Characterization and anaerobic digestion of highly concentrated Mexican wine by-products and effluents

M. Vital-Jacome, M. Cazares-Granillo, J. Carrillo-Reyes and G. Buitron

ABSTRACT

Wine production has increased in recent years, especially in developing countries such as Mexico. This increase is followed by an increase of winery effluents that must be treated to avoid environmental risks. However, little information is available about the characteristics of these effluents and the possible treatments. This paper aimed to characterize the effluents and by-products generated by the Mexican winery industry and to evaluate the performance and stability of the anaerobic treatment using a single-stage and a two-stage process. Results showed that the winery effluents had a high content of biodegradable organic matter, with chemical oxygen demand (COD) values ranging from 221 to 436 g COD/L. The single-stage anaerobic process was able to treat an organic loading rate of 9.6 kg COD/(m$^3$ d); however, it was unstable and highly dependent on the addition of bicarbonate alkalinity (0.31 g NaHCO$_3$/g COD removed). The two-stage process was more stable working at a higher organic load (12.1 kg COD/(m$^3$ d)) and was less dependent on the addition of bicarbonate (0.17 g NaHCO$_3$/g COD removed). The results highlight the potential of the winery effluents to produce methane through anaerobic digestion in a two-stage process, making wine production more sustainable.

Key words | alkalinity, anaerobic digestion, methane, winery wastewater

INTRODUCTION

One of the most important alcoholic beverages around the world is grape wine, with a global estimated demand of 25 billion litres, and a continuously growing market (Zacharof 2017). European countries have traditionally been the top wine-producing countries of the world, but with an increasing population interested in wine consumption, other countries have increased their production significantly in recent years. Mexico is no exception since its wine industry annually produces around 200,000 hectolitres of wine, and its demand has grown by 184% since 2000 (OIV 2017). As a result of this boost, the amount of winery wastes produced by these industries has also increased. In European countries such as France, producers are forced to deliver winery wastes to wine distilleries, in order to obtain by-products such as grape seeds, standardized fertilizers, alcohols, and calcium tartrate (Lempereur & Penavayre 2004). In contrast, in countries such as Mexico, winery industries must take care of their wastes, which are often used directly in agriculture as organic fertilizers. However, due to their composition, the use of these wastes without any pretreatment can cause significant environmental risks (Bustamante et al. 2005).

Among winery wastes, winery effluents and by-products are some of the most important sources of pollution. Winery effluents are the wastewater that comes from various operations during wine production, especially washing operations during vintage and grape reception, rinsing and sanitizing activities, tank washing, cleaning of filters, barrel washing and product losses. Winery effluents are characterized by a high concentration of chemical oxygen demand (COD), ranging from 0.8 to 296 g COD/L (Bustamante et al. 2005), and a high concentration of suspended solids, ethanol, organic acids, phenols, and polyphenols among other recalcitrant compounds.

Several technologies have been proposed to treat winery effluents, including physicochemical and biological processes (Ioannou et al. 2015). Among biological processes,
anaerobic digestion is the most attractive option, due to its capacity to treat highly concentrated effluents and the recovery of energy in the form of biogas (Moletta 2005), which can be used to generate electricity and heat power, making wine production more sustainable.

However, selecting the most suitable and cost-effective anaerobic technology is a complicated task, because each wine industry produces effluents with unique characteristics, making it impossible to agree on the most suitable treatment. Moreover, reports of winery wastewater treatments dealing with high influent concentrations are somewhat limited, even when these types of effluents are the most common in industries.

Recently in Buitrón et al. (2019), noteworthy results were achieved concerning the anaerobic treatment of highly concentrated winery effluents through a two-stage process, in which each stage was optimized independently. It was argued that the use of a two-stage system was preferred over single-stage systems due to instability issues of single-stage systems, commonly associated with reduced buffering capacity and accumulation of volatile fatty acids (VFAs). However, before taking the process to a larger scale, it is essential to evaluate the real feasibility of the single-stage process, in order to avoid a possible increase in technical complexity, as well as higher capital and operational costs related to the two-stage process, which does not always lead to more significant economic gains.

