Waste Management 139 (2022) 279–289

Contents lists available at ScienceDirect

Waste Management

journal homepage: www.elsevier.com/locate/wasman

Establishment of the upstream processing for renewable production of hydrogen using vermicomposting-tea and molasses as substrate

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ARTICLE INFO

Keywords:
Agro-industrial wastes
Biohydrogen
Operational conditions
Optimization
Vermicompost

ABSTRACT

This study aimed to establish the optimal operational conditions for hydrogen production using vermicomposting-tea and sugarcane molasses as substrate. The experiments were carried out by triplicate in 110 ml serological bottles, a Box-Behnken design of experiments was performed in anaerobic dark conditions. The maximal hydrogen production (HP), hydrogen production rate (HPR), and hydrogen yield (HY) attained were 1021.0 mlL^{-1}, 5.32 $mlL^{-1}h^{-1}$, and 60.3 mL_{H2}^{-1}/g_{TCC}, respectively. The statistical model showed that the optimal operational conditions for pH, molasses concentration, and temperature were 6.5; 30 % (v/v) and 25 °C. The bioreactor run showed 17.202 L of hydrogen, 0.58 Lh^{-1}, and 77.2 $ml_{H2}g_{TCC}^{-1}$ For HP, HPR, and HY. Chemometric analysis for the volatile fatty acids obtained at the fermentation showed that only two principal components are required to explain 90 % of the variance. The representative pathways for hydrogen production were acetic and butyric acids. This study established the operational conditions for the upstream processing amenable to pilot and industrial-scale operations. Our results add value to molasses within the circular economy for hydrogen production using a novel consortium from vermicompost.

1. Introduction

It is now globally accepted that the world is facing critical environmental issues that call for immediate actions. The utilization of hydrocarbons as the primary energy source has brought a very drastic climate change. Consequently, there is a worldwide effort to reduce greenhouse gases emission (Almer and Winkler, 2017). However, until the earth's inhabitants start using environmentally friendly energies, at best, the *status quo* could be maintained with the current environmental problems. Energy consumption in the non-OECD countries began to exceed OECD (Organization for economic cooperation and development) consumption and is projected to reach nearly two-thirds of the 793 quadrillions Btu (British thermal unit) global energy consumption in 2040 (Newell et al., 2018), contributing very likely to an aggravation of the environmental problems. Hydrogen as fuel or converted to electricity has been proposed as a promising alternative energy source to diminish the global environmental impact. Hydrogen might be generated from biomass, and there are two main ways to produce hydrogen:

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https://doi.org/10.1016/j.wasman.2021.12.027
Received 5 July 2021; Received in revised form 14 December 2021; Accepted 16 December 2021
Available online 5 January 2022

photosynthetic process or fermentative production. The latter can produce hydrogen continuously without light using different organic compounds as substrate. There is a significant trend to use dark fermentation of organic compounds to produce hydrogen (Yang and Wang, 2018). Mexico has a long tradition in sugarcane cropping, going back to its introduction by the Spaniards during the 16th century. This industry is one of the most important due to its economic and social importance. Sugar cane agroindustry generates up to 440 thousand direct jobs and directly benefits around 2.2 million people. For example, only in 2012, 5.1 million tons of sugar were produced, representing 4.7 % of the gross internal product (SAGARPA, 2014).

Furthermore, 1.5 Mt of molasses were produced, of which only 3.6 % was utilized to produce bioethanol (UNC, 2018); the rest is sold as a complement either for soils or cattle. Considering these figures, molasses need a new strategy to exploit their value in the cicular economy, such as a substrate for producing hydrogen. Currently, the high production and accumulation of organic wastes have surpassed the inherent capacity of nature to degrade these compounds, and there are limited surface areas to eliminate these compounds (Qasim and Chiang, 2017). For these reasons, the global scientific community seeks and develops technologies that should be economically viable, environmentally sustainable, and socially acceptable for humanity (Sinha et al., 2010). Modern technologies based on the use of certain types of earthworms combine all these advantages and benefits. This innovative technology effectively transforms organic waste bioconversion into environmentally friendly, practical, and valuable biological products for modern agrobiology and biological agriculture. In this scenario, vermicomposting has emerged as an excellent tool to degrade, eliminate, and recycle agro-industrial waste (Domínguez, 2018).

Vermicomposting is a technological process in which a population of earthworms processes organic-containing materials (usually organic waste) into a humus-like material known as vermicompost. In recent decades, vermicompost, produced due to earthworm activity (*Eisenia fetida*) in organic wastes, is considered biologically active and highly effective organic fertilizers. Although other species have been used to treat organic wastes (Ramesh et al., 2020), the use of epigeic earthworm *Eisenia fetida* in vermicomposting is well documented for industrial waste (Castillo et al., 2013). For instance, Karmegam et al. (2019) used vermicomposting to treat paper mill sludge with cow dung and mature green plants. The results showed that the total microbial population increased utilizing this combination, and the enzyme activity was increased at the beginning and then decreased, indicating vermicompost maturity. Another waste management using vermicomposting is the brewer's by-product, the brewer's spent grain (BSG). Saba et al. (2019) showed that BSG could be used either for animal feeding or exploited in biochemical processes such as vermicomposting to increase the physicochemical parameters at the end of the vermicompost recovery.

This earthworm has a diverse pool of digestive enzymes, a wide variety of mesophilic microorganisms. It is the favorite earthworm species for laboratory experiments due to its tolerance to environmental variables (e.g., pH, moisture content, temperature). Furthermore, *E. fetida* is small in size and has a uniformly pigmented body, and is characterized by a short life cycle and a high reproductive rate. This earthworm is an efficient biodegrader, nutrient releaser, and efficient compost producer. Therefore, it aids in litter comminution and earlier decomposition (Saba et al., 2019). In addition, it contains nutritious macro-and microelements in forms available for plants, phytohormones, and humic substances acting as plant growth (Edwards and Arancon, 2004; Sherman, 2010). There is a way to produce an enriched solution comprised of vermicompost plus vermicompost leachate or water called vermicompost-tea. This solution is made either under anaerobic or aerobic conditions to increase the biomass present in the solution; typically, the fermentation process uses some agro-industrial waste as substrate, *i.e.*, sugar cane molasses (Gómez-Brandon et al., 2014).

Although there is complete literature on vermicomposting and the management of waste, only two works in the scientific literature use a consortium from vermicomposting to produce hydrogen (Oceguera-Contreras et al., 2019; Pascualone et al., 2019). Whereas these two works analyzed only one factor, in this work, the combination of three variables was analyzed, and the operational conditions by mean a design of experiments (DoE) were established, taking into consideration the results published elsewhere (Oceguera-Contreras et al., 2019). To our knowledge, this is the first work to develop the operational conditions amenable for a further scaling-up.

Silva Benavides et al. (2019) demonstrated fertilizer utilization as the substrate to decrease the operational costs, diminishing the investment during the scale-up processes. Vermicompost is an enriched organic fertilizer that contains essential molecules to aid the growth and maintenance of microorganisms. Moreover, the earthworm leachate is cost-effective; the cost per gallon is around 2.00 USD, whereas enriched vermicompost-tea is around 7.00 USD per gallon (Ranch, 2021). This study aimed to optimize the operational conditions (pH, temperature, and substrate) using molasses, a substrate, and a vermicomposting consortium to establish the upstream processing.

2. Material and methods

2.1. Feedstock acquisition

The Earthworm leachate (EL) and solid vermicompost (SV) were kindly provided by Humic Solutions SAS de CV, located in Jalisco, México. Ten liters of vermicompost leachate and 5 kg of solid vermicompost were stored until utilization at 4 and 25 °C for EL and SV, respectively. Before starting the fermentation, the EL was mixed with 20 % (w/v) of solid vermicompost and stirred until reaching a homogenous solution, also called vermicomposting tea (VT). Sugarcane molasses were provided by Beta San Miguel sugarcane industry located in Ameca, Jalisco, México.

2.2. Experimental design

In this study, a Box-Behnken design (BBD) and response surface methodology (RSM) were used, where the effect of three independent variables (pH of 5–8; a temperature of 25–45 °C and substrate concentration of 10–30 % v/v) with the output variables, hydrogen production (HP), hydrogen production rate (HPR), and hydrogen yield (HY) were investigated to determine the statistical points for the optimization of this bioprocess. The experiments were conducted in 120 ml serological bottles under anaerobic conditions with 100 ml of working volume using sugarcane molasses as substrate and VT as inoculum. Before the fermentations started, all the experiments were heat pretreated at 90 °C by 24 h to eliminate hydrogen consuming microorganisms and only keep spore-forming microorganisms. Experiments were established based on the BBD with three factors at three levels. Each independent variable was coded at three levels between −1, 0, and + 1.

The experimental design consists of 18 experiments with six center points (to allow the estimation of pure error), calculates the response function at intermediate levels, and enables assessment of the system performance at any experimental point within the studied range (Hamed and Sakr, 2001).

All the experiments were carried out in triplicate, and the average of HP, HPR, and HY obtained was taken as the response (Table 1). A non-linear regression method was used to fit the second-order polynomial (Eq. (1)) to the experimental data and identify the relevant model terms. Considering all the linear terms, square terms, and interaction items, the quadratic response model can be described as follow:

$$Y = \beta_0 + \sum_{i=1}^{k} \beta_i x_i + \sum_{i=1}^{k} \beta_{ii} x_i^2 + \sum_{i<j}^{k} \beta_{ij} x_i x_j \quad (1)$$

Where Y is the response, $\beta 0$ is the constant, βi is the linear coefficient, βii is a quadratic coefficient, βij is the interactive coefficient, xi is the

Table 1
Design of experiment and its respective output value.

