A Comprehensive Overview of the Potential of Tequila Industry By-Products for Biohydrogen and Biomethane Production: Current Status and Future Perspectives

Octavio García-Depraect, Daryl Rafael Osuna-Laveaga and Elizabeth León-Becerril

Abstract

Nowadays, the use of agro-industrial by-products as alternative sustainable resources to generate bioenergy and high-value bioproducts is one of the most important research topics to tackle environmental concerns related to the excessive consumption of fossil-based fuels and rapid urbanization and industrialization. This chapter provides a broad overview of the potential of the main tequila industry by-products, agave bagasse and tequila vinasse, for biohydrogen (bioH₂) and biomethane (bioCH₄) production via dark fermentation and anaerobic digestion, respectively. First, pretreatment or conditioning steps commonly applied to tequila by-product streams before downstream biological processes are highlighted. The operational performance of bioH₂- and bioCH₄-producing reactors is subsequently reviewed, with a focus on reactor configuration and performance, microbial metabolic pathways, and the characterization of microbial communities. Additionally, the development of multi-stage anaerobic digestion processes is comprehensively discussed from a practical point of view. Finally, limitations and potential improvements in the field of bioH₂ and bioCH₄ production are presented.

Keywords: agave bagasse, tequila vinasse, dark fermentation, anaerobic digestion, biofuels

1. Tequila production process and its main by-products: agave bagasse and tequila vinasse

Tequila is a Mexican alcoholic beverage obtained from the distillation of fermented juice of the mature stems of *Agave tequilana* Weber var. azul. It possesses appellation of origin since 1974 and has received international recognition in the market. As an example, tequila-processing plants produced around 309 million liters of tequila in 2018, of which ~72% were exported, highlighting its international demand [1]. Thus, tequila production represents one of the most important activities for Mexico. In general, there are three major stages in the tequila production...
process, namely agave juice (must) extraction, fermentation, and distillation. In the first stage, the agave juice containing fermentable sugars is first obtained either through cooking or not-cooking processes. In the former, agave stems are cooked in ovens or autoclaves at high temperatures (95–120°C) for a long time (usually 8–12 h). Once cooked, the water-soluble carbohydrates are extracted by simultaneous shredding and pressure washing followed by pressing. In the latter, raw agave juice is obtained from previously shredded raw agave stems using hot water (80°C) through the use of equipment called diffuser. Afterward, the carbohydrates contained in the raw agave juice are hydrolyzed for 4–6 h under acidic conditions (pH 1.8–3) at high temperatures (80–85°C) [2, 3]. In the second stage, the agave juice is subjected to an alcoholic fermentation process, wherein agave sugars are transformed to ethanol, carbon dioxide, and other compounds (e.g. aldehydes, esters, furans, and ketones) by the action of different microorganisms, particularly yeasts [2, 3]. In the third stage, the fermented must is subjected to a two-step distillation process to obtain tequila [2, 4].

At this point, it must be noted that enormous quantities of solid (Agave tequilana bagasse, hereinafter referred to as AB) and semi-liquid (tequila vinasse, hereinafter referred to as TV) by-products are generated each year during the process of tequila manufacturing, particularly after the stages of agave juice extraction and distillation, respectively (Figure 1). It has been estimated that 1.4 kg of AB and 10–12 L of TV are obtained by each liter of tequila produced [4, 5]. Considering the tequila production of 264.9 ± 31.2 million liters reported in the last lustrum (2014–2018) by

![Figure 1](image_url)
the Tequila Regulatory Council [1], the generation of AB and TV is equivalent to 370,916 ± 43,701 tons and 2914.3 ± 343.3 million liters per year, respectively. The physicochemical composition of a given stream of AB and TV may change from batch to batch, depending mainly on the raw materials used (e.g. maturity of agave), juice extraction process (cooked and uncooked agave), and the prevailing conditions of fermentation and distillation in the case of TV [3, 6–9]. Despite such influential factors, there are some general features that can be distinguished between AB and TV. Concerning AB, it is a lignocellulosic material with a composition of 11–57% hemicellulose, 31–53% cellulose, 7–15% lignin, and 19–57% extractives [4, 8, 9]. Extractives are the nonstructural components of lignocellulose, including fats, phenolics, resin acids, waxes, and inorganics [10]. Regarding TV, it is a brown and acidic wastewater (pH of 3.4–4.5, total acidity of 1500–6000 mg-CaCO₃/L) containing high chemical oxygen demand (COD) concentration of 40–100 g/L, as well as high total solids (25–50 g/L), salts, metal ions, organic acids, phenolic compounds, and melanoidins [3, 5, 7, 11].

Regarding the management and final disposition of AB and TV, it must be highlighted that only a small part of the whole AB generated is used in the manufacturing of different products such as animal feeds, fertilizers, bricks, mattresses, furniture, and packing materials [12, 13]. Therefore, most of AB is treated as waste and returned to the fields in the form of piles that are directly exposed to outdoor conditions, where they may cause leachates, odor generation, and atmospheric pollution [12, 14]. In the case of TV, it has been reported that approximately 80% of the total volume of TV generated is discharged without receiving adequate treatment into receiving water bodies (e.g. rivers, lakes, and sewer system) or directly onto soil, which in turn can result in adverse environmental and human health impacts [5]. To valorize AB and TV and to face such disposal problems, nowadays, engineers and scientists are focusing on using them as potential substrates for the production of biofuels and value-added products in a tequila biorefinery framework. However, there are still several challenges that must be overcome before full-scale facilities could be implemented. This chapter provides an extended insight on (i) the pretreatment or conditioning steps of tequila by-product streams; (ii) the use of AB and TV to produce biogenic hydrogen (bioH₂) and methane (bioCH₄) via anaerobic fermentation processes, with a special emphasis on reactor configuration and operation, producing/competing metabolic pathways and the characterization of microbial communities; (iii) the development of multi-stage anaerobic digestion (AD) processes; and (iv) limitations and avenues for future research toward improving bioH₂ and bioCH₄ production.

2. Pretreatment/conditioning of agave bagasse and tequila vinasse

AD is the core technology for the treatment of several biodegradable organic wastes with concomitant bioenergy recovery in the form of biogas that is rich in bioCH₄, although bioH₂ may also be recovered. Besides bioCH₄ recovery, AD is advantageous due to low energy and nutrient requirements, low sludge production, and high organic loading capacity (20–35 g-COD/L-d) [15]. From a biochemical point of view, AD consists of four successive steps, namely hydrolysis, acidogenesis, acetogenesis and methanogenesis [15, 16].

It is worth mentioning that in the case of AB, the low biodegradability due to its lignocellulosic structure constitutes one of the main barriers to accelerate hydrolysis and enhance the recovery of bioH₂/bioCH₄. In the case of TV, its complex composition such as high COD, high solids content, unbalanced nutrient, presence of putative toxicants (e.g. organic acids, phenols, melanoidins) and the negligible
alkalinity along with the high concentration of components with a tendency to suffer very rapid acidification constitutes the major limitations for bio\textsubscript{H}_2/bio\textsubscript{CH}_4 production. Thus, in practice, before the feedstock (AB or TV) is sent to either the hydrogenogenic or the methanogenic stage, a pretreatment/conditioning step is commonly performed as a prerequisite to improve its biodegradability as well as to prevent DF/AD processes from potential toxicants, elevated solids, and organic overloading (Figure 2). Unlike AB, TV is only subjected to one or more conditioning steps. Commonly, they consist of lowering temperature, rising pH (adding alkalinity), diluting, adding complementary nutrients, and removing suspended solids (Figure 2). In contrast, AB is exposed to a drying step to prevent fungal and bacterial growth, mainly for long-time storage. Once AB is dried, it is subjected to a mechanical milling step devoted to reducing particle size, thereby increasing surface area, which makes carbohydrates more easily available for downstream processes. The mechanical fractionation also makes AB more homogeneous and easier to handle. After milling, the pretreatment applied to AB for either bio\textsubscript{H}_2 or bio\textsubscript{CH}_4 production may differ. For such purposes, dilute acid, alkaline hydrogen peroxide, detoxification and enzymatic hydrolysis have been evaluated in detail. Arreola-Vargas et al. [8] pretreated cooked and uncooked AB through a dilute acid hydrolysis at 5% (w/v), 56.4–123.6°C, 1.2–2.8% HCl, and 0.3–3.7 h reaction time, finding temperature as the principal factor which could increase the hydrolysis yield. Total sugars concentrations obtained were 27.9 and 18.7 g/L for cooked and uncooked AB hydrolysates, respectively. The higher yield of cooked AB was attributed to the fact that during the elaboration of tequila using cooking process, agave stems receives an in situ thermal treatment. Nevertheless, high concentrations (up to 1200 mg/L) of hydroxymethylfurfural (HMF) were detected in the cooked AB. In a further study, Arreola-Vargas et al. [17] pretreated AB through either acid or enzymatic hydrolysis for bio\textsubscript{CH}_4 and bio\textsubscript{H}_2 production. Acid hydrolysis was carried out for 1.3 h at 5% (w/v) of AB, 2.7% HCl and 124°C, while enzymatic hydrolysis was performed at 4% (w/v) of AB in 50 mM citrate buffer at pH 4.5 with Celluclast 1.5 L at 40 filter paper units (FPU) for 10 h at 45°C. As a result, 17.3 and 8.9 g-total sugars/L were obtained from acid and enzymatic hydrolysis, respectively. However, unlike enzymatic hydrolysates, acid hydrolysates promoted the generation of potential inhibitors such as formic acid (HFor), acetic acid (HAc), and phenolic and furanic compounds. In another study, Breton-Deval et al. [18] compared the type of acid catalyst (HCl vs. H\textsubscript{2}SO\textsubscript{4})
on the chemical composition of hydrolysates of AB. Overall, results showed that the use of HCl induced higher sugar recoveries than the use of H2SO4, 0.39 versus 0.26 g-total sugars/g of AB. Furthermore, the H2SO4 hydrolysate contained higher concentrations of HAc and furans. To remove undesirable compounds derived from acid hydrolysis of AB (30 g AB, HCl 1.9%, 130°C, 132 min reaction time), Valdez-Guzmán et al. [19] performed detoxification of acid AB hydrolysates using 1% (w/v) powdered coconut shell-activated carbon. Under batch conditions (pH 0.6, 20 min reaction time, 150 rpm, room temperature), the highest removal of HAc and phenols obtained were 89 and 21%, respectively, with minimal losses of fermentable sugars (3.6%). Besides, during acid hydrolysis, a hydrolysis yield of almost 40% of total sugars, a delignification of 44%, complete hydrolysis of hemicellulose, and no detection of furfural or HMF in the hydrolysate was obtained. In another study, Contreras-Dávila et al. [20] pretreated AB for bioH2 production using Celluclast 1.5 L during 10 h, obtaining sugar yields in the range of 0.19–0.38 g-total sugars/g of AB. Montiel and Razo-Flores [21] also pretreated AB by enzymatic hydrolysis to produce bioH2 and bioCH4. The conditions were 3.5% (w/v) of AB with Celluclast 1.5 L at 18 FPU/g of AB at 40°C during 12 h. The resulting hydrolysate had 27.2 g/L of total COD with 5.3 ± 0.8 g/L of total sugars (0.15 g-total sugars/g of AB) which contributed to 20% of the total COD, citrate buffer with 26%, enzyme with 38%, and other non-determined components with 16%. In the same year, Galindo-Hernández et al. [22] used alkaline hydrogen peroxide (AHP) as a pretreatment to remove lignin before enzymatic hydrolysis of AB. Under the experimental conditions tested (5% w/v of AB, 2% w/v of AHP, 50°C, pH 11.5 using NaOH, 120 rpm, 1.5 h reaction time), 97% of the lignin was removed and 88% of holocellulose (cellulose and hemicellulose) was recovered, promoting that the polysaccharide fractions are more available or exposed to a further enzymatic attack. The authors also demonstrated, in delignification terms, that it is better to use hydrogen peroxide and NaOH solution in a combined form than in a separate or sequential way and that using binary enzymatic hydrolysis (cellulases and hemicellulases) may improve the yield, percentage, and productivity of saccharification, which were 0.19 g-total sugars/g of AB, 26.7% and 17.1 g-total sugars/g of AB-h, respectively. The synergistic effect of using binary enzymatic hydrolysis was verified by Montoya-Rosales et al. [23], who compared the enzymatic hydrolysis of AB using a binary enzyme preparation that is composed of Celluclast 1.5 L and Viscozyme L with a single enzyme, that is, Stonezyme, which is a commercial cellulase preparation. The results showed that hydrolysis yields were higher with the binary enzymatic hydrolysis, 0.27 versus 0.22 g-carbohydrates/g of AB and 0.5 versus 0.28 g-COD/g of AB.

