

Hydrogen production from coffee pulp by dark fermentation

Raciel Miñón-Fuentes and Oscar Aguilar-Juárez

ABSTRACT

Coffee pulp (C.P.) is a waste of coffee production that needs to be controlled. Due to its high moisture and sugar content, a diagnostic study that characterizes the pulp was conducted and the potential for hydrogen production was evaluated. Subsequently, the kinetics of hydrogen production in a bioreactor were evaluated. A biodegradability index of 0.91 (DBO₅/DQO) was calculated, initial pH of the sample was 4.16 ± 0.05 , a concentration of total volatile solids (TVS) of 58.1 ± 0.94 [g/L], and total sugar of 19.6 ± 0.79 [g Dextrose/L]. The yield was at 49.2 [NmL H₂/g DQO_{initial}], the hydrogen production per fresh coffee pulp kilogram was 4.18 [L H₂/kg C.P.], the energy density was determined at 0.045 [MJ/kg C.P.]. Modified Gompertz parameters were 585 [NmL] for H_{max}, 4.1 [NmL H₂/g DQO-h] for R_{max} and a lag phase (λ) of 92.70 [h]. Because the yield of hydrogen production of coffee pulp estimated was similar to complex substrates like tequila vinasses, and there was a DQO reduction of 13.58%, based on some substrate restrictions, dark fermentation could be a stage of pretreatment of wastewater with coffee pulp in a biogas process to produce two relevant economic and energy products (hydrogen and biogas).

Key words | agroindustrial, biofuels, biohydrogen, biomass, coffee, fermentation

Raciel Miñón-Fuentes

Master in Renewable Energy,
Luz Torres Escamilla #1661, Col. Paseos del Sol,
C.P. 45079, Zapopan, Jalisco,
Mexico

Oscar Aguilar-Juárez (corresponding author)

Centro de Investigación y Asistencia en Tecnología
y Diseño del Estado de Jalisco A.C.,
Av. de los Normalistas #800, Col. Colinas de la
Normal, C.P. 44270, Guadalajara, Jalisco,
Mexico
E-mail: oaguilar@ciatej.mx

INTRODUCTION

The economic importance of coffee is due to the beverage that is prepared with its seed, an infusion from the roasted and ground grains of the coffee cherry. Most of the coffee drink consumed in the world is produced by the species *Coffea arabica* (Arabica) and *Coffea canephora* (Robusta) (Esquivel & Jiménez 2012), species that compete in the international markets of the coffee industry. In this study, the Arabica variety was studied.

Coffee production is an important economic activity in Mexico, where the associated waste needs to be treated properly (Bonilla-Hermosa *et al.* 2014). Such coffee production has environmental repercussions in the areas where coffee is processed. It is documented that during the production of coffee different organic wastes are generated, such as husks, coffee pulp and fermentation waters (wastewater), among others, which generate direct and indirect alterations in the environment (Alfaro & Rodriguez 1994). In the particular case of coffee pulp, it is the first waste generated during coffee processing, and represents approximately 29% of the total fruit (cherry) in dry weight (Braham & Bressani 1979), so in each production cycle it generates

considerable volumes of pulp that must be properly processed for disposal. The use of coffee pulp has been documented as raw material in different processes for energy purposes, such as the production of bioethanol (0.53 MJ/kg of fresh pulp), solid fuels (15.88 MJ/kg of dry pulp) and biogas (0.54 MJ/kg of fresh pulp) (Rodríguez & Zambrano 2010). In addition to giving a wastewater treatment, the present work aims to provide tools for energy production where hydrogen production is explored as an initial alternative to a larger process.

COFFEE PULP

The production of coffee (11,723,240 tons per year in Mexico) would allow a supply of the substrate with high availability, low acquisition costs (agro-industrial waste) and its use does not directly compete with the human or animal food supply (Bonilla-Hermosa *et al.* 2014), which would keep food security intact in coffee producing regions. Regarding energy security, since the coffee pulp is an

agro-industrial waste, the production of biohydrogen does not depend on an external raw material that can be affected by a supply or distribution chain, which would maintain an energy independence in the region, and consequently there would be a diversification of the energy mix in electricity generation.

