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# Biorefinery concept comprising acid hydrolysis, dark fermentation and anaerobic digestion for co-processing of fruit and vegetable wastes and corn stover

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28 **Biorefinery concept comprising acid hydrolysis, dark fermentation and anaerobic  
digestion for co-processing of fruit and vegetable wastes and corn stover**

30 **Abstract**

32 A new biorefinery conceptual process is proposed for biohydrogen and biomethane production from a  
combination of fruits and vegetables wastes (FVW) and corn stover (CS). The objective of this work was  
to perform the acid hydrolysis (HCl 0.5 % v/v, 120 °C, 2 h) of the FVW and CS at 3:1 dry basis ratio, and  
34 to process its main physical phases, liquid hydrolyzates (LH) and hydrolyzed solids (HS), by mesophilic  
dark fermentation (DF) and anaerobic digestion (AD), respectively. In DF of LH as carbon source,  
36 hydrogen was produced at maximum rate of 2.6 mL H<sub>2</sub>/(g<sub>glucose</sub> h) and maximum accumulation of 223.8  
mL H<sub>2</sub>/g<sub>glucose</sub>, equivalent to 2 mol H<sub>2</sub>/mol<sub>glucose</sub>, in a butyric-pathway driven fermentation. HS were  
38 digested to methane production assessing inoculum to substrate ratios in the range 2 – 4 g<sub>inoculum</sub>/g<sub>vs</sub>. The  
main results in AD were, 14 mmol CH<sub>4</sub>/g<sub>vs</sub>. The biorefinery demonstrated the feasibility to integrate the  
40 acid hydrolysis as pretreatment and subsequently use the LH for hydrogen production, and the HS for  
methane production, with an energy yield recovery of 9.7 kJ/g<sub>vs</sub>, being the energy contribution from  
42 anaerobic digestion 8-fold higher than of dark fermentation.

44  
**Keywords:** Biohydrogen; Furfural; Hydrochloric acid; Lignocellulosic waste; Methane; Multisubstrate;  
46 Phenolic compounds

48

## Introduction

50 Mexico is committed to reducing its greenhouse gases (GHG) by the year 2030 to 22%, whereas emissions  
of short-lived climate pollutants (black carbon) are aimed to 51% reduction (Gobierno de México 2015).  
52 The main proposals for obtaining energy from renewable sources are bioenergy, solar energy, geothermal  
energy, hydroelectric energy, wind energy, and tidal energy. The biomass as a source for bioenergies, i.e.  
54 biohydrogen, biomethane, bioethanol, and biobutanol (Mahlia et al. 2019), is most promising in the  
energetic share it could reach; according to some estimates, in Mexico it could supply ca. 46% of the  
56 annual energy, yet currently, only 5% is supplied (International Energy Agency 2016).

Residual biomass can be classified by its origin in forestry residues, agricultural residues, agro-  
58 industrial wastes and organic fraction of municipal solid waste (Li et al. 2016). Residual biomass  
exhaustive use has led to the development of the biorefinery of residues.

60 The biorefinery concept is associated with the intensive conversion of organic matter into added-value  
products (Escamilla-Alvarado et al. 2016; Moncada-Botero et al. 2016). Biorefineries are outlined by the  
62 following sections: *i*) pretreatment section to fractionate biomass, *ii*) section of bioprocesses (e.g.  
fermentation, digestion), *iii*) purification of bioproducts to discard impurities and pollutants, and *iv*) energy  
64 conversion section (Giuliano et al. 2016; Hernández-Flores et al. 2017).

The pretreatment with the highest efficiency of fermentable sugars release is diluted acid hydrolysis  
66 (Gonzales et al. 2016; Joglekar et al. 2019). It employs high temperatures and acids in low concentrations  
to hydrolyze macromolecules such as cellulose and hemicellulose into monomeric sugars such as glucose,  
68 xylose, arabinose, and others (Kumar et al. 2015). Such soluble hydrolyzate is prone to any kind of  
fermentation, yet the insoluble fraction remaining from the pretreatment (hydrolyzed solids) is also  
70 susceptible for downstream processing. In the biorefinery perspective, the use of both hydrolyzates would  
be very attractive to increase the productivities of the installation.

72 Fermentable sugars in liquid hydrolyzates (HL) can be used as a carbon source to produce hydrogen  
through dark fermentation (DF) bioprocess (Roy and Das 2016). On the other hand, the insoluble biomass,  
74 or hydrolyzed solids (HS), may be converted into a bioenergetic such as methane through anaerobic  
digestion (AD) (Oliwit et al. 2019). Actually, only few works have dealt with the either DF or AD of  
76 hydrolyzates with interesting results. For instance, Datar et al. (2007) applied steam explosion hydrolysis  
followed by the separated fermentation of the liquid hydrolyzates and the solid hydrolyzates for hydrogen  
78 production. Curiously, the dark fermentation of solid hydrolyzates did not produce hydrogen during the  
first 21 d, which was attained only after 20 h since the addition of cellulases. On the other hand, Tapia-  
80 Rodríguez et al. (2019) evaluated the parallel production of hydrogen and methane from  
enzymatic hydrolyzates of agave bagasse, however the HS were not included in the biorefining process.

82 The sources of biomass for biorefineries are numerous and in order to not compromise the food supply,  
biowastes should be mainly used as its substrates (Romero-Cedillo et al. 2016). Considering that Mexico  
84 was the 11<sup>th</sup> agriculture producer worldwide with an annual production of 210 million tons in 2018  
(SADER 2019), its wastes generation along the food supply chains (production, postharvest, processing,  
86 distribution, consumption) are also considerable. Indeed, as corn is the second most important crop in  
Mexico, the wastes associated to the over  $26 \times 10^6$  metric tons production (SADER 2019) are corn stover  
88 (CS) and corn cobs. Since close to 82% of the CS generated is used for animal feed, the remaining 18%  
does not have a defined use (ca.  $4.7 \times 10^6$  metric tons in dry base) and is therefore underused (Hernández  
90 et al. 2019). Currently, CS is the most promising lignocellulosic waste for biofuels production due to its  
high cellulose (23-40%) and hemicellulose (12-32%) contents, and its low-cost abundant biomass (Kim  
92 et al. 2019; Tan et al. 2019).

Another underused biowaste in Mexico are the fruits and vegetable wastes (FVW), which compose *ca.* 44% of municipal solid wastes and that are disposed in landfills and open dumps (Taboada-González et al. 2011; Díaz et al. 2017; Gavilán et al. 2018).

Even though worldwide distribution of biowaste has raised attractiveness on biorefineries, some traits of these substrates such as its recalcitrant nature and seasonal availability are some of the most important challenges for the development of biorefineries at large scale (Sultana and Kumar 2011; Giuliano et al. 2016). It has been proposed that the combination of multiple lignocellulosic biomass would be a feasible solution as it also improves biofuels yields, complements the nutritional balance and reduces delivery costs (Sultana and Kumar 2011; Wang et al. 2011). Therefore, this work aimed to evaluate a biorefinery concept for biohydrogen and biomethane production, as well as the resulting energy potential, from a combination of fruits and vegetables wastes (FVW) and corn stover (CS). The biorefinery concept (*h-H-M*) integrated the diluted acid hydrolysis (*h*) as pretreatment and the parallel production of hydrogen by DF (*H*) from liquid hydrolysates and methane (*M*) from hydrolyzed solids.