Experiment	pH	Temperature (°C)	Substrate % (v/v)	HP[a]	Hpmax[b]	Experimental vs modeled (R^2)	HP[c]	HPR[d]	HPRmax[e]	Lag phase[f]	HPR[c]	HY[g]	HY[c]
1	6.5	45	10	529.0	500.0	0.99	0.0435	2.75521	20.0	5	0.60245	18.66	0.23150
2	6.5	25	30	1021.0	1014.1	0.99	0.0313	5.31771	11.5	18.9	0.43365	60.30	0.12878
3	6.5	35	20	169.0	472.9	0.99	0.0769	0.88021	9.3	3.1	1.06588	4.23	0.48622
4	5	35	10	390.3	394.1	0.99	0.0506	2.03299	3.6	28.9	0.70135	6.94	0.37959
5	6.5	35	20	171.3	255.3	0.99	0.0764	0.89236	9.2	18.4	1.05859	4.56	0.46829
6	5	25	20	140.3	105.2	0.99	0.0844	0.7309	5.8	12.6	1.16969	3.48	0.53606
7	5	35	30	592.0	192.3	0.99	0.0411	3.08333	10.2	65.7	0.56949	21.21	0.21713
8	8	45	20	614.0	608.8	0.99	0.0404	3.19792	12.3	3.9	0.55920	21.35	0.21642
9	6.5	35	20	178.0	214.7	0.99	0.0750	0.92708	10.1	18.4	1.03858	4.96	0.44901
10	8	25	20	552.7	958.4	0.96	0.0425	2.87847	13.1	20.5	0.58941	15.43	0.25458
11	6.5	35	20	184.0	168.1	0.92	0.0737	0.95833	9.8	10	1.02151	5.43	0.42914
12	6.5	35	20	185.0	192.8	0.99	0.0735	0.96354	9.3	10.9	1.01874	5.64	0.42108
13	6.5	45	30	249.7	168.3	0.97	0.0633	1.30035	1.4	13.6	0.87694	6.15	0.40324
14	8	35	30	200.0	195.8	0.99	0.0707	1.04167	4.4	60.3	0.97980	6.11	0.40456
15	6.5	35	20	173.7	526.2	0.99	0.0759	0.90451	9.8	77.1	1.05146	4.57	0.46778
16	8	35	10	478.3	607.3	0.99	0.0457	2.49132	11.8	79.4	0.63356	7.84	0.35714
17	6.5	25	10	554.0	409.5	0.92	0.0425	2.88542	11.9	1.5	0.58870	20.89	0.21879
18	5	45	20	188.0	183.4	0.97	0.0729	0.97917	2.2	21.5	1.01058	5.90	0.41169

***a*:** Hydrogen Production; the data represent the average of the triplicate in mLL^{-1}.
***b*:** The data show the modeled-maximum Hydrogen Production according to the modified Gompertz equation
***c*:** Inverse square root transformation.
***d*:** Hydrogen Production rate $\left(mLL^{-1}h^{-1}\right)$.
***e*:** The data represent the maximum Hydrogen Production Rate attained according to the Gompertz equation.
***f*:** The data represent the last of Lag pHase according to the Gompertz Equation.
***g*:** The data represent the Hydrogen Yield $\left(mLL_{H2}^{-1}/g_{TCC}\right)$; TCC = Total Consumed Carbohydrates.

coded factor level, and k is the independent parameters ($k = 3$ in this study); (Wang and Yin, 2017). Analysis of variance (ANOVA) was used to determine the quality of the fit of the quadratic model expressed by the coefficient of regression R^2, and the F-test checked its statistical significance. Furthermore, the dataset was modeled with the Gompertz modified equation previously described elsewhere (Wang and Wan, 2009).

2.3. Analytical methods

To quantify the hydrogen production, an inverted burette was used to measure the volumetric displacement with NaOH 1 N. A direct method to detect hydrogen was carried out in a gas chromatograph (Perkin Elmer Claurus 580, Perkin Elmer Life and Analytical Sciences, Shelton, CT, USA) equipped with a thermal conductivity detector. Moreover, solvents and volatile fatty acids (VFA) were measured at the end of the fermentation. Both temperatures of the injector and flame ionization detector (FID) were 250 °C. Nitrogen was used as carrier gas at a flow rate of 10 ml/min. The analyses were performed with a split ratio of 10:1, and the temperature program was 45 °C × 1.5 min, with a gradual increase until 200 °C. To analyze the total carbohydrates content through fermentation, total carbohydrates were measured according to the method described by Dubois et al. (1956). Multivariate analyses, *i.e.*, principal component analysis (PCA) and hierarchical cluster analysis (HCA), were performed to find the correlation among the by-products obtained at the end of the fermentation. The bioreactor production was carried out in a 3 L batch bioreactor (Biostat A; Sartorius) with 2.5 L of working volume during 45 h in anaerobic conditions at 150 rpm and pH-controlled. The experimental data were used to compare the kinetic behavior of the model by mean Gompertz modified equation.

3. Results and discussion

3.1. Influence of the operational conditions on hydrogen production (HP)

The first analysis for the dataset employed was the Box-Cox model, where the λ value was calculated. The lambda outcome for the three output variables HP, HPR, and HY was calculated where $\lambda = -0.5$, which implies that an inverse square root transformation must be performed; for this reason, the dataset was calculated and analyzed using the transformed data (Table 1).

3.1.1. Optimization of the hydrogen production (HP)

The minimal and maximal amounts of hydrogen attained were experiments 6 and 2 (140.3 and 1021.0 mlL^{-1}) **(**Table 1**).** Likewise, according to the Gompertz modeling, these experiments reached the minimum and maximum amount with 105.2 and 1014.1 mlL^{-1} **(**Table 1**).** The correlation between the experimental vs the modeled data was evaluated, where most of the data fit almost perfectly with an $R^2 = 0.99$. Only five experiments had a minimal R^2 **(**Table 1**).** Fig. 1 shows the response surface methodology (RSM) with its respective surface contour plot (SCP) for the output variable HP, where the behavior of HP can be observed when interacting with the variables temperature vs pH **(**Fig. 1**A)** and temperature vs substrate **(**Fig. 1**C).** The maximal amount of hydrogen attained was located at the model's edges, with an average of 567.0 and 395.2 mlL^{-1} With 25 and 45 °C; on the other hand, for the center points (35 °C), the average amount attained was 272.2 mlL^{-1}. The effects of pH and temperature **(**Fig. 1**A)** and pH and substrate **(**Fig. 1**B)** during the HP are shown in their respective SRM and SCP. For instance, the average amounts reached were 341.5 and 461.3 mlL^{-1} for pH 6.5 and 8, whereas the minimal point (pH of 5) attained the minimal amount with 327.7 mlL^{-1}. Concerning substrate, the maximal amounts achieved were at the edges of the model, *i.e.,* 487.9 and 515.7 mlL^{-1} with 10 and 30 % of molasses, and the average amount attained for the minimal amount was 255.6 mlL^{-1} using 20 % of molasses; the statistical analysis **(Table A.1)** shows that the model was not significant; however, the quadratic term for the variable substrate had a significant effect ($p = 0.02$).

Equation (2) shows the final equation in terms of coded factors to fit the experimental data of hydrogen production according to the transformed dataset, which was solved by mean Eq. (1)**:**

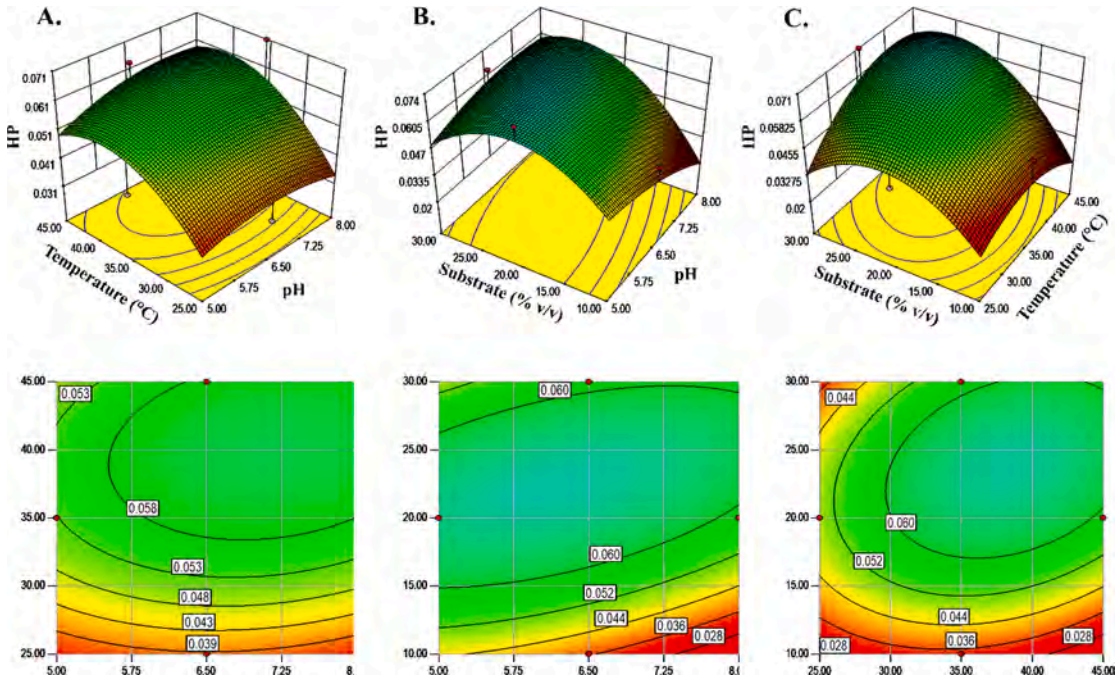

Fig. 1. Surface response plots for Hydrogen production (HP). The three variables are shown with their respective surface response **(Top)** and the surface contour plots **(Bottom). A)** Shows the variables Temperature vs pH, where the Substrate concentration was kept constant; **B)** Shows the variables substrate vs pH, where the temperature was kept constant; **C)** Shows substrate vs temperature, where the pH was kept constant. The units are in mlL^{-1} With the transformed data $\frac{1}{\sqrt{HP}}$. The substrate, temperature, and pH were kept constant to 30 gL^{-1} 45 °C, and 8.

