Vol. 18, No. 3 (2019) 1073-1083
Revista Mexicana de Ingeniería Química

CO-DIGESTION OF *Agave angustifolia* HAW BAGASSE AND VINASSES FOR BIOGAS PRODUCTION FROM MEZCAL INDUSTRY

CO-DIGESTIÓN DE BAGAZO Y VINAZAS DE *Agave angustifolia* HAW PARA LA PRODUCCIÓN DE BIOGÁS DE LA INDUSTRIA MEZCALERA

A.V. Gómez-Guerrero[1], I. Valdez-Vazquez[2], M. Caballero-Caballero[1], F. Chiñas-Castillo[3]*, R. Alavéz-Ramírez[1], J.L. Montes-Bernabé[1]

[1]*Instituto Politécnico Nacional, CIIDIR Unidad Oaxaca, Hornos No. 1003, Col. Noche Buena, Santa Cruz Xoxocotlán, Oaxaca, C.P. 71230, Oaxaca, México.*
[2]*Universidad Nacional Autónoma de México, Instituto de Ingeniería, Unidad Académica Juriquilla, Blvd. Juriquilla 3001, 76230 Querétaro, México.*
[3]*Tecnológico Nacional de México/Instituto Tecnológico de Oaxaca, Department of Mechanical Engineering, Calz. Tecnológico No. 125, Oaxaca, Oax., C.P. 68030, México.*

Received: October 10, 2018; Accepted: November 27, 2018

Abstract

Mezcal is produced mostly from an *Agave angustifolia* Haw var. Espadin and generates large quantities of bagasse and vinasses that contaminate the environment. This paper presents a study on the co-digestion of *Agave angustifolia* Haw bagasse and vinasses for biogas production under minimal operation requirements (water and nutrients), typical in mezcal production facilities. Methane production results from *Agave angustifolia* Haw bagasse (AAHB) are compared to *Agave tequilana* Weber bagasse var. Azul (ATWB) from the Tequila industry at the same tested conditions. Small fibers of AAHB are more effective for producing methane than large fibers but adding high concentrations of vinasses stops the generation of methane. The resistance of fiber to biodegradation by microorganisms is partly observed in AAHB but not in ATWB. The type of inoculum had a strong effect on the methane yield. ATWB plus granular sludge gave rise to a potential methane production 65% more than AAHB plus granular sludge.

Keywords: *Agave angustifolia* Haw, bagasse, methane, co-digestion, vinasses.

Resumen

El mezcal se produce principalmente a partir del *Agave angustifolia* Haw var. Espadín, y genera grandes cantidades de bagazo y vinazas que contaminan al medio ambiente. Este artículo presenta un estudio sobre la co-digestión del bagazo de *Agave angustifolia* Haw y vinazas para la producción de biogás bajo requerimientos mínimos de operación (agua y nutrientes) típico en las instalaciones de producción de mezcal. Los resultados de la producción de metano del bagazo de *Agave angustifolia* Haw (AAHB) son comparados con la producción de metano del bagazo de *Agave tequilana* Weber var. Azul (ATWB) de la industria del Tequila, bajo las mismas condiciones de prueba. Las fibras pequeñas de AAHB son más efectivas para producir biogás metano que las fibras largas pero al agregar vinazas a altas concentraciones se detiene la generación de metano. La resistencia a la biodegradación de la fibra por microorganismos se observa parcialmente en AAHW pero no en ATWB. El tipo de inóculo tuvo un fuerte efecto en la producción de metano. ATWB más lodos granulares dio lugar a una producción de metano del 65% más que AAHB más lodos granulares.

Palabras clave: *Agave angustifolia* Haw, bagazo, metano, co-digestión, vinazas.

1 Introduction

For many centuries, the Agave plant has been a source of economic, cultural and ecological benefits for people in Mexico and, recently it has become a potential feedstock for generation of bioenergy (Pérez-Pimienta *et al*., 2017). *Agave angustifolia* Haw var. Espadin (AAH) has a high drought resistance due to the efficient uptake of water and humidity of the environment (Rivera-Lugo *et al*., 2018, Somerville *et al*., 2010). In Oaxaca, there is a planted area of

* *Corresponding author. E-mail*: fernandochinas@gmail.com
https://doi.org/10.24275/uam/izt/dcbi/revmexingquim/2019v18n3/Gomez
issn-e: 2395-8472

15,503 Ha of AAH with an annual production of 130,240 tons and nearly 13,800 farmers are employed (SIAP-SAGARPA, 2016). The AAH stem or "piña" are processed with traditional methods in small-scale facilities to make the alcoholic beverage called mezcal (Silva *et al*., 2009). Produced in a very similar manner to Tequila industry, mezcal has four main stages: 1) cooking of Agave stem, 2) milling and juice extraction of the cooked stem, 3) fermentation of sugars and 4) one distillation. For each liter of mezcal produced, 15 kg (wet basis) of a lignocellulosic waste called bagasse is generated, this represents about 688,000 tons of dry fibers per year (CRM, 2018, Chávez-Guerrero & Hinojosa, 2010). The bagasse is discarded in croplands causing soil contamination, generating harmful pests and bad odors, affecting the surrounding populations to the final disposal area (Martínez-Gutiérrez *et al*., 2015). At present, bagasse is used as brick kilns for solid fuel and additive for soil stabilization (Narváez-Zapata & Sánchez-Teyer, 2010).