The coffee seed is obtained by extracting it from the coffee cherry, which makes its processing complex and generates a wide variety of wastes (Heeger *et al.* 2017). In the specific case of coffee pulp, this is the first by-product that is obtained in the processing of coffee fruit and represents, in wet form, approximately 43.58% of the weight of the fresh fruit (Rodríguez & Zambrano 2010).

There are two methods for obtaining coffee seed; the first is a dry method that is usually used with the Robusta variety and the second process is the wet method, which is associated with the Arabica variety (Vincent 1987).

Biohydrogen could be used as a substitute for fossil fuels in power generation due to its high calorific value, 120 MJ/kg (Buitrón & Carvajal 2009), so its use would increase the energy efficiency of processes of electricity generation by decreasing the amount of fuel used, since in comparison with other fuels such as gasoline or natural gas, hydrogen has a greater energy potential (2.48 and 2.73 times greater than gasoline and natural gas respectively).

In the wet method, coffee pulp is the first by-product that is obtained and this contains several organic compounds such as cellulose (63%), lignin (17%), proteins (11.5%), hemicellulose (2.3%), tannins (1.80 to 8.56%), pectic substances (6.5%), reducing sugars (12.4%), caffeine (1.3%), chlorogenic acid (2.6%) and caffeic acid (1.6%) (Corro *et al.* 2013). The pulp also contains phenolic compounds, known as potent inhibitors of anaerobic fermentation, because they cause a loss in the integrity of the biological membrane of microorganisms, especially low molecular weight compounds (Menezes *et al.* 2013).

Coffee pulp is processed in the creation of fertilizers, animal feed, and compost; however, these applications only use a fraction of the pulp generated and are generally technically inefficient processes (Menezes *et al.* 2013). Therefore, it is necessary to valorize these wastes through other techniques or processes, which can complement the wastewater treatment systems through energy production from the high organic load they contain, as this research suggests.

The main objective of this study is to determine the yield of hydrogen production from coffee pulp (Arabica variety) by dark fermentation in a batch reactor. The scope covers the physicochemical characterization

of the sample from a growing region of Coatepec, in the state of Veracruz, México, quantification of hydrogen production in a batch reactor, and calculation of yield and energy density per kilogram of fresh coffee pulp.

Because of the composition of the coffee pulp and its origin as organic waste (agricultural by-product), the coffee pulp has been used as supplement in animal feed, to produce compost, and as a substrate for biofuels like biogas and bioethanol (Rodríguez & Zambrano 2010). The outlook of production for Mexico and Central America in 2017 is 21,147 thousand coffee bags, which represent a 4.4% increase in production from 2016 to 2017 (Organización Internacional del Café 2017). The coffee berry has many constituents that must be removed to obtain the seed that will be dried and ground to be pack and sold as the primary product. One of the first waste products generated during the processing of the berry, when the wet method is used, is the coffee pulp which could represent, in dry weight, about 29% of the weight of the whole berry (Braham & Bressani 1979). Because the coffee pulp is an organic waste with high humidity, microorganisms grow quickly causing the degradation of the coffee pulp and environmental pollution (Roussos *et al.* 1995). Dark fermentation could be used as a biological process to generate hydrogen and treat the organic waste produced at each coffee production cycle. Nowadays, the biggest hydrogen production comes from methane reformation, which has high environmental repercussions (Cardoso *et al.* 2014). This is why there is a great interest in a biological process to produce hydrogen in a more sustainable way.

METHODS

It is important to note that the methodology implies the required process will provide an alternative to water treatment with an energy use, where biohydrogen will be considered as an alternative under a scheme of minimum unit operations and use of inoculum to maximize the benefit at a lower cost.

The experiment was divided into two phases, the first one consisting of the pretreatment and physicochemical characterization of the samples. In the second phase, the coffee pulp was fermented in a discontinuous reactor with a heat treated inoculum from an anaerobic vinasse treatment plant in order to quantify the amount of gas produced.

Pretreatment and physicochemical characterization

Coffee pulp was pretreated to homogenize the sample by a mechanical grinding and sieving with a standard test sieve number 5 (4 mm/0.157 in). The aqueous sample was collected and characterized by the methods and norms expressed in Table 1.