3. Biohydrogen production from agave bagasse and tequila vinasse

H2 is one of the most promising alternative energy carriers to partly fulfill the growing energy demands and overcome fossil fuel dependency and has attracted global attention for its highest energy content per unit weight (142 kJ/g) and carbon-free nature since it generates only water vapor during combustion. It can be used for a variety of purposes either alone to produce energy in fuel cells and combustion engines or blended with CH4 to produce a superior fuel known as hythane [24]. Comparing thermochemical, electrochemical, and biological ways of producing H2, the latter is considered the most sustainable because it is eco-friendlier and less energy intensive. Among biological processes, dark fermentation (DF) is thought to be practically applicable at large commercial scales in a near future owing to its capability of producing bioH2 at higher rates and versatility of
utilizing several different types of carbohydrate-rich wastes as substrate [25]. In this connection, since AB and TV are abundantly available, renewable, and have a high content of carbohydrates, they have been considered as suitable feedstocks for bioH$_2$ production. In the following sections, the operational performance, metabolic pathways, and microbial communities of DF systems treating either AB or TV are extensively reviewed.

### 3.1 Operational performance

Regarding the use of AB for bioH$_2$ production (Table 1), the first systematic study dealing with bioH$_2$ production from AB was conducted by Arreola-Vargas et al. (2016) [17], who assessed the use of AB hydrolysates obtained either from acid or enzymatic pretreatment for bioH$_2$ production. To the end, different proportions of hydrolysate (20, 40, 60, 80, and 100% v/v) were tested in an automatic methane potential test system (AMPTS II provided by Bioprocess control) at 37°C, 120 rpm, initial pH of 7, and using 10 g-volatile suspended solids (VSS)/L of heat-pretreated anaerobic granular sludge. Overall, the best bioH$_2$ production performance was achieved in the assays with enzymatic hydrolysate, obtaining the maximal bioH$_2$ yield (HY$_2$) and volumetric bioH$_2$ production rate (VHPR) of 3.4 mol-H$_2$/mol-hexose and 2.4 NL-H$_2$/L-d, respectively, both with the hydrolysate at 40% (v/v). The lower values observed with the acid hydrolysate were attributed to the feedstock composition in terms of sugar profile, weak acids, furans, and phenolics.

In another work, Contreras-Dávila et al. [20] used an enzymatic AB hydrolysate for bioH$_2$ production in a continuously stirred tank reactor (CSTR) and a trickling bed reactor (TBR), which were operated up to 87 days under different organic loading rates (OLR, 17–60 g-COD/L-d) obtained by varying hydrolysate concentration and/or hydraulic retention time (HRT). The reactor configurations showed different performances. In the CSTR, the VHPR and HY$_2$ displayed an inverse correlation with maximum values of 2.53 L-H$_2$/L-d and 1.35 mol-H$_2$/mol-substrate, attained at OLR of 52.2 and 40.2 g-COD/L-d, respectively, both with 6 h HRT. The bioH$_2$ concentrations of the produced gas were between 18 and 35% (v/v). In contrast, in the TBR, increasing OLR up to 52.9 g COD/L-d (4 h HRT)

| Pretreatment                        | Feeding     | T (°C) | pH  | YH$_2$ (NL/kg AB) | VHPR (NL/L-d) | H$_2$ (% v/v) | Ref. |
|-------------------------------------|-------------|--------|-----|------------------|---------------|---------------|------|
| Acid hydrolysis                     | Batch       | 37     | 7   | 1.6$^b$          | 2.4           | NR            | [17] |
| Individual enzymatic hydrolysis     | Batch       | 37     | 7   | 140, 3.4$^b$     | 2.4           | NR            | [17] |
| Individual enzymatic hydrolysis     | Continuous  | 37     | 5.5 | 67               | 3.45          | 26–52         | [20] |
| Individual enzymatic hydrolysis     | Continuous  | 35     | 5.5 | 105              | 6             | 56            | [21] |
| Alkaline hydrogen peroxide + binary enzymatic hydrolysis | Batch | 37 | 7.5$^a$ | 215 | 0.93 | NR | [22] |
| Individual enzymatic hydrolysis     | Semi-continuous | 37 | 4.8 | 1.6$^c$ | 0.6 | 49.3$^d$ | [26] |
| Acid hydrolysis + detoxification    | Batch       | 37     | 8.2$^a$ | 56.2 | 1.51 | NR | [19] |
| Binary enzymatic hydrolysis         | Continuous  | 37     | 5.5 | 117.8            | 13            | 51–60         | [23] |

Notes: All studies were conducted using thermally treated anaerobic granular sludge; $^a$Initial pH value; $^b$mol-H$_2$/mol hexose; $^c$mol-H$_2$/mol of consumed sugar; $^d$Value measured during the starting period; NR: not reported.

Table 1. Comparison of the literature data on biohydrogen production efficiency using pretreated agave bagasse as feedstock.
simultaneously enhanced VHPR and HY₂, attaining values of 3.45 L-H₂/L-d and 1.53 mol-H₂/mol-substrate, respectively, with bioH₂ concentrations of the produced gas between 26 and 52% (v/v). The observed bioH₂ production performances were explained by differences in the liquid and gas flow rates, agitation speed, and liquid-gas interface between the CSTR and TBR configurations, which in turn may have caused distinct bioH₂ concentrations in the liquid phase.

In a further study which set up to assess the batch bioH₂ production from pretreated AB with AHP followed by binary enzymatic saccharification (hemicellulases + cellulases), Galindo-Hernández et al. [22] performed a series of experiments in the AMPTS II system at 37°C, 150 rpm, initial pH of 7.5, and using an organic load of 5 g-COD/L and 13.5 g-volatile solid (VS)/L of thermally treated anaerobic sludge. The results suggested that delignification of AB and subsequent hydrolysis with a synergistic enzymatic mixture had a beneficial effect on bioH₂ production, obtaining a YH₂ of 3 mol-H₂/mol-hexose and a VHPR of 0.93 NL-H₂/L-d.

In an investigation on the effect of OLR and agitation speed on the continuous bioH₂ production from enzymatic hydrolysates of AB, Montiel and Razo-Flores [21] operated for 84 days a mesophilic (35°C) CSTR reactor (with a working volume of 1 L) inoculated with 4.5 g-VS/L of heat-treated anaerobic granular sludge and operated at different OLRs (40–52 g-COD/L-d), which were achieved by varying hydrolysate concentration. The evaluated stirring speeds were in the range of 150–300 rpm, while the HRT was maintained at 6 h during the whole operation. The authors observed that the strategy of increasing the agitation speed from 150 to 300 rpm favored both the VHPR and bioH₂ content in the gas phase, obtaining 6 NL-H₂/L-d and 55% (v/v), respectively, at an OLR of 44 g-COD/L-d. Such results indicated that the increase of the agitation speed in the CSTR improved the transfer of dissolved bioH₂ from the liquid to the reactor gas phase, overcoming one of the limitations for bioH₂ production previously observed by [21].

In another study, Toledo-Cervantes et al. [26] addressed the bioH₂ production from enzymatic hydrolysates of AB using an anaerobic sequencing batch reactor (AnSBR) with a working volume of 1.25 L. The reactor was inoculated with 10 g-VS/L of heat-treated anaerobic granular sludge and operated at 37°C, pH 4.8, and at four OLR (10.6–21.3 g-COD/L-d), which were modified by decreasing the cycle time (from 24 to 12 h) and increasing the COD concentration (from 8 to 12 and 16 g/L). Results showed that the highest OLR promoted the highest VHPR of 0.6 NL-H₂/L-d. Conversely, the YH₂ remained constant at 1.6 mol-H₂/mol of consumed sugar.