$$\frac{1}{\sqrt{HP}} = 0.075 - 6.236E^{-3} \times A + 2.415E^{-3} \times B + 3.021E^{-3} \times C + 2.338E^{-3} \times A \times 8.646E^{-3} \times A \times C + 7.763E^{-3} \times B \times C - 4.148E^{-3} \times A^2 - 0.011 \times B^2 - 0.019 \times C^2 \quad (2)$$

Ghimire et al. (2015) claimed the importance of pH parameters during hydrogen production by dark fermentation, where they found the maximal hydrogen production ranges from 5.5 to 4. Comparing the results obtained in this work with the published reports indicates that a pH of 4.17 could support hydrogen production; however, below 4, several microorganisms could be in a spore-forming latent state due to the acidic microenvironment. Other authors also underlined the importance of pH during hydrogen production using different substrates. For example, Pason et al. (2020) studied hydrogen production from cassava pulp using different initial pH values from 5.0 to 8.0. They found the pH of 7 as the optimum condition of hydrogen production (230.12 ml). The hydrogenase activity is also affected by alkaline and acid conditions. The control of pH throughout the process is mandatory to promote hydrogen generation and avoid enzymatic inhibition. In another study of agro-industrial wastes using sugarcane bagasse and pineapple peeling, the effect of pH was studied from 6.5 to 7.5. As pH increased, the amount of hydrogen decreased. At pH 7, the revival of methanogens might have occurred, inhibiting the hydrogen production (Robledo-Narváez et al., 2013). Hu et al. (2020) studied the effect of initial pH on hydrogen production using potato residues as substrate. They also underline the necessity of controlling the pH for getting higher yields. The experiments were performed from 5.0 to 9.0, where they found the optimal hydrogen production at pH 7.0, and again, the higher was the pH, the lower production of hydrogen was attained. Mirza et al. (2019) studied the hydrogen production using sugarcane bagasse as substrate using initial pH ranging between 5.0 and 9.0; a pH of 7.0 was established as the optimal for the hydrogen production. The considerable variability might explain these differences among the studies where the temperature, substrate, inoculum type, and pre-treatment methods differed.

The higher loads of organic matter, the higher hydrogen production is attained. This condition is preferred for a better and efficient fermentation and aid in minimizing the energetic requirements to operate during the process. However, a minimum amount of substrate is essential to activate the germination and prevent the bacteria from re-sporulating spore-forming microorganisms (Kim et al., 2006; Van Ginkel and Logan, 2005). Increasing the substrate concentration within an optimum range typically increases the hydrogen production during the dark fermentation process. Nevertheless, maximal substrate concentration could be unfavorable for hydrogen production because the microorganisms producing hydrogen could be inhibited in different ways, including the negative accumulation of VFAs, minimal intracellular pH, and maximal hydrogen partial pressure (Ciranna et al., 2014). For this reason, the optimization of substrate concentration for this consortium is critical for avoiding the inhibition mediated by the substrate. For instance, using the wheat powder in a range of 5–35 g COD/L, the author claims 32 g as the optimal substrate concentration and 35 g being the inhibitory concentration (Argun et al., 2008).

The process of biohydrogen production primarily depends on the nutrient content of the substrates; the carbon source is essential, and certain trace elements are necessary for the microorganism growth and maintenance. For instance, Abdullah et al. (2019) studied the influence of the C/N ratio on hydrogen production from palm oil effluent. Hydrogen production increased by 28 % at C/N ratio of 140. C/N ratio of 190 decreased the hydrogen production. In the same study, the influence of ferric ions was also explored because this is a cofactor necessary for hydrogenase activity. Ferric ions concentration of 100 mgL^{-1} produced the highest yield of hydrogen.

Braga et al. (2019) investigated hydrogen production from sugarcane bagasse by using specific salt concentrations and vitamin solutions to the reactor. Higher hydrogen production was observed with 2.5 % salts and vitamin solutions. Saidi et al. (2018) studied the effect of the C/N ratio on hydrogen production from anaerobic co-digestion of fruits, vegetable wastes, and fish wastes. Higher hydrogen production was attained with a C/N ratio of 12. Yeast extract was also used as a nitrogen source; they

also provided vitamins, amino acids, minerals, peptides, and other growth compounds presented in the vermicomposting-tea previously described elsewhere (Oceguera-Contreras et al., 2019). For these reasons, it is critical to find the proper substrate concentration for this bioprocess; for instance, the maximum amount of substrate for reaching the maximal hydrogen production in this study was 30 % (v/v). Although several authors pretreat the inoculum only for 15 min at 100 °C (Baghchehsaraee et al., 2008), here, we wanted to stress-up the consortium (90 °C by 24 h) to analyze the capability of producing hydrogen with the minimum amount of microorganisms and thus, corroborate the high amounts of spore-forming microorganisms present in this consortium. Thermodynamically, high temperatures in thermophilic microorganisms help break down biological structures and increase the enzymatic activity; hence, the microorganisms can assimilate the fermentable carbohydrates. However, Alvarez-Guzmán et al. (2017) set up 26.3 °C as the optimal temperature operating with psychrophilic bacteria. After the statistical analysis of the dataset, the optimal temperature for this model was 25 °C. Regarding temperature, Domínguez (2018) showed that the optimal temperature for the growth of *Eisenia fetida* is from 20 to 25 °C, which implies a microbiome evolutionary adaptation for living in that range of temperature; however, in the same work, the author also underlines the wide range of temperature supported by this species. For instance, there are several reports on producing hydrogen from different manures at different temperatures. For example, Yokoyama et al. (2007) used cow slurry without pre-treatment of the substrate in a batch culture at 37 °C, where he reached 0.7 $ml_{H2}g_{VS}^{-1}$; Concetti et al. (2013) used Buffalo manure in a batch culture at 37° C where he reached 10.4 $ml_{H2}g_{VS}^{-1}$; Xing et al. (2010) used dairy manure in a batch culture at 36.7° C, where he reached 18.1 $ml_{H2}g_{VS}^{-1}$; Wang et al. (2013) used cow manure slurry at 60 °C in a batch culture reaching 10.25 mLg_{VS}^{-1}. According to Kamaraj et al. (2019), the most utilized manure with maximal hydrogen production was Buffalo manure due to the high hydrogen yields with this consortium using organic wastes. Until this point, we have realized that the consortium has at least two different sub-populations of hydrogen-producing microorganisms, with other operating conditions, as seen at the model's edges. For this reason, a phylogenetic analysis of the consortium to elucidate the population dynamic is necessary to isolate and characterize the best hydrogen-producing microorganisms as a single entity or in a selected combined mix, capable of degrading agro-industrial wastes and producing hydrogen with high yields.

3.1.2. Optimization of the hydrogen production rate (HPR)

The fermentations were followed during 192 h; for hydrogen production rate, the maximal and the minimal amounts attained were for experiments 6 and 2 with 0.73 and 5.32 $mlL^{-1}h^{-1}$.

For instance, the effect of pH on the adaptability of this consortium could be seen in Table 1**,** where the duration of the lag phase (average) for pH 5, 6.5, and 8 was 32.1, 17.7, and 41.0 h, respectively. This result can be explained that the production rate will be lower because the lag phase duration is longer, but occurred the opposite. There is no significance among the variables, as shown in the ANOVA (**table A.2**); however, preference for a slightly acidic environment instead of basics could be observed.

Regarding temperature, the extent of the lag phase was 13.4, 37.2, and 11 h, for 25, 35, and 45 °C, respectively; the fastest adaptation is on the model's edges. As aforementioned, we suppose at least two subpopulations with different operational conditions, working better at 25 and 45 °C, respectively.

On the other hand, the substrate is of utmost importance because there could be inhibition if the substrate increase substantially; the last of the lag phase was 28.7, 19.6, and 39.6 h, for 10, 20, and 30 % (v/v). It could be seen better adaptability for hydrogen production at the center of the model. However, a single variable could not determine the behavior of hydrogen production; for example, the lag phase of experiment 13 lasted 13.6 h at 45 °C and pH 6.5 using 30 % (v/v) of molasses. Another example is experiment 17 with the fastest lag phase (1.5 h) with 25 °C and 10 % (v/v) conditions. The importance of this kind of DoE lies in the combination of the variables plus how open you can set the edges of your model.

Fig. 2 shows the SRM with its respective surface contour plot for the output variable HPR. It can be seen the behavior of HPR when interacting with the variables temperature vs pH **(**Fig. 2**A)** and temperature vs substrate **(**Fig. 2**C)**. The maximal average amount attained was located at the edges of the model with 2.95 and 2.06 $mlL^{-1}h^{-1}$ For 25 and 45 °C; in this case, the center points attained the minimal amount with 1.42 $mlL^{-1}h^{-1}$. For pH, the maximal amount of hydrogen acquired was located at the edge (pH of 8) of the model with an average of 2.40 $mlL^{-1}h^{-1}$; in this case, the center points and the minimal point had the minimal average amount attained with 1.78 and 1.71 $mlL^{-1}h-1$ for pH of 6.5 and 5. The effect of pH and temperature **(**Fig. 2**A)** and pH and substrate **(**Fig. 2**B)** over the HPR is shown in its respective SRM and SCP. Concerning substrate, the maximal amounts attained were at the edges of the model, *i.e.,* 2.69 and 2.54 $mlL^{-1}h^{-1}$ with 10 and 30 % of molasses, and the average amount attained for the minimal value was 1.33 $mlL^{-1}h^{-1}$ using 20 % of molasses, the statistical analysis **(Table A.2)** shows that the model was insignificant; however, the quadratic term for the variable substrate had a significant effect ($p = 0.02$).

The amounts obtained for HPR were 2.5, 1.3, and 2.7 $mlL^{-1}h^{-1}$, for 10, 20 and 30 % (v/v) of substrate. According to the Gompertz modeling, the amounts obtained are far away from the experimental results; for example, for 10 %, the model shows an HPR of 11.8 $mlL^{-1}h^{-1}$, neither the experiments with 30 % reach the amount from the model. The reason why all the outcomes must be optimized is due to not only to obtain higher hydrogen production and yield but also to achieve that the enzymes adapt more quickly.