In the specific case of mechanical treatments, it has been reported that the reduction in particle diameter increases the performance of biogas production processes by 20%, when particle diameters are reduced to 10 mm (Hajji & Rhachi 2013), so that methods such as maceration, grinding, and chipping are usually used in reducing the particle size and creating a larger contact surface (Wang & Yin 2017), facilitating the interactions of the substrate with the inoculum.

Inoculum

The inoculum (HPB) can be obtained from almost any anaerobic environment (Ginkel Sung & Lay 2001), such as sludge from anaerobic digesters. However, it is necessary to perform a mud treatment to reduce the presence of hydrogen consumers (methanogenic bacteria). A species usually used for the production of H₂ is *Clostridium* sp., which are anaerobic spore-forming organisms capable of converting hexoses to hydrogen with a yield of 2 mol of hydrogen per mole of hexose, a yield that is usually higher than other species such as the *Enterobacter* sp., which have a yield of 1 mole of hydrogen per mole of hexose (Patra *et al.* 2008).

There are pretreatments to the inoculum for the elimination of H₂-consuming bacteria, such as thermal, chemical (acid/basic) and ultrasonic treatments (Venkata Mohan

2009). Pretreatments and the use of mixed cultures of HPB allow a higher yield in hydrogen production to be obtained; Table 2 summarizes the accumulated production (mmol/day) and volumetric flow of hydrogen (mmol H₂/m³ day) for HPB with and without pretreatment.

The pretreatment applied to the consortium reported in Table 2 was a combined treatment with a thermal and chemical treatment. The difference in cumulative hydrogen production is significant, 24.5 times greater with pretreatment compared to the use of substrate without pretreatment.

On the other hand, pH is one of the most significant process variables; different values and optimal pH ranges have been reported in hydrogen production because there is also a dependence on the type of substrate used in digestion. According to Fang and Liu, an optimum pH of 9 is documented for batch reactor fermentation, pH varies from 4 to 4.5 for fermentation in continuous reactors, and ranges from 4.7 to 5.7 for starch fermentation in continuous reactors (Fang & Liu 2002).

The increase in pH increases the presence of methane in the biogas, so for the production of hydrogen it is necessary to maintain acid levels to increase the concentration of H₂ in the gas mixture during fermentation, since at these acidic conditions there is suppression of methanogenic activities (Fang & Liu 2002).

Preparation of the first fermentation inoculum

Because the inoculum must be free of methanogenic microorganisms that can decrease the concentration of hydrogen, a thermal and physical pretreatment was performed for its use. Anaerobic sludge was treated based on the methodology set forth by Moreno *et al.* (2015); Figure 1 summarizes the pretreatment. Different inoculum conditions were tested (wet, dry, and thermal treatment). To obtain the wet and dry inoculums it was necessary to take aliquots of the continuous stirred tank reactor (CSTR), which were centrifuged for a time of 8 min at 10,000 RPM. In the case of the dry inoculum, moisture was removed from the centrifuged inoculum for 12 hours at a temperature of 45 °C, the inoculum with heat treatment was obtained based on the process specified in Figure 1.

Table 1 | Methods and norms for physicochemical characterization of coffee pulp

Parameter	Units	Method/Norm
Total solids (TS)	[mg/L]	NOM-AA-034-SCFI-2015
Total volatile solids (TVS)	[mg/L]	NOM-AA-034-SCFI-2015
Chemical oxygen demand	[mg O ₂ /L]	HACH 8000
Total nitrogen	[mg N/L]	HACH TNT 828
Total sugar	[mg dextrose/L]	Dubois <i>et al.</i> (1956)
Total carbon	[mg C/L]	5310 B (Shimadzu Corporation)
Total phenols	[mg gallic acid/L]	Folin Ciocalteu

Table 2 | Hydrogen production in trials with and without pretreatment

Trials HPB	H ₂ (mmol/day)	H ₂ (mmol H ₂ /m ³ day)
Without pretreatment	0.97	0.24
With pretreatment	23.81	5.95

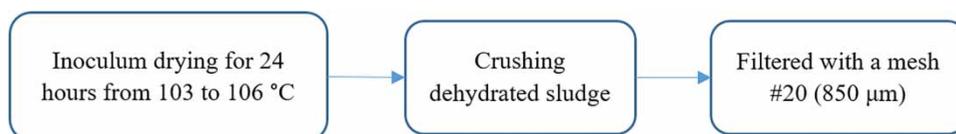


Figure 1 | Inoculum pretreatment.