In a similar study, Valdez-Guzmán et al. [19] showed the importance not only of optimizing pretreatment but also of removing several compounds (e.g. furfural, HMF, phenolic compounds, and organic acids) that are generated during its application. They compared the bioH₂ production potential of undetoxified and detoxified acid hydrolysates from AB. The authors reported ~39 and ~9% increases on YH₂ and VHPR, respectively, comparing detoxified AB with activated carbon and undetoxified AB, 1.71 versus 1.23 mol-H₂/mol of consumed sugar and 1.51 versus 1.38 NL-H₂/L-d. Such increments were correlated to changes in the fermentation by-products suggesting the occurrence of different pathways or changes in the microbial community, since the detoxified hydrolysate produced HAc and butyric acid (HBu), while lactic acid (HLac) was found in the undetoxified hydrolysate.

Most recently, Montoya-Rosales et al. [23] compared and evaluated the continuous bioH₂ production from individual and binary enzymatic hydrolysates of AB in two different configurations, that is, CSTR and TBR. The experiments were carried out at 37°C and pH 5.5 and at various OLRs 36–100 g-COD/L-d, which were achieved by increasing the influent concentration, while keeping the HRT constant.
at 6 h. The results showed that the performance was highly dependent on the type of reactor and OLR. Regarding the CSTR configuration, in general, the higher OLR resulted in higher VHPR. Nonetheless, the bioH2 production efficiency using individual enzymatic hydrolysate (0.72–2.25 NL-H2/L-d and 11.8–20.4 NL-H2/kg of AB) was lower compared to that obtained with the binary enzymatic hydrolysate (3.9–13 NL-H2/L-d and 83.3–117.9 NL-H2/kg of AB), with the maximum VHPR and YH2 at 100 and 60 g-COD/L-d and 90 and 52 g-COD/L-d, respectively. Regarding the TBR configuration, the binary enzymatic hydrolysate also outperformed the individual one, obtaining the maximum VHPR of 5.76 NL-H2/L-d at an OLR of 81 g-COD/L-d and YH2 of 72.4 NL-H2/kg of AB at an OLR of 69 g-COD/L-d. The enhancement was attributed, on one hand, to the use of binary hydrolysis that could have contributed to produce a higher proportion of monomers of easy degradation by bioH2-producing bacteria (HPB) and to avoid the formation/release of potential inhibitors; on the other hand, to the differences of substrate availability given by the mode of growth in each reactor.

Concerning the use of TV for bioH2 production (Table 2), there are a few studies in the literature, with a particular focus on (i) optimizing pretreatments to further enhance bioH2 production [27]; (ii) testing the effect of different operational conditions such as pH [28, 29], temperature [28, 30], substrate concentration [28, 30, 31], solid content [22, 31], nutrient formulation [22, 31], inoculum addition [22, 31], HRT [22, 30, 32], and OLR [22, 32]; (iii) producing bioH2 in different systems, such as serum bottle [33], fixed bed reactor (FBR) [34], and CSTR [35];

| Pretreatment/conditioning | Feeding | T (°C) | pH | YH2 * | VHPR (NL/L-d) | H2 (%) v/v | Ref. |
|---------------------------|---------|-------|----|-------|---------------|------------|------|
| Alkalization              | Batch   | 35    | 6.5–7.5 | 1.5a, 2.8b,d | NR           | NR         | [27] |
| None                      | Semi-continuous | 55 | 5.5 | 13.8b,d | 2.8               | NR         | [28] |
| Dilution, nutrient supple-| Semi-continuous | 35 | 5.5 | NR | 2.2                      | 29.2       | [30] |
| mentation                 |         |       |     |     |               |            |      |
| Dilution                  | Continuous | 35 | 4.7 | 1.3a, 1.36c | 1.7                       | 64         | [34] |
| Dilution, nutrient supple-| Semi-continuous | 35 | 5.5 | 0.12b     | 1.4                      | NR         | [35] |
| mentation                 |         |       |     |     |               |            |      |
| Dilution                  | Batch   | 36    | 5.5e | 0.7b        | 0.5                       | NR         | [33] |
| Co-fermentation           | Batch   | 35    | 5.5 | 1.1b       | 2.6                      | 71         | [11] |
| Nutrient supplementation  | Batch   | 35    | 6.5–5.8 | 4.8c, 0.12e | 3.8                      | 70         | [37] |
| Solid removal (centrifuga-| Batch   | 35    | 6.5–5.8 | 4.3b, 0.11c | 5.4                      | 71         | [31] |
| tion)                    |         |       |     |     |               |            |      |
| Co-fermentation           | Batch   | 35    | 5.5 | 1.2b        | 2.4                      | 68         | [36] |
| Co-fermentation           | Batch   | 35    | 6.5–5.8 | 2.5b, 2.7c | 3.7                      | 73         | [29] |
| Solid removal (centrifuga-| Continuous | 35 | 5.8 | 3.4c | 12.3          | 90         | [38] |

Notes: Inoculum: anaerobic digester sludge [27, 28], thermally treated anaerobic granular sludge [11, 29–31, 33–38]; *Units: “mol-H2/mol glucose”; ‡NL-H2/L of reactor; †NL-H2/L of TV; ¶NL-H2/g-COD; ‡‡NL-H2/g-VSfed; ‡Calculated from provided information; †Initial pH value; NR: not reported.

Table 2. Comparison of the literature data on biohydrogen production efficiency using tequila vinasse as feedstock.
(iv) evaluating the feasibility of co-fermentation [11, 36]; and (v) exploring the microbial ecology of the process [32, 36, 37].

More particularly, Espinoza-Escalante et al. [27] evaluated the effect of three pretreatments, that is, alkalinization, cavitation, and thermal pretreatment, on the metabolic profile and the increments of COD and total reducing sugars (TRS) of TV, as well as on its bioH₂ production potential. From that study, it can be concluded that the application of such pretreatments to raw TV resulted in different degrees of solubilization of COD and TRS, depending on the applied pretreatment and combinations thereof. However, there was no apparent relation in the consumption of TRS and COD with bioH₂ production. Indeed, the optimal conditions that led to the highest solubilization of both COD and TRS did not result in a significant improvement in the YH₂, which was about 2.8 NL-H₂/L of reactor, indicating that compounds other than TRS could be involved in the mechanism of bioH₂ production.

In another report, Espinoza-Escalante et al. [28] studied the effect of pH (4.5, 5.5, and 6.5), HRT (1, 3, and 5 d), and temperature (35 and 55°C) on the semi-continuous production of bioH₂ from TV. The experiments were performed in 1-L glass vessels inoculated with 10% (v/v) of mesophilic anaerobic digester sludge. The results showed that all factors studied had an important effect on bioH₂ production. The highest efficiency in terms of bioH₂ production was achieved at a pH of 5.5, an HRT of 5 d and a temperature of 55°C. Based on constructed mathematical models, pH was the most influential parameter.

In a similar study, Buitrón and Carvajal [30] investigated the effect of temperature (25 and 35°C), HRT (12 and 24 h), and substrate concentration on bioH₂ production from TV using a 7-L AnSBR, with a working volume of 6 L. The exchange volume was 50% with a reaction time of 11.3 or 5.3 h depending on the applied HRT, while pH and mixing were controlled at 5.5 and 153 rpm, respectively, in all cases. It was evidenced that all parameters studied affected the efficiency of bioH₂ production. The HRT had a major influence on bioH₂ production. It was found that the shorter the HRT, the higher the bioH₂ production. Overall, the maximum VHPR of 2.2 NL-H₂/L-d and an average bioH₂ content in the biogas of 29.2 ± 8.8% (v/v) were obtained at 35°C, 12 h HRT, and 3 g-COD/L OLR.

Later, Buitrón et al. [34] evaluated the performance of an FBR to produce bioH₂ in a continuous mode from TV. The reactor had a working volume of 1.7 L and was packed with polyurethane rings for biomass immobilization. The temperature, pH, HRT, and OLR were kept constant at 35°C, 4.7, 4 h, and 2.15 g-COD/L-d (influent concentration of 8 g-COD/L), respectively. After an initial acclimatization period of HPB to TV, the FBR exhibited a VHPR of 1.7 NL-H₂/L-d and a YH₂ of 1.36 NL-H₂/L of TV. In a follow-up study conducted by the same research group, by using a 0.6-L AnSBR operated under mesophilic and acidophilic conditions at an HRT of 6 h, it was observed that increasing substrate concentration from 2 to 16 g-COD/L increased the VHPR up to 1.4 NL-H₂/L-d. Hence, the use of TV for bioH₂ production did not result in inhibition [35].

Another interesting advance was made by García-Depraect et al. [11], who studied the technical feasibility of using a co-fermentation approach to produce bioH₂ from TV in a well-mixed reactor operated under batch mode. Nixtamalization wastewater (NW) was chosen as the complementary substrate based on its wide availability in Mexico and high alkalinity. The TV:NW ratio of 80:20 (w/w) resulted in the highest VHPR of 2.6 NL-H₂/L-d with a bioH₂ content in the gas phase of 71% (v/v). Interestingly, the co-fermentation study allowed the identification of iron and nitrogen as essential nutrients which may be limiting in TV-fed DF reactors. This identification becomes significant to avoid nutrient-limited conditions and to prevent excessive nutrient supplementation that has been
occurring in several studies at bench scale, but its practice may be prohibited on larger scales.

In this field of progressive research, the effect of pH on the bioH\(_2\) production efficiency was subsequently studied by García-Depraect et al. [29] through macro- and micro-scale behavior analysis approaches. It was found that fixed pH of 5.8 showed a longer lag phase compared with fixed pH of 6.5, but the latter promoted bioH\(_2\) sink through propionogenesis. Based on the above observations, a two-stage pH-shift control strategy was devised to further increase bioH\(_2\) production. The strategy entailed the control of pH at 6.5 for first \(\sim 29\) h of culture to decrease the lag time, and then the pH was maintained at 5.8 to increase the bioH\(_2\) conversion efficiency by inhibiting the formation of propionic acid (HPr). The pH-shift strategy reduced running time and enhanced bioH\(_2\) production by 17\%, obtaining 2.5 NL-H\(_2\)/L of reactor. In a further study, the use of TV as the sole carbon source in the batch bioH\(_2\)-yielding process was evaluated through a comprehensive approach entailing the operational performance, kinetic analysis, and microbial ecology [37]. A YH\(_2\) of 4.3 NL-H\(_2\)/L of reactor and a peak VHPR of 3.8 NL-H\(_2\)/L-d were obtained.