Equation **(3)** shows the final equation in terms of coded factors for hydrogen production rate according to the transformed data:

$$\frac{1}{\sqrt{HPR}} = 1.04 - 0.086 \times A + 0.033 \times B + 0.042 \times C + 0.032 \times A \times B + 0.12 \times A \times C + 0.11 \times B \times C - 0.057 \times A^2 - 0.15 \times B^2 - 0.26 \times C^2 \quad (3)$$

During the hydrogen production rate, time is crucial to have maximal values where the microorganism's adaptability is essential. The hydrogenases play a fundamental role during electron bifurcation (Buckel and Thauer, 2013). In many systems, these reactions of bifurcation are critical points for controlling and balancing the electron flow in the metabolism. A better understanding of these mechanisms could potentially provide the platform for managing biocombustible production in metabolic engineering (Peters et al., 2015).

Moreover, it is crucial to understand how microbial consortia's ecological features are related to the stability and yield and how they are affected by the pre-treatment and the culture conditions. Some of these environmental features include functional redundancy and its relationship with the diversity (Tracy et al., 2012) and the biotic interactions among the community (Proulx et al., 2005; Shade et al., 2012). The ecological attributes of any biological system are in function of the population dynamics, directly impacting the evolutionary processes that can address fluctuations during the bioreactor process (Goldman and Brown, 2009).

3.1.3. Optimization of hydrogen yield (HY)

Another critical output to consider during the fermentation is the yield. As previously mentioned in the results, an essential factor is a substrate considering that not all microorganisms can produce hydrogen with high yields using agro-industrial wastes. For instance, the results showed that the min and max amount attained was with experiments 6 and 2 with 3.48 and 60.3 mlL_{H2}^{-1}/g_{TCC} **(**Table 1**).** The effect of substrate

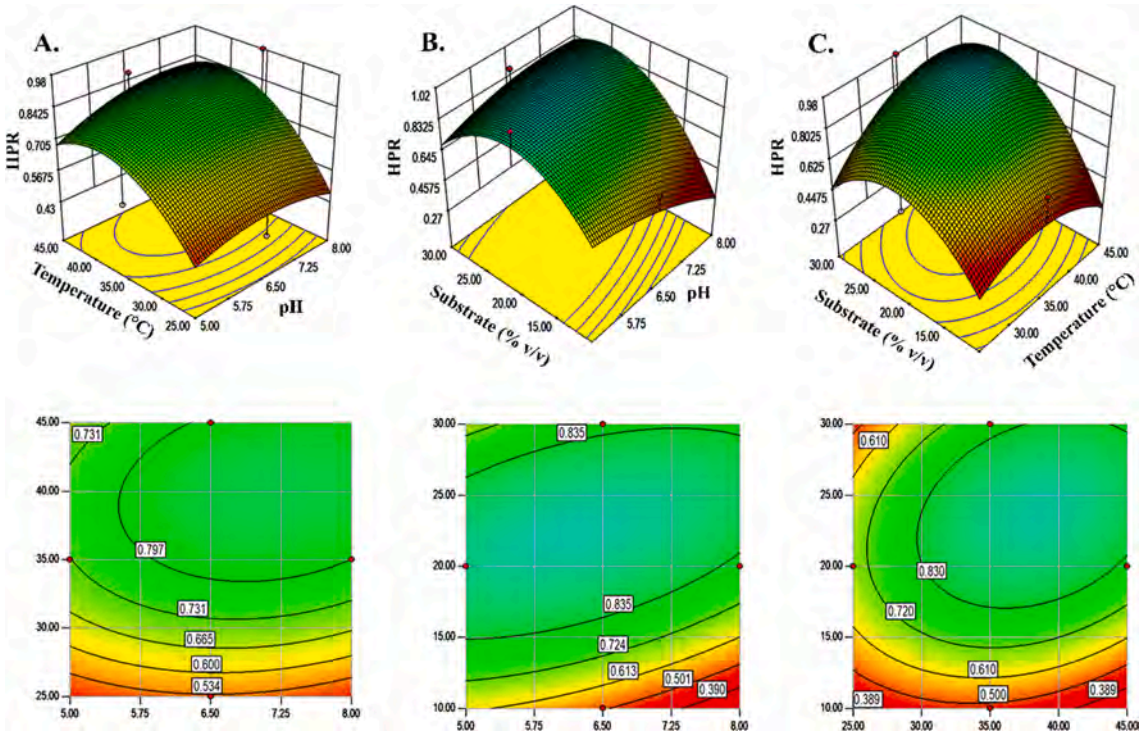

Fig. 2. Surface response plots for Hydrogen production rate (HPR). The three variables are shown with their respective surface response **(Top)** and the surface contour plots **(Bottom). A)** Shows the variables temperature vs pH, where the Substrate concentration was kept constant; **B)** shows the variables substrate vs pH, where the temperature was kept constant; **C)** shows substrate vs temperature, where the pH was kept constant. The units are $mlL^{-1}h^{-1}$ In the transformed data $\frac{1}{\sqrt{HPR}}$. The substrate, temperature, and pH were kept constant to 30 gL^{-1}, 45 °C, and 8.

and pH (Fig. 3**B)** and substrate and temperature (Fig. 3**C)** is shown on their respective SRM and SCP for the output variable pH. For this variable, the maximal amounts attained (average) were 11.2 and 13.3 mlL_{H2}^{-1}/g_{TCC} for 30 and 20 % of molasses where the minimal amount attained was with the model's minimal point 4.9 mlL_{H2}^{-1}/g_{TCC}. Secondly, the effect of temperature and pH (Fig. 3**A)** and temperature and substrate (Fig. 3**C)** is shown on their respective SRM and SCP; it can be seen at the model's edges. For instance, the average amounts of 16.2 and 25.6

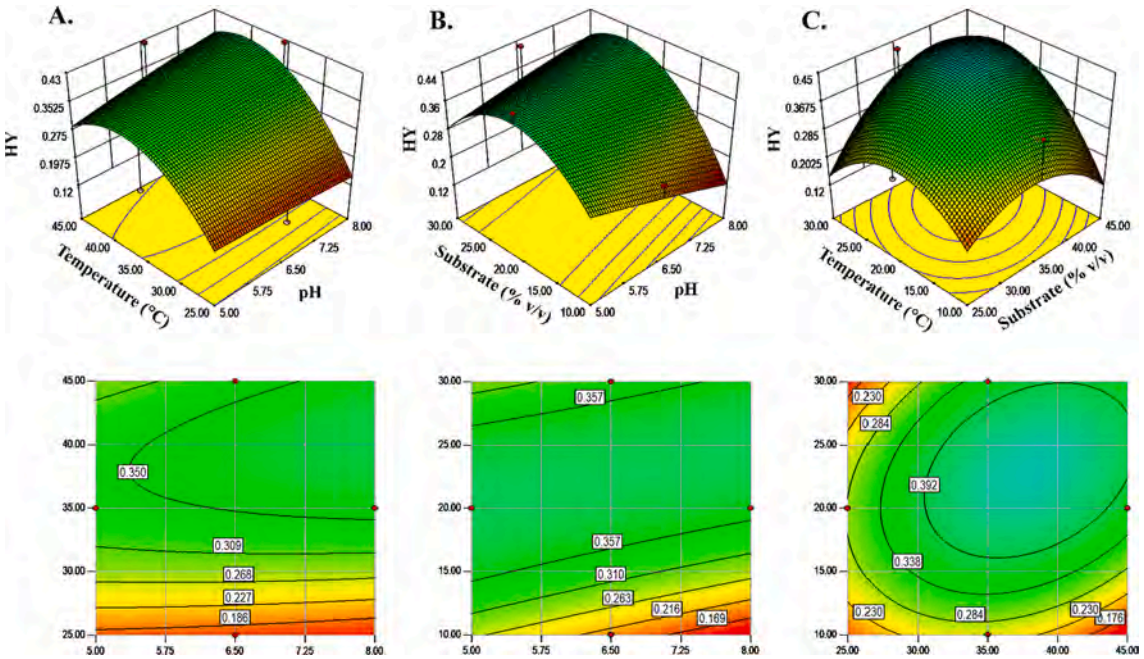

Fig. 3. Surface response plots for Hydrogen Yield (HY). The three variables are shown with their respective surface response **(Top)** and the surface contour plots **(Bottom). A)** Shows the variables Temperature vs pH, where the Substrate concentration was kept constant; **B)** shows the variables substrate vs pH, where the temperature was kept constant; **C)** shows substrate vs temperature, where the pH was kept constant. The units are $mlL_{H2}^{-1}G_{TCC}^{-1}$ In the transformed data $\frac{1}{\sqrt{HY}}$. The substrate, temperature, and pH were kept constant to 30 gL^{-1}, 45 °C, and 8.

were attained with 25 and 45 °C, and the center points reached the minimal amount with an average of 5.6 mlL_{H2}^{-1}/g_{TCC}. The last variable considered for this model was pH, where the effect of this variable with temperature (Fig. 3**A)** and Substrate (Fig. 3**B)** is shown in their respective SRM and SCP. For example, the maximal amounts attained were at the edges of the model pH 5 and 8 with 13.7 and 25.6 mlL_{H2}^{-1}/g_{TCC}, and the minimal amount attained was 5.6 mlL_{H2}^{-1}/g_{TCC} for the center points. Moreover, the variance analysis shows that only the substrate's square term had a significant effect ($p = 0.04$) **(Table A.3).**

Equation (4) shows the final equation in terms of coded factors for hydrogen production rate according to the transformed data:

$$\frac{1}{\sqrt{HY}} = +0.48 - 0.044 \times A + 0.014 \times B - 3.415E^{-3} \times C + 0.027 \times A \times B + 0.057 \times A \times +0.070 \times B \times C - 3.762E^{-3} \times A^2 - 0.10 \times B^2 - 0.12 \times C^2 \quad (4)$$

As mentioned earlier, in developing countries (e.g., México), molasses are used only to produce bioethanol in a low proportion. However, the circular economy of this by-product must not finish there; other strategies could be implemented. Álvarez-Cao et al. (2019) described a two-step system for the production of bioethanol from molasses. The high content of carbohydrates, such as sucrose, raffinose, glucose, and fructose, serves mainly as a substrate for industrial bioethanol production using the yeast *Saccharomyces cerevisiae* (Akbas and Stark, 2016). The use of food industry wastes as sustainable substrates for the microbiological synthesis of other biotechnological products besides ethanol, such as enzymes and other active ingredients, is an increasingly gaining field in the circular economy context (da Silva, 2016). From this point, molasses provides a good carbon source for Embden-Meyerhof Parnas pathways to produce hydrogen with high yields.

