Acclimation of microorganisms for an efficient production of volatile fatty acids and biogas from mezcal vinasses in a dark fermentation process

S. A. Díaz-Barajas, M. A. Garzón-Zúñiga, I. Moreno-Andrade,
J. M. Vigueras-Cortés and B. E. Barragán-Huerta

ABSTRACT

Mezcal is an alcoholic artisanal drink made from agave plants in Mexico. Its production causes the generation of wastewater called vinasses, which are highly polluting residues due to its concentration of organic matter as chemical oxygen demand (COD) (35,000–122,000 mg/L) and acidity (pH < 4). Due to their organic content, these residues can be used in dark fermentation to obtain biogas, which is rich in hydrogen. In this work, the acclimation of inoculum by means of a dark fermentation process, in the presence of toxic compounds from mezcal vinasses was studied. The strategy of increasing the initial concentration of vinasse in each treatment cycle in a sequencing batch reactor (SBR) reactor was applied. It was possible to obtain a maximum biogas production of 984 ± 187 mL/L, from vinasses (18,367 ± 1,200 mg COD/L), with an organic matter removal efficiency of 20 ± 1%. A maximum generation of volatile fatty acids (VFA) of 980 ± 538 mg/L equivalent to a production of 74 ± 21% of the influent concentration and removal rate of organic matter of 1,125 ± 234 mg COD/L d^{-1} equivalent to a removal efficiency of 20 ± 4% was obtained from vinasses with a concentration of 19,648 ± 1,702 mg COD/L.

Key words | acclimation, dark fermentation, inoculum activation, mezcal vinasses, substrate for sustainable energy production

S. A. Díaz-Barajas
M. A. Garzón-Zúñiga (corresponding author)
J. M. Vigueras-Cortés
National Laboratory of Water Integral Management.
Instituto Politécnico Nacional. CIIDIR, Unidad Durango. Calle Sigma 119, 20 de Noviembre II, 34220 Durango, Dgo, Mexico
E-mail: *marco.cuerna@gmail.com*

I. Moreno-Andrade
Laboratory of Research on Advanced Processes for Water Treatment,
Unidad Académica Juriquilla, Instituto de Ingeniería, Universidad Nacional Autónoma de México,
Blvd. Juriquilla 3001, 76230 Querétaro, México

B. E. Barragán-Huerta
Escuela Nacional de Ciencias Biológicas, Instituto Politécnico Nacional,
Avenida Wilfrido Massieu s/n, Unidad Profesional Adolfo López Mateos, Mexico City, 07738, México

HIGHLIGHTS

- Mezcal vinasses can be used as substrate for sustainable energy production.
- Residues of mezcal production can be used in a dark fermentation to obtain biogas rich in hydrogen.
- An efficient VFA production and maximum COD removal was obtained from an influent with 75% of mezcal vinasses.

INTRODUCTION

Mezcal is an alcoholic beverage obtained from the distillation of agave fermented juices produced in Mexico, for which demand has increased from 980,375 L/year in 2011 to 7,145,039 L/year in 2019 (CRM 2020). Mezcal production generates a liquid waste called 'vinasse' which presents high concentrations of organic matter (22,500–35,000 mg BOD_5/L, and 35,000–122,000 mg COD/L), electric conductivity (2.6–5.81 mS/cm), phenols (58–542 mg/L), total solids (45,000–96,000 mg/L), total suspended solids (8,400–83,130 mg/L),

doi: 10.2166/wst.2021.176

volatile fatty acids (9,615 mg VFA/L), acidity (pH 3.5–3.94), and a high discharge temperature (70–90 °C) (Robles-González *et al.* 2012). The documented treatments for mezcal vinasses showing the best results are fungal and anaerobic treatments, with efficiencies of chemical oxygen demand (COD) removal higher than 80%. However, an approach on the management of this waste that has not been studied is the appreciation of mezcal vinasses using a dark fermentation (DF) process for power generation in the hydrogen form.

In a DF process, the removal of organic matter (as COD) is not the main purpose, but the production of VFA and hydrogen as an alternate energy source. In DF processes, 12–17% of the COD of the influent is used in the production of hydrogen-rich biogas, and part of the COD remains in the effluent in the form of by-products such as volatile fatty acids (VFA) and alcohols (Wang & Zhao 2009; García-Depraect *et al.* 2019). Vinasses are a substrate with the potential to be used in this process due to their high concentration of COD (35,000–122,000 mg/L). However, their high content of toxic and recalcitrant compounds such as phenols (478–542 mg gallic acid/L) or sulfates (308–947 mg/L) can inhibit the microbial activity of the DF process. Therefore, the biomass must be acclimated to the presence of toxic compounds of the vinasses (García-Depraect & León-Becerril 2018). In this work, the activation of inoculum in a dark fermentation process and its acclimation to mezcal vinasses to produce VFA and biogas as substrates for sustainable energy production were studied, applying a strategy to gradually increase the concentration of vinasse in each treatment cycle, in a sequencing batch reactor (SBR).