In this regard, this research had two main objectives: first, to characterize the effluents from Mexican wine industries, since there are no previous studies about their characteristics in the medium term (4 years); second, to assess the stability and feasibility of the anaerobic treatment of these effluents, using a single-stage and two-stage anaerobic process.

The second objective was achieved following the through the following experimental strategy: first, a single-stage anaerobic process was operated, consisting of a methanogenic continuous stirred tank reactor (CSTR) treating winery effluents, operating the system without and with alkalinity control; second, the methanogenic reactor was coupled to an acidogenic stage (another CSTR), converting the system into a two-stage anaerobic process. Conventional CSTR configurations were chosen due to the high solids content in the winery effluents. The stability of both systems was assessed by evaluating the methane production, the COD removal, the alkalinity index and the accumulation of VFAs during operation. The performance of the single-stage process was compared with the two-stage coupled process and with other previous reports in the literature.

MATERIALS AND METHODS

Winery effluents

Ten samples of winery effluents were collected over 4 years from two different wine industries located in the state of Querétaro, México. These effluents come from the production of white and red wine and were analyzed in terms of pH, total COD, total and volatile solids (TS, VS), total and volatile suspended solids (TSS, VSS), total phosphorus (P-PO$_4^{3-}$), ammonium nitrogen (N-NH$_4^+$), total carbohydrates, phenols, ethanol and VFAs concentrations.

Anaerobic processes

The single-stage anaerobic process consisted of a methanogenic CSTR of 4 L working volume (Applikon Biotechnology) operated in mesophilic conditions (35 °C) with a mixing speed of 150 rpm. Mixing was achieved by using the Rushton impellers and baffles included by the bioreactor manufacturer. The pH was controlled during reactor operation at 7.5 ± 0.1 using 3 M NaOH and HCl and the alkalinity was controlled by the addition of sodium bicarbonate. The inoculum used for the anaerobic treatment was granular anaerobic sludge from an industrial upflow anaerobic sludge blanket (UASB) reactor treating brewery wastewater. This inoculum was selected because previous experiments in the laboratory showed good methanogenic activity (500 ± 100 mL CH$_4$/(g VS d), and 330 ± 19 mL CH$_4$/g COD in glucose). The methanogenic reactor was inoculated with 16 g VS/L of anaerobic granular sludge (800 mL). The reactor was operated in batch mode during the start-up, where two batch cycles were performed using an initial concentration of 20 g COD/L of winery effluents and 4 L working volume. The two start-up batch cycles lasted for 20 days each, at the end of each batch the solids were settled, and 50% of the supernatant was renewed.