The effects of total solids content, substrate concentration, nutrient formulation, and inoculum addition on bioH\(_2\) production performance from TV have been also investigated in batch experiments [31]. It was observed a consistent bioH\(_2\) production which was primarily influenced by inoculum addition followed by substrate concentration, nutrient formulation, and solids content. Maximum VHPR (5.4 NL-H\(_2\)/L-d) and YH\(_2\) (4.3 NL-H\(_2\)/L of reactor) were achieved by removing suspended solids and enhancing nutrient content, respectively [31]. Finally, the highest VHPR (12.3 NL-H\(_2\)/L-d, corresponding to \(\sim 3.4\) NL-H\(_2\)/L of TV) up to date has been achieved via a novel multi-stage process operated under continuous mode for 6 h HRT, which also resulted in high stability (VHPR fluctuations <10\%) and a high bioH\(_2\) content in the gas phase of \(\sim 90\%\) (v/v) [38].

3.2 Metabolic pathways

Following the by-products formed during fermentation is of utmost importance to understand, predict, control, and optimize the behavior of DF processes. It is well known that the distribution of the fermentation by-products may change depending on culture conditions. Low bioH\(_2\) productions matched with the presence of undesired electron sinks, such as HLac, HPr, iso-butyrate, valerate, iso-valerate, and solvents (e.g. ethanol, acetone, and butanol). For instance, the production of HPr reduces the amount of bioH\(_2\) that may be produced, as shown in reactions 1–3 (Table 3). Biomass growth also represents an electron sink. Commonly bioH\(_2\) production is growth-associated. However, higher biomass growth does not necessarily imply the achievement of the best bioH\(_2\) production [29]. Thus, a proper balance between biomass growth and bioH\(_2\) production is desirable. On the other hand, bioH\(_2\) sink through the formation of bioCH\(_4\) via the hydrogenotrophic pathway (reaction 4) seems to be less problematic in DF processes due to the application of inoculum pretreatments together with biokinetic control such as acidic pH and low HRT, even using attached-growth reactors [34]. The formation of HLac can also lead to stuck DF fermentations, as shown in reactions 5–7. Acetogenesis (reaction 8) and homoacetogenesis (reaction 9) may also occur during the process, decreasing the bioH\(_2\) production efficiency. It has been reported that the consumption of bioH\(_2\) and carbon dioxide due to homoacetogenesis depends on the type of reactor and OLR, being its occurrence accentuated in suspended growth systems and high OLR [20, 23].

Contrarily, bioH\(_2\) production via DF is typically related to HBu and HAc production from carbohydrates degradation, as shown in reactions 10 and 11, respectively. Theoretically, 4 and 2 mol of H\(_2\) derive from 1 mol of glucose when HAc and
HBu are the end-products, respectively. However, from published studies in the field of DF, it seems reasonable to conclude that, in mixed cultures, a high bioH2 production efficiency is rather related with the formation of HBU than HAc because the latter may come from acetogenesis/homoacetogenesis.

At this point, it must be noted that bioH2 can also come from the degradation of HLac, as shown in reactions 12–14 [37]. The HLac-type fermentation could provide the basis for the design of stable bioH2-producing reactors whose feedstocks are rich in HLac and HAc such as distillery wastewater (including TV), food waste, dairy wastewater, ensiled crops, lignocellulosic residues, and their hydrolysates (including AB), among others [36]. The amount of bioH2 obtained from the HLac-type fermentation may vary significantly depending on several factors such as pH, temperature, HRT, OLR, operation mode, substrate type, mixing, and prevailing microorganisms [31]. Also, it has been observed that the HLac-type fermentation in vinasse-fed DF reactors could be induced by low carbohydrate-available conditions [31, 36, 37]. On the other hand, the formation of HFor also can yield bioH2 (reaction 15) via the action of HFor hydrogenase complexes [37]. In addition, ethanol-type fermentation (reaction 16) generates ethanol, HAc, bioH2, and carbon dioxide. According to Ren et al. [39], the ethanol-type fermentation is favored by a pH of 4.0–5.0 and oxidation-reduction potential (ORP) of $< -200 \text{ mV}$. In comparison to the HAc-HBu-mixed type fermentation, which has been ascertained as the most common bioH2-producing pathway, the latter two reactions have been less frequently found in DF reactors fed with AB/TV.

### 3.3 Microbial communities

Another pertinent point is that the performance of bioH2-producing reactors strongly depends on the selection and maintenance of HPB. However, this is a
difficult task because DF processes treating unsterilized feedstocks under continuous conditions are open systems, meaning that several microbial interactions may take place. In the literature, it has been used defined mixed cultures to inoculate DF reactors treating complex feedstocks such as AB and TV. In most cases, heat-shock pretreatment has been used as the selective method for the enrichment of HPB (based on their ability in forming spores), while killing bioH₂ consumers. However, other aspects such as biological/physiological (e.g. growth rate, microbial interactions, auto/allochthonous bacteria, adaptation to environmental stress conditions, and nutrients requirements), the composition of broth culture (e.g. availability of substrate/nutrients, organic acids, and toxicants), process parameters (e.g. pH, temperature, HRT, OLR, and ORP) and reactor configurations (e.g. suspended and attached biomass, mixing, and liquid-gas interface mass transfer capacity) are also selective pressure factors to determine prevailing microbial community structure during operation. At this point, it must be noted that the application of the heat-shock pretreatment decreases the diversity eliminating not only microorganisms with a negative effect on the overall bioH₂ production, but also with a potentially positive role. Besides having a high capacity to produce bioH₂, the biocatalyst must be able to thrive on the presence of putative toxic by-products such as HFor, HAc, phenols, and furans which are commonly detected in pretreated AB and raw TV.

Interestingly, molecular biology tools reveal that HPB (e.g. Clostridium, Klebsiella, and Enterobacter) are, in almost all DF systems, accompanied by lactic acid bacteria (LAB) (e.g. Lactobacillus and Sporolactobacillus) [40]. This co-occurrence could be attributed to the fact that LAB are ubiquitous in the environment, the physicochemical characteristics of feedstocks could sustain the proliferation of LAB, and LAB possess complex adaptation mechanisms that confer their ecological advantages over other bacteria [31]. Streptococcus and Lactobacillus have actually been detected in TV [31]. Bearing in mind such explanations, it is reasonable to assume that DF reactors fed with TV will naturally undergo the proliferation of LAB. Indeed, this assumption was verified by [11, 29, 31, 36, 37].

Except for capnophilic HLac pathway, it is well known that HLac is produced through zero-bioH₂-producing pathways. Moreover, the proliferation of LAB is commonly associated with the deterioration of bioH₂ production, mainly due to substrate competition, acidification of cultivation broth, and excretion of antimicrobial peptides known as bacteriocins [41]. At this point, another important constraint to be mentioned is that methods devoted to preventing the growth of LAB such as pretreatment of inoculum and sterilization of feedstock may be expensive, thus imposing a high economic burden on the process. Besides, the application of pretreatments does not always hinder the proliferation of LAB [42]. Therefore, there is an urgent need for novel technical solutions to ensure a maximum VHPR and YH₂.

Fortunately, the activity of LAB may also have positive effects on the overall DF process, mainly through the aforementioned HLac-type fermentation (HLac-driven bioH₂ production). Indeed, it is noteworthy mentioning that, under certain conditions, a DF process mediated by beneficial trophic links between HPB and LAB may be highly stable and consequently of high relevance for practical applications. In this case, LAB may help in the production of bioH₂ by pH regulation, substrate hydrolysis, biomass retention, oxygen depletion, and substrate detoxification [36]. Nevertheless, to exploit these advantages, a thorough understanding of the mechanisms underlying the HLac-type fermentation is essential. In this context, molecular analyses have depicted a possible syntrophy between LAB, acetic acid bacteria (AAB) and HPB [11, 29, 31, 36, 37]. For instance, Illumina MiSeq sequencing has revealed that Clostridium beijerinckii, Streptococcus sp., and Acetobacter lovaniensis were the most abundant species at the highest bioH₂ production activity [37].
possible changes of metabolites and microbial communities through time have also been investigated to understand the potential mechanism of bioH₂ production from HLac and HAc [36]. In this regard, the microbial structure showed coordinated dynamic behavior over time, identifying three stages throughout the process: (i) a first stage (corresponding to the lag phase in relation to bioH₂ production) in which the major part of TRS were consumed by dominant LAB and AAB, (ii) a second stage (corresponding to the exponential bioH₂ production phase) during which the HLac-type fermentation was catalyzed by emerging HPB, and (iii) a third stage (corresponding to the stationary bioH₂ production phase) in which non-HPB regrown while HPB became subdominant [36]. Interestingly, it has been also shown that an operating strategy based on pH-control may stimulate the syntrophy between *Clostridium* and *Lactobacillus*, and reduced the proliferation of *Blautia* and *Propionibacterium* (which are undesirable microorganisms due to their homoacetogenic and propionogenic activity, respectively), trending bioH₂ production to enhanced efficiency [29].

4. Biomethane production from agave bagasse and tequila vinasse

The operational performance, metabolic pathways, and microbial communities of the AD of AB and TV are extensively reviewed in the following sections.

4.1 Operational performance

In recent years, there have been several efforts to improve the AD performance of AB and TV (Table 4). Regarding the use of AB, the first study reported in this

| Pretreatment                          | Feeding   | Stage   | T (°C) | pH   | YCH₄ * | VMPR (NL/L-d) | CH₄ (% v/v) | Ref. |
|--------------------------------------|-----------|---------|--------|------|--------|---------------|-------------|------|
| Acid hydrolysis                      | Semi-continuous | Single  | 32     | 7.5  | 0.26ᵇ   | 0.3           | 70–74       | [8]  |
| Acid hydrolysis                      | Batch     | Single  | 37     | 8ᵃ   | 0.16ᵇ   | 0.78ᵈ         | NR          | [17] |
| Individual enzymatic hydrolysis      | Batch     | Single  | 37     | 8ᵃ   | 0.09ᵇ   | 0.6ᵈ          | NR          | [17] |
| Acid hydrolysis                      | Batch     | Two     | 37     | 8ᵃ   | 0.24ᵇ   | 0.75ᵈ         | NR          | [17] |
| Individual enzymatic hydrolysis      | Batch     | Two     | 37     | 8ᵃ   | 0.24ᵇ   | 0.96          | NR          | [17] |
| Individual enzymatic hydrolysis      | Semi-continuous | Two     | 37     | 7    | NR      | 0.41          | NR          | [7]  |
| Acid hydrolysis                      | Semi-continuous | Single  | 35     | 7    | 0.28ᵇ,c, 130ᶜ | NR          | NR          | [18] |
| Alkaline hydrogen peroxide + binary enzymatic hydrolysis | Batch | Single  | 37     | 7.5ᵃ | 0.2ᵇ,c, 393ᶜ | 0.67        | NR          | [22] |
| Individual enzymatic hydrolysis      | Continuous | Two     | 22–25 | 7.5  | 0.32ᵇ,c, 225ᶜ | 6.4         | 70–76       | [21] |

Notes: All studies were conducted using anaerobic granular sludge; *Initial pH value; *Units:ᵇNL-CH₄/g-COD removed;ᶜNL-CH₄/kg of AB;ᵈCalculated from provided information; NR: not reported.