## **Materials and methods**

### *Biorefinery h-H-M set up*

The FVW and CS were used as substrates to test the *h-H-M* biorefinery concept as shown in Fig 1. The co-substrates were combined in dry basis (db) at the ratio 3:1 FVW:CS according to Rodríguez-Valderrama (2018). The co-substrates were subjected to hydrolysis pretreatment (*h-stage*) using HCl as the catalyst acid. After the dilute acid hydrolysis, the liquid and solid phases were separated by centrifugation. Liquid hydrolysates (LH) were limed to decrease the concentration of inhibitory compounds, and used as a carbon source in an H<sub>2</sub>-producing dark fermentation stage (*H-stage*) at mesophilic temperature. The hydrolyzed solids (HS) were washed to remove the catalyst acid excess and

116 used as carbon source in a methane producing anaerobic digestion bioprocess (*M-stage*). Each of the three  
stages will be described in detail in the following sections.

118

INSERT FIG. 1

120

#### *Co-substrates*

122 Corn stover (collected from Cuencamé, Durango, Mexico) and FVW (collected from a local cafeteria  
Chemical Sciences Faculty, Universidad Autónoma de Nuevo León, Nuevo León, Mexico) were  
124 separately dried in an oven at 85 °C for 24 h, grinded to 180 µm particle size of using a manual mill, and  
stored in distinct tightly closed plastic bags at room temperature. FVW and CS were physico-chemically  
126 characterized (Table 1). The empirical molecular formulas and the heat power value were  $\text{CH}_{4.31}\text{O}_{0.68}\text{N}_{0.01}$ ,  
3606 cal/g<sub>db</sub>, and  $\text{CH}_{0.8}\text{O}_{0.62}\text{N}_{0.04}$ , 2712 cal/g<sub>db</sub> for CS and FVW, respectively.

128

130 INSERT TABLE 1

#### *Diluted acid hydrolysis and overliming*

The acid hydrolysis was carried out using dilute HCl (0.5% v/v) in 0.5 L Schott bottles. The reaction  
134 volume was 0.3 L and the solid content was 6.6% of reaction volume. The co-substrates ratio was 3:1  
FVW:CS (g:g). The hydrolysis reaction was performed in an autoclave (121 °C) for 120 min (Yan et al.  
136 2009; Kumar et al. 2015). After the hydrolysis, liquid hydrolyzates (LH) were separated by centrifugation  
(10 000 g, 10 min) and characterized in terms of reducing and monomeric sugars and the inhibitory

138 compounds, i.e. acetic acid, formic acid, furfural, 5- hydroxymethyl-furfural (HMF), and total phenolic  
compounds (TPC).

140 Overliming treatment was used applied to the LH in a two-step approach (Chang et al. 2011). Firstly, the  
pH of LH was adjusted to 10 by adding powder  $\text{Ca}(\text{OH})_2$  in continuous stirring for one hour. Secondly,  
142 the pH was reduced to to 7 by 6 M HCl addition, following a centrifugation (10 000 g, 15 min) and the  
separation of liming precipitates and supernatant. Sample were retrieved from the LH to analyze the  
144 removal of inhibitory compounds and sugars.

#### 146 *Dark fermentation*

DF was carried out by duplicate in 0.5 L Schott bottles with 0.4 L of reaction volume. The methanogenic  
148 anaerobic sludge was heat-treated to inhibit methane-producing microflora in water bath at 96° C for 2 h.  
The initial reducing sugars (RS) were adjusted to 13 g/L and the amount of substrate and inoculum were  
added according to the inoculum to substrate ratio (ISR) of 1.2 (VS basis). The fermentation volume was  
150 supplemented with 4 mL of 200 fold mineral medium previously reported by Rodríguez-Valderrama et  
al. (2019). The medium was supplemented with nitrogen source (1 g  $\text{NH}_4\text{Cl}/\text{L}$ ) and buffer medium (3 g  
152  $\text{K}_2\text{HPO}_4/\text{L}$ , 1.5 g  $\text{KH}_2\text{PO}_4/\text{L}$ ). The experimental units were stirred at 150 rpm in a multiple magnetic stirrer  
154 inside an incubator at 35 °C.

#### 156 *Anaerobic digestion*

HS from the separation of LH were washed twice with distilled water (0.03 L of water per 10  $\text{g}_{\text{wb}}$  of HS),  
158 vortexed and sedimented for 10 min, to remove the residual acid catalyst. Afterwards, HS were recovered



by centrifugation (15 min at 10 000 g), dried at 80 °C and characterized (Table 1). HS empirical molecular  
160 formula based on elemental composition was  $\text{CH}_{2.30}\text{O}_{0.42}\text{N}_{0.03}$ .

AD was carried out in 0.120 L serum bottles with 0.08 L of work volume. The inoculum was anaerobic  
162 sludge from a semi-continuous digester fed with FVW at 30 d of hydraulic retention time. The inoculum  
was degassed during 3 d and then used for HS anaerobic digestion. The inoculum had the following  
164 characteristics: 7.81 pH, 93.64%<sub>wb</sub> moisture, 6.36%<sub>wb</sub> TS, 51.46%<sub>db</sub> VS, 48.54%<sub>db</sub> ashes. The alkalinity  
and total VFA were 12 300 mg  $\text{CaCO}_3/\text{L}$  and 8 040 mg VFA/L, respectively. Its empirical molecular  
166 formula based on elemental composition was  $\text{CH}_{1.34}\text{O}_{2.02}\text{N}_{0.10}$ .

Inoculum to substrate ratios (ISR) were assayed in batch mode: 2, 2.5, 3, 3.5 and 4 g<sub>vs</sub> inoculum/g<sub>vs</sub>  
168 HS. A control was run with fresh FVW at ISR 3. A blank was loaded only with inoculum and water to to  
determine the methane production by the organic matter present in the inoculum. The methane production  
170 (2.16 mmol  $\text{CH}_4$ ) from blanks was subtracted from the methane produced by HS. All the experimental  
units were kept constant in its inoculum content at 2.5 g<sub>vs</sub>, the substrate amounts were adjusted according  
172 to each ISR. The anoxic environment in each bottle was promoted by flushing  $\text{N}_2$  during 3 min.  
Afterwards, the bottles were sealed with a rubber stopper and aluminum rings. The operation temperature  
174 and stirred velocity were  $35 \pm 1$  °C and 150 rpm, respectively. All the experiments were carried out by  
duplicate.