For the single-stage operation, the reactor was fed with wine effluents diluted to a concentration of 73.7 ± 7.6 g COD/L, and the hydraulic residence time (HRT) was 8 days with constant organic loading rate (OLR) of 9.6 kg COD/(m$^3$ d). After start-up, the anaerobic reactor was operated in continuous mode for 111 days, during which two phases can be distinguished: in phase I, from day 0 to 72, only pH was controlled while alkalinity was only monitored; in phase II, from day 72 to 111, pH was controlled, and alkalinity was also controlled by the addition of sodium bicarbonate to values below 0.6.
For the two-stage anaerobic treatment, the methanogenic reactor was restarted, using the same inoculum as for the one-stage treatment: anaerobic granular sludge at a concentration of 16 g VS/L. The methanogenic reactor was coupled to an acidogenic CSTR of 1 L working volume (Applikon Biotechnology) operated in mesophilic conditions (35 °C) with a mixing speed of 150 rpm. The pH of the acidogenic reactor was controlled at 5.5 ± 0.1 using 3 M NaOH and HCl. This reactor had an endogenous inoculum, consisting of the microorganisms already present in the winery effluent, which was previously enriched and acclimated. Acclimation was performed by operating the acidogenic reactor as a sequencing batch reactor for 30 days, with winery effluents diluted to a concentration of 75.5 ± 4.3 g COD/L. At the end of each cycle, the solids were settled, and 25% of the supernatant was renewed. During this acclimation, the HRT in the reactor was reduced from 8 to 4 to 2 days; after that, the reactor was operated in continuous mode. The two-stage process operated with a fixed HRT of 2.1 days for the acidogenic reactor and 13.7 days for the methanogenic reactor (15.81 days combined). This selection derives from the best conditions found in Buitrón et al. (2019), where the two stages were optimized independently. During the two-stage operation, the effluent of this reactor was directly fed to the methanogenic reactor, and two phases (named phase III and phase IV) can also be distinguished: in phase III, an OLR of 35.4 kg COD/(m³ d) was applied to the acidogenic reactor, resulting in an OLR of 4.2 kg COD/(m³ d) in the methanogenic reactor; in phase IV, an OLR of 99.2 kg COD/(m³ d) was applied to the acidogenic reactor, resulting in an OLR of 12.1 kg COD/(m³ d) in the methanogenic reactor. Throughout the experimental phases, the volume of biogas produced was quantified with a continuous flow meter (μFlow, Bioprocess Control, Sweden), and the methane concentration was measured by gas chromatography (see analytical methods).

Alkalinity

Liquid samples of 10 mL were taken from the reactor effluent and the alkalinity was determined by direct titration using 0.1 N HCl. First, each sample was titrated from their initial pH to pH 5.75 (alkpH 5.75) and then from pH 5.75 to pH 4.3 (alkpH 4.3) (Ripley et al. 1986). The alkalinity index (α) was selected as the control parameter as suggested by Rojas (1987) and according to Equation (1). This index corresponds to the ratio between the alkalinity due to the concentration of VFAs with respect to the total alkalinity. The suggested range of variation for this index is between 0.2 and 0.4.

\[
\alpha = \frac{\text{alkpH 4.3} - \text{alkpH 5.75}}{\text{alkpH 4.3}}. \tag{1}
\]

Analytical methods

The total and soluble COD were measured using the colorimetric closed reflux method (Hach 435, range from 0 to 15,000 mg COD/L). The content of solids as TS, VS, TSS, and VSS was measured using standard gravimetric methods (APHA 2005). Soluble concentrations of N-NH₄⁺, P-PO₄⁻, phenols and total carbohydrates were measured by the salicylate method, the molybdovanadate colorimetric method, and the colorimetric phenolsulfuric acid method, respectively (Dubois et al. 1956; APHA 2005). Liquid samples for soluble COD, ethanol and VFAs were first centrifuged at 3,500 rpm for 10 min and then filtered using 0.45 μm nylon membranes before analysis. Ethanol and VFAs concentrations in the liquid were measured by gas chromatography (Agilent Tech 6890N, Varian 330 C) according to Carrillo-Reyes et al. (2019). Gas samples of 10 mL were taken from the headspace of the reactor to characterize the biogas produced. Methane and carbon dioxide in biogas were measured by gas chromatography (SRI model 8610C) according to Carrillo-Reyes et al. (2019).
The amount of wine lees also had an impact on the high ethanol content of the winery effluents, which can represent from 49 to 92% of the total COD, corresponding to an ethanol concentration from 60 to 120 g/L. In this evaluation, ethanol was not determined in all samples because the analytical technique was not ready during the first years, but due to the high COD content (Table 1) and considering that those effluents were not distilled, it can be assumed that residual ethanol was a common characteristic in all samples. The presence of this ethanol must be carefully considered because it can be inhibitory for anaerobic digestion processes (Camarillo & Rincón 2009). The low content of nitrogen in the effluents could also cause some problems for anaerobic digestion, due to an imbalance concerning the carbon source (Chen et al. 2008). Phenolic compounds, which were found in more significant proportion in red wine effluents than in white wine effluents, could also be inhibitory to methanogenic microorganisms (Chen et al. 2008).