Table 4. Comparison of the literature data on biomethane production efficiency using pretreated agave bagasse as feedstock.

---

*A Comprehensive Overview of the Potential of Tequila Industry By-Products for Biohydrogen...*  
DOI: http://dx.doi.org/10.5772/intechopen.88104
field was conducted by Arreola-Vargas et al. [8], who evaluated the feasibility of producing bioCH₄ from acid uncooked AB hydrolysates under two conditions, that is, with and without nutrient addition. The experiments were conducted in a mesophilic (32°C) AnSBR (with recirculation) at an OLR of 1.3 g-COD/L-d (influent concentration of 5 g-COD/L). The reactor had a working volume of 3.6 L and was inoculated with 5.8 g-VSS/L of anaerobic granular sludge collected from a full-scale UASB reactor treating brewery wastewater. The total cycle time was 72 h with a reaction time of 71 h and an exchange ratio of 80% (v/v). Unexpectedly, the best performance was obtained without additional supplementation of nutrients, achieving a volumetric bioCH₄ production rate (VMPR) of 0.3 NL-CH₄/L-d and a bioCH₄ yield (YCH₄) of 0.26 NL-CH₄/g-CODremoved with a CH₄ content in the biogas of 70–74% (v/v).

In a later study, Arreola-Vargas et al. [17], assessed the use of AB hydrolysates (20, 40, 60, 80, and 100% v/v) obtained either from acid or enzymatic pretreatment for bioCH₄ production in single- and two-stage AD processes. The experiments were conducted in the AMPTS II system at 37°C, 120 rpm, initial pH of 8, and using 10 g-VSS/L of anaerobic granular sludge collected from a full-scale UASB reactor treating TV as inoculum. The highest VMPR for single- (0.84 NL-CH₄/L-d) and two-stage (0.96 NL-CH₄/L-d) processes were achieved in the assays with enzymatic hydrolysates at 100% and 20%, respectively. Regarding YCH₄ results, the highest value with the single-stage process of 0.16 NL-CH₄/g-CODremoved was obtained in the assays with 20% hydrolysate from enzymatic pretreatment, while the two-stage process attained up to 0.24 NL-CH₄/g-CODremoved also at 20% hydrolysate regardless of the type of pretreatment used. Although both hydrolysates harbor potential fermentation inhibitors (i.e. organic acids, furan derivatives, and polyphenols) in different concentrations, results showed no negative effects in the AD performance. Toledo-Cervantes et al. [7] also evaluated the bioCH₄ production from the spent medium of DF of enzymatic hydrolysate of AB. The authors found that bioCH₄ production in an AnSBR was severely inhibited likely because the remaining catalytic activity of the enzyme used may have contributed to the degradation of CH₄ biocatalyst. In the same year, Breton-Deval et al. [18] contrasted the bioCH₄ production from acid AB hydrolysates previously obtained using two different acid catalysts, that is, HCl and H₂SO₄. The experiments were carried out in the AMPTS II at 35°C, 120 rpm, initial pH of 7.5, an organic load of 8 g-COD/L, and using 10 g-VSS/L of anaerobic granular sludge collected from a full-scale UASB reactor treating TV as inoculum. The results showed that HCl hydrolysate outperformed the H₂SO₄ one by obtaining a four-fold increase on YCH₄ that is, 0.17 versus 0.04 NL-CH₄/g-CODremoved, respectively. The impairment of the methanogenic activity was attributed to the fact that the addition of sulfate ions favored the activity of sulfate-reducing bacteria (SRB). However, when using optimized HCl hydrolysates based on bioCH₄ production (1.8% HCl, 119°C, and 103 min) rather than sugar recovery (1.9% HCl, 130°C, and 133 min), the highest YCH₄ of 0.19 NL-CH₄/g-CODremoved (0.09 NL-CH₄/g-VS of AB) was obtained indicating that other components of the hydrolysates besides sugars may influence bioCH₄ production, for example, extractives, potential microbial inhibitors.

In another study, Galindo-Hernández et al. [22] evaluated the bioCH₄ production potential from AB previously pretreated with AHP followed by enzymatic saccharification with hemicellulases and cellulases. The experiments were performed in the AMPTS II system at 37°C, 150 rpm, initial pH of 7.0, and using an organic load of 5 g-COD/L, 10 g-VS/L of inoculum (anaerobic granular sludge from a mesophilic full-scale TV treatment plant) and a defined mineral solution. Under such conditions, the YCH₄ and VMPR were found as 0.2 NL-CH₄/g-CODremoved.
(0.39 NL-CH$_4$/g of AB) and 0.67 NL-CH$_4$/L-d, respectively, indicating the potential advantage of integrating a delignification pretreatment and the use of synergistic enzymatic mixtures before the AD process.

Regarding continuous processes, Montiel and Razo-Flores [21] studied the effect of OLR on the VMPR using a mesophilic (23–25°C) 1.5-L UASB reactor (with a working volume of 1.25 L) feeding with diluted (and supplemented with nutrients) acidogenic effluent generated during the DF of enzymatic hydrolysates of AB. The reactor was inoculated with 20 g-VS/L of anaerobic granular sludge from a full-scale UASB reactor treating TV and operated for 80 d to achieve OLRs between 1.35 and 24 g-COD/L-d by increasing the COD concentration of the influent and then by decreasing the HRT from 21 to 10 h. The highest VMPR and YCH$_4$ of 6.4 NL-CH$_4$/L-d and 0.32 NL-CH$_4$/g-COD$_{fed}$ (225 NL-CH$_4$/kg of AB) were achieved at an OLR of 20 g-COD/L-d (14 h HRT). Under such conditions, the COD removal efficiency was above 90% and the CH$_4$ content in the gas phase was of 73% (v/v).

Regarding the use of TV for bioCH$_4$ production (Table 5), Méndez-Acosta et al. [43] assessed the mesophilic AD of TV in a lab-scale CSTR reactor for 250 d at HRTs of 14–5 d corresponding to increments in the OLR from 0.7 to 6 g-COD/L-d (influent COD concentrations of 10–33 g/L). The highest YCH$_4$ of 0.32 L-CH$_4$/g-COD$_{removed}$ and VMPR of 2.8 L-CH$_4$/L-d with bioCH$_4$ concentrations in the biogas greater than 65% (v/v) and COD removal efficiencies over 90% were obtained, even with an unbalanced COD/N/P ratio, at 6 g-COD/L-d OLR. However, a relatively long start-up of 50 d and continuous supplementation of external alkalinity were needed in order to provide stability to the process.

With the aim of enhancing the stability of the AD of TV, López-López et al. [44] investigated the influence of alkalinity and volatile fatty acids (VFAs) on the performance of a 2-L UASB reactor. The UASB reactor was inoculated with anaerobic granular sludge and operated under mesophilic conditions during 235 d at OLRs from 2.5 to 20 g-COD/L-d with recirculation of the treated effluent at recycling flow rate to influent flow rate ratios of 1:1 to 10:1 in one-unit increments. In that study, it was found that, by maintaining a VFAs to alkalinity ratio $\leq$ 0.5 with recirculation 1:10, the recirculation of the effluent could induce stable performances by reducing the impact of VFAs and organic matter concentration present in the effluent, attaining a COD removal efficiency higher than 75% with a YCH$_4$ of 0.33

| Pretreatment/ conditioning | Feeding | Stage T (°C) | pH | YCH$_4$ (NL/g-COD$_{removed}$) | VMPR (NL/L-d) | CH$_4$ (%) (v/v) | Ref. |
|----------------------------|---------|-------------|----|-----------------------------|--------------|----------------|------|
| Dilution | Continuous | Single | 35 | 7.4 | 0.32* | 1.7* | 65 | [43] |
| Dilution | Continuous | Single | 35 | 7.4 | 0.32 | 1.9* | 75 | [45] |
| Dilution, nutrient supplementation | Semi-continuous | Two | 35 | 6.8–7.5 | 0.26 | 0.29 | 68 | [35] |
| Dilution, solid removal (centrifugation) | Continuous | Single | 35 | ~7 | 0.33 | NR | 60–65 | [44] |
| Dilution | Semi-continuous | Single | 32 | 8 | 0.28 | 2.3* | 90 | [46] |
| Dilution | Continuous | Single | 35 | 7 | 0.24 | 3.03 | 65 | [47] |
| Dilution | Continuous | Two | 35 | 7.7 | 0.29 | 2.3* | 80 | [7] |

Notes: All studies were conducted using anaerobic granular sludge; *Calculated from provided information; NR: not reported.

Table 5. Comparison of the literature data on biomethane production efficiency using tequila vinasse as feedstock.
NL-CH₄/g-COD_removed. However, even though the high recirculation ratio led to the recovery of alkalinity without any addition of external alkalinity, the granular sludge tended to become flocculent with a reduction in the average size from 2.5 to 1.5 mm.

In another study conducted by Jáuregui-Jáuregui et al. [45], after a start-up period of 28 d, a mesophilic up-flow FBR inoculated with anaerobic granular sludge withdrawn from a full-scale UASB reactor treating brewery wastewater exhibited a YCH₄ of 0.27 NL-CH₄/g-COD_removed with a CH₄ content of 75% (v/v) and COD removal efficiencies of up to 90% under an OLR of 8 g-COD/L-d and an HRT of 4 d. However, the authors also reported the inhibition of biogas production due to digester clogging, which led to an excessive VFAs accumulation. In the same year, Buitrón et al. [35] reported the performance of a UASB reactor treating the resulting effluent of a DF stage at three different COD concentrations, that is, 0.4, 1.08, and 1.6 g/L, and two HRTs, that is, 24 and 18 h. The maximal content of CH₄ in the gas phase (68% v/v) and COD removal (67%) were achieved at the concentration of 1.6 g-COD/L with an HRT of 24 h. A further decrease in HRT resulted in lower efficiencies, that is, 40% CH₄ content and 52% removal efficiency.