The vinasses are wastewater resulting from the distillation stage, for each liter of mezcal produced, 8 to 15 L of vinasses are generated and according to estimations 43 x 106 L are produced annually (CRM, 2018, Robles-González *et al*., 2012). These vinasses are discarded directly into rivers and agricultural lands causing aquatic life death, loss of soil fertility (España-Gamboa *et al*., 2012), groundwater contamination and bad odors that give rise to pests (insects and rodents) (López-López *et al*., 2010, Moran-Salazar *et al*., 2016). The organic pollutants are commonly expressed by the biochemical oxygen demand (BOD_5), varying from 22,000 to 33,600 mg/L and chemical oxygen demand (COD) oscillate from 56,000 to 123,000 mg/L and pH values from 3 to 4 (Moran-Salazar *et al*., 2016).

A process commonly utilized to treat vinasses is anaerobic digestion (AD) and has been applied frequently in the tequila industry. For example, Méndez-Acosta *et al*. (2010) operated an anaerobic digester for 200 days to stabilize diluted tequila vinasses with an initial COD of 10,000 mg/L. They reached efficiencies of 90-95% in COD removal and a methane yield of 910 mL CH_4/g COD_{added}. Espinoza-Escalante *et al*. (2009) evaluated the effects of hydraulic retention time (HRT) for 1.3 and 5.0 days, three pH values (4.5, 5.5 and 6.5) and two temperatures (35 and 55 °C) on methane production from tequila vinasses in a semi-continuous reactor with an initial COD of 64,000 mg/L. They reported that the best conditions (HRT of 5 days, pH of 6.5 and 35 °C) yielded a methane production of 44 mL CH_4/g COD_{added}. Arreola-Vargas *et al*. (2016b) evaluated on an anaerobic sequencing batch reactor (AnSBR) the effects of pH (7 and 8) and temperature (32 °C and 38 °C) on methane production from tequila vinasses with an initial COD of 8000 ± 500 mg/L. The AnSBR reached efficiencies higher than 85% of COD removal, and the highest methane production of 290 mL CH_4/g COD_{added} at pH 7 and 38 °C. Jiménez *et al*. (2006) studied the effect of organic loading rate and performance on methane production using continuous mesophilic bioreactors loaded with fermented tequila vinasses (from 1.5 to 7.5 g COD/L·d). The continuous bioreactor operated at an organic loading rate of 6.5 g COD/L·d, had a methane production rate higher than untreated vinasses (174 mL CH_4/g COD_{added} and 104 mL CH_4/g COD_{added}, respectively).

Earlier studies also reported the use of AD to treat Agave bagasse to produce second-generation biofuels even though this residue had a low-biodegradability due to high levels of recalcitrance (resistance to biodegradation) (Pérez-Pimienta *et al*., 2018, Palomo-Briones *et al*., 2017). Physical and chemical pre-treatments have successfully been applied to increase the biodegradability of Agave bagasse, for instance, Mshandete *et al*. (2006) reported that a particle size reduction of the Agave sisalana var. Armata bagasse from 100 mm to 2 mm gave rise to a 40% of biodegradation and 23% more methane production and a maximum methane yield of 220 mL CH_4/gVS_{added}. Enzymatic and chemical pre-treatments yielded Agave hydrolyzates with fermentable sugars ready to be methanized. Arreola-Vargas *et al*. (2015) hydrolyzed *Agave tequilana* Weber var. Azul bagasse (ATWB) (cooked and uncooked) with diluted acid to extract sugars and produce methane in an AnSBR reactor finding a maximum yield of 260 mL CH_4/g COD_{added}. Arreola-Vargas *et al*. (2016a) also tested a two-stage anaerobic process to get methane from enzymatic hydrolysis of ATWB for a maximum yield of 240 mL CH_4/g COD_{added}. Recent studies have focused on the valorization of waste coming mainly from the tequila industry located in the state of Jalisco, Mexico because tequila industry is financially and technologically stronger than mezcal infrastructure (Davis *et al*., 2011). However, in the last few years, the mezcal global market has gained popularity with an annual economic growth of 120% from 2011 to 2015. Despite this growth, most of the mezcal produced in Oaxaca uses traditional methods, scarce technology, and small production facilities (Álvarez del Castillo-Romo *et al*., 2018).

This paper aims to evaluate the potential methane production by anaerobic co-digestion of *Agave angustifolia* Haw bagasse (AAHB) and mezcal vinasses under minimal operation requirements of water and nutrients, operating conditions typical in mezcal production facilities. Methane production results from AAHB are compared to ATWB from Tequila industry at the same conditions. Additionally, the effect of inocula (pig manure and activated granular sludge) and fiber bagasse on biogas co-generation is discussed. A severity factor (R_0) using data from previous studies that use only lignocellulosic substrates for methane production was calculated and compared to the results obtained in this work to know which process requires more energy to generate methane.

2 Materials and methods

2.1 *Inoculum and substrate*

The inoculum for anaerobic co-digestion treatments in this work consisted of a) pig manure and b) granular sludge. Pig manure came from a municipal slaughterhouse (Oaxaca City, Mexico) all pigs were fed with a corn-soybean during the growth phase and stored at 4°C. This inoculum was mixed with tap water in 1:1 ratio (wt/wt), the chemical composition after dilution was 75 mg/g of total solids (TS), 43 mg/g of volatile solids (VS) and an initial pH of 7.2, carried out according to previously reported methods in APHA (2012). Granular sludge was obtained from an up-flow anaerobic sludge blanket (UASB), the chemical composition was 43 mg/g of TS, 73.7 mg/g of VS and an initial pH of 7.2, determined according to APHA (2012).