MATERIAL AND METHODS

Vinasses and composition

The vinasses were collected from the sedimentation tank of an artisanal mezcal factory located in Nombre de Dios, Durango, Mexico, and preserved in 20 L capacity plastic containers at 4 °C. Before using the vinasses to feed the reactor or to characterize the influent, they were sedimented for 24 h, diluted with tap water in order to adjust the initial concentration of organic matter in the influent, then the pH was adjusted to 5.5 with a solution of $Ca(OH)_2$ (100 g/L). Vinasses were then characterized according to the parameters presented in Table 1, which were carried out according to the standard and HACH methods (APHA 2005; HACH 2005). VFA determination by the Hach method (8196) reports the total VFA concentration as their equivalent in mg/L of acetic acid.

Table 1 | Characterization of mezcal vinasse

Parameter	Mezcal (Durango)
pH	3.82 ± 0.16
Temperature (°C)	68.67 ± 3.21
VFA (mg/L)	5,815 ± 714
COD (mg /L)	32,966 ± 3,088
BOD_5 (mg /L)	11,700 ± 1,272

Reactor and experimental setup

An 800 mL SBR made of glass was used with an operating volume of 600 mL and an exchange volume of 50%. The temperature was maintained at 35 °C with a coiled thermal jacket. The system was mixed with a recirculation of 216 mL/min. The volumetric production of biogas was determined using the inverted test tube technique (Figure 1).

Sludge from an anoxic stage of the south municipal wastewater treatment plant in the city of Durango (Mexico) was used as inoculum, which was thermally treated at 105 °C for 24 h, following the methodology described by Buitrón & Carvajal (2010) to select the presence of heat-resistant spores of hydrogen-producing bacteria. For the activation of the spores, the SBR was inoculated with 20 g/L of dry mud, using 2 g/L of glucose

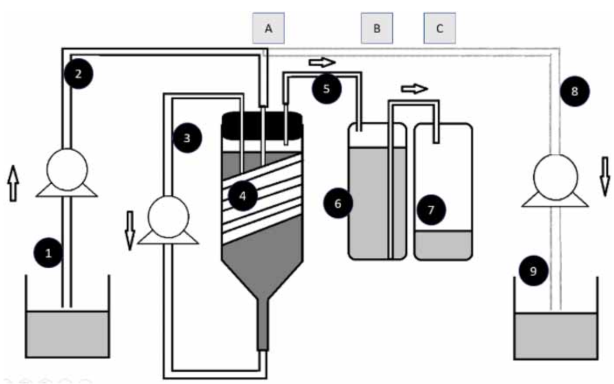

Figure 1 | Experimental setup. (a) SBR; (b) gas collector 'Inverted test tube'; (c) displaced liquid collector. (1) Substrate; (2) influent; (3) recirculation of mixed liquor; (4) thermal jacket; (5) biogas collected; (6) acid solution; (7) displaced acidic solution; (8) effluent; (9) exchange volume.

as an easily assimilable substrate. A modification was made to the methodology of Buitrón & Carvajal (2010), which consisted of adding 1% mezcal vinasse and only macronutrients (250 mg of K_2HPO_4 and 520 mg of NH_4Cl) as a feedstock. The pH was maintained at 5.5 ± 0.2, and retention time (RT) of 1 d was applied (equivalent to a hydraulic retention time (HRT) of 2 d, considering the exchange volume of the reactor). The inoculum was considered activated in the DF process once the system reached a constant removal efficiency of COD and a stable production of VFA.

Biomass acclimation

Once the inoculum was activated, the biomass acclimation was carried out in seven steps, by gradually increasing the initial concentration of vinasse and simultaneously decreasing the glucose (Table 2) so that the tolerance of the microorganisms to the presence of recalcitrant compounds increased and the need for an easily assimilable carbon source (glucose) decreased. The adaptation steps were performed by operating the reactor with treatment cycles of four days (HRT = 8 d). The other operating conditions of the SBR remained constant. The acclimation of the biomass to the recalcitrant compounds was measured indirectly as a function of the COD removal, the production of VFA and biogas. If a dark fermentation is being carried out, the VFAs would accumulate in the reactor, but if they are being consumed with other organic compounds, an anaerobic/methanogenic process is likely to take place (Elbeshbishya *et al.* 2017). At each stage, a minimum of three treatment cycles was carried out or until the system was stabilized at a new concentration of vinasse, which was determined when a constant COD removal, VFA, and biogas production was achieved.