The substrate/inoculum ratio should be maintained at 2.7 g/g TVS as suggested by the H₂ Specific Production Protocol (Carrillo-Reyes *et al.* 2017).

AMPTS tests

With the use of the Automatic Methane Potential Test System (AMPTS), three reactors with an operating volume of 360 mL were used, a substrate concentration of 5.94 ± 4.0 g COD/L and the solution of minerals and nutrients described in Table 3 with an operating temperature of 37 °C and an initial pH of 7. In these tests, the effect of the inoculum was evaluated in the lag phase by the use of sludge collected from the Applikon reactor; this sludge was use as the inoculum, since it was already adapted to the coffee pulp after the dark fermentation.

Dark fermentation

The aqueous sample was fermented in an Applikon batch reactor (3 liters) with temperature, pH and mixing control. The operating conditions were 250 RPM for mixing, a temperature of 35.5 °C and pH of 5.5, the operating level of the reactor was 2 liters where 140 grams of coffee pulp were dissolved with distilled water to obtain a COD concentration of 5.94 ± 0.4 (g COD/L). Regarding the inoculum, 27.40 g of inoculum (84.14 ± 1.95% TVS) was added to the reactor.

The inoculum used was a sludge from an anaerobic tequila vinasses treatment plant, so it was thermally treated by heating it for 24 hours at 105 ± 5 °C to avoid the presence of methanogenic bacteria in the reactor (Moreno *et al.* 2015). A solution of minerals and buffer 'MES' (2-(N-morpholino)

ethane sulfuric acid) were added to the reactor, their concentrations are showed in Table 3.

The reactor was sealed and bubbled for a time of one minute with nitrogen to ensure anaerobic conditions and purge as much oxygen as possible. The kinetic of hydrogen production was estimated by the modified Gompertz model expressed in terms of R_{max}, H_{max}, lag phase (λ) and time (h); the mathematical expression of the model is shown in Equation (1).

$$H = H_{max} \left\{ -exp \left[\frac{R_{max} \cdot e}{H_{max}} (\lambda - t) + 1 \right] \right\} \quad (1)$$

where:

H_{max}: is the maximum hydrogen production (NmL)

R_{max}: is the hydrogen rate of production (NmL/h)

e: is the Euler number (2.7183)

λ: is the lag phase (h)

t: is the fermentation time.

RESULTS AND DISCUSSION

Pretreatment and physicochemical characterization

After grinding and sieving, the density of the coffee pulp was calculated at 1.04 (kg/L); the results from the physicochemical characterization can be seen in Table 4.

Table 3 | Mineral and buffer concentration in reactor

Compound	Concentration	Unit
NH ₄ Cl	41.60	[g/L]
MES	19.52	[g/L]
MgCl ₂ ·6H ₂ O	2.00	[g/L]
FeSO ₄ ·7H ₂ O	1.60	[g/L]
MnCl ₂ ·4H ₂ O	0.04	[g/L]
KI	0.04	[g/L]

Table 4 | Physicochemical characterization of coffee pulp

Parameter	Value	Units
Total solids (TS)	66,533 ± 809	[mg/L]
Total volatile solids (TVS)	58,100 ± 939	[mg/L]
Chemical oxygen demand (COD)	88,333 ± 4,401	[mg O ₂ /L]
Total nitrogen	1,205 ± 133	[mg N/L]
Total sugar	19,688 ± 793	[mg dextrose/L]
Total carbon	40,800	[mg C/L]
Total phenols	0.305 ± 0.05	[mg gallic acid/L]
pH	4.16 ± 0.05	–

The TS concentration is above the range reported for hydrogen production, which is 1.3–50 [g/L] (Ramos *et al.* 2012), so the inoculum-substrate interaction may be affected, reducing the H₂ production with an impact on the increase of the lag phase during the production of hydrogen by hindering the interrelation between the inoculum and the substrate. This fact may be a limitation in the use of these wastes due to the energy consumption required for particle size reduction. The COD has a low concentration in contrast with the fermentation waters from the Arabica coffee variety from the wet process (77,875.00 ± 5.31 [mg O₂/L]) (Lepe 2017). The carbon to nitrogen ratio was estimated as 30.85:1, which is below the limit reported of 35:1 (Varnero 2011), where a slow growing bacteria may present due to the lack of nitrogen, which could affect the reproduction of the inoculum.