The substrates, 10 kg of AAHB and 10 L of vinasses, were obtained from a facility that produces 5000 L of mezcal per year, located in San Baltazar Chichicapam, Oaxaca, Mexico (16º 46´ NL, 96º 29´ WL, 1,540 MASL). In this facility, the process starts when the Agave stems are baked in a preheated and sealed conical underground oven over 48 hours. The cooked stems are then crushed by circular grinders and introduced into wooden tubs to be fermented over one week. The fermented product is then deposited in a copper pot still for distillation to obtain mezcal as the main product and bagasse with vinasses as waste. The collected bagasse was washed with tap water, sun dried over 72 hours and conditioned in two particle sizes: 100 mm (manually) and 1 mm ± 0.5 using a universal cutting mill (Model Pulverisette-19 Fritsch). The ATWB was supplied by a local producer located in Amatitan, Jalisco, Mexico (20°42´NL, 103°37´, 1220 MASL) this substrate was treated by the same procedure of AAHB.

The AAHB chemical composition was determined on a per kilogram basis using methods previously reported (APHA, 2012, Sanchez-Herrera *et al*., 2018): 960 g of total solids (TS), 943 g of volatile solids (VS), 229 g of extractives, 121 g of hemicellulose, 474 g of cellulose, 118 g of lignin, and 17 g of ash. The ATWB chemical composition was also determined on a per kilogram basis: 910 g TS, 880 g (VS), 250 g of extractives, 133 g of hemicellulose, 430 g of cellulose, 105 g of lignin and 20 g of ash. Vinasses were stored in disinfected plastic containers and refrigerated at 4 ºC for further use. The chemical composition of vinasses was carried out according to previously reported methods (AOAC, 2012): 91.7 g/L of TS, 72.2 g/L of VS, 8,700 mg/kg of BOD_5, 107,000 mg/kg of COD, and pH of 3.8.

2.2 *Experimental design and statistical analysis*

Glass containers of 1000 mL served as bioreactors, the working volume was 700 mL, containers were loaded with 170 g of pig manure, 20 g of AAHB with a particle size of 1 mm (group A tests) and 100 mm (group B tests) and vinasses according to table 1, tap water was added to fix the working volume for each essay. Four controls were prepared: the endogenous control (CE) which indicated the methane potential of the inoculum, raw vinasses, bagasse at 1 mm and bagasse at 100 mm particle size. A water bath kept the bioreactors at 35 ± 0.5 °C for 20 days with manual agitation for 1 minute twice a day. Essays were carried out with no further addition of nutrients or pH adjustment.

In order to compare the methane potential of AAHB and ATWB at the same test conditions, complementary tests were carried out. Samples of AAHB and ATWB were inoculated with granular sludge and with pig manure as indicated in table 2. For these complementary tests, glass containers were loaded with 700 mL of a nutrient solution (composition per liter according to Angelidaki *et al*. (2009)) with pH adjusted to 7.3. The prepared samples were inoculated with a substrate/inoculum (S_0/X_0) ratio of 0.5 gVS/gVS keeping the substrate fixed at 3 g of VS (see Table 2), for which the highest methane yields have been obtained in past studies in ranges

Table 1. Central experimental design for co-digestion of AAHB and vinasses from mezcal industry.

Treatments	**Particle size**	**Crude vinasses (mL)**
A1	1 mm	0
A2	1 mm	85
A3	1 mm	170
A4	1 mm	255
A5	1 mm	340
B1	100 mm	0
B2	100 mm	85
B3	100 mm	170
B4	100 mm	255
B5	100 mm	340
Controls		
CE	170 g of pig manure	
Vinasse	340 mL of vinasse	
1 mm AAHB	20 g of 1 mm AAHB	
100 mm AAHB	20 of 100 mm AAHB	

Table 2. Complementary experimental design for AAHB and ATWB.

Treatments	Inoculum gVS	Substrate gVS
$AAHB_{Granular\ Sludge}$	6	3
$ATWB_{Granular\ Sludge}$	6	3
$AAHB_{Pig\ manure}$	6	3
$ATWB_{Pig\ manure}$	6	3

Table 3. Methane production from different essays with AAHB and ATWB.

	Treatments	**Initial pH**	**Final pH**	**MMC (%)**	**MY (NmL/g SVadded)**	**MPR (NmL/kg·d)**	**PMP (NmL)**
Central experimental design	A1	6.3	6	30	8.3	15	150
	A2	6.2	5.8	35	3	6	73
	A3	5.8	5.4	22	1.5	4	43
	A4	4.8	5.2	22	0.2	0.2	4
	A5	4.5	4.8	16	0.1	0.1	2
	B1	6.3	5.8	31	6.6	11	125
	B2	6.1	5.7	13	1	3	29
	B3	5.8	5.2	16	0.6	1.5	17
	B4	4.9	4.2	14	0.2	0.3	4
	B5	4.6	4.1	12	0	0.1	1
Controls	CE	7.2	6.8	62	19	11	140
	Vinasse	3.9	3.9	0	0	0	0
	1 mm AAHB	6.3	6	0	0	0	0
	100 mm AAHB	6.3	6	0	0	0	0
Complementary experimental design	$AAHB_{Granular\ Sludge}$	7.3	7	55	193.6	54.4	910
	$ATWB_{Granular\ Sludge}$	7.3	7	66	323.6	83.3	1521
	$AAHB_{Pig\ manure}$	7.3	6.5	37	95.7	72.3	450
	$ATWB_{Pig\ manure}$	7.3	6.8	44	135.1	100.5	635

MMC maximum methane content; MY methane yield; MPR methane production rate; PMP potential methane production. Note: CE, endogenous control. Standard deviations were lower than 10%.

between 0.5 and 0.9 gVS/gVS ratios with different lignocellulosic substrates (Pellera & Gidarakos, 2016, Moset *et al.*, 2015). All experiments were conducted in triplicate.