Table 2 | Steps of biomass acclimation of the vinasses

Step	Vinasses (%)	Glucose (g/L)	Initial COD (mg/L)
1	7	0.75	2,422 ± 124
2	15	0.5	4,972 ± 1,119
3	45	0.125	12,639 ± 797
4	55	0.1	14,206 ± 419
5	65	0.05	17,066 ± 922
6	75	0.05	19,648 ± 1,702
7	75	0	21,810 ± 583

Analytical methods

Organic matter (as BOD_5 and COD) and pH were determined according to standard methods (APHA 2005). The VFA content was measured using the 8196 Hach method (HACH 2005). The composition of the biogas (CH_4, CO_2, and H_2) was analyzed using a SRI 8610C gas chromatograph equipped with a thermal conductivity detector and a 30-m-long (0.53 mm ID) Carboxen-1010 PLOT column. The operating conditions were set as follows: the carrier gas was nitrogen at a flow rate of 4.5 mL/min; the temperature of the injector was 200 °C; the column temperature was 100 °C, and the temperature of the detector was fixed at 230 °C.

Statistical analysis

A Tukey test ($p > 0.05$) was performed applying the software GraphPAD 8 with the aim of determining if the initial concentration of vinasses feeding the reactor had a statistical effect over the concentration of VFAs generated at the end of each treatment cycle and the accumulated generation of biogas.

RESULTS AND DISCUSSION

Inoculum activation

Figure 2 shows that during the first four days of operation, there was a progressive decrease in the initial concentration of COD on the SBR, which went from 4,125 to 1,475 mg COD/L. This is because a new substrate, a mixture of vinasses, glucose, and macronutrients were introduced every day (influent or exchange solution) and it took this time for the exchange solution and the mixed liquor remaining in the reactor (with the settled sludge) to reach a state of equilibrium at the initial concentration. During these first four days, an average minimum removal of COD was observed between the influent and the effluent (4.41 ± 3%) and an intermittent generation of VFA from 4.5 to 394 mg VFA/L. From operation day 5 on, a constant removal of COD (435 ± 158 mg/L) was observed, equivalent to a removal efficiency of 25 ± 6%. The SBR reactor presented a constant production of VFA of 94 ± 45 mg VFA/L, equivalent to a production of 52 ± 28%. The inoculum was considered activated to a DF process (Figure 2) since the generation of VFA indicates that a fermentation process is

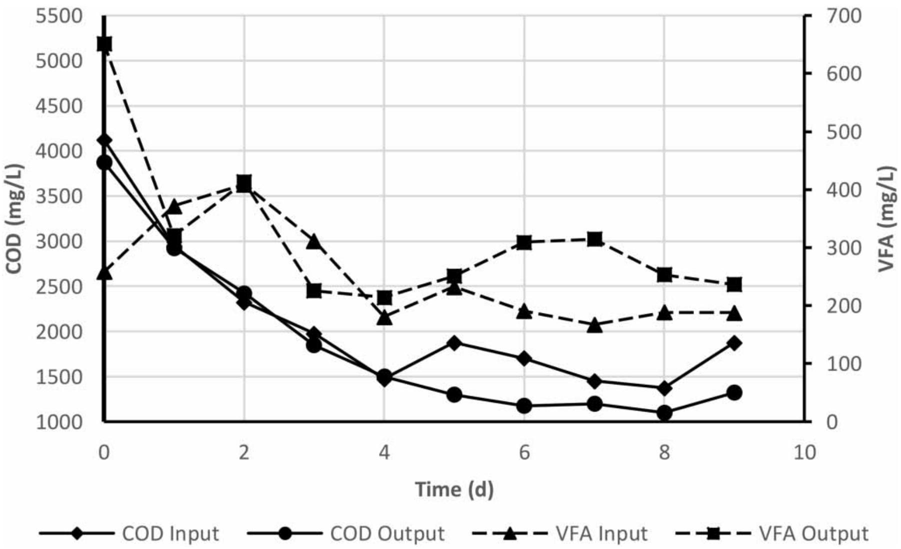

Figure 2 | Inoculum activation by applying RT of 1 d, a pH of 5.5 at 35 °C.

occurring, and its accumulation indicates that an anaerobic/methanogenic process is not being carried out.