176

### *Analytical methods*

178 The pH was determined according to the procedure described by NMX-AA-25-1984 (1992). Solids profile  
was measured according to standard methods (APHA/AWWA/WEF 2005). Cellulose and acid-insoluble  
180 lignin contents were determined by the gravimetric method base on AOAC methods (AOAC 1992).  
Hemicellulose amount was determined subtracting the cellulose content from holocellulose content after

182 by lignin oxidation by NaClO (Escamilla-Alvarado et al. 2015). The extractives in CS and FVW were  
determined by differential weight after extraction in a water bath at 60 °C 24 h (Sluiter et al. 2008). The  
184 elemental characterization (C, H, O, N) was determined by an elemental analyzer (Thermo Scientific Flash  
2000, U.S.A), O<sub>2</sub> was used as combustion gas and He was used as carrier gas.

186 The reducing sugars (RS) in hydrolyzate were determined by the 3,5-dinitrosalicylic acid method (DNS)  
using dextrose for the calibration curve (Miller 1959). Glucose, xylose, and arabinose were quantified by  
188 high performance liquid chromatograph (LDC Analytical, U.S.A.) equipped with a Rezex RHM-  
Monosaccharide (300mm X 7.8 mm) column and a refractive index detector (Varian Prostar, U.S.A.). The  
190 column temperature was 65 °C, whereas the mobile phase (H<sub>2</sub>O) flow rate was maintained at 0.6 mL/min.

The total amount of biogas produced in DF and AD was determined by the acid-brine displacement  
192 method (Escamilla-Alvarado et al. 2013). Hydrogen and methane gas contents were determined in a gas  
chromatograph (Thermo Scientific Trace 1310, U.S.A.); the gas chromatograph was equipped with a  
194 thermal conductivity detector and a molecular sieve column (TG-BOND Msieve 5A, 30 m x 0.33 mm).  
The operating temperatures were 100 °C, 150 °C, and 200°C for the oven, injector, and detector,  
196 respectively. Nitrogen gas was used as a carrier gas with a flow rate of 3 mL/min.

The total phenolic compounds (TPC) were determined by the Folin-Ciocalteu method proposed by  
198 Blainski et al. (2013) using tannic acid as the standard. Furfural, HMF, formic acid, acetic acid, propionic  
acid, succinic acid, and lactic acid were determined by gas chromatography (Varian CP 3380, U.S.A.) with  
200 a column ZB-FFAP (15m x 0.53 x 1 µm) and flame ionization detector. The injector and detector  
temperatures were 230 and 280 °C, respectively. The temperature program for the column initiated at 90  
202 °C for 3 min, then increased to 200 °C at 20 °C/min rate and maintained for 3 min, and finally raised to  
250 °C at 30 °C/min, which was maintained for 4 min.

204 Total volatile fatty acids (TVFA) and alkalinity concentration were determined by a titration method  
(Anderson and Yang 1992). Acetic acid, propionic acid, butyric acid, and ethanol were quantified using a  
206 gas chromatograph according to the method described in our previous work (Rodríguez-Valderrama et al.  
2019).

208

### *Calculations*

210 A set of response variables was calculated according to Table 2 to analyze the production of hydrogen,  
methane and energy potential. The cumulative specific hydrogen production  $H(t)$  (mL H<sub>2</sub>/g<sub>glucose</sub>) was used  
212 for describing the accumulated production of hydrogen in time and to obtain kinetic parameters from  
fitting the results by the Gompertz equation (Eq. 1). Thus, the maximum cumulative specific hydrogen  
214 production  $H_{max}$  (mL H<sub>2</sub>/g<sub>glucose</sub>), the maximum specific hydrogen production rate  $r_{max,H}$  (mL H<sub>2</sub>/(g<sub>glucose</sub>  
h)) and the adaptation time  $\lambda$  (h) were determined.

216 The hydrogen molar pseudoyield,  $Y'_{H_2}$  (mol H<sub>2</sub>/mol<sub>glucose</sub>) in Eq. 2 was obtained from the  $H_{max}$  (mL  
H<sub>2</sub>/g<sub>glucose</sub>) as a means to compare the system to the maximum theoretical hydrogen yield (2 and 4 mol  
218 H<sub>2</sub>/g<sub>glucose</sub> for butyrogenic and acetogenic pathways, respectively).

Other variables in the equations listed in Table 2 used in Eq. 1 or Eq. 2 are  $t$  is fermentation time (h),  $e$  is  
220 2.718,  $C_{RS,0}$  and  $C_{RS,f}$  (g/L) are the concentration of RS at the beginning and at the end of DF, and  $MW_{glucose}$   
is the glucose molar weight (180.16 g/mol).

222 Regarding the AD equations and parameters (Table 2), the cumulative methane production  $B(t)$  (mmol  
CH<sub>4</sub>) and cumulative specific methane production  $b(t)$  (mmol CH<sub>4</sub>/g<sub>vs</sub>) were used to calculate the kinetic  
224 parameters by two methods: an adaptation of the Gompertz equation (Lo et al. 2010, Eq. 3 and Eq. 4), and  
the first-order model proposed by Hashimoto (1989, Eq. 5 and Eq. 6). Through Eq. 3 and Eq. 5 the  
226 maximum cumulative methane production  $B_{max}$  is obtained, whereas through Eq. 4 and 6 the maximum

228 cumulative specific methane production  $b_{max}$  is estimated. The other parameters estimated through these  
equations are the maximum methane production rate  $R_{max,M}$  (mmol CH<sub>4</sub>/ d), the maximum specific  
methane production rate  $r_{max,M}$  (mmol CH<sub>4</sub>/(g<sub>vs</sub> d)),  $\lambda$ , and the methane production rate  $k$  (1/d).

230 The specific gross energy potential  $\hat{E}_p$  (kJ/g<sub>vs</sub>) was used to compare our *h-H-M* biorefinery against  
other biorefinery models in the literature, either serial where DF is followed by AD (Eq. 7), or in parallel  
232 DF and AD systems (Eq. 8). In these equation, the hydrogen high heating value  $HHV_{H_2}$  is 282.8 kJ/mol,  
the methane high heating value  $HHV_{CH_4}$  is 889.9 kJ/mol,  $V_M$  is the molar volume of an ideal gas at standard  
234 conditions (22.4 L/mol), 1000 is the mL to L conversion factor,  $\eta_{DF}$  is the quotient of VS consumed and  
VS fed in the DF units, and  $\eta_{AH}$  is the quotient of glucose released and VS fed in acid hydrolysis  
236 experiments.