Furthermore, the use of vermicomposting is an attractive alternative not only as a consortium but also contains certain minerals that can act as a cofactor for the activity of the hydrogenase. For example, Kumar et al. (2019) described different metallic nanoparticles for enhancing hydrogen production. He underlined that either the size or the shape of the nanoparticle is crucial for hydrogen production. Moreover, he shows that the best nanoparticles for hydrogen production are iron and nickel, which is understandable because these two metals act physiologically as a cofactor for hydrogenases.

For decades it was assumed that only aerobic organisms were capable of oxidizing methane; however, there are several inorganic terminal acceptor electrons (TEAs), which in turn support anaerobic methane oxidation (AOM) by specialized microorganisms (Segarra et al., 2015). Recently, a non-organic material, also known as humic substances (HS), has been studied to demonstrate the AOM activity (Valenzuela et al., 2019). In this scenario, it is essential to start looking at biotic and abiotic compounds present in soils. Also, Valenzuela et al. (2019) showed that HS could oxidize methane and N_2O; both are important greenhouse gases (GHG). He also underlines the critical role of wetlands during the oxidation of these two compounds and suppresses the GHG until 200 Tg methane per year. Under this premise, the consortium could be excellent hydrogen-producing moisture full of microorganisms and other essential molecules such as HS capable of shuttling electrons and acting as a hydrogen source. Coupled with this, soil microorganisms' degradation capacity of recalcitrant compounds is surprising, considering that they can utilize and incorporate atmospheric gases into their metabolism. For example, it is well known that soil microorganisms can act either as a source or sink for trace gases (Conrad, 1996).

For instance, methane, nitrogen, and hydrogen cycles pass through soil microorganisms and are converted into macromolecules or decomposed and released into the atmosphere. Moreover, the oxidation of hydrogen in soils is carried out by abiotic hydrogenases, and anaerobic chemolithotrophic microorganisms (methanogens, homoacetogens, and sulfate reducers) scavenge hydrogen to incorporate it into their metabolism. On the other hand, nitrogen fixers release hydrogen into the atmosphere during the nitrogen cycle. From an evolutionary standpoint, this consortium has evolved to either oxidize or reduce hydrogen for many years, since the origin of earthworms during the lower Cretaceous (Domínguez et al., 2015) until now. According to Rhee et al. (2006), soil microorganism contributes significantly to incorporating hydrogen into the atmosphere during the biogeochemical cycles (88 ± 11 Teragrams per year). The hydrogen can have two paths, one for the soil and the other for the atmosphere. During the first path, the hydrogen-consuming microorganisms incorporate this element into their metabolism; if these microorganisms are eliminated, they can release hydrogen into the atmosphere. The main reason to overheat the consortium in this study was to get only spore-forming microorganisms and eliminate the hydrogen-consuming microorganisms. The consortium was stressed for an entire day. We could see the speed during the adaptation. Despite the overheat, the microorganisms were capable of starting the hydrogen production in a few hours as seen in the experiments 17, 3 and 8, with 1.5, 3.1 and 3.9 h. Until here, according to the statistical model, the operational conditions are as follows 25 °C, 30 % (v/v), and 6.5 for temperature, substrate concentration, and pH, respectively.

3.2. VFAs at the end of the fermentation

At the end of the fermentation, the pH of all the experiments was measured. The average pH was 4.17 ± 0.23, corresponding to an acidic microenvironment and the termination of hydrogen production. Anaerobic dark fermentations go together with acid production. Fig. 4 shows the scores and the loadings of the principal component analysis (PCA), where it can be seen that only 2 PCAs are necessary to explain 90 % of the variance observed (Fig. 4**A-B**). Moreover, two branches (**a** and **b**) and three sub-groups are formed; one accounts for acetic acid, the second group for butyric acid and ethanol, and the last sub-group for isobutyric, propionic acids, 1-butanol, and acetone (Fig. 4**C).** The amount of VFAs accumulated at the end of each fermentation is represented in Fig. 4**D**, where it can be seen the amount in g L^{-1} of VFAs at the end of the fermentation in a matrix plot. The primary biochemical hydrogen production pathways were acetic and butyric acid. The maximal acetic acid amounts accumulated 5.86 and 4.17 gL^{-1} in experiments 7 and 4, respectively; whereas the maximal butyric acid amounts attained were 2.33 and 3.60 gL^{-1} in experiments 8 and 18, respectively **(Table A.4).**

Dark fermentation of mixed cultures is a natural process that evolved to increase cellular growth but not the hydrogen yield (Hallenbeck and Benemann, 2002). By-products (hydrogen, VFAs, and alcohols) are converted to methane. During fermentation of balanced mixed cultures, hydrogen is produced through hydrogenases as an intermediary by-product and butyrate and acetate (Hallenbeck and Benemann, 2002). Hydrogen transfer in anaerobic mixed cultures follows many pathways until reaching thermodynamically favorable reactions (Xu et al., 2010). Unless methanogenic bacteria are inhibited, the hydrogen will be consumed to produce methane to maintain a low partial pressure of hydrogen (PaH_2). Theoretically, when hydrogen consuming bacteria is entirely inhibited, hydrogen will accumulate until reaching 2 or 4 $mol_{H2}mol_{glucose}^{-1}$ using acetate or butyrate as the final products. Acetate formation is preferable because this regenerates the reducing molecules and allows ATP synthesis by microorganisms (Xu et al., 2010). Nevertheless, under high PaH_2 (>60 Pa), butyrate is produced to avoid the accumulation of reducing molecules (Angenent et al., 2004).

3.3. Run in bioreactor

The optimized variables to obtain the maximum hydrogen production, hydrogen production rate, and hydrogen yield were a pH of 6.5, a temperature of 25 °C, and a substrate concentration of 30 % (v/v). The

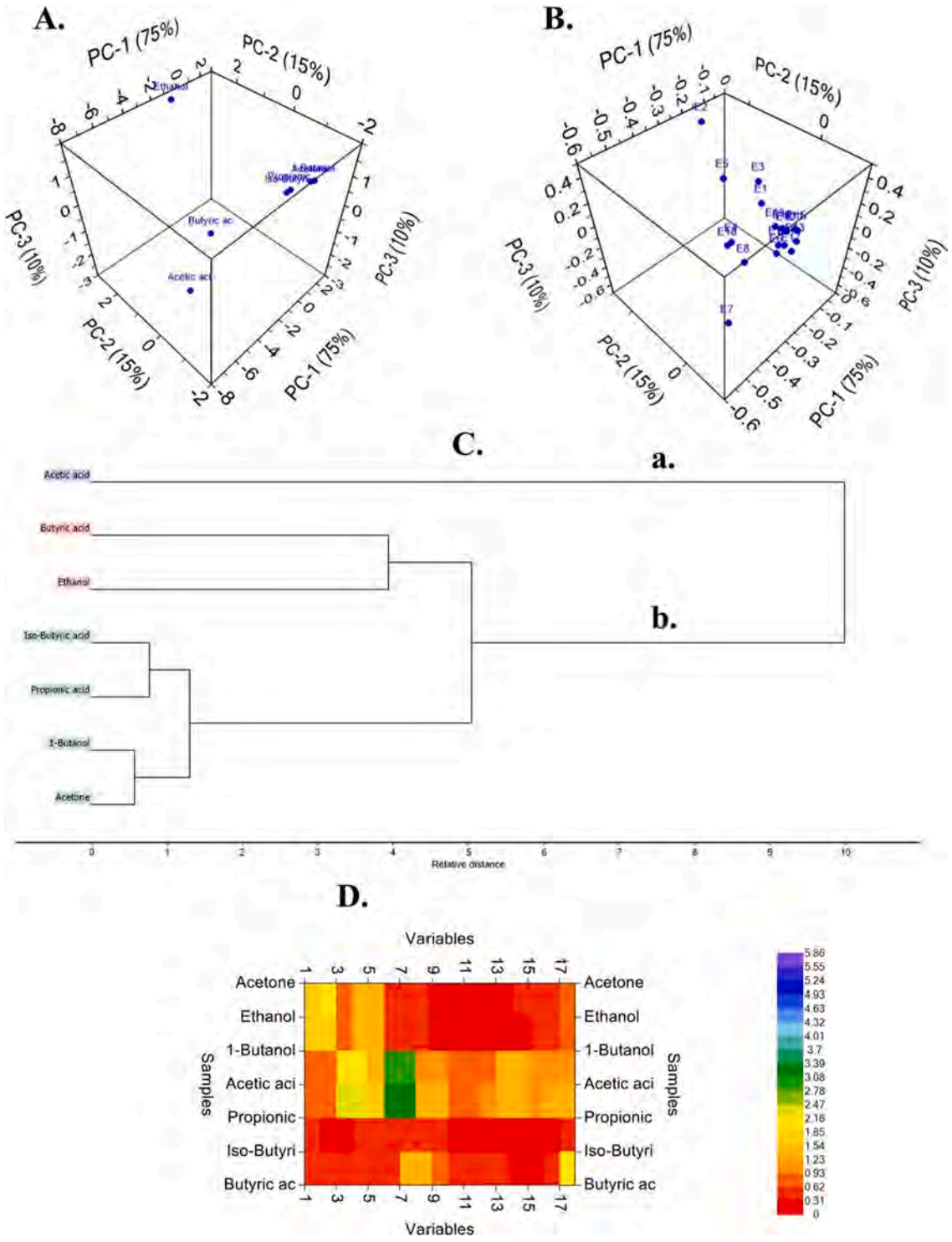

Fig. 4. Chemometric analysis of VFAs at the end of the fermentation. A and B) Multivariate Analysis (PCA); **C)** Hierarchical Cluster Analysis by mean Ward's method and Squared Euclidian Distance; and **D)** Matrix plot of the VFAs at the end of the fermentation in gL^{-1}.