The content of total volatile solids (TVS) can be attributed to the content of organic matter present in the sample since, during the incineration, the residual products (ashes) represent an inorganic or mineral phase that cannot be volatilized; in the case of the coffee pulp, 92.92% of the sample was volatilized, so a high biodegradability can be inferred. Another indication of the amount of organic compounds present in the sample is directly reflected in the total organic carbon content, which represents for the coffee pulp 98% of the total carbon in the sample, which can be interpreted as a higher content of organic compounds susceptible to degradation by biological means, due to the organic nature of carbon.

The COD and BOD₅ can be directly evaluated using the biodegradability index; this gives us a quantitative approximation of the biological degradability of the sample and, in the case of coffee pulp, it was estimated at 0.91, greater than 0.4, which is the minimum value for biodegradability according to Ahn *et al.* (Ahn *et al.* 1999).

The total phenols content was estimated at 0.305 [mg of gallic acid/L] (0.305 ppm), which indicates that the concentration of phenols for the sample is relatively low, favoring the metabolic activities of microorganisms in hydrogen production, since it has been reported that there is a reduction in the production of H₂ in the presence of phenolic compounds with a concentration of 5 g/L (Sharma & Melkania 2017), with the concentration of total phenols being less than the concentration reported by Sharma and Melkania.

The sugar content was estimated at 0.021–0.022 kg dextrose/kg of dry sample, based on calculation of 1 kg of dry sample and a concentration of 19.688.33 ± 793.29 [mg/L] determined in the physicochemical characterization, representing a percentage. From 2.05 to 2.23% of the total

sugar content, this percentage could impact on the hydrogen production yield by decreasing H₂ productivity compared to other substrates, although a percentage of total sugars of 9.7% has been reported for the coffee pulp (Bonilla-Hermosa *et al.* 2014), so the difference in sugar content reported in the literature and the sample may be due to the origin of the pulp (geographical conditions, type of crop, type of soil, use of agrochemicals in its cultivation, etc.).

Regarding the C:N ratio, it has been reported that microorganisms generally use C:N ratios of 25:1 to 30:1 (Ward *et al.* 2008); in the case of coffee pulp, the C:N ratio is 30.85:1. Although it is above that reported by Ward and collaborators, it does not exceed the superior value of 35:1 where the decomposition of matter with high content of carbon occurs more slowly, because the multiplication and development of bacteria is low as a result of the lack of nitrogen (Varnero 2011).

A minimum hydrogen production rate in the presence of vanillin and syringaldehyde of 5 [g/L] has been reported due to the toxicity of those compounds (Sharma & Melkania 2017). In the case of coffee pulp, the amount of total phenols was estimated at 0.305 ± 0.05 [mg of gallic acid/L], so intoxication of bacteria by the phenols concentration may be rejected. In the case of the total sugars concentration, the quantification in terms of dextrose was estimated for the coffee pulp in a 20.5–2.23% dry base.

Reaction systems and AMPTS tests

The fermentation maintained a behavior similar to that described by the Gompertz model, although the lag phase (λ) was prolonged (92.70 hours or 3.86 days); this can be attributed mainly to the fact that the inoculum used had to adapt not only to the operating conditions (36 °C and pH 5.5), but also to the substrate, since as was mentioned the inoculum was obtained from an anaerobic treatment plant of tequila vinasses, so the adaptation time was expected to be prolonged. Additionally, the substrate/inoculum ratio (S/I) was lower than that recommended by the Latin American Biohydrogen Network, who suggest an S/I of 2.7 g substrate/g TVS inoculum (where the weight in grams of the substrate refers to the grams of anhydrous glucose), (Carrillo-Reyes *et al.* 2017), which could have caused a slow stage of adaptation to the inoculum.