238 INSERT TABLE 2

## 240 **Results and Discussion**

### *Diluted acid hydrolysis and overliming*

242 The RS concentration after co-substrates hydrolysis was 23.49 g/L, containing high amounts of glucose  
(10.36 g/L) followed by xylose (8.61 g/L) and arabinose (0.39 g/L). The RS production yield was 48.54%  
244 (calculated as the amount of RS produced divided by the sum of volatile solids added) for the 3:1 FVW:CS  
combination. This yield is comparable to those reported in the literature for acid hydrolysis of either FVW  
246 or CS. For instance, Datar et al. (2007) obtained a hydrolysis yield of 47% in the steam-explosion  
treatment of acid impregnated CS. Cao et al. (2009) managed to extract the 35.20% of sugars in the CS  
248 acid hydrolysis. On the other hand, Díaz et al. (2017) extracted 35.9% of the reducing sugars available in  
tomato wastes through acid hydrolysis. Additionally, one of the main benefits of the co-substrates acid

250 hydrolysis is the improvement of the monomeric sugars distribution. For instance, in our 3:1 FVW:CS  
experiment the monosaccharide distribution in the liquid hydrolyzates was 53.5% glucose, 44.5% xylose  
252 and 2% arabinose (Table 1). In contrast, the main monomeric sugars distribution from hydrolysis of only  
CS were 9.08% of glucose, 83.08% of xylose and 7.84% of arabinose (Datar et al. 2007), whereas FVW  
254 were only composed of 100% hexoses (Del Campo et al. 2006).

The overliming treatment of acid hydrolyzates successfully reduced the inhibitory compounds as TPC  
256 in 33.86%, although 10.05% RS were also lost (Table 3). RS loss is commonly expected in such treatments  
(Saha et al. 2005), for instance, Chang et al. (2011) reported 9% RS loss after the overliming of rice husk  
258 hydrolyzates, and Purwadi et al. (2004) had 8.42% RS loss from detoxification by  $\text{Ca}(\text{OH})_2$  of Swedish  
forestry residues hydrolyzates. The concentration of TPC, HMF, furfural, and acetic acid, did not surpass  
260 the concentrations known to inhibit hydrogen production, which are in the following ranges: 0.8-2.28 g/L  
for TPC, 0.86-1.89 g/L for HMF, 0.8-3.41 g/L for furfural and 0.6-7.80g/L for acetic acid (Ren et al. 2008;  
262 Gonzales et al. 2016).

264 INSERT TABLE 3

### 266 *Dark fermentation*

After overliming pretreatment, the LH were used for hydrogen production at initial pH of 7 and 35 °C.  
268 After 150 h of fermentation, maximum experimental cumulative biogas and maximum cumulative specific  
hydrogen production were 2717 mL and 223.8 mL  $\text{H}_2/\text{g}_{\text{glucose}}$ , respectively (Fig. 2). The hydrogen average  
270 content in biogas was 50.89%, whereas methane was not detected. According to the Gompertz parameters  
(Table 4), the production of hydrogen from the acid hydrolyzates of the co-substrates mixtures (3:1  
272 FVW:CS) showed a  $r_{\text{max},H}$  of 2.60 (mL  $\text{H}_2/(\text{g}_{\text{glucose}} \text{ h})$ ) and 19.25 h of adaptation time.

$r_{max,H}$  is comparable to other studies presented in literature where hydrogen production from acidic hydrolyzates of organic waste has been studied. Zhang et al. (2015) reported a  $r_{max,H}$  of 0.92 (mL H<sub>2</sub>/(g<sub>glucose</sub> h)) in a 100 mL serum vials with acid hydrolyzates (1.7% v/v H<sub>2</sub>SO<sub>4</sub>, 120 min, 120 °C) from CS produced by activated sludge. The reason for their low  $r_{max,H}$  compared to our study might be attributed to the low initial sugars concentration (5 g/L against 13 g/L, respectively) and the low initial ISR (0.19 against 1.2, respectively), as such parameters are directly related to hydrogenogenic performance (Fan et al. 2006; Ozmihci et al. 2011). In addition, the hydrogen batch fermentation at ISR lower than 0.16 could have presented inhibition due to the high amounts of substrate (Wong et al. 2014).

INSERT FIG. 2

The maximum productivity of hydrogen from LH was 2909 mL H<sub>2</sub>/L<sub>reactor</sub>, which can be compared with other studies also using LH for hydrogen production. In the case of Datar et al. (2007), they reported hydrogen productivity of 3310 mL H<sub>2</sub>/L<sub>reactor</sub> from CS hydrolyzates in a CSTR reactor using a pre-treated anaerobic sludge as an inoculum; the volumetric productivity reported in our work was 12.1% lower than theirs. However, the adaptation time reported by Datar et al. (2007) was 49.5% higher due to the initial concentrations of inhibitors present in their hydrolyzates.

290

INSERT TABLE 4

292

The molar pseudoyield obtained at 35°C was 2.02 mol H<sub>2</sub>/mol<sub>glucose</sub>, comparable to those using pure and mixed cultures from acid hydrolyzates. Biohydrogen production from starch hydrolyzates led to a maximum  $Y_{H_2}$  of 1.28 and 0.85 mol H<sub>2</sub>/mol<sub>glucose</sub> by either *Clostridium pasteurianum* CH5 or by

296 consortium from dark fermentation sludge, respectively (Chen et al. 2008). Both experiments were carried  
out at 37 °C and initial sugars concentration of 26.7 g/L. Using acid hydrolyzates from wheat starch (121  
298 °C, 15 min, pH 2.5, H<sub>2</sub>SO<sub>4</sub>), Cakr et al. (2010) obtained a  $Y'_{H_2}$  of 2.4 mol H<sub>2</sub>/mol<sub>glucose</sub> at 55 °C and initial  
sugars concentration of 18.5 g/L using heat-treated anaerobic sludge. This value was 1.2-fold higher than  
300 ours, however, the use of thermophilic temperatures may decrease the energy gains from biofuels  
production (Rodríguez-Valderrama et al. 2019).

302 The final pH in hydrogen production was 5.34 (Table 4), an adequate value for hydrogen  
production as reported in literature (Braguglia et al. 2018). In our experiments, the pH<sub>0</sub> was adjusted to 7  
304 as commonly done in free fermentation experiments. For instance, Liu et al. (2013) observed a  
considerable hydrogen yield increase from 0.02 mol H<sub>2</sub>/mol<sub>sugars</sub> at pH<sub>0</sub> 5, to 0.44 mol H<sub>2</sub>/mol<sub>sugars</sub> at pH<sub>0</sub>  
306 7 when using rice straw acid hydrolyzates.

The main metabolites in DF were butyric acid (6.13 g/L), acetic acid (1.11 g/L), propionic acid  
308 (0.36 g/L) and a small amount of ethanol (0.07 g/L), which indicates that the fermentation was carried  
out in greatly by butyric acid metabolic pathway (Chen et al. 2006; Wang and Wan 2009). These results  
310 demonstrated the good performance of dark fermentation from acid hydrolyzates obtained from organic  
substrates mixtures by anaerobic sludge.