Biomass acclimation

Once the inoculum was activated, the acclimation strategy was applied. The acclimation was evaluated based on the concentration of the removed organic matter (i.e., COD), production of VFA, as well as the generation and composition of the biogas production. By increasing the initial concentration of vinasses during the inoculum activation from 1% (1,220 ± 92 mg COD/L) to 7% (2,422 ± 124 mg COD/L) in the first stage of the acclimation process, a decrease in COD removal efficiency was observed from 25 ± 6% to 11 ± 4%. The increase of the concentration of mezcal vinasses in the influent could increase the concentration of toxic compounds and a possible microbial inhibition could be observed (Buitrón & Carvajal 2010). However, the increase of the concentration of vinasse from 7% to 75% (21 810 ± 583 mg COD/L) gradually increased the COD removal rate from 68 ± 22 mg/L d^{-1} to 1,125 ± 234 mg/L d^{-1} (equivalent to 11 ± 4% to 20 ± 4% of the initial concentration) at the end of the acclimation process (Figure 3). The efficiency observed during the acclimation process agrees with that reported by previous works in which tequila vinasses were treated by DF systems, obtaining COD removal efficiencies between 9% and 17%, because in these systems the organic matter is mainly transformed into VFA instead of being removed (Buitrón *et al.* 2014; García-Depraect & León-Becerril 2018). Therefore, it can be inferred that the microorganisms in the reactor were adapted to the toxic compounds of mezcal vinasses throughout the different stages of the acclimation process.

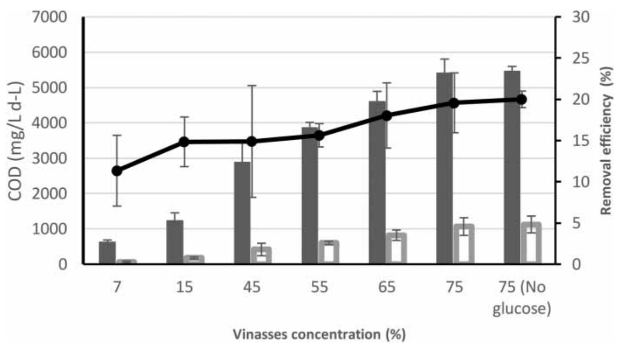

Figure 3 | Organic matter removal (COD) during acclimation.

Regarding the evolution of COD removal kinetics during the biomass acclimation process, when the SBR was operated with different vinasses concentration, it was observed that when the SBR was fed with 15% vinasses, it was possible to remove the highest concentration of COD between days 1 and 3 of operation, having approximately 40% of the initial substrate as glucose and 60% provided by the carbon compounds of the vinasses. By increasing the vinasse content to 45% and decreasing the glucose concentration by 84.4%, the highest concentration of COD was removed at the end of the operating cycle between days 3 and 4 of operation. This can be explained given that the concentration of the more complex substrate (vinasse) was increased, which became the main source of COD (approximately 97%) and there was a period of acclimation to achieve the hydrolysis and removal of this substrate. It can be observed that as the biomass was acclimated to the carbohydrates present in the vinasses, the removal of the COD was faster, occurring more and more towards the first days of the operation cycle. This occurred in such a way that when feeding the system with 75% vinasse and reducing the glucose concentration by 93.4%, COD was mostly removed during days 1 and 2 of the treatment cycle, in which 11 ± 3% of the COD was removed.

Volatile fatty acids production

When increasing the initial concentration of vinasses from 1% (197 ± 32 mg VFA/L), during the inoculum activation process, to 7% (400 ± 122 mg VFA/L) in the first acclimation stage, there were no significant differences ($p < 0.05$) in the production of VFA generation efficiencies, which were of 52 ± 28% and 49 ± 16%, respectively. As the vinasses concentration increased from 7% to 75% (6,275 ± 42 mg VFA/L), a gradual increase in the production efficiency of accumulated VFAs of 49 ± 16% (215 ± 35 mg VFA/L) was observed at 74 ± 21% (980 ± 538 mg AGV/L), respectively (Figure 4). This would indicate that the microorganisms inside the reactor adapted to assimilate higher concentrations of the carbohydrates present in the vinasses and transform them into VFA through a fermentation process in such a way that, towards the end of the acclimation process, the biomass is adapted to produce and accumulate 50% more VFAs from complex substrates than those produced from glucose, which shows the success of the acclimation process.