The generated biogas was measured periodically using the displacement of a lubricated syringe. Biogas composition (O_2, CO_2, and CH_4) was analyzed using an infrared gas detector Model PGD-IR3 (Status Scientific Controls, Nottinghamshire, United Kingdom). Statistical data analysis was carried out by ANOVA (95% significance level) and Tukey´s test (Rice, 1989).

3 Results and discussion

3.1 Particle size effect on methane production

The production of biogas from AAHB with a particle size of 100 mm resulted in a potential methane production (the highest amount of methane produced in a test) of 125 NmL (B1) (units according to Wang (2016)). However, adding 85 mL of vinasses (sample B2) to the digester reduced by 76.8% this potential methane production to 29.0 NmL. The samples with higher content of vinasses shown in table 1 (B3, B4, and B5) had a stronger inhibitory effect on the methane production (see Table 3).

Bagasse fibers of 1 mm that were inoculated with pig manure (sample A1) gave rise to 150 NmL CH_4 potential methane production (about 7% higher than CE) and 15.0 NmL CH_4/kg·d in methane production rate, that is 36% higher than CE (140 NmL and 11 NmL CH_4/kg·d) as shown in Table 3. When vinasses were added, a strong inhibitory effect was observed on the kinetic parameters ($p<0.05$), for instance sample A2, $p<0.05$, the methane production rate was reduced from 15 to 6 NmL CH_4/kg·d (60% less) and the potential methane production decreased from 150 to 73 (50% less). At higher volumes of vinasses (samples A3, A4, and A5 in Table 3) the process was inhibited almost completely.

From these results, it can be observed in figure 1 that using large fibers (100 mm) of AAHB to produce methane is not as effective as small fibers (1 mm). As vinasses are added in higher concentrations they cause an inhibitory effect of the AD methane production.

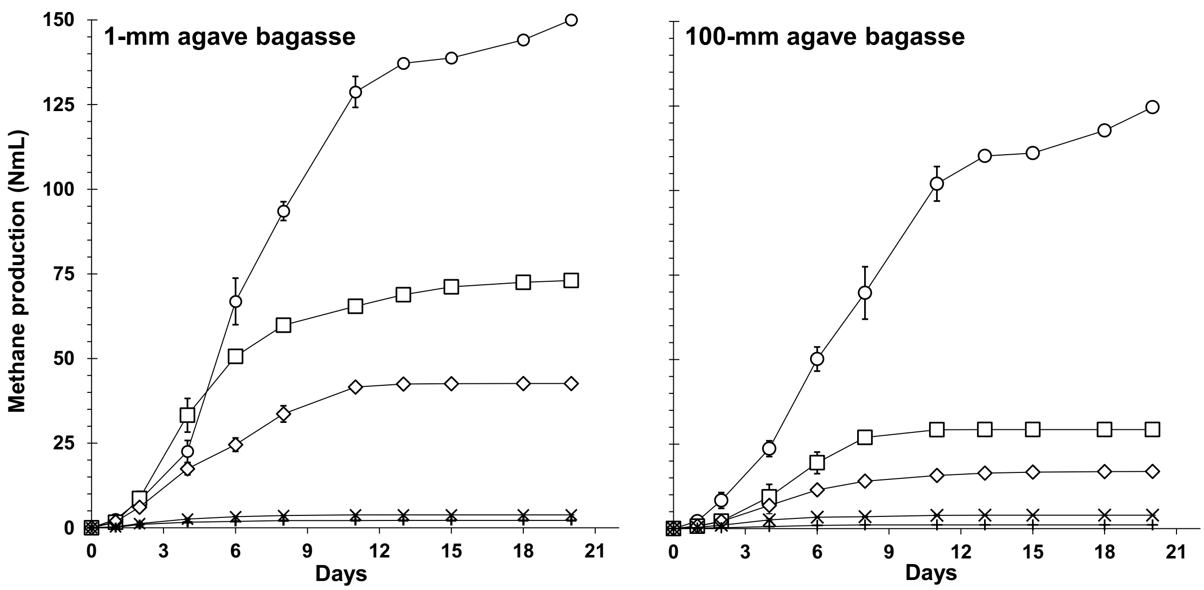

Fig. 1. Methane production kinetic behavior from co-digestion of Agave angustifolia Haw bagasse (1 mm and 100 mm) with increasing volume of raw vinasses: A1-B1 (o), A2-B2 (□), A3-B3 (◊), A4-B4 (×), and A5-B5 (+). Results are average measurements based on triplicates per treatment (n = 3). Errors bars represent the standard deviation of the measurements.

These results agree with past studies where they indicated that fibers of small particle sizes increased the methane production in lignocellulosic substrates. Herrmann *et al.* (2012) studied a wide range of lignocellulosic substrates to assess the effect of particle size on methane yield. In those experiments, the production increased from 11% to 13% for sizes from 33 mm to 6 mm respectively. Mshandete *et al.* (2006) showed that small fibers (2 mm) of bagasse from *Agave sisalana* Perrine leaves increased the methane yield of 216 $mLCH_4/gSV_{added}$ by 19% more than larger fibers (>100 mm).

In this work, 1 mm AAHB fibers yielded 20% more methane production than fibers of 100 mm. The highest methane production potentials from lignocellulosic substrates have been reported using fibers of 1mm size (this work) up to 6 mm. The reduction in particle size increases the available surface area for enzymatic hydrolysis and reduces the crystallinity of the cellulose (Herrmann *et al.*, 2012), but excessive grinding leads to the formation of much smaller fibers (< 1 mm) with low biodegradability reducing the action of microorganisms in the methanization process (Lara-Vázquez *et al.*, 2014).