In a further study, Arreola-Vargas et al. [46] achieved YCH₄ ranging from 0.25 to 0.29 NL-CH₄/g-COD_removed with 75–90% (v/v) CH₄ content and 85% COD removal using a bench scale AnSBR inoculated with anaerobic granular sludge and fed with diluted TV (8 g-COD/L), the reaction time varied within 3–9 d. Interestingly, later, the same research group performed a pilot scale study for the mesophilic AD treatment of TV using a 445-L packed bed reactor (PBR) which was operated for 231 d under increasing OLRs, from 4 to 12.5 g-COD/L-d [47]. The PBR showed a stable performance exhibiting COD removals and YCH₄ in the range of 86–89% and 0.24–0.28 NL-CH₄/g-COD_removed, respectively. Meanwhile, the highest VMPR of 3.03 NL-CH₄/L-d was reached at the highest OLR of 12.5 g-COD/L-d [47].

More recently, in two-stage PBRs operated over 335 d, Toledo-Cervantes et al. [7] achieved the highest YCH₄ of 0.29 NL-CH₄/g-COD_removed at OLRs in the range of 2.7–6.8 g-COD/L-d (6–2.4 d HRT) with COD removal efficiencies between 81 and 95%, and with average CH₄ contents around 80% (v/v). However, further increasing the OLR to 12 g-COD/L-d (2.2-d HRT) decreased the removal efficiency of COD (from 81 to 74%) accompanied with HAc and HPr accumulation.

### 4.2 Metabolic pathways

As shown in Table 6, the majority of bioCH₄ produced in AD systems occurs from the use of HAc and bioH₂ via acetoclastic (reaction 17) and hydrogenotrophic (reaction 4) pathways, respectively. However, bioCH₄ can also be evolved from HFor (reaction 18), compounds with the methyl group like methanol (reaction 19),

| Reaction | Products |
|----------|----------|
| 4H₂ + CO₂ → CH₄ + 2H₂O | (4) |
| HAc → CH₄ + CO₂ | (17) |
| 4HFor → CH₄ + 3CO₂ + 2H₂O | (18) |
| 3CH₃OH + H₂ → CH₄ + H₂O | (19) |
| 4HPr + 2H₂O → 4HAc + CO₂ + 3CH₄ (syntrophic conversion) | (20) |
| HBu + 2H₂O → 4HAc + CO₂ + CH₄ (syntrophic conversion) | (21) |

Table 6. Biomethane-producing reactions.
and from the syntrophic degradation of HBu (reaction 20) and HPr (reaction 21) [48]. Thus, an even production and consumption rate of organic acids is a sign of healthy single-stage AD processes. Contrarily, excessive accumulation of organic acids in the effluent has been related to reactor upset and failure, causing a drop in biogas production and COD removal efficiency. For instance, the presence of HPr in a HPr/HAc ratio \( \geq 1 \) is usually matched with operational instability [43]. The alkalinity ratio, \( \alpha = \frac{\text{intermediate alkalinity (pH = 5.75)}}{\text{partial alkalinity (pH = 4.3)}} \), roughly relates the amounts of VFAs and bicarbonate alkalinity in anaerobic reactors, measuring the buffer potential of the systems [49]. Values \( \leq 0.3 \) are reported as adequate for achieving stable operation; however, in the case of TV-fed anaerobic reactors, stable processes have been achieved at slightly higher range of \( \alpha \) between 0.2 and 0.5 [44, 47]. Moreover, bioCH\(_4\) production can be disrupted by the formation of certain by-products such as long chain fatty acids or solvents, which may jeopardize the suitable availability of bioCH\(_4\) precursors. In this regard, in the case of integrated DF-AD schemes, special attention must be also paid to the concentration and composition of organic acids coming from the DF stage. At this point, it should be mentioned that the redirection of carbon through HLac has been reported as a strategy to enhanced AD processes due to its thermodynamic advantages [50–52].

### 4.3 Microbial communities

AD reactors contain mixed microbial populations [15]. BioCH\(_4\) formation from AB and TV has been related with the coexistence of syntrophic bacteria (Anaerolineaceae, Candidatus, Cloacamonas, Syntrophobacter, Syntrophomonas, and Syntrophus), hydrogenotrophic (Methanobacterium and Methanocorpusculum) and acetoclastic (Methanoseta and Methanosarcina) methanogens [7, 18, 47]. It has been previously observed that the two-stage AD of TV at low concentrations of VFAs (low OLRs) favored the acetoclastic pathway, in contrast, hydrogenotrophic methanogens enriched at high concentrations (high OLRs) [7]. This change in diversity has been also observed in an AnSBR digester fed with acid AB hydrolysates [53]. However, the opposite trend was observed during the single stage AD of TV using a pilot-scale PBR [47]. Regardless of the tequila by-product used, loss of syntrophic relationships for interspecies \( \text{H}_2/\text{HFor} \) transfer and interspecies HAc transfer has been associated with microbial imbalance, which subsequently affects negatively bioCH\(_4\) production [8, 53]. However, in the case of multi-stage AD processes, unsuitable concentrations of hydrolytic/acidogenic bacteria in DF effluent may be quite detrimental for the granular methanogenic sludge [15]. In addition, other bacteria which can compete with the methanogens for bioCH\(_4\) precursors may also be present in AD reactors, for example, SRB [15, 18].

### 5. Multi-stage anaerobic digestion

Since TV has negligible levels of alkalinity and high concentrations of components with a tendency to suffer very rapid acidification [43, 44], two-stage AD processes have emerged as important operational strategies to provide enhanced stability of the CH\(_4\)–producing stage [7, 24]. However, the multi-stage AD approach seems to be also applicable for pretreated AB [17, 21]. In fact, a two-stage AD process fed with AB hydrolysates showed up to 3.3-fold higher energy recovery than a single-stage process [17]. Indeed, according to Lindner et al. [16], two-stage systems seem to be only recommendable for digesting sugar-rich feed stocks, which undergo a quick hydrolysis/acidogenesis. This approach allows to provide optimal
environmental conditions for the different groups of microorganisms which have differences in terms of physiology, nutrient intake, nutritional requirements, growth rate, optimum growth conditions such as pH, and adaptation to environmental stress conditions [16]. The acidogenesis and methanogenesis separated in space may also produce bioH₂ via DF process [17, 24, 35]. However, it is not necessarily desirable to produce bioH₂ in all cases [7]. In the latter case, a stream rich in HLac can be obtained through the HLac-type fermentation which can be further fed to the methanogenic stage [36, 37], where hydrogenotrophic may be benefited for the conversion of HLac to HAc by consuming the intermediate bioH₂ gas immediately [52]. The possibility of operating at higher organic loading capacity (in the methanogenic stage), reducing alkali addition, and increasing COD removal efficiency are additional advantages of the two-stage AD as compared to single-stage AD [7, 21, 24]. A small number of reactor configurations devoted to bioH₂/bioCH₄ production from AB/TV can be found in the literature (Figure 3). Among them, for both AB and TV, the CSTR and UASB configurations have shown the highest performance to date for producing bioH₂ and bioCH₄, respectively, that is,

![Diagram](image1)

**Figure 3.** Types of reactor configurations used for biohydrogen and biomethane production from tequila processing by-products. (a) Batch reactor, (b) continuously stirred tank reactor (CSTR) with recirculation, (c) CSTR, (d) anaerobic sequencing batch reactor (AnSBR), (e) trickling bed reactor with recirculation, (f) packed bed reactor, (g) up-flow anaerobic sludge blanket (UASB) reactor. AnSBR can integrate mechanical or hydraulic mixing. UASB can operate with effluent recycle.
13 NL-H$_2$/L-d from AB [23] and 12.3 NL-H$_2$/L-d from TV [38] and 6.4 NL-CH$_4$/L-d from AB [21] and 3.5 NL-CH$_4$/L-d from TV [54].

6. Current limitations and potential improvements

Notwithstanding the enormous efforts made to achieve a better understanding of the DF/AD process of AB/TV, it is still necessary to improve not only bioH$_2$ or bioCH$_4$ productivities and yields but also the (long-term) stability of processes for commercialization purposes. TV is a highly complex wastewater that besides high COD and negligible alkalinity, harbors recalcitrant compounds such as phenols, which may act as inhibitors in DF/AD. While the main limitation to use AB as the feedstock is its recalcitrant structure. As mentioned earlier, some of the pretreatment/conditioning steps used in AB have been optimized not only in terms of hydrolysis yield, reaction time, the generation/release and effect of putative fermentation inhibitory compounds, cost-effectiveness but also in terms of bioH$_2$/bioCH$_4$ production efficiency. However, there is still a need to explore other pretreatments that have not been yet embraced in the field of DF/AD of AB but they have been ascertained as potentially useful in releasing sugars for other applications like the production of bioethanol, such as ammonia fiber explosion (AFEX), autohydrolysis, organosolv, high-energy radiation, ozonolysis, alkaline, ionic liquids, or any combination of those pretreatments. It could be also interesting to explore consolidated processes (direct fermentation) which combine into a single operation the enzymatic hydrolysis of (pretreated) biomass and biological conversion to the desired by-product (in this case bioH$_2$/bioCH$_4$) by mixed consortia.

Besides the features described before, from practical purposes, the highly variable composition of AB/TV constitutes another constraint to produce bioH$_2$ since DF systems are commonly unable to overcome perturbations in feedstock composition. One of the most significant challenges is to assure consistency in the prevailing metabolic pathways during the DF process and favor bioH$_2$-producing pathways over other unwanted routes, for example, homoacetogenesis and methanogenesis. Very little is known about the microbial community structure of DF/AD processes treating AB/TV. In this regard, it is not clear the role of microorganisms and their association with operational parameters (e.g. pH, HRT, and OLR) and process indicators (e.g. VHPR, VMPR, and metabolic composition). Also, much less is known about how microbial assemblage may change through time, and what factors (operating parameters) govern its dynamics. It is worth noticing that HLac monitoring has been disregarded limiting the understanding of integrated DF-AD processes since it, as an intermediate, has a vital role in the carbon flux.