The use of these winery effluents is not recommended for direct application in agriculture, due to the high organic matter content and the low pH, which can increase soil salinity and could cause phytotoxic effects on crop growth (Bueno et al. 2009; Mosse et al. 2010). On the other hand, the characteristics of these effluents make them suitable candidates for an anaerobic digestion treatment. The effluents treated in this research corresponded to the red wine effluents from the winery B of the year 2018.

### Single-stage process

In the single-stage process, the reactor was fed with a winery effluent concentration of 73.7 g COD/L, HRT of 8 days and with a constant OLR of 9.6 kg COD/(m³ d). During phase I, pH was controlled while alkalinity was only monitored, and the CSTR was operated in the optimal conditions of pH and temperature for methanogenesis; however, the biogas production was low and intermittent during this phase, with values below 1 m³/(m³ d) as observed in Figure 1. The

### Table 1 | Characterization of red and white winery effluents produced by two wineries in México for 4 years

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<td>pH</td>
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<td>Total COD (g/L)</td>
<td>436 ± 1</td>
<td>270 ± 14</td>
<td>268 ± 5</td>
<td>252 ± 9</td>
<td>389 ± 17</td>
<td>375 ± 1</td>
<td>221 ± 1</td>
<td>250 ± 1</td>
<td>273 ± 1</td>
<td>223 ± 1</td>
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<tr>
<td>TS (g/L)</td>
<td>231 ± 3</td>
<td>82 ± 1</td>
<td>–</td>
<td>58 ± 0</td>
<td>197 ± 4</td>
<td>132 ± 0</td>
<td>154 ± 2</td>
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<td>73 ± 0</td>
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<td>VS (g/L)</td>
<td>209 ± 2</td>
<td>69 ± 0</td>
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<td>51 ± 0</td>
<td>21 ± 1</td>
<td>28 ± 0</td>
<td>24 ± 1</td>
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<td>49 ± 1</td>
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<td>TSS (g/L)</td>
<td>199 ± 3</td>
<td>36 ± 1</td>
<td>60 ± 0</td>
<td>35 ± 0</td>
<td>103 ± 4</td>
<td>93 ± 6</td>
<td>66 ± 2</td>
<td>37 ± 2</td>
<td>52 ± 1</td>
<td>8 ± 1</td>
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<td>VSS (g/L)</td>
<td>183 ± 3</td>
<td>32 ± 1</td>
<td>51 ± 0</td>
<td>33 ± 0</td>
<td>6 ± 0</td>
<td>18 ± 1</td>
<td>5 ± 1</td>
<td>31 ± 1</td>
<td>42 ± 3</td>
<td>6 ± 1</td>
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<tr>
<td>P-PO₄³⁻ (g/L)</td>
<td>6 ± 0</td>
<td>2.3 ± 0.0</td>
<td>3.8 ± 0.0</td>
<td>0.3 ± 0.0</td>
<td>24 ± 0</td>
<td>31 ± 0</td>
<td>18 ± 1</td>
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<td>N-NH₄⁺ (g/L)</td>
<td>0.1 ± 0</td>
<td>0.02 ± 0.00</td>
<td>0.1 ± 0.00</td>
<td>0.5 ± 0.00</td>
<td>0.7 ± 0.1</td>
<td>0.2 ± 0.00</td>
<td>1.3 ± 1</td>
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<td>Total carbohydrates (g/L)</td>
<td>91 ± 1</td>
<td>32 ± 1</td>
<td>22 ± 0</td>
<td>33 ± 0</td>
<td>65 ± 3</td>
<td>58 ± 1</td>
<td>50 ± 4</td>
<td>5.1 ± 1</td>
<td>25 ± 1</td>
<td>6 ± 3</td>
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<td>Phenols (g/L)</td>
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<td>–</td>
<td>0.2 ± 0.0</td>
<td>116 ± 2</td>
<td>20 ± 1</td>
<td>85 ± 1</td>
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<td>CODₑq ethanol (g/L)</td>
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<td>125 ± 3</td>
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<td>142 ± 12</td>
<td>210 ± 5</td>
<td>182 ± 14</td>
<td>207 ± 4</td>
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<td>CODₑq acetate (g/L)</td>
<td>–</td>
<td>–</td>
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<td>–</td>
<td>–</td>
<td>–</td>
<td>4 ± 1</td>
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<td>9 ± 2</td>
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<td>CODₑq propionate (g/L)</td>
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<td>CODₑq butyrate (g/L)</td>
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<td>–</td>
<td>4 ± 1</td>
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*TS, total solids; VS, volatile solids; TSS, total suspended solids; VSS, volatile suspended solids. ‘–’ stands for not determined parameters.
maximum methane yield reached during phase I was 0.08 m$^3$ CH$_4$/kg COD fed. The methane content in the biogas during this phase had large fluctuations, the minimum recorded value was 23%, the maximum value was 75% and the average was 62.6 ± 10.3%. The low biogas production and the high fluctuations in methane content were clear signs of process instability during this phase.