However, the S/I ratio was not that recommended in the reaction system and during the AMPTS tests, hydrogen concentrations suggested that the inoculum had the necessary conditions (sugars) to generate biomass (increase in the

populations of HPB), which allowed continuation of the consumption of the substrate for the generation of hydrogen, which was represented in the hydrogen content, which remained above 85% in volume in the tests carried out in the AMPTS. Also during the operation of the AMPTS, it was observed that the lag phase was reduced to 24 hours, which could be attributed to the use of the sludge generated in the Applikon reactor as inoculum in the AMPTS tests, since conditions of operations, mineral solutions and substrate were the same, the adapted inoculum from the dark fermentation may be the cause of the lag phase reduction. Hydrogen gas was measured during these test at 93.88 ± 13.55 NmL.

Dark fermentation

Hydrogen gas produced by the dark fermentation was graphed in terms of NmL and time (hours) to determine the parameters of the modified Gompertz model. The hydrogen production behavior can be seen in Figure 2.

H_{\max} and λ were determined directly by Figure 2, R_{\max} was estimated by a nonlinear solving method and a solver app from Excel; the result is shown in Table 5.

With the parameters of Table 5, the modified Gompertz model for hydrogen production was graphed along with the experimental data and the R^2 coefficient was determined for the adjustment of the model to the experimental data, which was calculated at 0.9955. The Gompertz model and experimental data are shown in Figure 3.

Table 6 shows the yield and Gompertz parameters for the dark fermentation of coffee pulp and tequila vinasses (with and without treatment for the removal of phenol compounds) (Rodríguez 2017).

Yield for the coffee pulp is comparable to the results for tequila vinasses in dark fermentation (4.6–6.4 mL H_2 /g DQO-h). In the case of the rate of hydrogen production, the results were similar to the tequila vinasses. The lag phase was the only Gompertz parameters with different results in comparison with the tequila vinasses; it could be attributed to the acid pH (5.5) where dark fermentation was done and to the fact that there was no adaptation time before the use of the inoculum to the coffee pulp.

The gas produced at the reactor was analyzed using a gas chromatographer (PerkinElmer Clarus 580); no methane was identified, only hydrogen and a fraction of carbon dioxide, which are expected as the main products of dark fermentation processes. Regarding the power density for hydrogen production with the use of coffee pulp as substrate, a value of 0.045 MJ/kg of fresh coffee pulp was estimated.

The COD content for vinasses is reported in a range of 18–45 [g COD/L]; in the case of coffee pulp its COD content was estimated at 80.4 ± 8.8 [g COD/L], the COD content being higher in the coffee pulp, with these concentrations it could be attributed that the higher COD content in the substrate, the greater the production of hydrogen; however, in the case of the coffee pulp the final COD concentration for the coffee pulp was estimated at 76.3 ± 2.1 [g COD/L], that is, the system was able to obtain a 13.58% reduction

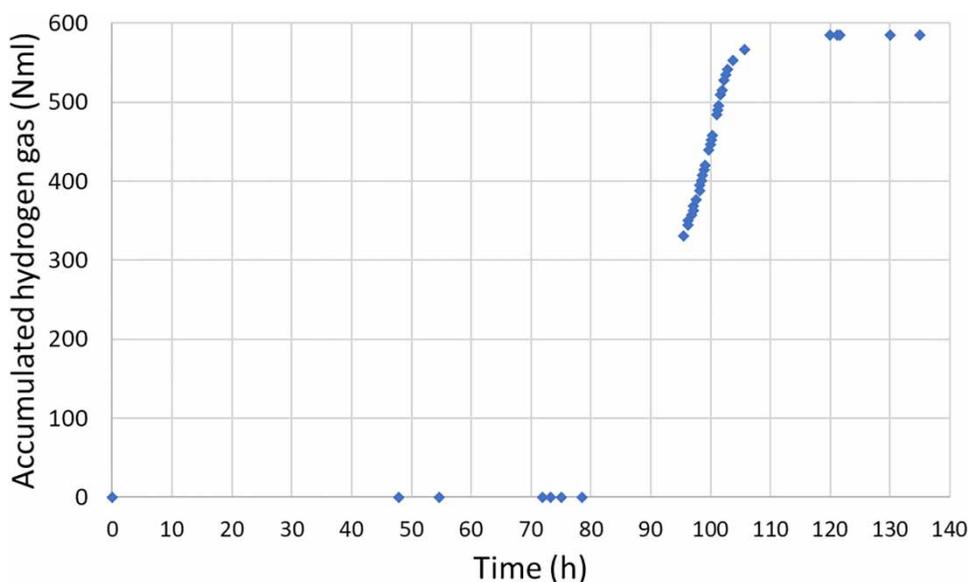


Figure 2 | Accumulated hydrogen gas NmL.