Another concern worth to mention is that most of the previous studies were carried out in batch or semi-continuous reactors. Thus, it is vital to transfer the kinetic knowledge gained from such studies to the expansion of continuous systems. In this context, the development of integrated DF-AD schemes for the continuous production of bioH$_2$ and bioCH$_4$ using AB/TV as feedstocks requires intensive research on interlinking side streams for producing high added-value bioproducts in a biorefinery framework (e.g. HLac-bioH$_2$/bioCH$_4$) for better sustainability of the existing tequila industries.

7. Conclusions

Tequila industry generates huge amounts of AB and TV, which could be subjected to integrated DF-AD processes to produce bioH$_2$ and bioCH$_4$, while reducing their pollution potential. This chapter focused on the state-of-the-art of...
configurations and process parameters, metabolic pathways, and microbial ecology of bioH₂- and bioCH₄-producing reactors. The pretreatment/conditioning steps applied to enhance the valorization of AB/TV were also reviewed. It has been suggested that the HLac-type fermentation coupled to DF and AD can boost the development of cascading design in multi-stage AD processes. This multiproduct approach using AB/TV as resources in the biorefinery scheme may facilitate sustainability to the tequila industry.

Acknowledgements

This work was financially supported by Consejo Nacional de Ciencia y Tecnología (CONACYT) through the Project-PN-2015-2101-1024. Osuna-Laveaga D.R. acknowledges CONACYT for the Ph.D. scholarship: 267499.

Conflict of interest

The authors declare no conflict of interest.

Acronyms and abbreviations

| Acronym | Description |
|---------|-------------|
| HAc     | acetic acid |
| AAB     | acetic acid bacteria |
| AB      | agave bagasse |
| AHP     | alkaline hydrogen peroxide |
| AD      | anaerobic digestion |
| AnSBR   | anaerobic sequencing batch reactor |
| AMPTS II| automatic methane potential test system |
| bioH₂   | biohydrogen |
| YH₂     | biohydrogen yield |
| HPB     | biohydrogen-producing bacteria |
| bioCH₄  | biomethane |
| YCH₄    | biomethane yield |
| HBu     | butyric acid |
| COD     | chemical oxygen demand |
| CSTR    | continuously stirred tank reactor |
| DF      | dark fermentation |
| FPU     | filter paper units |
| FBR     | fixed bed reactor |
| HFor    | formic acid |
| HRT     | hydraulic retention time |
| HPB     | hydrogen-producing bacteria |
| HMF     | hydroxymethylfurfural |
| HLac    | lactic acid |
| LAB     | lactic acid bacteria |
| NW      | nixtamalization wastewater |
| ORP     | oxidation-reduction potential |
| OLR     | organic loading rate |
| PBR     | packed bed reactor |
| HPr     | propionic acid |
| SRB     | sulfate-reducing bacteria |
| Acronym   | Abbreviation                        |
|-----------|------------------------------------|
| VFAs      | volatile fatty acids               |
| VS        | volatile solid                    |
| VSS       | volatile suspended solids          |
| VHPR      | volumetric biohydrogen production rate |
| VMPR      | volumetric biomethane production rate |
| TV        | tequila vinasse                    |
| TRS       | total-reducing solids              |
| TBR       | trickling bed reactor              |
| UASB      | up-flow anaerobic sludge blanket reactor |

**Author details**

Octavio García-Depraect, Daryl Rafael Osuna-Laveaga and Elizabeth León-Becerril*
Department of Environmental Technology, Centro de Investigación y Asistencia en Tecnología y Diseño del Estado de Jalisco, A.C., Guadalajara, Jalisco, México

*Address all correspondence to: eleon@ciatej.mx

**IntechOpen**

© 2019 The Author(s). Licensee IntechOpen. This chapter is distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/3.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.
References

[1] CRT. Producción total Tequila y Tequila 100% [Internet]. 2019. Available from: https://www.crt.org.mx/EstadisticasCRTweb [Accessed: 14-05-2019]

[2] Villanueva-Rodríguez SJ, Rodríguez-Garay B, Prado-Ramírez R, Gschaedler A. Tequila: Raw material, classification, process, and quality parameters. Encyclopedia of Food and Health. Academic Press; 2016:283-289. DOI: 10.1016/B978-0-12-384947-2.00688-7

[3] Rodríguez-Félix E, Contreras-Ramos SM, Davila-Vazquez G, Rodríguez-Campos J, Marino-Marmolejo EN. Identification and quantification of volatile compounds found in vinasses from two different processes of tequila production. Energies. 2018;11:1-18. DOI: 10.3390/en11030490

[4] Cedeño-Cruz M. Tequila production from agave: Historical influences and contemporary processes. In: Jacques KA, Lyons TP, Kelsall DR, editors. The Alcohol Textbook. 4th ed. Oxford UK: Nottingham University Press; 2003. pp. 223-245

[5] López-López A, Davila-Vazquez G, León-Becerril E, Villegas-García E, Gallardo-Valdez J. Tequila vinasses: Generation and full scale treatment processes. Reviews in Environmental Science and Biotechnology. 2010;9:109-116. DOI: 10.1007/s11157-010-9204-9

[6] del Real-Olvera J, López-López A. Biogas production from anaerobic treatment of agro-industrial wastewater. In: Kumar S, editor. Biogas. Rijeka: InTech; 2012. pp. 91-112

[7] Toledo-Cervantes A, Guevara-Santos N, Arreola-Vargas J, Snell-Castro R, Méndez-Acosta HO. Performance and microbial dynamics in packed-bed reactors during the long-term two-stage anaerobic treatment of tequila vinasses. Biochemical Engineering Journal. 2018;138:12-20. DOI: 10.1016/j.bej.2018.06.020

[8] Arreola-Vargas J, Ojeda-Castillo V, Snell-Castro R, Corona-González RI, Alatriste-Mondragón F, Méndez-Acosta HO. Methane production from acid hydrolysates of Agave tequilana bagasse: Evaluation of hydrolysis conditions and methane yield. Bioresource Technology. 2015;181:191-199. DOI: 10.1016/j.biortech.2015.01.036

[9] Hernández C, Escamilla-Alvarado C, Sánchez A, Alarcón E, Ziarelli F, Musle R, et al. Wheat straw, corn Stover, sugarcane, and agave biomasses: Chemical properties, availability, and cellulosic-bioethanol production potential in Mexico. Biofuels, Bioproducts & Biorefining. 2019:1-17. DOI: 10.1002/bbb.2017

[10] Pecha B, García-Perez M. Pyrolysis of lignocellulosic biomass: Oil, char, and gas. In: Dahiya A, editor. Bioenergy Biomass to Biofuels. Oxford UK: Academic Press; 2015. pp. 413-442. DOI: 10.1016/B978-0-12-407909-0.00026-2

[11] García-Depraect O, Gómez-Romero J, León-Becerril E, López-López A. A novel biohydrogen production process: Co-digestion of vinasse and Nejayote as complex raw substrates using a robust inoculum. International Journal of Hydrogen Energy. 2017;42:5820-5831. DOI: 10.1016/j.ijhydene.2016.11.204

[12] Iñiguez-Covarrubias G, Lange SE, Rowell RM. Utilization of byproducts from the tequila industry: Part 1: Agave bagasse as a raw material for animal feeding and fiberboard production. Bioresource Technology. 2001;77:25-32. DOI: 10.1016/S0960-8524(00)00137-1

[13] Moran-Salazar RG, Marino-Marmolejo EN, Rodríguez-Campos J,
Dávila-Vázquez G, Contreras-Ramos SM. Use of agave bagasse for production of an organic fertilizer by pretreatment with *Bjerkandera adusta* and vermicomposting with *Eisenia fetida*. Environmental Technology. 2015;37:1-12. DOI: 10.1080/09593330.2015.1108368

[14] Palomo-Briones R, López-Gutiérrez I, Islas-Lugo F, Galindo-Hernández KL, Munguía-Aguilar D, Rincón-Pérez JA, et al. Agave bagasse biorefinery: Processing and perspectives. Clean Technologies and Environmental Policy. 2018;20:1423-1441. DOI: 10.1007/s10098-017-1421-2

[15] van Lier JB, Mahmoud N, Zeeman G. Anaerobic wastewater treatment. In: Henze M, Loosdrecht v, Ekama GA, Brdjanovic, editors. Biological Wastewater Treatment: Principles Modeling and Design. London UK: IWA Publishing; 2008. pp. 401-442

[16] Lindner J, Zielonka S, Oechsner H, Lemmer A. Is the continuous two-stage anaerobic digestion process well suited for all substrates? Bioresource Technology. 2016;200:470-476. DOI: 10.1016/j.biortech.2015.10.052

[17] Arreola-Vargas J, Flores-Larios A, González-Álvarez V, Corona-González RI, Méndez-Acosta HO. 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. 2016;41:897-904. DOI: 10.1016/j.ijhydene.2015.11.016

[18] Breton-Deval L, Méndez-Acosta HO, González-Álvarez V, Snell-Castro R, Gutiérrez-Sánchez D, Arreola-Vargas J. *Agave tequilana* bagasse for methane production in batch and sequencing batch reactors: Acid catalyst effect, batch optimization and stability of the semi-continuous process. Journal of Environmental Management. 2018;224:156-163. DOI: 10.1016/j.jenvman.2018.07.053

[19] Valdez-Guzmán BE, Rios-Del Toro EE, Cardenas-López RL, Méndez-Acosta HO, González-Álvarez V, Arreola-Vargas J. Enhancing biohydrogen production from *Agave tequilana* bagasse: Detoxified vs. Undetoxified acid hydrolysates. Bioresource Technology. 2019;276:74-80. DOI: 10.1016/j.biortech.2018.12.101

[20] Contreras-Dávila CA, Méndez-Acosta HO, Arellano-García LA, Alatriste-Mondragón F, Razo-Flores E. Continuous hydrogen production from enzymatic hydrolysate of *Agave tequilana* bagasse: Effect of the organic loading rate and reactor configuration. Chemical Engineering Journal. 2017;313:671-679. DOI: 10.1016/j.cej.2016.12.084

[21] Montiel CV, Razo-Flores E. Continuous hydrogen and methane production from *Agave tequilana* bagasse hydrolysate by sequential process to maximize energy recovery efficiency. Bioresource Technology. 2018;249:334-341. DOI: 10.1016/j.biortech.2017.10.032