During phase II, when pH and alkalinity were controlled simultaneously, a rapid increase in biogas production was observed, which stabilized after day 90. The biogas production rate reached an average value of 3.5 ± 0.4 m$^3$/m$^3$ d, corresponding to an average methane yield of 0.24 m$^3$ CH$_4$/kg COD fed. The biogas produced during phase II had a more stable methane composition compared to phase I, with lower fluctuations and average composition of 64.8 ± 1.7% of CH$_4$. The results showed that despite an unstable operation during phase I, the complete failure of the process was avoided by maintaining the pH control, while a significant increase in production was achieved by adding alkalinity control without reducing the organic load.

Regarding the COD removal, in phase I, a gradual increase in the COD removal efficiency was observed (Figure 2). However, in parallel to the low methane production, the process was never able to achieve COD removal efficiencies above 36%, indicating an unstable operation. During phase II, after the addition of bicarbonate, the COD removal efficiency was rapidly increased, going from 36% to 85% in only 16 days, equivalent to two HRT. The maximum COD removal efficiency reached was 94.4%, with a reactor effluent concentration of 5.9 g COD/L.

During phase I, when alkalinity was only monitored without making any correction, $\alpha$ values oscillated between 0.7 and 0.9 (Figure 2). High $\alpha$ values usually indicate instability of the anaerobic system, which is attributed to acidification due to the accumulation of VFAs, causing a decrease in the buffer capacity of the medium. The accumulation occurs because the reaction rate of the hydrolytic and acidogenic bacteria in anaerobic digestion is usually higher than the rate of consumption of VFAs by the methanogenic bacteria. At the beginning of phase II, a strong correction of alkalinity was made by the addition of sodium bicarbonate, bringing the $\alpha$ index to values below 0.6. During phase II, a total of 315 g of sodium bicarbonate was added to the system, corresponding to an alkalinity/COD ratio of 0.31 g NaHCO$_3$/g COD removed, which is within the range of values recommended by Boncz et al. (2012) (0.6) and González et al. (1998) (0.4), for anaerobic digestion of vinasses and sugar cane molasses respectively.

In phase II, the $\alpha$ index was maintained below 0.6 but above the recommended values (0.2–0.4); the reason for this was that the addition of bicarbonate in excess increases the pH in the medium above 7.5, the neutralization of the latter causes the release of CO$_2$, decreasing the methane content in the biogas and producing a false measurement of biogas production. Besides, this neutralization can also cause the release of significant amounts of Na$^+$ cations to the medium, and consequently, a possible cation inhibition (Chen et al. 2008). Nevertheless, the bicarbonate addition during this phase was enough to allow the system to maintain an adequate buffer capacity and a stable operation, which was reflected in a significant increase in the biogas production rate and the COD removal efficiency.