Table 5 | Modified Gompertz parameters for coffee pulp at dark fermentation

H_{\max}	585.00	NmL
R_{\max}	48.92	NmL/h
λ	92.70	h

in terms of the initial COD. As dark fermentation progresses, the higher the concentration of inhibitor compounds caused by the metabolism of fermenting bacteria such as volatile fatty acids (Intanoo & Chavadej 2012). It should be remembered that at this stage of the fermentation the molecules of great molecular weight are partially oxidized, so a high percentage of removal is not expected.

In terms of hydrogen production, the coffee pulp obtained a production of 49.2 mL H₂/g COD, which compared to tequila vinasses, the production derived from the use of coffee pulp. This is within the production range generated by tequila vinasses (26.6–92 mL H₂/g COD). Regarding the yields of hydrogen production per kg of substrate used in fermentation, Table 7 summarizes the yields reported by different authors and in this work, for agroindustrial substrates.

The performance for the coffee pulp was the lowest according to the data reported in Table 7, which can be attributed to the high content of hemicellulose, lignin and other complex organic compounds that hinder the hydrolysis of coffee pulp; however, the availability of the coffee pulp makes said substrate technically viable since an estimated 2,958,978 tonnes per year in Mexico of coffee pulp are

generated in each coffee production cycle, according to data from the SIAP (2018).

In energy terms, the coffee pulp has been used in the production of solid, liquid and gaseous biofuels (Rodríguez & Zambrano 2010), its energy yields per unit mass are reported in Table 8, together with the energy density obtained in this work, which was determined based on the energy density of H₂ (120.9 kJ/g) and the yield obtained in hydrogen production in the CSTR (4.18 NmL H₂/g C.P.).

The energy density of the biohydrogen produced through dark fermentation, is lower than the density of biogas (mainly methane), reported in the literature, however the biohydrogen can be used as a biofuel in processes of electricity generation or as a fuel in transport systems.

CONCLUSIONS

Coffee pulp is a substrate with a high content of total solids and chemical oxygen demand, nevertheless, it could be degraded by dark fermentation to obtain a yield at hydrogen production comparable with substrates as complex as tequila vinasses. During this fermentation process, a long lag phase was observed which could be attributed to acid pH at the reactor, and due to the fact that no adaptation of the inoculum to the coffee pulp was done. Although a 13.58% of COD reduction was obtained by the dark fermentation process, so it could be used as a pretreatment step in a biogas production system reducing the impact of COD

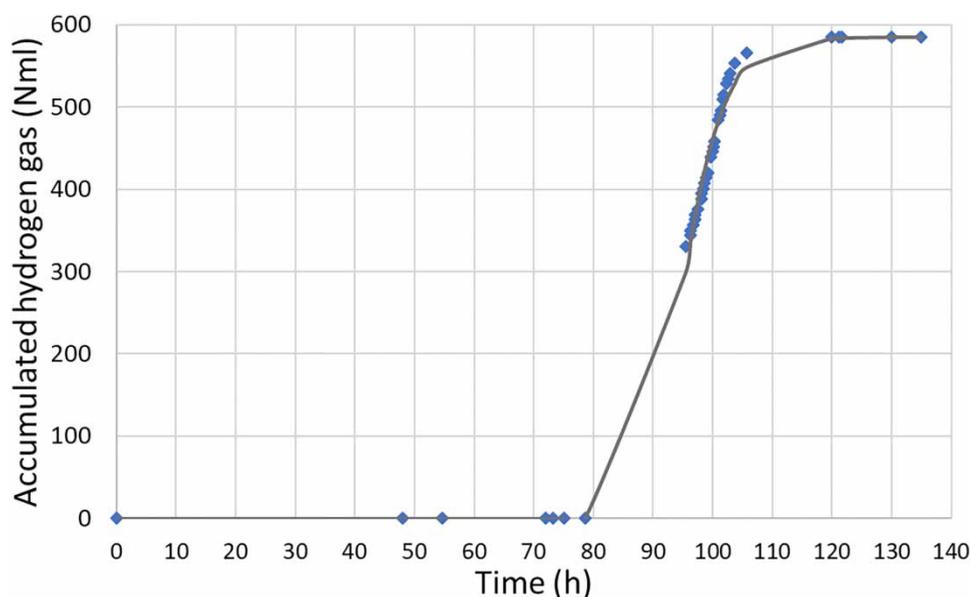
**Figure 3** | Modified Gompertz model for hydrogen production experimental data and estimated model.