[22] Galindo-Hernández KL, Tapia-Rodríguez A, Alatriste-Mondragón F, Celis LB, Arreola-Vargas J, Razo-Flores E. Enhancing saccharification of *Agave tequilana* bagasse by oxidative delignification and enzymatic synergism for the production of hydrogen and methane. International Journal of Hydrogen Energy. 2018;43:22116-22125. DOI: 10.1016/j.ijhydene.2018.10.071

[23] Montoya-Rosales JJ, Olmos-Hernández DK, Palomo-Briones R, Montiel-Corona V, Mari AG, Razo-Flores E. Improvement of continuous hydrogen production using individual and binary enzymatic hydrolysates of agave bagasse in suspended-culture and biofilm reactors.
[24] Ruggeri B, Tommasi T, Sanfilippo S. Two-step anaerobic digestion process. In: Ruggeri B, Tommasi T, Sanfilippo S, editors. BioH2 & BioCH4 through Anaerobic Digestion. From Research to Full-Scale Applications. Green Energy and Technology. Springer London Heidelberg New York Dordrecht; Springer-Verlag London: 2015. pp. 161-191. DOI: 10.1007/978-1-4471-6431-9

[25] Ghimire A, Frunzo L, Pirozzi F, Trably E, Escudie R, Lens PNL, et al. A review on dark fermentative biohydrogen production from organic biomass: Process parameters and use of by-products. Applied Energy. 2015;144:73-95. DOI: 10.1016/j.apenergy.2015.01.045

[26] Toledo-Cervantes A, Arreola-Vargas J, Elias-Palacios SE, Marino-Marmolejo EN, Davila-Vazquez G, González-Álvarez V, et al. Evaluation of semi-continuous hydrogen production from enzymatic hydrolysates of Agave tequilana bagasse: Insight into the enzymatic cocktail effect over the co-production of methane. International Journal of Hydrogen Energy. 2018;43:14193-14201. DOI: 10.1016/j.ijhydene.2018.05.134

[27] Espinoza-Escalante FM, Pelayo-Ortiz C, Gutiérrez-Pulido H, González-Álvarez V, Alcaraz-González V, Bories A. Multiple response optimization analysis for pretreatments of Tequila’s stillages for VFAs and hydrogen production. Bioresource Technology. 2008;99:5822-5829. DOI: 10.1016/j.biortech.2007.10.008

[28] Espinoza-Escalante FM, Pelayo-Ortiz C, Navarro-Corona J, González-García Y, Bories A, Gutiérrez-Pulido H. 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. 2009;33:14-20. DOI: 10.1016/j.biombioe.2008.04.006

[29] García-Depraect O, Rene ER, Gómez-Romero J, López-López A, León-Becerril E. Enhanced biohydrogen production from the dark co-fermentation of tequila vinasse and nixtamalization wastewater: Novel insights into ecological regulation by pH. Fuel. 2019;253:159-166. DOI: 10.1016/j.fuel.2019.04.147

[30] Buitrón G, Carvajal C. Biohydrogen production from tequila vinasses in an anaerobic sequencing batch reactor: Effect of initial substrate concentration, temperature and hydraulic retention time. Bioresource Technology. 2010;101:9071-9077. DOI: 10.1016/j.biortech.2010.06.127

[31] García-Depraect O, Rene ER, Diaz-Cruces VF, León-Becerril E. Effect of process parameters on enhanced biohydrogen production from tequila vinasse via the lactate-acetate pathway. Bioresource Technology. 2019;273:618-626. DOI: 10.1016/j.biortech.2018.11.056

[32] Marino-Marmolejo EN, Corbalá-Robles L, Cortez-Aguilar RC, Contreras-Ramos SM, Bolaños-Rosales RE, Davila-Vazquez G. Tequila vinasses acidogenesis in a UASB reactor with Clostridium predominance. Springerplus. 2015;4:1-8. DOI: 10.1186/s40064-015-1193-2

[33] Moreno-Andrade I, Moreno G, Kumar G, Buitrón G. Biohydrogen production from industrial wastewaters. Water Science and Technology. 2014;71:105-110. DOI: 10.2166/wst.2014.471

[34] Buitrón G, Prato-Garcia D, Zhang A. Biohydrogen production from tequila
vinasses using a fixed bed reactor. Water Science and Technology. 2014; 70:1919-1925. DOI: 10.2166/wst.2014.433

[35] Buitrón G, Kumar G, Martinez-Arce A, Moreno G. Hydrogen and methane production via a two-stage processes (H₂-SBR + CH₄-UASB) using tequila vinasses. International Journal of Hydrogen Energy. 2014;39:19249-19255. DOI: 10.1016/j.ijhydene.2014.04.139

[36] García-Depraect O, Valdez-Vázquez I, Rene ER, Gómez-Romero J, López-López A, León-Becerril E. Lactate- and acetate-based biohydrogen production through dark co-fermentation of tequila vinasse and nixtamalization wastewater: Metabolic and microbial community dynamics. Bioresource Technology. 2019;282:236-244. DOI: 10.1016/j.biortech.2019.02.100

[37] García-Depraect O, León-Becerril E. Fermentative biohydrogen production from tequila vinasse via the lactate-acetate pathway: Operational performance, kinetic analysis and microbial ecology. Fuel. 2018;234:151-160. DOI: 10.1016/j.fuel.2018.06.126

[38] García-Depraect O, van Lier JB, Muñoz R, Rene ER, Díaz-Cruces VF, León-Becerril E. Interlinking lactate-type fermentation in a side stream anaerobic digestion process of tequila vinasse: An alternative for highly stable and efficient biohydrogen production. (Unpublished)

[39] Ren N, Zhao D, Chrn X, Li J. Mechanism and controlling strategy of the production and accumulation of propionic acid for anaerobic wastewater treatment. Science In China. 2002;45:319-327

[40] Sikora A, Błaszczzyk M, Jurkowski M, Zielenkiewicz U. Lactic acid bacteria in hydrogen-producing consortia: On purpose or by coincidence? In: Kongo J, editor. Lactic Acid Bacteria. R & D for Food, Health and Livestock Purposes. Rijeka: InThech; 2013. pp. 487-514. DOI: 10.5772/50364

[41] Elbeshbishy E, Dhar BR, Nakhla G, Lee HS. A critical review on inhibition of dark biohydrogen fermentation. Renewable and Sustainable Energy Reviews. 2017;79:656-668. DOI: 10.1016/j.rser.2017.05.075

[42] Cabrol L, Marone A, Tapia-Venegas E, Steyer JP, Ruiz-Filippi G, Trably E. Microbial ecology of fermentative hydrogen producing bioprocesses: Useful insights for driving the ecosystem function. FEMS Microbiology Reviews. 2017;41:158-181. DOI: 10.1093/femsre/fuw043

[43] Méndez-Acosta HO, Snell-Castro R, Alcaraz-González V, González-Álvarez V, Pelayo-Ortiz C. Anaerobic treatment of tequila vinasses in a CSTR-type digester. Biodegradation. 2010;21:357-363. DOI: 10.1007/s10532-009-9306-7

[44] López-López A, León-Becerril E, Rosales-Contreras ME, Villegas-García E. Influence of alkalinity and VFAs on the performance of an UASB reactor with recirculation for the treatment of tequila vinasses. Environmental Technology. 2015;36:2468-2476. DOI: 10.1080/09593330.2015.1034790

[45] Jáuregui-Jáuregui JA, Méndez-Acosta HO, González-Álvarez V, Snell-Castro R, Alcaraz-González V, Godonc JJ. Anaerobic treatment of tequila vinasses under seasonal operating conditions: Start-up, normal operation and restart-up after a long stop and starvation period. Bioresource Technology. 2014;168:33-40. DOI: 10.1016/j.biortech.2014.04.006

[46] Arreola-Vargas J, Jaramillo-Gante NE, Celis LB, Corona-González RI, González-Álvarez V, Méndez-Acosta HO. Biogas production in an anaerobic
sequencing batch reactor by using tequila vinasses: Effect of pH and temperature. Water Science and Technology. 2016;73:550-556. DOI: 10.2166/wst.2015.520

[47] Arreola-Vargas J, Snell-Castro R, Rojo-Liera NM, González-Álvarez V, Méndez-Acosta HO. Effect of the organic loading rate on the performance and microbial populations during the anaerobic treatment of tequila vinasses in a pilot-scale packed bed reactor. Journal of Chemical Technology & Biotechnology. 2018;93:591-599. DOI: 10.1002/jctb.5413

[48] Gerardi MH. Anaerobic food chain. In: Gerardi MH, editor. The Microbiology of Anaerobic Digesters. Canada: John Wiley & Sons; 2003. p. 39-41

[49] Vuitik GA, Fuess LT, Del Nery V, Bañares-Alcántara R, Pires EC. Effects of recirculation in anaerobic baffled reactors. Journal of Water Process Engineering. 2019;28:36-44. DOI: 10.1016/j.jwpe.2018.12.013

[50] Detman A, Mielecki D, Pleśniak Ł, Bucha M, Janiga M, Matyasik I, et al. Methane-yielding microbial communities processing lactate-rich substrates: A piece of the anaerobic digestion puzzle. Biotechnology for Biofuels. 2018;11:1-18. DOI: 10.1186/s13068-018-1106-z

[51] Wu Y, Wang C, Liu X, Ma H, Wu J, Zuo J, et al. A new method of two-phase anaerobic digestion for fruit and vegetable waste treatment. Bioresource Technology. 2016;211:16-23. DOI: 10.1016/j.biortech.2016.03.050

[52] Pipyn P, Verstraete W. Lactate and ethanol as intermediates in two-phase anaerobic digestion. Biotechnology and Bioengineering. 1981;23:1145-1154

[53] Snell-Castro R, Méndez-Acosta HO, Arreola-Vargas J, González-Álvarez V, Pintado-González M, González-Morales MT, et al. Active prokaryotic population dynamics exhibit high correlation to reactor performance during methane production from acid hydrolysates of Agave tequilana var. Azul bagasse. Journal of Applied Microbiology. 2019;126:1618-1630. DOI: 10.1111/jam.14234

[54] Díaz-Cruces VF, García-Depraect O, León-Becerril E. Performance of two-stage anaerobic digestion of tequila vinasse with acidogenic lactate-type fermentation. (Unpublished)