The accumulation of VFAs is another indicator of process stability, and they were measured together with the concentration of ethanol during reactor operation (Figure 3). At the beginning of phase I (day 3–13), VFAs such as acetic, propionic and butyric acid accumulated in the medium, indicating that the instability of the process was mainly
due to this accumulation. However, from day 19 to 43, the concentration of VFAs decreased significantly, not exceeding 6 g COD\(_{Eq}/L\), indicating that the system instability was due to other reasons than VFAs accumulation. Interestingly, this decrease in VFAs was not adequately reflected in the \(\alpha\) index, showing that this index is not the only indicator necessary to evaluate the stability of the process. During this period, a significant amount of ethanol accumulated in the system, as observed in Figure 3, which could cause the inhibition of the anaerobic digestion process (Camarillo & Rincón 2009). However, it is worth noting what happened from day 47 to 71, when an important accumulation of VFAs and a decrease in ethanol concentration was observed again. This result suggested that after day 47, the system overcame the inhibition by ethanol, probably by adaptation of the microorganisms to the high concentration of this solvent.

Despite overcoming the inhibition, the system remained unstable until the addition of alkalinity in phase II. From day 72 to 110, the concentration of VFAs and ethanol significantly decreased, due to the increase in the buffer capacity of the medium that promoted the activity of the methanogenic microorganisms, indicating an improvement in the stability. During this period, the removal of ethanol from the winery effluents reached 100% after day 101, equivalent to a removal rate of 6.6 g COD\(_{Eq}/(L \cdot d)\), which is a significant result given the high initial concentration of this solvent.

Two-stage process

The two-stage coupled process was operated under two OLR conditions. In phase III, the OLR was 35.6 kg COD/(m\(^3\) d) in the acidogenic stage and 4.2 kg COD/(m\(^3\) d) in the methanogenic stage, corresponding to an influent concentration close to the concentration used during the single-stage operation (75.5 ± 4.3 g COD/L). In the acidogenic reactor, the VFA production was dominated by acetate (99%) with an acidification degree (percentage ratio of VFA as COD divided by the soluble COD) of 3%. In the methanogenic reactor, the average biogas production rate was 1.3 ± 0.3 m\(^3\)/(m\(^3\) d), with a stable methane content of 57.4 ± 0.2% (Figure 4(a)) and a methane yield of 0.17 m\(^3\) CH\(_4)/kg COD. During this phase, the total COD removal was 96% (Figure 4(b)), while 21% of the COD in the influent was removed in the acidogenic reactor. The \(\alpha\) index remained at values around 0.25 during phase III (Figure 4(b)), and there was no need for the addition of bicarbonate, which remarkably reflects greater stability of the process.

In phase IV, the OLR was 99.2 kg COD/(m\(^3\) d) in the acidogenic stage and 12.1 kg COD/(m\(^3\) d) in the methanogenic stage, corresponding to an influent concentration of 202.1 ± 14.1 g COD/L of undiluted winery effluents. In the acidogenic reactor, the VFA production was still dominated by acetate (99%) with an acidification degree of 8%. In the methanogenic reactor, the average biogas production rate was 5.0 ± 0.4 m\(^3\)/(m\(^3\) d) (Figure 4(a)), with a stable methane content of 62.1 ± 3.2% (Figure 4(a)) and a methane yield of 0.26 m\(^3\) CH\(_4)/kg COD. During phase IV, the COD removal was 92% (Figure 4(b)), and the COD removed in the acidogenic reactor was around 19%. The \(\alpha\) index in phase IV ranged between 0.3 and 0.5 (Figure 4(b)), and bicarbonate was added to keep an \(\alpha\) value close to the suggested values. The alkalinity/COD ratio during this phase was 0.17 g NaHCO\(_3)/g COD\) removed, this is 46% lower than the same ratio during single-stage operation, despite operating at a higher OLR and with a higher COD concentration in the influent.

