization and pre-treatment on total bio-methane yield and energy performance. Biotechnol Adv 34:1289–1304. https://doi.org/10.1016/0079-6700(94)90033-7
40. Badshah M, Lam DM, Mattiasson B (2012) Use of an automatic methane potential test system for evaluating the biomethane potential of sugarcane bagasse after different treatments. Bioresour Technol 114:262–269. https://doi.org/10.1016/j.biortech.2012.02.022
41. Schroyen M, Vervaeren H, Vandepitte H, Van Hulle SWH, Raes K (2015) Effect of enzymatic pretreatment of various lignocellulosic substrates on production of phenolic compounds and biomethane potential. Bioresour Technol 192:696–702. https://doi.org/10.1016/j.biortech.2015.06.051
42. Vivekanand V, Olsen EF, Eijsink VGH, Horn SJ (2013) Effect of different steam explosion conditions on methane potential and enzymatic saccharification of birch. Bioresour Technol 127:343–349. https://doi.org/10.1016/j.biortech.2012.09.118
43. Perez J, Muñoz-Dorado J, de la Rubia T, Martínez J (2002) Biodegradation and biological treatments of cellulose, hemicellulose and lignin: an overview. Int Microbiol 5:53–63. https://doi.org/10.1007/s10123-002-0062-3
44. Theuretzbacher T, Lizasoain J, Lefever C, Saylor MK, Enguidanos R, Weran N, Gronauer A, Baiuer A (2015) Steam explosion pretreatment of wheat straw to improve methane yields: investigation of the degradation kinetics of structural compounds during anaerobic digestion. Bioresour Technol 179:299–305. https://doi.org/10.1016/j.biortech.2014.12.008
45. Weiland P (2010) Biogas production: current state and perspectives. Appl Microbiol Biotechnol 4:849–860. https://doi.org/10.1007/s00253-009-2246-7
46. Nieto-Delgado C, Rangel-Mendez JR (2013) In situ transformation of agave bagasse into activated carbon by use of and environmental scanning electron microscope. Microporous Mesoporous Mater 167:249–253. https://doi.org/10.1016/j.micromeso.2012.09.014
47. Speece RE (1996) Anaerobic biotechnology for industrial wastewaters. Ingram International Inc, La Vergne
48. Perez-Pimienta JA, Poggi-Varaldo HM, Ponce-Noyola T, Ramos-Valdivia AC, Chavez-Carvayar JA, Stavila V, Simmons BA (2016) Fractional pretreatment of raw and calcium oxalate-extracted agave bagasse using ionic liquid and alkaline hydrogen peroxide. Biomass Bioenergy 91:48–55. https://doi.org/10.1016/j.biombioe.2016.05.001