All previous studies have reported diluted vinasses with an initial organic load in the range of 8,000 to 64,000 mg COD/L (Mendez-Acosta *et al.* 2010; Espinoza-Escalante *et al.* 2009; Arreola-Vargas *et al.* 2016), resulting in COD removals of 95%. Fungal pre-treatment has also been reported to reduce the organic load of vinasses, improving the performance of the AD (Jimenez *et al.* 2006). In this work, raw and undiluted vinasses with an initial COD of 104,000 mg/L co-digested with bagasse fibers at different bagasse/vinasses ratios were used (see Table 1). The addition of vinasse strongly inhibited the anaerobic digestion in those tests with a high concentration of this substrate. Thus the idea that diluted vinasses must be used to get satisfactory methane yields through co-digestion with AAHB is strengthened. Co-digestion of alkaline residues, such as maize processing wastewaters (called Nejayote) with a pH value of 12 ± 0.2, could improve methane production (García-Depraect *et al.*, 2017, Ferreira-Rolón *et al.*, 2014).

3.2 Angustifolia Haw vs tequilana Weber on methane production

Agave tequilana Weber bagasse (ATWB) is currently a substrate of high interest in AD processes due to its high production from tequila industry (Arreola-Vargas *et al.* 2016; Arreola-Vargas *et al.* 2015; Montiel Corona and Razo-Flores 2017). For this reason, the potential methane production (PMP) using ATWB was compared to AAHB at the same test conditions. Results shown in Table 3 indicate that the sample of ATWB plus granular sludge had a PMP of 1521 NmL while AAHB plus granular sludge gave rise to a PMP of 910 NmL; that is 40% less than ATWB plus granular sludge. ATWB plus pig manure had a PMP of 635 NmL and finally, AAHB plus pig manure had a PMP of 450 NmL, all tested under the same conditions.

From these results, it is very interesting that despite ATWB and AAHB share many similarities, because they are close species of Agave (Rivera-Lugo *et al.*, 2018), a higher level of resistance to biodegradation than ATWB in terms of methane potential is observed (Pérez-Pimienta *et al.*, 2018). Best performance samples (A1>A2>B1) had a total accumulated methane production between 73 to 150 NmL CH_4, that yielded 3 to 6 $NmLCH_4/gSV$. Performance of sample A1 was hardly better than CE due to the minimal conditions of the AD process (without nutrients addition or pH adjustment), however, as soon as AAHB digestion was supplied with nutrients (in the complementary experiments granular sludge or pig manure) and pH adjusted to 7.3, methane potential markedly increased (see Figure 2).

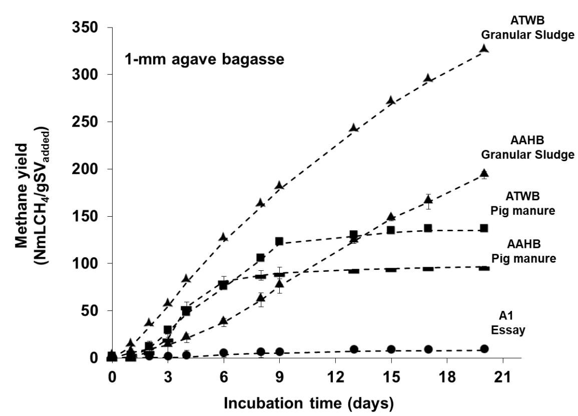

Fig. 2. The methane production kinetic behavior of 1 mm agave bagasse. Inoculum type / best performance: anaerobic granular sludge with ATWB (▲), anaerobic granular sludge with AAHB (▲), pig manure with ATWB (■), pig manure with AAHB (▬) and A1 test (●). Average measurement results are based on triplicates per treatment (n = 3). Errors bars represent the standard deviation of the measurements (>10%).

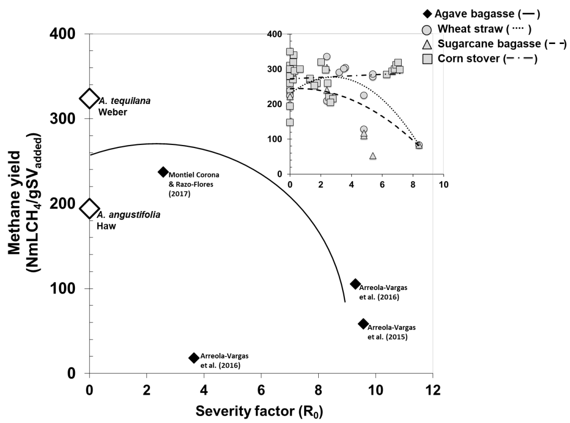

Figure 3. Methane yield from different lignocellulosic substrates as a function of severity factor (R0) (data from [Arreola-Vargas *et al*. 2015, Arreola-Vargas *et al*. 2016, Montiel Corona & Razo-Flores 2017, Abdolali *et al*., 2014; Mshandete *et al*., 2006; Hu and Ragauskas 2012; Bolado-Rodríguez *et al*. 2016; Ferreira *et al*. 2014; Hassan *et al*., 2017; Hassan *et al*., 2016]).

The addition of pig manure to AAHB gave an enhancement in the potential of methane production (PMP) of 300% reaching a final value of 450 NmL CH_4 compared to test sample A1. The use of anaerobic granular sludge increased the PMP by 600% (910 NmL CH_4). Comparing ATWB to AAHB, results indicate that ATWB was better than AAHB.