The above results suggest greater process stability of the two-stage coupled process compared with the single-stage, which was reflected in constant biogas production and COD removal, while no accumulation of VFAs was observed in the process effluent. The improvement in stability was mainly due to the efficient hydrolysis of the
particulate organic matter in the acidogenic reactor, which provides soluble substrates to the methanogenic stage, preventing the accumulation of VFAs in the medium with a reduced buffer capacity (Banks & Humphreys 1998).

In Buitrón et al. (2019) the methanogenic and acidogenic stages of a two-stage process were optimized separately; in that research, the red wine effluents from the winery B of the year 2017 were used (Table 1). Similar results were obtained in terms of stability concerning this investigation since there was no need for the addition of bicarbonate even at higher organic loads such as 26.6 kg COD/(m$^3$ d).

Table 2 presents the results for some selected reactor technologies used to treat highly concentrated winery effluents. It should be noted that the effluents used in this research are among the most concentrated effluents reported in the literature. The OLR used in the single-stage process (9.6 kg COD/(m$^3$ d)) was also high compared with the recommended OLR for anaerobic processes of suspended biomass, which is between 1 and 5 kg COD/(m$^3$ d) (Moletta 2005), although this OLR is in the range of other anaerobic technologies suggested to treat winery effluents, such as the UASB reactors and anaerobic filters (5–20 kg COD/(m$^3$ d)) (Andreottola et al. 2009). However, these configurations are not suitable in the case of highly concentrated effluents, due to the high concentration of solids, which can cause several problems; for example, solids can lead to the formation of low settling granules in UASB reactors or severe clogging in anaerobic filters (Fernández et al. 2001). In this regard, conventional suspended growth systems are more suitable for high solids wastewater treatment (Akunna 2008), which justifies the use of a CSTR reactor in this work.

It is worthwhile to mention that the use of anaerobic granular biomass as inoculum may have contributed to the resistance of the single-stage process since it was able to recover after operating in unstable conditions, and it is well recognized that biofilm-based systems are highly resistant compared with suspended biomass (Andreottola et al. 2009). Other parameters such as the COD removal and the methane yield were also high compared with other single-stage anaerobic treatment technologies, but the two-stage processes overcame them.

As shown in Table 2, two stage-processes can treat higher influent concentrations at higher organic loading rates. The HRT of two-stage processes is higher because of the treatment of more organic matter, and the HRT of the acidogenic stage should also be considered. This configuration allows high removal efficiencies (above 90%) and methane yields closer to the theoretical value of 0.35 m$^3$/kg COD to be achieved. The results of this research also showed that a much smaller addition of chemicals is required to maintain the stability of the two-stage process. Finally, it is important to mention that despite the good results of the anaerobic treatment and the energy recovery, a post-treatment is still needed to meet the levels required for wastewater discharges, which can be achieved in combination with aerobic systems (Bolzonella et al. 2019).

### CONCLUSIONS

The present study showed that Mexican winery effluents are characterized by a high content of biodegradable organic matter, composed mainly of solids, ethanol, and carbohydrates. Due to these characteristics, these by-products can be treated by anaerobic digestion to produce energy in the form of methane. The results showed that a single-stage anaerobic process could treat such effluents; however, this process is not suitable in terms of stability. The process
was susceptible to changes in the buffer capacity of the medium, since the biogas production, as well as the COD removal, was closely related to the variation of the $\alpha$ index and the addition of chemicals to control alkalinity.

Two-stage anaerobic processes were more suitable for the treatment of the highly concentrated effluents. Higher organic loads can be applied, and a higher methane production can be achieved using this configuration. Also, the two-stage process was more stable compared to the single-stage process and required less addition of chemicals.

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