initial substrate concentration was 224 g of total carbohydrates; the experimental data showed that the hydrogen production lasted only 36 h. Three more h (39 h), the hydrogen production was zero, despite that the fermentation was followed until 45 h with the same results (zero of hydrogen production). This result addressed the ending of the fermentation. The total hydrogen production attained was 17.202 L of hydrogen with a Hydrogen production rate of 0.58 Lh^{-1} And Hydrogen yield of 77.2 $ml_{H2}G_{TCC}^{-1}$ **(**Fig. 5**A).** Both temperature and pH were controlled for this experiment according to the parameters established by the statistical model **(**Fig. 5**B-C).** Regarding to the Gompertz equation previously described, these datasets were also modeled, where the lag phase lasted 1.48 h with a maximum productivity of 0.66 Lh^{-1} and 19.33 L of hydrogen production. Moreover, experimental and modeled data are highly correlated (R^2 = 0.985), indicating that the data fits almost perfectly to the statistical model. With these parameters operated in a bioreactor was possible to reach a monoauxic kinetic, typically reached only with single cultures. This kinetic behavior and the total consumption of the carbohydrates show that the conditions are

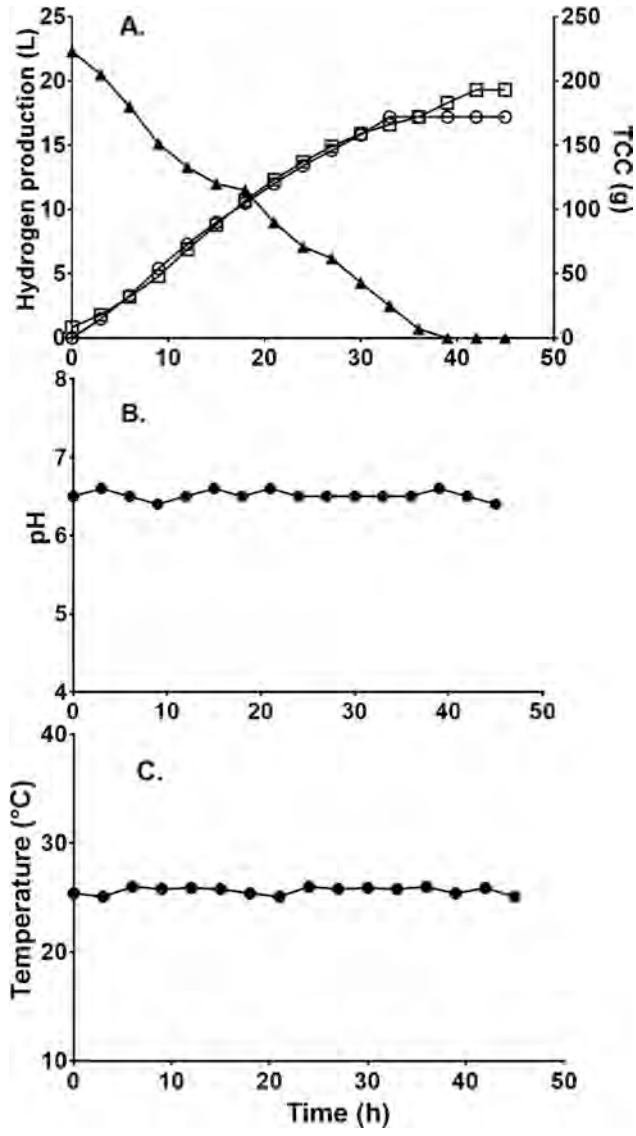

Fig. 5. Run in a 3 L bioreactor. A) Hydrogen production kinetics according to the experimental data (), Gompertz modeling () and the Total Carbohydrate consumed (); **B)** pH, and **C)** Temperature.

favorable for the microorganism's growth, hydrogenase activity, and hydrogen production—for instance, Wang and Jin. (2009) used a single strain of *Clostridium butyricum* and molasses as substrate; they attained up to 10 L in 36 h. A monoauxic behavior was observed due to the previous description mentioned above. In addition, The level of hydrogen production compares favorably with yields reported by Concetti et al. (2013), Marone et al. (2015), and Ghimire et al. (2017) using agroindustrial wastes as substrate. The bioreactor production was monitored for only 48 h because the hydrogen production ended at hour 33 and plateaued without observable gas production.

Optimization of operational conditions is of utmost importance when it comes to scaling-up the process. For example, establishing the pH conditions is one of the pivotal factors that affects the hydrogen production from agro-industrial waste by suitable microorganisms. Maintaining optimum pH is essential to attain maximum hydrogen yield (Arimi et al., 2015). The pH of the substrate can affect the chemical reactions by which hydrogen production occurs. Furthermore, suitable pH conditions also determine microbial growth and enzyme activity (Bolatkhan et al., 2019). Many studies claim that acidic conditions are favorable because it is addressed by butyrate instead of acetate production. However, in some cases, neutral pH was optimal, where 6.5 obtained in this work would be set up (Bhatia et al., 2021; Soares et al., 2020).

Regarding temperature is another critical factor influencing the bioconversion of agro-industrial waste into hydrogen. The temperature opted for hydrogen production depends on substrate and microorganisms (Ziara et al., 2019). High volumetric hydrogen production rates are achieved with mesophilic organisms due to low cell densities and slow rate proliferation, while higher yields are attained with thermophilic organisms (Łukajtis et al., 2018). According to Saravanan et al. (2021), bioconversion of agro-industrial waste can be intervened under different temperatures, and 25 °C obtained in this work fits the psychrophilic conditions. The process of biohydrogen production primarily depends on the nutrient content substrates. Besides carbon, other nutrients such as nitrogen, phosphorous, and micronutrients are responsible for the microorganism's propagation and hydrogen metabolism. Some agro-industrial substrates such as wheat straw, sugarcane bagasse, and corn stover might be deficient in micronutrients; hence, adding these is necessary. The physicochemical properties of the vermicompost-tea were previously studied elsewhere (Oceguera-Contreras et al., 2019); since vermicompost contains these micronutrients, the advantage of using this consortium is that the addition of micronutrients is not necessary.

4. Conclusions

The study findings showed that inoculation of vermicomposting-tea into molasses was suitable for producing hydrogen at dark anaerobic conditions and is a feasible approach for giving an extra value to molasses. This substrate/inoculum combination can tolerate excessive heat pre-treatment (around 24 h × 90 °C). The experimental conditions showed values ranging from 140.3 to 1021.0 mlL^{-1} of hydrogen. The statistical model showed that the optimal operational conditions were pH 6.5, molasses concentration 30 % (v/v), and temperature 25 °C. The main metabolic pathways were acetic and butyric acid. The run in bioreactor showed a cumulative production of 17.202 L of hydrogen over 33 h in an operational volume of 2.5 L. The statistical model fitted adequately to the experimental data according to the Gompertz modeling.

The bioreactor production runs proved the principle of successfully producing hydrogen with the selected consortium and demonstrating the potential scaling-up process. It is essential to underline that the inoculum was pretreated similarly for all the experiments, including the bioreactor run. For large-scale operations, inoculum pre-treatment should be limited to15 min at 100 °C. The critical process parameters and the operational space for a robust upstream process with this consortium using molasses as a substrate for hydrogen production at either pilot or industrial scale were identified.

"E-supplementary data of this work can be found in the online version of the paper."

CRediT authorship contribution statement

Edén Oceguera-Contreras: Conceptualization, Methodology, Validation, Software, Resources, Writing – original draft, Funding acquisition. **Oscar Aguilar-Juarez:** Conceptualization, Methodology, Validation, Software, Resources, Writing – original draft, Funding acquisition. **David Oseguera-Galindo:** Formal analysis, Investigation, Resources. **José Macías-Barragán:** Formal analysis, Investigation, Resources. **Gerardo Ortiz-Torres:** Formal analysis, Investigation, Resources. **María Luisa Pita-López:** Formal analysis, Investigation, Resources. **Jorge Domínguez:** Methodology, Supervision, Writing – review & editing. **Igor Titov:** Methodology, Supervision, Writing – review & editing. **Amine Kamen:** Methodology, Supervision, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

This work was supported by CONACYT Grant: Problemas Nacionales with expedient number 2015-01-1214. We thank CIATEJ A.C. and Roberto Bolaños for the technical assistance. EOC wants to thank Dr. Amine Kamen for the scholarship granted during his postdoctoral position in the Department of Bioengineering at Mcgill University.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.wasman.2021.12.027.

References

Abdullah, B., Syed Muhammad, S.A.F. ad, Shokravi, Z., Ismail, S., Kassim, K.A., Mahmood, A.N., Aziz, M.M.A., 2019. Fourth generation biofuel: A review on risks and mitigation strategies. Renew. Sustain. Energy Rev. 10.1016/j.rser.2019.02.018.

Akbas, M.Y., Stark, B.C., 2016. Recent trends in bioethanol production from food processing by-products. J. Ind. Microbiol. Biotechnol. https://doi.org/10.1007/s10295-016-1821-z.

Almer, C., Winkler, R., 2017. Analyzing the effectiveness of international environmental policies: The case of the Kyoto Protocol. J. Environ. Econ. Manage. 82, 125–151. https://doi.org/10.1016/j.jeem.2016.11.003.