3.3 *Calculation of the severity factor (R_0)*

The severity factor (R_0) is generally used for comparison of the efficiencies of various treatment strategies in preparing the cellulosic biomass for conversion. This factor Ro introduces an integration of the time period in the treatment done at a certain temperature, where t is the holding time of treatment in min, T(t) is the treatment temperature, 100 is the reference temperature. The fitted value (14.75) is based on the activation energy when assuming pseudo-first-order kinetics (Carvalheiro *et al*., 2009). In this work, the severity factor was calculated using Eq. (1) where time (t) and temperature (T) are measured in minutes and degrees Celsius respectively:

$$\text{Severity factor}(R_0) = \int_a^b \frac{(T(t)-100)}{14.75} = t \times exp((T(t)-100)/14.75) \quad (1)$$

Past studies from wheat straw and sugarcane bagasse (Bolado-Rodríguez *et al*., 2016, Ferreira *et al*., 2014), had methane yields of 270 and 250 NmLCH_4/g SV respectively, at R_0 values between 2 and 3 (see Figure 3) and corn stover had high methane yields between 320 to 350 NmL CH_4/g SV at R_0 values from 0.0 to 7.5 (Hassan *et al*., 2016, Hassan *et al*., 2017, Lizasoain *et al*., 2017). Previous studies on ATWB used enzymatic and acidic hydrolysates with R_0 values between 2.6 and 9.5 but had their maximum peak of methane yields at R_0 close to 3.0 (Arreola-Vargas *et al*., 2016a, Arreola-Vargas *et al*., 2016b, Montiel Corona & Razo-Flores, 2017).

This work reports a methane yield of 194 NmL CH_4/g SV added and R_0 equal to zero from AAHB as shown in Figure 3. However, ATWB showed a methane yield of 324 NmL CH_4/g SV_{added} and R_0 equal to zero (see Figure 3). Both AAHB and ATWB methane yields overcome acidic hydrolysates, being ATWB methane yield higher than AAHB, however, a resistance to biodegradation, also called recalcitrance, was observed in AAHB that limited its full methanation potential.

Lignocellulosic biomass is an alternative to fossil fuels because of its abundance and versatility for many processes (Hou *et al*., 2017). It is already known that lignin is one of the major causes of recalcitrance due to the barrier created causing limited accessibility to microorganisms and that agaves have high levels of lignin (18-20%) to overcome making polysaccharides easily available for saccharification and fermentation for biofuels production i.e. methane, hydrogen, and bioethanol, but it seems that this is not the only reason for recalcitrance to take place (Perez-Pimienta *et al*., 2013; Trajano *et al*., 2013; Pérez-Pimienta *et al*., 2018). At present, tests are currently underway by the authors of this paper to identify other mechanisms that inhibit the process of the AD when AAHB is used as a substrate and be able to suggest how to tackle this drawback so as the AAHB reaches its full potential to generate biogas. If the mezcal industry optimizes this process, it could increase the productivity into a biorefinery scheme for the production of biofuels and value-added products.

Conclusions

From this study, the following main conclusions are:

- Smaller fibers yield more methane than large fibers. Large fibers (100 mm) of AAHB plus vinasses at high concentrations stop the AD process to produce methane.

- Also, AAHB tested in this study and compared to ATWB under the same conditions showed partial recalcitrance reducing the process of anaerobic co-digestion.
- ATWB plus granular sludge produced 65% more potential methane production than AAHB plus granular sludge. AAHB plus pig manure only produced about 50% less than AAHB plus granular sludge under the same conditions.
- Methane production from wastes generated in the mezcal industry is feasible but, recalcitrance phenomena needs to be explored further and identify the mechanisms that reduce the anaerobic digestion effectiveness when AAHB is used as a substrate.

Acknowledgements

The authors wish to express their sincere thanks to Instituto Politécnico Nacional (Project No. IPN/SIP: 20151315), TECNM/Instituto Tecnológico de Oaxaca (Project No. 5813.16-P), SENER - CONACyT Mexico (Project No. 247006 Gaseous Biofuels Cluster) for the financial support to carry out the present work.

References

Álvarez Del Castillo-Romo, A., Morales-Rodriguez, R. & Román-Martínez, A. (2018). Multiobjective optimization for the socio-eco-efficient conversion of lignocellulosic biomass to biofuels and bioproducts. *Clean Technologies and Environmental Policy 20*, 603-620.

Angelidaki, I., Alves, M., Bolzonella, D., Borzacconi, L., Campos, J. L., Guwy, A. J., Kalyuzhnyi, S., Jenicek, P. & Van Lier, J. B. (2009). Defining the biomethane potential (BMP) of solid organic wastes and energy crops: a proposed protocol for batch assays. *Water Science and Technology 59*, 927-934.

Aoac (2012). Official Methods of Analysis of AOAC International. AOAC International, Gaithersburg, Maryland, USA.

Apha (2012). *Standard Methods for the Examination of Water and Wastewater*. Washington, DC, USA

Arreola-Vargas, J., Flores-Larios, A., González-Álvarez, V., Corona-González, R. I. & Méndez-Acosta, H. O. (2016a). Single and two-stage anaerobic digestion for hydrogen and methane production from acid and enzymatic hydrolysates of *Agave tequilana* bagasse. *International Journal of Hydrogen Energy 41*, 897-904.

Arreola-Vargas, J., Jaramillo-Gante, N., Celis, L., Corona-González, R., González-Álvarez, V. & Méndez-Acosta, H. (2016b). Biogas production in an anaerobic sequencing batch reactor by using tequila vinasses: effect of pH and temperature. *Water Science and Technology 73*, 550-556.