Table 6 | Yield and Gompertz parameters for hydrogen production from coffee pulp and tequila vinasses

	Yield	R_{max}	λ
Substrate	mL H ₂ /g DQO	mL H ₂ /g DQO-h	h
VAC	26.6	4.6	28
VASC	41	6.4	28
C.P.	49.2	4.1	92.7
VAC/R	63.6	4.7	9
VASC/R	92	5.7	8

VAC: agave vinasse with cooking, VASC: agave vinasse without cooking, C.P.: coffee pulp, R: resin treatment for phenol removal.

Table 7 | Yields of hydrogen production via A.D. with different substrates

Substrate	Yield L H ₂ /kg substrate	Reference
Coffee pulp	4.18	This work
Sweet sorghum	10.4	Antonopoulou <i>et al.</i> (2008)
Starch	106	Kapdan & Kargi (2006)
Wheat straw	44.17	Ntaikou <i>et al.</i> (2010)

Table 8 | Biofuel energy density produced with coffee pulp

		Units	Coffee pulp
Bioethanol	0.53	[MJ/kg]	Fresh
Solid	15.88	[MJ/kg]	Dry
Biogas	0.54	[MJ/kg]	Fresh
Biohydrogen	0.045	[MJ/kg]	Fresh

contact to the environment. There by, two economical and energetic significant products will be generated with the same substrate.

The yield for hydrogen production per gram of initial COD obtained for tests with coffee pulp is consistent with what has been reported in the production of H₂ through the use of tequila vinasses; however, the yield per kg of fresh pulp is low compared to other agroindustrial wastes. It is necessary to optimize the production of hydrogen through an alkaline hydrolysis that allows increasing the sugar content on the coffee pulp as well as decrease the solids content in the sample.

The adaptation of the inoculum to the substrate is of vital importance to reduce the conditioning times in the fermentation systems, this would imply an optimization of the resources used for the control of the temperature, agitation,

pH, etc., reducing the operating costs of hydrogen production systems.

The high concentrations of total solids present in the substrate can be a limiting factor for the production of hydrogen, especially in the lag phase, since it has been reported that high concentrations of TS can limit the mass transfer between the substrate and the microorganisms (Ramos *et al.* 2012), so it is suggested to carry out an additional treatment to the maceration to reduce the content of TS.

Perspectives

In order to give continuity to this research, the following points are raised as part of future research projects, which allow the addition of value to the guideline of this work:

Obtaining performance parameters and productivity indices for a continuous hydrogen production system, which maximizes the production speed of H₂, as well as increasing the volume and concentration of said gas, optimizing the operating parameters such as temperature and pH.

Continuous system scaling at pilot level.

Integration of an electric power generation system that allows hydrogen to be used as fuel continuously.

Evaluation of the integration of the hydrogen production system as part of a pretreatment in methane production processes.

ACKNOWLEDGEMENTS

This work was realized at the 'Investigation and Assistance Center for Technology and Design of the State of Jalisco A.C.' (CIATEJ), we thank CONACYT for the financing of the Project number 2015-01-1214 on National Problems 'Utilization of agroindustrial effluents for the continuous production of sustainable energy channels: a prospecting and optimization study' and to the Ibero-American Program of Science and Technology for Development (CYTED) and CONACYT for the financing of the Project number 918PTE0539 'Use of biomass residues in the production of transportation fuels'.

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First received 13 June 2019; accepted in revised form 6 December 2019. Available online 20 December 2019