312

### *Anaerobic digestion*

314 The cumulative methane production and specific methane production as a function of time for different  
ISR are shown in Fig 3. The best results for each parameter were obtained at ISR 2.5 and 4, respectively.  
316 Indeed, all the experiments evaluated with HS presented methane productions and specific methane  
productions higher than the experiments with FVW (control) during the first five days due to the readily

318 biodegradable organic matter in the HS. Still, at the end the control produced as good results as most of  
them due to the RIS used and the good biodegradability of FVW at high digestion times (Li et al. 2011).  
320 The cumulative methane production ( $B_{max}$ ) at the different studied ISR (2-4) was in the range 6.37 to 8.78  
mmol CH<sub>4</sub>. The best results were obtained in the order ISR 2.5>4>3>2>3.5 (Table 5, Fig 3A). On the  
322 other hand, the cumulative specific methane production ( $b_{max}$ ) was in the range 5.84-13.64 mmol CH<sub>4</sub>/g<sub>vs</sub>,  
obtained from highest to lowest in the order ISR 4>3>3.5>2.5>2. This was in agreement to reports in  
324 literature that have ascribed this phenomenon to the fact that a greater inoculum to substrate ratio allows  
a more exhaustive conversion of biomass into methane (Hashimoto 1989).

326 The predicted maximum cumulative methane production ( $B_{max}$ , 8.90 mmol CH<sub>4</sub>) and the lowest  
deviation in respect to the experimental value (1.30%) were presented with the first-order kinetic model  
328 for ISR=2.5, whereas Gompertz prediction had a deviation of 3.36% respect to the measured production.  
The coefficient of determination for this experiment (ISR = 2.5) was 0.99, slightly higher than the obtained  
330 by the Gompertz model (0.99). The maximum methane rates ( $k$ ) for each ISR evaluated were in the range  
of 0.19 to 0.28 (1/d). The maximum  $k$  (0.28 1/d) corresponded to ISR of 2.5, which could have reflected  
332 a positive interaction of a rapid substrate biodegradability due to pretreatment and an adequate inoculum  
load. Similarly, at 2.5 ISR Moset et al. (2015) registered a  $k$  of 0.16 (1/d) in the anaerobic digestion of  
334 corn stover silage (1 year), and 0.08 (1/d) for wheat straw without any previous treatment.

The predicted specific methane productions ( $b_{max}$ ) by the first-order kinetic and Gompertz models  
336 were 14 mmol CH<sub>4</sub>/g<sub>vs</sub> and 13.2 mmol CH<sub>4</sub>/g<sub>vs</sub>, respectively (Table 6). However, the first-order kinetic  
model presented the best fit to the experimental data since its error (2.62%) was lower than the respective  
338 of the Gompertz model (3.07%). These errors are usual when comparing the fits of mathematical models.  
As reported by Zhang et al. (2014) who obtained deviations from 1.5% for the first-order kinetic model



340 and 3.7% for the Gompertz model when they evaluated the anaerobic digestion of dewatered sewage  
sludge at ISR of 1 and 37 °C of fermentation temperature.

342

INSERT FIG. 3

344

INSERT TABLE 5

346

The maximum predicted  $b_{max}$  (14 mmol CH<sub>4</sub>/g<sub>vs</sub> or 342.3 L CH<sub>4</sub>/kg<sub>vs</sub>) is comparable to the maximum  
348 methane specific productions in literature, only 8% lower than the value of 372.4 L CH<sub>4</sub> /kg<sub>vs</sub>, from  
alkaline pretreated (5% NaOH) CS on solid-state (Zhu et al. (2010). This difference may be mainly due  
350 to the positive traits of alkaline treatment and its less severity compared to acid treatments. Besides lignin  
solubilization, alkaline pretreatments also provide neutralization of the different acids in lignocellulosic  
352 compounds degradation. However, the maximum methane production rate (9.3 L CH<sub>4</sub>/(kg<sub>vs</sub> d)) found by  
Zhu et al. (2010) after 40 d, was 6.2 fold lower than that obtained from HS during 15 d (2.35 mmol  
354 CH<sub>4</sub>/(g<sub>vs</sub> d); 57.4 L CH<sub>4</sub>/(kg<sub>vs</sub> d)), which indicates the rapid substrate degradation due to the acid  
pretreatment process.

356 The  $b_{max}$  and the  $r_{max,M}$  obtained in the control experiment with fresh untreated FVW were 10.01 mmol  
CH<sub>4</sub>/g<sub>vs</sub> (244.5 L CH<sub>4</sub>/ kg<sub>vs</sub>) and 1.29 mmol CH<sub>4</sub>/ (g<sub>vs</sub> d) (31.6 L CH<sub>4</sub>/(kg<sub>vs</sub> d)), respectively. This  $b_{max}$  was  
358 28.63% lower than that obtained with HS (342.3 L CH<sub>4</sub>/kg<sub>vs</sub>), because when pretreating a substrate, either  
by alkaline pretreatment or acidic pretreatment, the methane production is raised, since soluble organic  
360 matter was increased and then easily used by microorganisms (Abudi et al. 2016). The increase in methane  
production (28.63%) due to substrate pretreatment was also observed in the literature. For instance, Abudi  
362 et al. (2016) registered the increment of 6.69% in the methane production from rice straw without

pretreatment and 45.15% from alkali rice straw organic fraction of municipal solid wastes (OFMSW). On  
364 the other hand, both substrates (rice straw and pretreated rice straw) showed higher methane production  
when compared to OFMSW digestion, but this phenomenon seems to be related to the nature of the  
366 substrate rather than to the pretreatment applied.

368 INSERT TABLE 6

370 *Energy evaluation of the h-H-M concept biorefinery*

The energy yield from *h-H-M* biorefinery model and its comparison with two-stage systems from different  
372 biomasses as substrates are shown in Table 7. The energy yield for *h-H-M* biorefinery based on gaseous  
biofuels production was 9.7 kJ/g<sub>vs</sub>. The stage with the greatest contribution to the energy yield was the  
374 methane production (*M*) stage with a contribution near to 89.4% (8.7 kJ/g<sub>vs</sub>), whereas the contribution of  
hydrogen produced in dark fermentation (*H*) was the remaining 10.6% (1.0 kJ/g<sub>vs</sub>). Ghimire et al. (2015)  
376 calculated a maximum energy yield of 4.4 kJ/g<sub>vs</sub> for hydrogen and methane production from food waste.  
Energy contribution of DF was 29.6% (1.3 kJ/g<sub>vs</sub>) when the food wastes were fermented in a 1.5 L semi-  
378 continuous reactor at 55°C, whereas the maximum energy yield contribution was presented by AD (70.4%,  
3.1 kJ/g<sub>vs</sub>). The relatively low energy contribution of DF is ascribed to the inherent energy loss in the form  
380 of metabolites in the effluents (Xia et al. 2013).

382 INSERT TABLE 7

## **Conclusions**

384 This work demonstrated that a new biorefinery approach coined as *h-H-M*, was technically feasible for  
treating a preparation of fruits and vegetables wastes and corn stover through acid hydrolysis, dark

386 fermentation and anaerobic digestion. Acid hydrolysis of the preparation 3:1 fruit and vegetable wastes to  
corn stover produced hydrolyzates containing up to 10.4 g/L of glucose, 8.61 g/L of xylose and 0.39 g/L  
388 of arabinose.