Arreola-Vargas, J., Ojeda-Castillo, V., Snell-Castro, R., Corona-González, R. I., Alatriste-Mondragón, F. & Méndez-Acosta, H. O. (2015). Methane production from acid hydrolysates of *Agave tequilana* bagasse: Evaluation of hydrolysis conditions and methane yield. *Bioresource Technology 181*, 191-199.

Bolado-Rodríguez, S., Toquero, C., Martín-Juárez, J., Travaini, R. & García-Encina, P. A. (2016). Effect of thermal, acid, alkaline and alkaline-peroxide pretreatments on the biochemical methane potential and kinetics of the anaerobic digestion of wheat straw and sugarcane bagasse. *Bioresource Technology 201*, 182-190.

CRM (2018). Statistical report from regulatory council of mezcal. http://www.crm.org.mx/PDF/INF_ ACTIVIDADES/INFORME2017.pdf.

Chávez-Guerrero, L. & Hinojosa, M. (2010). Bagasse from the mezcal industry as an alternative renewable energy produced in arid lands. *Fuel 89*, 4049-4052.

Carvalheiro, F., Silva-Fernandes, T., Duarte, L.C., Gírio, F.M. (2009). Wheat straw autohydrolysis: process optimization and products characterization. *Applied Biochemistry and Biotechnology 153*, 84-93

Davis, S. C., Dohleman, F. G. & Long, S. P. (2011). The global potential for Agave as a biofuel feedstock. *GCB Bioenergy 3*, 68-78.

España-Gamboa, E. I., Mijangos-Cortés, J. O., Hernández-Zárate, G., Maldonado, J. a. D. & Alzate-Gaviria, L. M. (2012). Methane production by treating vinasses from hydrous ethanol using a modified UASB reactor. *Biotechnology for Biofuels 5*, 82. DOI: 10.1186/1754-6834-5-82.

Espinoza-Escalante, F. M., Pelayo-Ortíz, C., Navarro-Corona, J., González-García, Y., Bories, A. & Gutiérrez-Pulido, H. (2009). Anaerobic digestion of the vinasses from the fermentation of *Agave tequilana* Weber to tequila: The effect of pH, temperature and hydraulic retention time on the production of hydrogen and methane. *Biomass and Bioenergy 33*, 14-20.

Ferreira-Rolón, A., Ramírez-Romero, G. & Ramírez-Vives, F. (2014). Aumento de la actividad metanogenica en lodos granulares, precipitando calcio en el nejayote mediante el burbujeo de CO_2. *Revista Mexicana de Ingeniería Química 13*, 517-525.

Ferreira, L. C., Nilsen, P. J., Fdz-Polanco, F. & Pérez-Elvira, S. I. (2014). Biomethane potential of wheat straw: Influence of particle size, water impregnation and thermal hydrolysis. *Chemical Engineering Journal 242*, 254-259.

García-Depraect, O., Gómez-Romero, J., León-Becerril, E. & López-López, A. (2017). A novel biohydrogen production process: Co-digestion of vinasse and Nejayote as complex raw substrates using a robust inoculum. *International Journal of Hydrogen Energy 42*, 5820-5831.

Hassan, M., Ding, W., Bi, J., Mehryar, E., Talha, Z. a. A. & Huang, H. (2016). Methane enhancement through oxidative cleavage and alkali solubilization pre-treatments for corn stover with anaerobic activated sludge. *Bioresource Technology 200*, 405-412.

Hassan, M., Umar, M., Mamat, T., Muhayodin, F., Talha, Z., Mehryar, E., Ahmad, F., Ding, W. & Zhao, C. (2017). Methane enhancement through sequential thermochemical and sonication pretreatment for corn stover with anaerobic sludge. *Energy & Fuels 31*, 6145-6153.

Herrmann, C., Heiermann, M., Idler, C. & Prochnow, A. (2012). Particle size reduction during harvesting of crop feedstock for biogas production I: Effects on ensiling process and methane yields. *BioEnergy Research 5*, 926-936.

Hou, Q., Ju, M., Li, W., Liu, L., Chen, Y., Yang, Q. (2017). Pretreatment of lignocellulosic biomass with ionic liquids and ionic liquid-based solvent systems. *Molecules 22*, 490. DOI:10.3390/molecules22030490.

Jiménez, A. M., Borja, R., Martín, A. & Raposo, F. (2006). Kinetic analysis of the anaerobic digestion of untreated vinasses and vinasses previously treated with *Penicillium decumbens*. *Journal of Environmental Management 80*, 303-310.

Lara-Vázquez, A. R., Quiroz-Figueroa, F. R., Sánchez, A. & Valdez-Vazquez, I. (2014). Particle size and hydration medium effects on hydration properties and sugar release of wheat straw fibers. *Biomass and Bioenergy 68*, 67-74.

Lizasoain, J., Trulea, A., Gittinger, J., Kral, I., Piringer, G., Schedl, A., Nilsen, P. J., Potthast, A., Gronauer, A. & Bauer, A. (2017). Corn stover for biogas production: Effect of steam explosion pretreatment on the gas yields and on the biodegradation kinetics of the primary structural compounds. *Bioresource Technology 244*, 949-956.

López-López, A., Davila-Vazquez, G., León-Becerril, E., Villegas-García, E. & Gallardo-Valdez, J. (2010). Tequila vinasses: generation and full scale treatment processes. *Reviews in Environmental Science and Bio/Technology 9*, 109-116.