Álvarez-Cao, M.E., Cerdán, M.E., González-Siso, M.I., Becerra, M., 2019. Bioconversion of beet molasses to alpha-galactosidase and ethanol. Front. Microbiol. 10 https://doi.org/10.3389/FMICB.2019.00405/FULL.

Alvarez-Guzmán, C.L., Balderas-Hernández, V.E., González-García, R., Ornelas-Salas, J. T., Vidal-Limón, A.M., Cisneros-de la Cueva, S., De Leon-Rodriguez, A., 2017. Optimization of hydrogen production by the psychrophilic strain G088. Int. J. Hydrogen Energy 42, 3630–3640. https://doi.org/10.1016/j.ijhydene.2016.08.098.

Angenent, L.T., Karim, K., Al-Dahhan, M.H., Wrenn, B.A., Domíguez-Espinosa, R., 2004. Production of bioenergy and biochemicals from industrial and agricultural wastewater. Trends Biotechnol. https://doi.org/10.1016/j.tibtech.2004.07.001.

Argun, H., Kargi, F., Kapdan, I.K., Oztekin, R., 2008. Batch dark fermentation of powdered wheat starch to hydrogen gas: Effects of the initial substrate and biomass concentrations. Int. J. Hydrogen Energy 33, 6109–6115. https://doi.org/10.1016/j.ijhydene.2008.08.004.

Arimi, M.M., Knodel, J., Kiprop, A., Namango, S.S., Zhang, Y., Geißen, S.U., 2015. Strategies for improvement of biohydrogen production from organic-rich wastewater: A review. Biomass and Bioenergy. https://doi.org/10.1016/j.biombioe.2015.02.011.

Baghchehsaraee, B., Nakhla, G., Karamanev, D., Margaritis, A., Reid, G., 2008. The effect of heat pretreatment temperature on fermentative hydrogen production using mixed cultures. Int. J. Hydrogen Energy 33, 4064–4073. https://doi.org/10.1016/j.ijhydene.2008.05.069.

Bhatia, S.K., Jagtap, S.S., Bedekar, A.A., Bhatia, R.K., Rajendran, K., Pugazhendhi, A., Rao, C.V., Atabani, A.E., Kumar, G., Yang, Y.H., 2021. Renewable biohydrogen production from lignocellulosic biomass using fermentation and integration of systems with other energy generation technologies. Sci. Total Environ. https://doi.org/10.1016/j.scitotenv.2020.144429.

Bolatkhan, K., Kossalbayev, B.D., Zayadan, B.K., Tomo, T., Veziroglu, T.N., Allakhverdiev, S.I., 2019. Hydrogen production from phototrophic microorganisms: Reality and perspectives. Int. J. Hydrogen Energy. https://doi.org/10.1016/j.ijhydene.2019.01.092.

Braga, J.K., Abreu, A.A., Motteran, F., Pereira, M.A., Alves, M.M., Varesche, M.B.A., 2019. Hydrogen Production by Clostridium cellulolyticum a Cellulolytic and Hydrogen-Producing Bacteria Using Sugarcane Bagasse. Waste and Biomass Valorization 10, 827–837. https://doi.org/10.1007/s12649-017-0105-9.

Buckel, W., Thauer, R.K., 2013. Energy conservation via electron bifurcating ferredoxin reduction and proton/Na+ translocating ferredoxin oxidation. Biochim. Biophys. Acta - Bioenerg. 10.1016/j.bbabio.2012.07.002.

Castillo, J.M., Romero, E., Nogales, R., 2013. Dynamics of microbial communities related to biochemical parameters during vermicomposting and maturation of agroindustrial lignocellulose wastes. Bioresour. Technol. 146, 345–354.

Ciranna, A., Ferrari, R., Santala, V., Karp, M., 2014. Inhibitory effects of substrate and soluble end products on biohydrogen production of the alkalithermophile Caloramator celer: Kinetic, metabolic and transcription analyses. Int. J. Hydrogen Energy 39, 6391–6401. https://doi.org/10.1016/j.ijhydene.2014.02.047.

Concetti, S., Chiariotti, A., Patriarca, C., Marone, A., Varrone, C., Contò, G., Calì, M., Signorini, A., 2013. Biohydrogen production from buffalo manure codigested with agroindustrial by-products in an anaerobic reactor. Buffalo Bulletin. 1241–1244.

Conrad, R., 1996. Soil microorganisms as controllers of atmospheric trace gases (H2, CO, CH4, OCS, N2O, and NO). Microbiol. Rev. 60, 609–640.

da Silva, L.L., 2016. Adding Value to Agro-Industrial Wastes. Ind. Chem. 2 https://doi.org/10.4172/2469-9764.1000e103.

Domínguez, J., 2018. Earthworms and Vermicomposting. Earthworms - The Ecological Engineers of Soil. 63–77. https://doi.org/10.5772/intechopen.76088.

Domínguez, J., Aira, M., Breinholt, J.W., Stojanovic, M., James, S.W., Pérez-Losada, M., 2015. Underground evolution: New roots for the old tree of lumbricid earthworms. Mol. Phylogenet. Evol. 83, 7–19. https://doi.org/10.1016/j.ympev.2014.10.024.

Dubois, M., Gilles, K.A., Hamilton, J.K., Rebers, P.A., Smith, F., 1956. Colorimetric Method for Determination of Sugars and Related Substances. Anal. Chem. 28, 350–356. https://doi.org/10.1021/ac60111a017.

Edwards, C.A., Arancon, N.Q., 2004. Vermicomposts suppress plant pest and disease attacks. Biocycle 45, 51–54.

Ghimire, A., Frunzo, L., Pirozzi, F., Trably, E., Escudie, R., Lens, P.N.L., Esposito, G., 2015. A review on dark fermentative biohydrogen production from organic. Appl. Energy 144, 73–95.

Ghimire, A., Luongo, V., Frunzo, L., Pirozzi, F., Lens, P.N.L., Esposito, G., 2017. Continuous biohydrogen production by thermophilic dark fermentation of cheese whey: Use of buffalo manure as buffering agent. Int. J. Hydrogen Energy 42, 4861–4869. https://doi.org/10.1016/j.ijhydene.2016.11.185.

Goldman, R.P., Brown, S.P., 2009. Making sense of microbial consortia using ecology and evolution. Trends Biotechnol. 27, 3–4. https://doi.org/10.1016/j.tibtech.2008.10.003.

Gómez-Brandon, M., Vela, M., Martínez-Toledo, M.V., Insam, H., Domínguez, J., 2014. Effects of Compost and Vermicompost Teas as Organic Fertilizers. Advances in Fertilizer Technology: Synthesis. 300–318.

Hallenbeck, P.C., Benemann, J.R., 2002. Biological hydrogen production. Fundamentals and limiting processes, in: International Journal of Hydrogen Energy. 1185–1193. https://doi.org/10.1016/S0360-3199(02)00131-3.

Hamed, E., Sakr, A., 2001. Application of multiple response optimization technique to extended release formulations design. J. Control. Release 73, 329–338. https://doi.org/10.1016/S0168-3659(01)00356-X.

Hu, B., Li, Y., Zhu, S., Zhang, H., Jing, Y., Jiang, D., He, C., Zhang, Z., 2020. Evaluation of biohydrogen yield potential and electron balance in the photo-fermentation process with different initial pH from starch agricultural leftover. Bioresour. Technol. 305 https://doi.org/10.1016/j.biortech.2020.122900.

Kamaraj, M., Ramachandran, K.K., Aravind, J., 2019. Biohydrogen production from waste materials: benefits and challenges. Int. J. Environ. Sci. Technol. https://doi.org/10.1007/s13762-019-02577-z.

Karmegam, N., Vijayan, P., Prakash, M., John Paul, J.A., 2019. Vermicomposting of paper industry sludge with cowdung and green manure plants using Eisenia fetida: A viable option for cleaner and enriched vermicompost production. J. Clean. Prod. 228, 718–728. https://doi.org/10.1016/j.jclepro.2019.04.313.

Kim, S.H., Han, S.K., Shin, H.S., 2006. Effect of substrate concentration on hydrogen production and 16S rDNA-based analysis of the microbial community in a continuous fermenter. Process Biochem. 41, 199–207. https://doi.org/10.1016/j.procbio.2005.06.013.

Kumar, G., Mathimani, T., Rene, E.R., Pugazhendhi, A., 2019. Application of nanotechnology in dark fermentation for enhanced biohydrogen production using inorganic nanoparticles. Int. J. Hydrogen Energy. https://doi.org/10.1016/j.ijhydene.2019.03.131.

Łukajtis, R., Hołowacz, I., Kucharska, K., Glinka, M., Rybarczyk, P., Przyjazny, A., Kamiński, M., 2018. Hydrogen production from biomass using dark fermentation. Renew. Sustain. Energy Rev. 10.1016/j.rser.2018.04.043.

Marone, A., Varrone, C., Fiocchetti, F., Giussani, B., Izzo, G., Mentuccia, L., Rosa, S., Signorini, A., 2015. Optimization of substrate composition for biohydrogen production from buffalo slurry co-fermented with cheese whey and crude glycerol, using microbial mixed culture. Int. J. Hydrogen Energy 40, 209–218. https://doi.org/10.1016/j.ijhydene.2014.11.008.

Mirza, S.S., Qazi, J.I., Liang, Y., Chen, S., 2019. Growth characteristics and photofermentative biohydrogen production potential of purple non sulfur bacteria from sugar cane bagasse. Fuel 255, 115805. https://doi.org/10.1016/j.fuel.2019.115805.

Newell, R.G., Iler, S., Raimi, D., 2018. Global Energy Outlooks Comparison Methods: 2018 Update. Resour, Futur.