Significant statistical differences (values between α and $p < 0.05$ equals to 0.0125 y 0.0289) were found in the production of VFA at the end of three operational periods when feeding the reactor with three different concentrations (15, 45, and 75%) of vinasses. The increase in the initial concentration of vinasses in the influent benefits the production of VFA. After analyzing the behavior in the generation of VFA during the treatment cycles in the SBR throughout the acclimation process (Figure 5), it can be observed that by feeding the system with a larger proportion of easily assimilated organic matter composed of 40% glucose and 60% carbohydrates from mezcal vinasses (initial vinasses concentration of 15%), the main production of VFAs (291 ± 104 mg VFA/L) was obtained during the first 24 h of operation and a second increase of 69 ± 31 mg VFA/L appeared between days 2 and 4 of operation. The first increase in the generation of VFAs can be attributed to the degradation of the easily assimilable carbon source (glucose), while the second (of lower concentration), can be associated with the beginning of an adaptation process to the degradation of the more complex carbon compounds present in vinasses.

It was also observed that, as the acclimation process advanced and since carbohydrates from vinasses represent most of the available organic matter (fed with initial vinasses concentrations between 45 and 75%), the formation and accumulation of VFA occurred faster, at between 24 and 48 h of operation, showing that the hydrolysis and acidogenesis from the carbohydrates of the vinasses were carried out successfully after the acclimation process. However, this process took twice as long as having an easily assimilable soluble substrate as a carbon source. This is most likely because a previous hydrolysis process is necessary to transform complex carbohydrates into VFA, contrary to what happens when a simple carbohydrate such as glucose is used as the main substrate.

Biogas production

During the first two acclimation steps, with 7% and 15% vinasses concentration, biogas production was not detected. Figure 6 shows the volumetric production of biogas

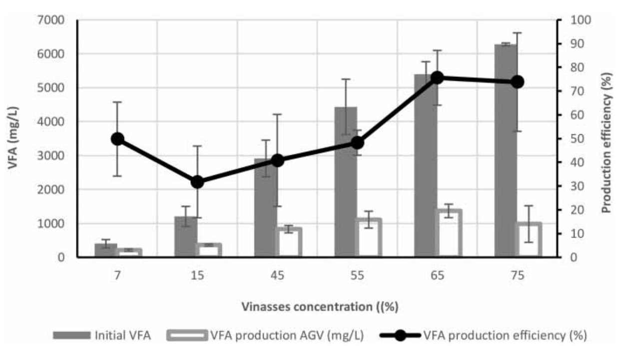

Figure 4 | Volatile fatty acid production during acclimation process.

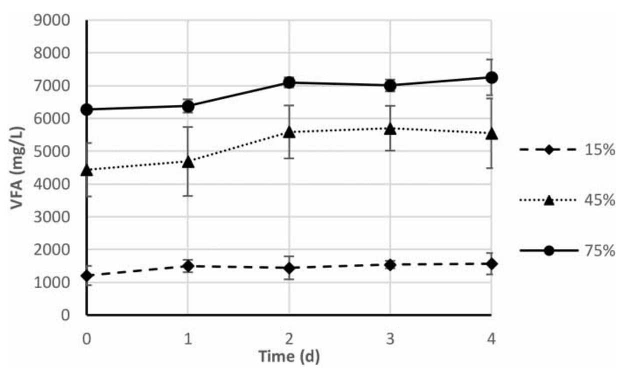

Figure 5 | Volatile fatty acid production kinetics with different vinasses concentration.

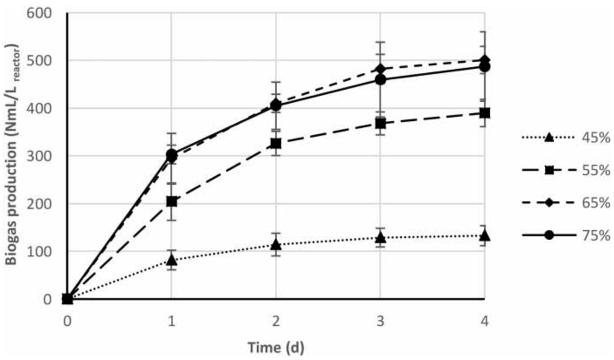

Figure 6 | Biogas production during acclimation with different vinasses concentration.

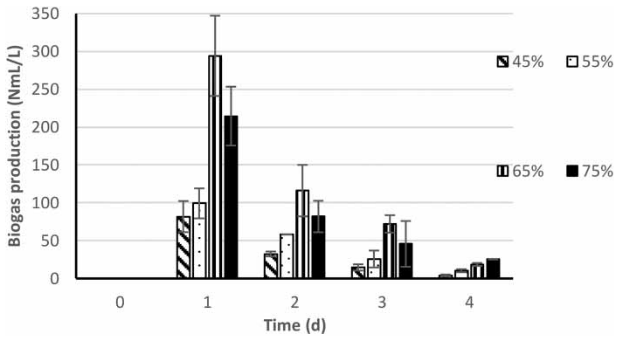

Figure 7 | Daily production of biogas by varying the concentration of vinasses.

accumulated as the initial concentration of vinasses increased from 45% to 75%. The COD present in the feeding solution with 45% vinasses had 97% of the COD coming from the carbohydrates present in the vinasses and only 3% as easily degradable organic matter from glucose. Under these conditions, biogas production was successfully achieved, proving that acidogenesis and acetogenic were occurring by degrading carbohydrates present in mezcal vinasses.