Martínez-Gutiérrez, G., Ortiz-Hernández, Y., Aquino-Bolaños, T., Bautista-Cruz, A. & López-Cruz, J. (2015). Properties of *Agave angustifolia* Haw. bagasse before and after its composting. *Comunicata Scientiae 6*, 418-429.

Méndez-Acosta, H. O., Snell-Castro, R., Alcaraz-González, V., González-Álvarez, V. & Pelayo-Ortiz, C. (2010). Anaerobic treatment of Tequila vinasses in a CSTR-type digester. *Biodegradation 21*, 357-363.

Montiel Corona, V. & Razo-Flores, E. (2017). Continuous hydrogen and methane production from *Agave tequilana* bagasse hydrolysate by sequential process to maximize energy recovery efficiency. *Bioresource Technology 249*, 334-341.

Moran-Salazar, R. G., Sanchez-Lizarraga, A. L., Rodriguez-Campos, J., Davila-Vazquez, G., Marino-Marmolejo, E. N., Dendooven, L. & Contreras-Ramos, S. M. (2016). Utilization of vinasses as soil amendment: consequences and perspectives. *SpringerPlus 5*, 1007. DOI: 10.1186/s40064?016?2410?3.

Moset, V., Al-Zohairi, N. & Moller, H. B. (2015). The impact of inoculum source, inoculum to substrate ratio and sample preservation on methane potential from different substrates. *Biomass and Bioenergy 83*, 474-482.

Mshandete, A., Björnsson, L., Kivaisi, A., Rubindamayugi, M. S. T. & Mattiasson, B. (2006). Effect of particle size on biogas yield from sisal fibre waste. *Renewable Energy 31*, 2385-2392.

Narváez-Zapata, J. & Sánchez-Teyer, L. (2010). Agaves as a raw material: recent technologies and applications. *Recent Patents on Biotechnology 3*, 185-191.

Palomo-Briones, R., López-Gutiérrez, I., Islas-Lugo, F., Galindo-Hernández, K. L., Munguía-Aguilar, D., Rincón-Pérez, J. A., Cortés-Carmona, M. Á., Alatriste-Mondragón, F. & Razo-Flores, E. (2017). Agave bagasse biorefinery: processing and perspectives. *Clean Technologies and Environmental Policy 20*, 1423-1441.

Pellera, F.-M. & Gidarakos, E. (2016). Effect of substrate to inoculum ratio and inoculum type on the biochemical methane potential of solid agroindustrial waste. *Journal of Environmental Chemical Engineering 4*, 3217-3229.

Perez-Pimienta, J.A., Lopez-Ortega, M.G., Varanasi, P., Stavila, V., Cheng, G., Singh, S., Simmons, B.A. (2013). Comparison of the impact of ionic liquid pretreatment on recalcitrance of agave bagasse and switchgrass. *Bioresource Technology 127*, 18-24.

Pérez-Pimienta, J. A., López-Ortega, M. G. & Sanchez, A. (2017). Recent developments in Agave performance as a drought-tolerant biofuel feedstock: agronomics, characterization, and biorefining. *Biofuels, Bioproducts and Biorefining 11*, 732-748.

Pérez-Pimienta, J. A., Mojica-Álvarez, R. M., Sánchez-Herrera, L. M., Mittal, A. & Sykes, R. W. (2018). Recalcitrance assessment of the agro-industrial residues from five agave species: Ionic liquid pretreatment, saccharification and structural characterization. *BioEnergy Research 11*, 551-561. DOI:10.1007/s12155-018-9920.

Rice, W. R. (1989). Analyzing tables of statistical tests. *Evolution 43*, 223-225.

Rivera-Lugo, M., García-Mendoza, A., Simpson, J., Solano, E. & Gil-Vega, K. (2018). Taxonomic implications of the morphological and genetic variation of cultivated and domesticated populations of the *Agave angustifolia* complex (*Agavoideae, Asparagaceae*) in Oaxaca, Mexico. *Plant Systematics and Evolution 304*. DOI:10.1007/s00606-018-1525.

Robles-González, V., Galíndez-Mayer, J., Rinderknecht-Seijas, N. & Poggi-Varaldo, H. M. (2012). Treatment of mezcal vinasses: A review. *Journal of Biotechnology 157*, 524-546.

Sanchez-Herrera, D., Sanchez, O., Houbron, E., Rustrian, R., Toledano, T., Tapia-Tussell, R. & Alzate-Gaviria, L. (2018). Biomethane potential from sugarcane straw in Veracruz, Mexico: combined liquid hot water pretreatment and enzymatic or biological hydrolysis. *Revista Mexicana de Ingeniería Química 17*, 1105-1120.

Siap-Sagarpa (2016). Agrifood and Fishery Information Service (Servicio de Informacion Agroalimentaria y Pesquera), Available at http://nube.siap.gob.mx/gobmx_publicaciones_siap/pag/2016/Atlas-Agroalimentario-2016.

Silva, S. L., Hernández, L. H., Caballero, M. & López, I. (2009). Tensile strength of fibers extracted from the leaves of the angustifolia haw agave in function of their length. *Applied Mechanics and Materials 15*, 103-108.

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

Trajano, H.L., Engle, N.L., Foston, M., Ragauskas, A.J., Tschaplinski, T.J., Wyman, C.E. (2013).

The fate of lignin during hydrothermal pretreatment. *Biotechnology for Biofuels 6*, 110. DOI: 10.1186/1754-6834-6-110.

Wang, B. (2016). Factors that Influence the Biochemical Methane Potential (BMP) Test: Steps towards the Standardisation of BMP Test, Biotechnology, Lund University.