Dark fermentation from liquid hydrolyzates performed competitively at inoculum to substrate ratio 1.2,  
390 reaching productivity of 2909.7 mL H<sub>2</sub>/L<sub>reactor</sub> and pseudoyield 2.02 mol H<sub>2</sub>/mol<sub>glucose</sub>.

The hydrolyzed solids were adequately used as carbon source for anaerobic digestion, promoting higher  
392 initial methane production when compared to compared to fresh substrate. The maximum cumulative  
methane production, 8.9 mmol CH<sub>4</sub>, and the highest methane specific production, 13.6 mmol CH<sub>4</sub>/g<sub>vs</sub>,  
394 were obtained at the inoculum to substrate ratios of 2.5 and 4, respectively.

The total energy potential from the *h-H-M* biorefinery concept in the form of hydrogen and methane  
396 reached 9.7 kJ/g<sub>vs</sub>, of which hydrogen contributed to 10.6 %.

Using a combination of co-substrates such as FVW and CS opens up the possibility of interesting  
398 configurations for real biorefineries, as this approach may provide an alternative to mono-substrate  
drawbacks.

400

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576

### Abbreviations

- 578 AD Anaerobic digestion
- $B(t)$  Cumulative methane production (mmol CH<sub>4</sub>)
- 580  $B_{max}$  Maximum cumulative methane production (mmol CH<sub>4</sub>)
- $b(t)$  Cumulative specific methane production (mmol CH<sub>4</sub>/g<sub>vs</sub>)
- 582  $b_{max}$  Maximum cumulative specific methane production (mmol CH<sub>4</sub>/g<sub>vs</sub>)
- CS Corn stover
- 584  $C_{RS,0}$  Initial reducing sugars concentration (g/L)
- $C_{RS,f}$  Final reducing sugars concentration (g/L)
- 586 DF Dark fermentation
- $e$  Euler number (2.718)
- 588  $\hat{E}_p$  Specific gross energy potential (kJ/g<sub>vs</sub>)
- FVW Fruits and vegetables wastes
- 590  $h$  Diluted acid pretreatment stage
- $H$  Hydrogen production stage by DF
- 592  $H(t)$  Cumulative hydrogen specific production (mL H<sub>2</sub>/g<sub>glucose</sub>)
- $H_{max}$  Maximum cumulative specific hydrogen production (mL H<sub>2</sub>/g<sub>glucose</sub>)
- 594  $h$ - $H$ - $M$  Biorefinery model
- $HHV_{H_2}$  High hydrogen heating value (282.8 kJ/mol)
- 596  $HHV_{CH_4}$  High methane heating value (889.9 kJ/mol)
- HMF 5-hydroxymethylfurfural
- 598 HS Hydrolyzed solids
- ISR Inoculum to substrate ratio
- 600  $k$  Methane production rate (1/d)
- LH Liquid hydrolyzates
- 602  $M$  Methane production stage by AD
- $MW_{glucose}$  Glucose molar weight (180.16 g/mol)
- 604 ND Not determined
- pH<sub>0</sub> Initial pH
- 606  $r_{max,M}$  Maximum specific methane production rate (mmol/(g<sub>vs</sub> d))
- $r_{max,H}$  Maximum specific hydrogen production rate (mL/(g<sub>glucose</sub> h)),
- 608  $R_{max,M}$  Maximum methane production rate (mmol/d)
- $R^2$  Coefficient of determination

610	RS	Reducing sugars
	T	Operational temperature
612	$t$	Time
	TPC	Total phenolic compounds
614	TS	Total solids
	TVFA	Total volatile fatty acids
616	$V_M$	Molar volume at standard conditions (22.4 L/mol H <sub>2</sub> or CH <sub>4</sub> )
	$V_O$	Operational volume
618	VS	Volatile solids
	$Y'_{H_2}$	Hydrogen molar pseudoyield (mol H <sub>2</sub> /mol <sub>glucose</sub> )
620		

622 **Subindices**

	db	dry basis
624	wb	wet basis

626 **Greek characters**

628	$\lambda$	adaptation time (h or d)
	$\eta_{AH}$	Acid hydrolysis efficiency (g <sub>glucose</sub> /g <sub>vs</sub> )
630	$\eta_{DF}$	Dark fermentation efficiency (g <sub>vs</sub> consumed/g <sub>vs</sub> added)



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652 **Table 1** Characteristic of co-substrates (FVW and CS) and HS

Parameter	FVW	CS	HS
pH	5.52	7.54	3.66
Moisture (%wb)	89.81	5.41	84.00
TS (%wb)	10.19	94.59	16.00
VS (%db)	87.66	89.78	93.43
Ashes (%db)	12.34	10.22	6.57
Alkalinity (mg CaCO <sub>3</sub> /L)	475	450	200
Total Volatile fatty acids (mg VFA/L)	570	420	220
Cellulose (%db)	12.80	33.25	24.86
Hemicellulose (%db)	24.40	24.35	17.81
Lignin (%db)	10.26	24.74	16.29
Protein (%db)	12.63	3.25	12.14
Extractives (%db)	38.11	10.19	ND
C (%db)	51.69	43.84	56.19
H (%db)	3.43	15.74	10.74
O (%db)	42.69	39.98	31.15
N (%db)	2.19	0.44	1.92

654 Abbreviations: CS, corn stover; db, dry base; FVW, fruits and vegetables wastes; ND, not determined; TS, total solids; VFA, volatile fatty acids; VS, volatiles solids; wb, wet base.

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**Table 2** Equations used to describe the  $H$  and  $M$  stages and the biorefinery performance

Response variable	Equation	Equation number
Cumulative hydrogen specific production	$H(t) = H_{\max} \cdot \exp \left\{ -\exp \left[ \frac{R_{\max, H} \cdot e}{H_{\max}} (\lambda - t) + 1 \right] \right\}$	(1)
Hydrogen molar pseudoyield	$Y'_{H_2} = \frac{H_{\max} \cdot C_{RS,0} \cdot (MW_{glucose})}{(C_{RS,0} - C_{RS,f}) \cdot (V_M) \cdot 1000}$	(2)
Cumulative methane production (Gompertz-modified model)	$B(t) = B_{\max} \cdot \exp \left\{ -\exp \left[ \frac{R_{\max, M} \cdot e}{B_0} (\lambda - t) + 1 \right] \right\}$	(3)
Cumulative specific methane production (Gompertz-modified model)	$b(t) = b_{\max} \cdot \exp \left\{ -\exp \left[ \frac{r_{\max, M} \cdot e}{b_0} (\lambda - t) + 1 \right] \right\}$	(4)
Cumulative methane production (first-order kinetic model)	$B(t) = B_{\max} \cdot (1 - \exp^{-k \cdot t})$	(5)
Cumulative specific methane production (first-order kinetic model)	$b(t) = b_{\max} \cdot (1 - \exp^{-k \cdot t})$	(6)
Specific gross energy potential for serial hydrogen and methane production	$\hat{E}_P = \frac{1}{V_M \cdot 1000} \cdot (H_{\max} \cdot HHV_{H_2} + (1 - \eta_{DF}) \cdot b_{\max} \cdot HHV_{CH_4})$	(7)
Specific gross energy potential for parallel hydrogen and methane production	$\hat{E}_P = \frac{1}{V_M \cdot 1000} \cdot (\eta_{AH} \cdot H_{\max} \cdot HHV_{H_2} + (1 - \eta_{AH}) \cdot b_{\max} \cdot HHV_{CH_4})$	(8)