The increase of the initial concentration of vinasses from 45% to 65% significantly increased the volumetric production of biogas. The initial COD increased from 12,639 ± 797 to 17,066 ± 922 mg COD/L generating 133 ± 21 and 501 ± 29 NmL/L of biogas, respectively. However, with the increase to 75% vinasses (19 648 ± 1 702 mg COD/L) no significant difference ($p > 0.05$) in biogas production was observed. It is possible that by feeding the system with a vinasses concentration higher than 70%, the maximum concentration of carbohydrates that biomass can degrade, has been reached in such a way that a substantial increase in biogas production was not generated by increasing the concentration of COD between these stages.

By analyzing the daily biogas generation for each acclimation step (Figure 7), it can be observed that for all cases the maximum volume of biogas generation was obtained during the first 24 h as higher concentrations of organic matter were available so that, as the substrate was exhausted, the volume of biogas generated progressively decreased.

Long-term operation of the SBR

Once the SBR was fed with an initial concentration of 75% vinasses and operated with an RT of 4 d, the system was operated for a long-term period (63 cycles). Operating the SBR under a RT of 4 d equivalent to an HRT of 8 d generated various changes in the system. (i) By analyzing the behavior of the reactor for VFAs and biogas generation, at the end of the treatment cycle, it can be observed that from cycle 46 onwards the concentration of VFAs decreased from 6,813 ± 418 to 5,640 ± 42 mg/L, coinciding with a decrease in the accumulated production of biogas (Figure 8(a) and 8(b)). (ii) The accumulated biogas production decreased by 11%, from 385 ± 31 to 341 ± 77 NmL/L d. (iii) After analyzing the representative operational cycle (56) during this period, it can be observed that regarding the COD removal kinetics, the COD was not only removed during the first 24 h, but presented a continuous removal during the 4 d of RT (Figure 8(c)), reaching an efficiency of 20 ± 1% removal. (iv) VFA kinetic before cycle 46 had a behavior as follows: they were generated and accumulated in the first 48 h (remaining stable from this period onwards) (Figure 5), but after 46 cycles presented the highest production, up to 7,570 ± 99 mg VFA/L, at 36 h of operation, and then suddenly decreased to between 36 and 48 h of the treatment cycle (Figure 8(d)). VFA did not remain accumulated and one hypothesis is that they were most likely consumed by acetotrophic/methanogenic microorganisms. In this respect, the biogas composition under these conditions before cycle 46 was 79 ± 4% H_2, 11 ± 1% CH_4, and 10 ± 2% CO_2, and the composition of biogas after cycle 46 was 5 ± 1% H_2, 28 ± 4% CH_4, and 67 ± 4% CO_2.

The composition of the biogas obtained after cycle 46 differed from what was expected for a DF in residues associated with the production of alcoholic beverages, in which it is possible to obtain from 31% to 72% hydrogen (Buitrón et al. 2014; Sydney et al. 2014; García-Depraect & León-Becerril 2018). These changes indicate that operating the SBR after biomass acclimation with an HRT of 8 d for a long-time period (46 operational cycles) favored the development of methanogenic populations, which consumed the VFAs generated to produce methane (Moraes et al. 2015). Methanogenic microorganisms could be fed into the system