Oceguera-Contreras, E., Aguilar-Juárez, O., Oseguera-Galindo, D., Macías-Barragán, J., Bolaños-Rosales, R., Mena-Enríquez, M., Arias-García, A., Montoya-Buelna, M., Graciano-Machuca, O., De León-Rodríguez, A., 2019. Biohydrogen production by vermihumus-associated microorganisms using agro industrial wastes as substrate. Int. J. Hydrogen Energy 44, 9856–9865. https://doi.org/10.1016/j.ijhydene.2018.10.236.

Pascualone, M.J., Gómez Costa, M.B., Dalmasso, P.R., 2019. Fermentative biohydrogen production from a novel combination of vermicompost as inoculum and mild heat-pretreated fruit and vegetable waste. Biofuel Res. J. 6, 1046–1053. https://doi.org/10.18331/BRJ2019.6.3.5.

Pason, P., Tachaapaikoon, C., Panichnumsin, P., Ketbot, P., Waeonukul, R., Kosugi, A., Ratanakhanokchai, K., 2020. One-step biohydrogen production from cassava pulp using novel enrichment of anaerobic thermophilic bacteria community. Biocatal. Agric. Biotechnol. 27, 101658 https://doi.org/10.1016/j.bcab.2020.101658.

Peters, J.W., Schut, G.J., Boyd, E.S., Mulder, D.W., Shepard, E.M., Broderick, J.B., King, P.W., Adams, M.W.W., 2015. [FeFe]- and [NiFe]-hydrogenase diversity, mechanism, and maturation. Biochim. Biophys. Acta - Mol. Cell Res. 10.1016/j.bbamcr.2014.11.021.

Proulx, S.R., Promislow, D.E.L., Phillips, P.C., 2005. Network thinking in ecology and evolution. Trends Ecol. Evol. 20, 345–353. https://doi.org/10.1016/j.tree.2005.04.004.

Qasim, S.R., Chiang, W., 2017. Sanitary landfill leachate: Generation, control and treatment, Sanitary Landfill Leachate: Generation, Control and Treatment. 10.1201/9780203740217.

Ramesh, M.K., Kalaivanan, K., Durairaj, S., Selladurai, G., 2020. Poultry waste management using earthworms e. Agric. Sci. Dig. 40, 323–327. https://doi.org/10.18805/ag.D-5116. Eugeniae, e. foetida and p. excavates.

Ranch, T.W., 2021. Worm Wine – Texas Worm Ranch [WWW Document]. URL http://txwormranch.com/worm-wine/ (accessed 11.9.21).

Rhee, T.S., Brenninkmeijer, C.A.M., Röckmann, T., 2006. The overwhelming role of soils in the global atmospheric hydrogen cycle. Atmos. Chem. Phys. 6, 1611–1625. https://doi.org/10.5194/acp-6-1611-2006.

Robledo-Narváez, P.N., Muñoz-Páez, K.M., Poggi-Varaldo, H.M., Ríos-Leal, E., Calva-Calva, G., Ortega-Clemente, L.A., Rinderknecht-Seijas, N., Estrada-Vázquez, C., Ponce-Noyola, M.T., Salazar-Montoya, J.A., 2013. The influence of total solids content and initial pH on batch biohydrogen production by solid substrate fermentation of agroindustrial wastes. J. Environ. Manage. 128, 126–137. https://doi.org/10.1016/j.jenvman.2013.04.042.

Saba, S., Zara, G., Bianco, A., Garau, M., Bononi, M., Deroma, M., Pais, A., Budroni, M., 2019. Comparative analysis of vermicompost quality produced from brewers' spent grain and cow manure by the red earthworm Eisenia fetida. Bioresour. Technol. 293 https://doi.org/10.1016/j.biortech.2019.122019.

SAGARPA, 2014. PRONAC [WWW Document]. Programa Nac. la Agroindustría la Caña Azúcar. URL http://www.cndsca.gob.mx/documentoseficproductiva/8. PRONAC/PRONAC 2014-2018.pdf (accessed 5.9.19).

Saidi, R., Liebgott, P.P., Hamdi, M., Auria, R., Bouallagui, H., 2018. Enhancement of fermentative hydrogen production by Thermotoga maritima through hyperthermophilic anaerobic co-digestion of fruit-vegetable and fish wastes. Int. J. Hydrogen Energy 43, 23168–23177. https://doi.org/10.1016/j.ijhydene.2018.10.208.

Saravanan, A., Kumar, P.S., Mat Aron, N.S., Jeevanantham, S., Karishma, S., Yaashikaa, P.R., Chew, K.W., Show, P.L., 2021. A review on bioconversion processes for hydrogen production from agro-industrial residues. Int. J. Hydrogen Energy. https://doi.org/10.1016/j.ijhydene.2021.08.055.

Segarra, K.E.A., Schubotz, F., Samarkin, V., Yoshinaga, M.Y., Hinrichs, K.U., Joye, S.B., 2015. High rates of anaerobic methane oxidation in freshwater wetlands reduce potential atmospheric methane emissions. Nat. Commun. 6 https://doi.org/10.1038/ncomms8477.

Shade, A., Peter, H., Allison, S.D., Baho, D.L., Berga, M., Bürgmann, H., Huber, D.H., Langenheder, S., Lennon, J.T., Martiny, J.B.H., Matulich, K.L., Schmidt, T.M., Handelsman, J., 2012. Fundamentals of microbial community resistance and resilience. Front. Microbiol. 3 https://doi.org/10.3389/fmicb.2012.00417.

Sherman, R.L., 2010. Vermicomposting for businesses and institutions, Vermiculture Technology: Earthworms, Organic Wastes, and Environmental. Management. 391–408. https://doi.org/10.1201/b10453.

Silva Benavides, A.M., Rudin, M.C., Villalobos, N., Touloupakis, E., Torzillo, G., 2019. Growth and hydrogen production by three Chlamydomonas strains cultivated in a commercial fertilizer. Int. J. Hydrogen Energy 44, 9849–9855. https://doi.org/10.1016/j.ijhydene.2018.11.209.

Sinha, R.K., Agarwal, S., Chauhan, K., Chandran, V., Soni, B.K., 2010. Vermiculture Technology: Reviving the Dreams of Sir Charles Darwin for Scientific Use of Earthworms in Sustainable Development Programs. Technol. Invest. 01, 155–172. https://doi.org/10.4236/ti.2010.13019.

Soares, J.F., Confortin, T.C., Todero, I., Mayer, F.D., Mazutti, M.A., 2020. Dark fermentative biohydrogen production from lignocellulosic biomass: Technological challenges and future prospects. Renew. Sustain. Energy Rev. 10.1016/j.rser.2019.109484.

Tracy, B.P., Jones, S.W., Fast, A.G., Indurthi, D.C., Papoutsakis, E.T., 2012. Clostridia: The importance of their exceptional substrate and metabolite diversity for biofuel and biorefinery applications. Curr. Opin. Biotechnol. https://doi.org/10.1016/j.copbio.2011.10.008.

UNC, 2018. Zafra Nacional 2018-2019 [WWW Document]. URL http://caneros.org.mx/ (accessed 5.9.19).

Valenzuela, E.I., Avendaño, K.A., Balagurusamy, N., Arriaga, S., Nieto-Delgado, C., Thalasso, F., Cervantes, F.J., 2019. Electron shuttling mediated by humic substances fuels anaerobic methane oxidation and carbon burial in wetland sediments. Sci. Total Environ. 650, 2674–2684. https://doi.org/10.1016/j.scitotenv.2018.09.388.

Van Ginkel, S.W., Logan, B., 2005. Increased biological hydrogen production with reduced organic loading. Water Res. 39, 3819–3826. https://doi.org/10.1016/j.watres.2005.07.021.

Wang, J., Wan, W., 2009. Kinetic models for fermentative hydrogen production: A review. Int. J. Hydrogen Energy. https://doi.org/10.1016/j.ijhydene.2009.02.031.

Wang, J., Xu, S., Xiao, B., Xu, M., Yang, L., Liu, S., Hu, Z., Guo, D., Hu, M., Ma, C., Luo, S., 2013. Influence of catalyst and temperature on gasification performance of pig compost for hydrogen-rich gas production. Int. J. Hydrogen Energy 38, 14200–14207. https://doi.org/10.1016/j.ijhydene.2013.08.075.

Wang, J., Yin, Y., 2017. Optimization of hydrogen production process, in: Green Energy and Technology. Springer Verlag, pp. 291–337. 10.1007/978-981-10-4675-9_7.

Wang, X., Jin, B., 2009. Process optimization of biological hydrogen production from molasses by a newly isolated Clostridium butyricum W5. J. Biosci. Bioeng. 107, 138–144. https://doi.org/10.1016/j.jbiosc.2008.10.012.

Xing, Y., Li, Z., Fan, Y., Hou, H., 2010. Biohydrogen production from dairy manures with acidification pretreatment by anaerobic fermentation. Environ. Sci. Pollut. Res. 17, 392–399. https://doi.org/10.1007/s11356-009-0187-4.

Xu, K., Liu, H., Li, X., Chen, J., Wang, A., 2010. Typical methanogenic inhibitors can considerably alter bacterial populations and affect the interaction between fatty acid degraders and homoacetogens. Appl. Microbiol. Biotechnol. 87, 2267–2279. https://doi.org/10.1007/s00253-010-2708-y.

Yang, G., Wang, J., 2018. Various additives for improving dark fermentative hydrogen production: A review. Renew. Sustain. Energy Rev. 10.1016/j.rser.2018.07.029.

Yokoyama, H., Waki, M., Moriya, N., Yasuda, T., Tanaka, Y., Haga, K., 2007. Effect of fermentation temperature on hydrogen production from cow waste slurry by using anaerobic microflora within the slurry. Appl. Microbiol. Biotechnol. 74, 474–483. 10.1007/s00253-006-0647-4.

Ziara, R.M.M., Miller, D.N., Subbiah, J., Dvorak, B.I., 2019. Lactate wastewater dark fermentation: The effect of temperature and initial pH on biohydrogen production and microbial community. Int. J. Hydrogen Energy 44, 661–673. https://doi.org/10.1016/j.ijhydene.2018.11.045.