662 **Table 3** Main sugar production and secondary products from acid hydrolysis pretreatment of co-substrates (3:1 FVW:CS)

Parameter	LH before overliming	LH after overliming
RS (g/L)	23.49	21.13
Glucose (g/L)	10.36	9.65
Xylose (g/L)	8.61	8.36
Arabinose (g/L)	0.39	0.07
HMF (g/L)	0.65	ND
Furfural (g/L)	0.14	ND
TPC (g/L)	1.14	0.76
Formic acid (g/L)	4.02	ND
Acetic acid (g/L)	0.53	ND

664 Abbreviations: HMF, 5-hydroxymethylfurfural; ND, not detected; LH, liquid hydrolyzates; RS, reducing sugars; TPC, total phenolic compounds.

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**Table 4** Gompertz coefficients and performance parameters from dark fermentation of LH

Parameter	Value
$H_{max}$ (mL H <sub>2</sub> /g <sub>glucose</sub> )	223.8 ± 5.2
$R_{max,H}$ (mL H <sub>2</sub> /(g <sub>glucose</sub> h))	2.60 ± 0.08
$\lambda$ (h)	19.25 ± 0.52
R <sup>2</sup>	0.99
Final pH	5.34 ± 0.60
Acetic acid (g/L)	1.11 ± 0.007
Propionic acid (g/L)	0.36 ± 0.011
Butyric acid (g/L)	6.13 ± 0.265
Ethanol (g/L)	0.07 ± 0.003
Sugars consumption (%)	89.30 ± 0.4
Volumetric productivity (mL H <sub>2</sub> /L <sub>reactor</sub> )	2909 ± 67.8
$Y'_{H_2}$ (mol H <sub>2</sub> /mol <sub>glucose</sub> )	2.02 ± 0.05

670 Abbreviations:  $H_{max}$ , maximum cumulative specific hydrogen production;  $R_{max,H}$ , maximum specific hydrogen production rate; R<sup>2</sup>, coefficient of determination;  $Y'_{H_2}$ , hydrogen molar pseudoyield;  $\lambda$ , adaptation time.

672 **Table 5** Kinetic parameters comparison between the first-order model and Gompertz model on the  
 maximum cumulative methane production ( $B_{max}$ ) during anaerobic digestion of HS and FVW

Model	ISR-substrate type					
	2-HS	2.5-HS	3-HS	3.5-HS	4-HS	3-FVW
Experimental $B_{max}$ (mmol CH <sub>4</sub> )	7.59	8.78	7.70	6.37	8.19	7.74
<i>First-order</i>						
$B_{max}$ (mmol CH <sub>4</sub> )	7.85	8.89	8.09	6.84	8.40	NA
$k$ (1/d)	0.23	0.28	0.23	0.19	0.25	NA
R <sup>2</sup>	0.99	0.99	0.99	0.99	0.99	NA
Error (%)	3.47	1.29	5.09	7.36	2.62	NA
<i>Gompertz</i>						
$B_{max}$ (mmol CH <sub>4</sub> )	7.39	8.49	7.69	6.32	7.93	8.01
$R_{max,M}$ (mmol CH <sub>4</sub> /d)	1.18	1.67	1.14	0.82	1.41	1.03
$\lambda$ (d)	0.00	0.00	0.00	0.00	0.00	1.46
R <sup>2</sup>	0.99	0.99	0.99	0.99	0.99	0.99
Error (%)	2.61	3.36	0.11	0.87	3.07	3.49

674 Abbreviations:  $B_{max}$ , maximum cumulative methane production;  $k$ , methane production rate; HS, hydrolyzed solids; FVW, fruits  
 676 and vegetables wastes; NA, not applicable;  $R_{max,M}$ , maximum methane production rate; R<sup>2</sup>, coefficient of determination;  $\lambda$ ,  
 adaptation time.

Note: range of final pH values 7.53-7.72.

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680 **Table 6** Kinetic parameters comparison between the first-order model and Gompertz model on the  
 maximum cumulative specific methane production ( $b_{max}$ ) during anaerobic digestion of HS and FVW

Model	ISR-substrate					
	2-HS	2.5-HS	3-HS	3.5-HS	4-HS	3-FVW
Experimental $b_{max}$ (mmol CH <sub>4</sub> /g <sub>VS</sub> )	5.84	8.78	9.63	9.10	13.64	9.67
<i>First-order</i>						
$b_{max}$ (mmol CH <sub>4</sub> / g <sub>VS</sub> )	6.04	8.89	10.12	9.77	14.00	NA
$k$ (1/d)	0.23	0.28	0.23	0.19	0.25	NA
R <sup>2</sup>	0.99	0.99	0.99	0.99	0.99	NA
Error (%)	3.47	1.29	5.09	7.36	2.62	NA
<i>Gompertz</i>						
$b_{max}$ (mmol CH <sub>4</sub> / g <sub>VS</sub> )	5.68	8.49	9.62	9.02	13.22	10.01
$r_{max,M}$ (mmol CH <sub>4</sub> / (g <sub>VS</sub> d))	0.90	1.67	1.43	1.18	2.35	1.29
$\lambda$	0.00	0.00	0.00	0.00	0.00	1.46
R <sup>2</sup>	0.99	0.99	0.99	0.99	0.99	0.99
Error (%)	2.61	3.36	0.11	0.87	3.07	1.46

682 Abbreviations:  $b_{max}$ , maximum cumulative specific methane production;  $k$ , methane production rate; HS, hydrolyzed solids;  
 684 FVW, fruits and vegetables wastes; NA, not applicable;  $r_{max,M}$ , maximum specific methane production rate; R<sup>2</sup>, coefficient of  
 determination;  $\lambda$ , adaptation time.

Note: range of final pH values 7.53-7.72.