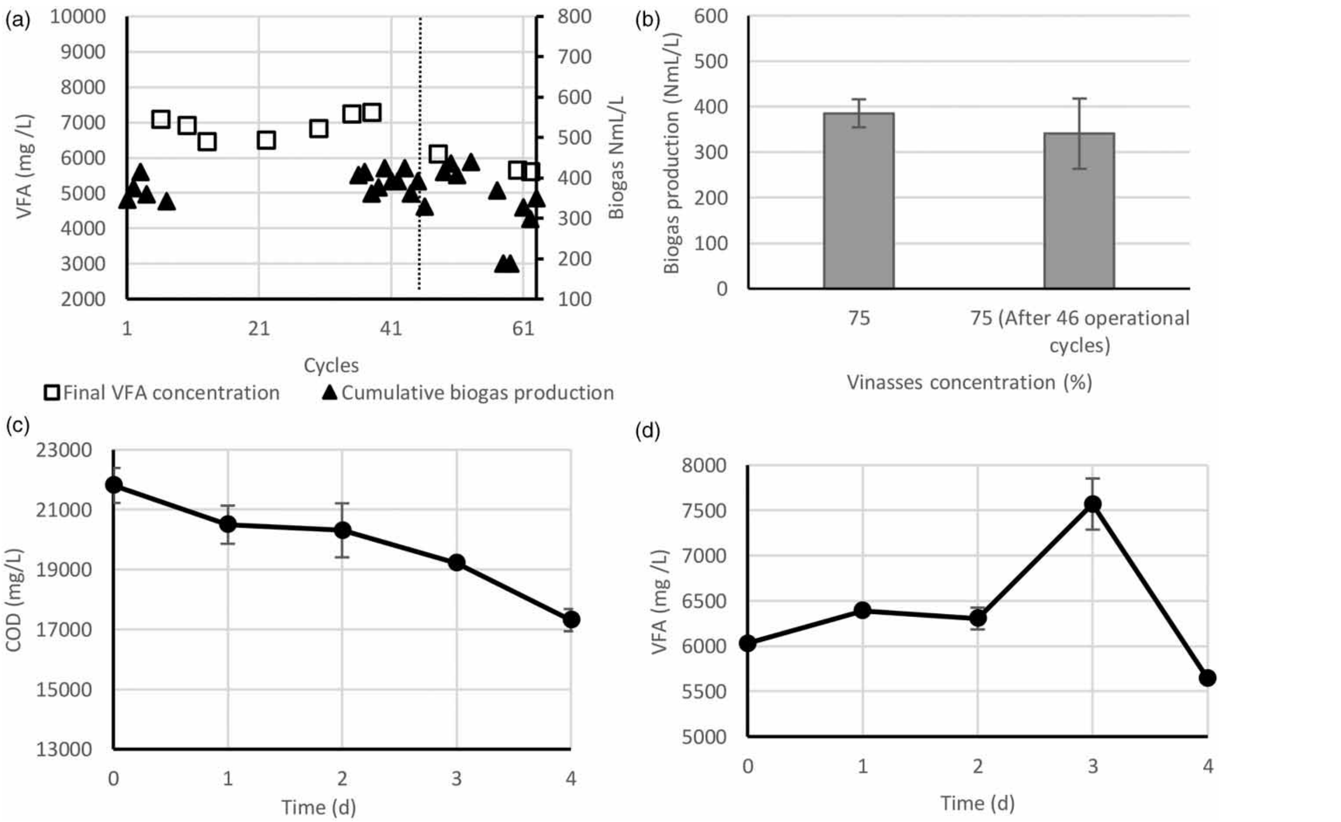

Figure 8 | Parameters analyzed during the long-term operation of the SBR, maintaining the initial concentration of vinasses at 75%: (a) final concentration of VFAs and biogas at the end of 60 operational cycles; (b) accumulated biogas production; (c) kinetic of COD removal during one operational cycle (after cycle 46); (d) VFA production kinetics during an operational cycle after (cycle 46).

in the vinasses and established in the reactor, using the VFAs produced by acidogenic bacteria as substrate, despite the acidic conditions for the hydrogen production in a dark fermentation that were maintained. That is, the average pH of the influent was 5.58 ± 0.10, and the average pH of the effluent was 5.18 ± 0.11 (Supplementary material) (Buitrón *et al.* 2014; Arreola-Vargas *et al.* 2016). In this regard, De Beer *et al.* (1996) reported that the biofilm could be 'heterogeneous', allowing the coexistence of different 'micro-ecosystems' with different pH environments. In these cases, the most acidic micro-ecosystems or those with higher resistance to environmental stress conditions are in the external part of the biofilm in contact with the mixed liquor, while the areas with a pH close to neutrality and less resistance to environmental stress (such as methanogenic bacteria) are in the internal part (Azeredo et al. 2016; Fulaz et al. 2019). Taking this into consideration, it is necessary to reduce the RT of the reactor, to periods ranging between 24 and 48 h, to favor the generation and accumulation of VFA and preventing the colonization of methanogenic microorganisms and thus guaranteeing a dark fermentation process.

CONCLUSIONS

The activation of a (heat-treated) inoculum, in a dark fermentation process, was achieved using glucose as an easily assimilable carbon source, after five days of operation. The biomass was successfully acclimated to mezcal vinasses with a concentration of 19,648 ± 1,702 mg COD/L (75% of mezcal vinasses), reaching a COD removal rate of organic matter of 1,125 ± 234 mg COD/L d-1 (equivalent to a removal efficiency of 20 ± 3%), together with a production of 1 390 ± 99 mg VFA/L. It was determined that acclimating the biomass, keeping the SBR operating with an RT of 4 (HRT of 8 d), favored the growth of methanogenic biomass that consumed the previously formed VFA, thus increasing the concentration of methane in the biogas. Possibly these methanogenic microorganisms entered the system in the influent, due to the use of a non-innocuous substrate (vinasses). Therefore, in order to maintain a fermentation process that promotes the generation and accumulation of VFAs, it is recommended to operate the reactor with a reaction time between 24 and 48 h.

ACKNOWLEDGEMENTS

The financial support granted by 'Fondo de SIP IPN (Mexico)' through project 2020 2000 energy production from liquid waste from the agroindustry is gratefully acknowledged. S.A. Díaz-Barajas thanks CONACYT for the award of a scholarship.

CONFLICTS OF INTEREST

The authors declare that they have no competing interests.

DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

REFERENCES

APHA 2005 *Standard Methods for the Examination of Water and Wastewater*, 23rd edn. American Public Health Association, Washington, DC, USA.

Arreola-Vargas, J., Jaramillo-Gante, N. E., Celis, L. B., Corona-González, R. I., González-Álvarez, V. & Méndez-Acosta, H. O. 2016 Biogas production in an anaerobic sequencing batch reactor by using tequila vinasses: effect of pH and temperature. *Water Science & Technology* **73** (3), 550–556.

Azeredo, J., Azevedo, N. F., Briandet, R., Cerca, N., Coenye, T., Costa, A. R. & Sternberg, C. 2016 Critical review on biofilm methods. *Critical Reviews in Microbiology* **43** (3), 313–351.

Buitrón, G. & Carvajal, C. 2010 Biohydrogen production from Tequila vinasses in an anaerobic sequencing batch reactor: effect of initial substrate concentration, temperature, and hydraulic retention time. *Bioresource Technology* **101** (23), 9071–9077.

Buitrón, G., Prato-Garcia, D. & Zhang, A. 2014 Biohydrogen production from tequila vinasses using a fixed bed reactor. *Water Science & Technology* **70** (12), 1919–1925.

Consejo Regulador del Mezcal (CRM) 2020 *Informe estadístico 2020*. México.

De Beer, D., Stoodley, P. & Lewandowski, Z. 1996 Liquid flow and mass transport in heterogeneous biofilms. *Water Research* **30** (11), 2761–2765.

Elbeshbishya, E., Dharb, B. R., Nakhlac, G. & Leed, H. S. 2017 A critical review on inhibition of dark biohydrogen fermentation. *Renewable and Sustainable Energy Reviews* **79**, 656–668.

Fulaz, S., Hiebner, D. W., Barros, C., Devlin, H., Vitale, S., Quinn, L. & Casey, E. 2019 Ratiometric imaging of the in-situ pH: distribution of biofilms by use of Fluorescent Mesoporous Silica Nanosensors. *ACS Applied Materials & Interfaces* **11**, 32679–32688.

García-Depraect, O. & León-Becerril, E. 2018 Fermentative biohydrogen production from tequila vinasse via the lactateacetate pathway: operational performance, kinetic analysis, and microbial ecology. *Fuel* **234**, 151–160.

García-Depraect, O., Rene, E. R., Gómez-Romero, J., López-López, A. & León-Becerril, E. 2019 Enhanced biohydrogen production from the dark co-fermentation of tequila vinasse and nixtamalization wastewater: novel insights into ecological regulation by pH. *Fuel* **253**, 159–166.

HACH 2005 *Hach Company. DR5000 Spectrophotometer: Procedures Manual*, 2nd edn. Hach Company, Duesseldorf, Germany.

Moraes, B. S., Zaiat, M. & Bonomi, A. 2015 Anaerobic digestion of vinasse from sugarcane ethanol production in Brazil: challenges and perspectives. *Renewable and Sustainable Energy Reviews* **44**, 888–903.

Robles-González, V. S., Galíndez-Mayer, J., Rinderknecht-Seijas, N. & Poggi-Valardo, H. N. 2012 Treatment of mezcal vinasses: a review. *Journal of Biotechnology* **157** (4), 524–546.

Sydney, E. B., Larroche, C., Novak, A. C., Nouaille, R., Sarma, S. J., Brar, S. K. & Soccol, C. R. 2014 Economic process to produce biohydrogen and volatile fatty acids by a mixed culture using vinasse from sugarcane ethanol industry as nutrient source. *Bioresource Technology* **159**, 380–386.

Wang, H. & Zhao, Y. 2009 A bench scale study of fermentative hydrogen and methane production from food waste in integrated two-stage process. *International Journal Hydrogen Energy* **34** (1), 245–254.

First received 15 February 2021; accepted in revised form 24 April 2021. Available online 6 May 2021