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**Table 7** Biorefinery process conditions and energy yields

Substrate	Substrate pretreatment	Process	Dark fermentation	Anaerobic digestion	$\hat{E}_p$ (kJ/g <sub>VS</sub> )	Reference
Rapeseed straw	Alkaline peroxide (3% v/v) and steam (18 °C, 10 min)	Serial two-stage: DF (batch) + AD (batch)	V <sub>0</sub> =60 mL T=55 °C pH <sub>0</sub> =5.5 H <sub>max</sub> =91 mL H <sub>2</sub> /g <sub>VS</sub>	V <sub>0</sub> =60 mL T= 55 °C pH <sub>0</sub> =7.5 b <sub>max</sub> =370 mL CH <sub>4</sub> /g <sub>VS</sub>	13.2	Luo et al. (2011)
<i>Nannochloropsis oceanica</i>	Microwave (140 °C, 15 min, 1.0% H <sub>2</sub> SO <sub>4</sub> , 50 g/l)	Serial two-stage: DF (batch) + AD (batch)	V <sub>0</sub> =250 mL T=35 °C pH <sub>0</sub> =6.0 H <sub>max</sub> = 39 mL H <sub>2</sub> /g <sub>VS</sub>	V <sub>0</sub> =250 mL T= 35 °C pH <sub>0</sub> =8.0 b <sub>max</sub> = 114 mL CH <sub>4</sub> /g <sub>VS</sub>	4.7	Xia et al. (2013)
Food waste	NA	Serial DF (semi-continuous) and AD (batch)	V <sub>0</sub> = 1500 mL T= 55 °C pH <sub>0</sub> = 7.0 H <sub>max</sub> =104 mL H <sub>2</sub> /g <sub>VS</sub>	V <sub>0</sub> = 600 mL T= 34 °C pH <sub>0</sub> = 7 b <sub>max</sub> = 99.3 mL CH <sub>4</sub> /g <sub>VS</sub>	4.4	Ghimire et al. (2015)
Organic fraction of municipal solid wastes	NA	Serial two-stage DF (semi-continuous) + AD (semi-continuous)	V <sub>0</sub> = 500 g T= 55 °C pH <sub>0</sub> = 8.0 H <sub>max</sub> = 33.3 mL H <sub>2</sub> /g <sub>VS</sub>	V <sub>0</sub> = 500 g T= 55 °C pH <sub>0</sub> = 8.0 b <sub>max</sub> = 353 mL CH <sub>4</sub> /g <sub>VS</sub>	13.5	Escamilla-Alvarado et al. (2014)
Sugarcane bagasse and water hyacinth (1:2 ratio)	Alkaline peroxide (2.5% v/v, 50°C, 150 min) and enzymatic hydrolysis ( <i>Cellulase</i> , 2 U/g <sub>db</sub> , 37 °C, 180 rpm, 48 h)	Serial two-stage DF (continuous) + AD (continuous)	V <sub>0</sub> = 500 mL T= 37 °C pH <sub>0</sub> =6.5 H <sub>max</sub> = 303 mL H <sub>2</sub> /g <sub>VS</sub>	V <sub>0</sub> = 4000 mL T= 37 °C pH <sub>0</sub> = 7.5 b <sub>max</sub> = 142 mL CH <sub>4</sub> /g <sub>VS</sub>	6.0	Kumari and Das (2019)
Water hyacinth	NA	Serial two-stage DF (semi-continuous) + AD (semi-continuous)	V <sub>0</sub> = 500 mL T= 37 °C pH <sub>0</sub> = 6.5 H <sub>max</sub> = 93 mL H <sub>2</sub> /g <sub>VS</sub>	V <sub>0</sub> = 500 mL T= 37 °C pH <sub>0</sub> = 7.5 b <sub>max</sub> = 270.5 mL CH <sub>4</sub> /g <sub>VS</sub>	2.6	Varanasi et al. (2018)
Corn stover	Steam-explosion (220 °C, 3 min) water-impregnated)	Parallel DF	V <sub>0</sub> = 1250 mL T= 35 °C pH <sub>0</sub> = 5.5 H <sub>max</sub> = 304.3 mL H <sub>2</sub> /g <sub>VS</sub>	V <sub>0</sub> = 1250 mL T= 35 °C pH <sub>0</sub> = 5.5 H <sub>max</sub> = 152.3 mL H <sub>2</sub> /g <sub>VS</sub>	2.24	Datar et al. (2007)
Organic wastes (FVW-40%, WAS-40%, OMW-10%, CM-10%)	NA	Serial two-stage DF (continuous) and AD (continuous)	V <sub>0</sub> = 800 mL T= 37 °C pH <sub>0</sub> = 5.2 H <sub>max</sub> = 79.4 mL H <sub>2</sub> /g <sub>VS</sub>	V <sub>0</sub> = 1200 mL T= 37 °C pH <sub>0</sub> = 7.2 b <sub>max</sub> = 410 mL H <sub>2</sub> /g <sub>VS</sub>	14.69	Farhat et al. (2018)
Preparation of FVW:CS 3:1	Acid hydrolysis (0.5% HCl, 120 °C, 120 min)	Parallel DF and AD	V <sub>0</sub> = 400 mL T= 35°C pH <sub>0</sub> =7.0 H <sub>max</sub> = 223.8 mL H <sub>2</sub> /g <sub>VS</sub>	V <sub>0</sub> = 80 mL T= 35 °C pH <sub>0</sub> = 7.6 b <sub>max</sub> = 342.3 mL CH <sub>4</sub> /g <sub>VS</sub>	9.7	This work



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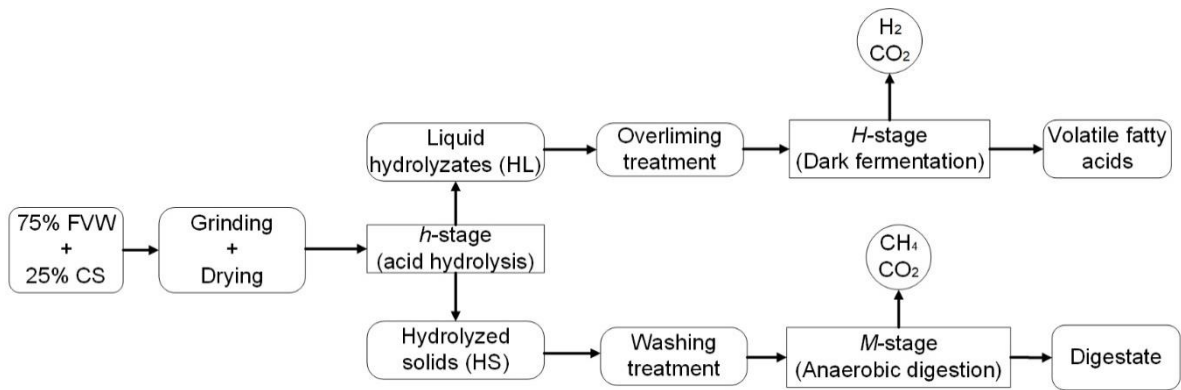
692 Abbreviations: AD, anaerobic digestion;  $b_{max}$ , Maximum cumulative specific methane production; DF, dark fermentation;  
694 CM, cattle manure NA, not applicable; FVW, fruit and vegetable waste;  $H_{max}$ , maximum cumulative specific hydrogen  
production; OMW, olive mill wastewater;  $pH_0$ , initial pH; T, operational temperature;  $V_0$ , operational volume; WAS, waste-  
activated sludge.

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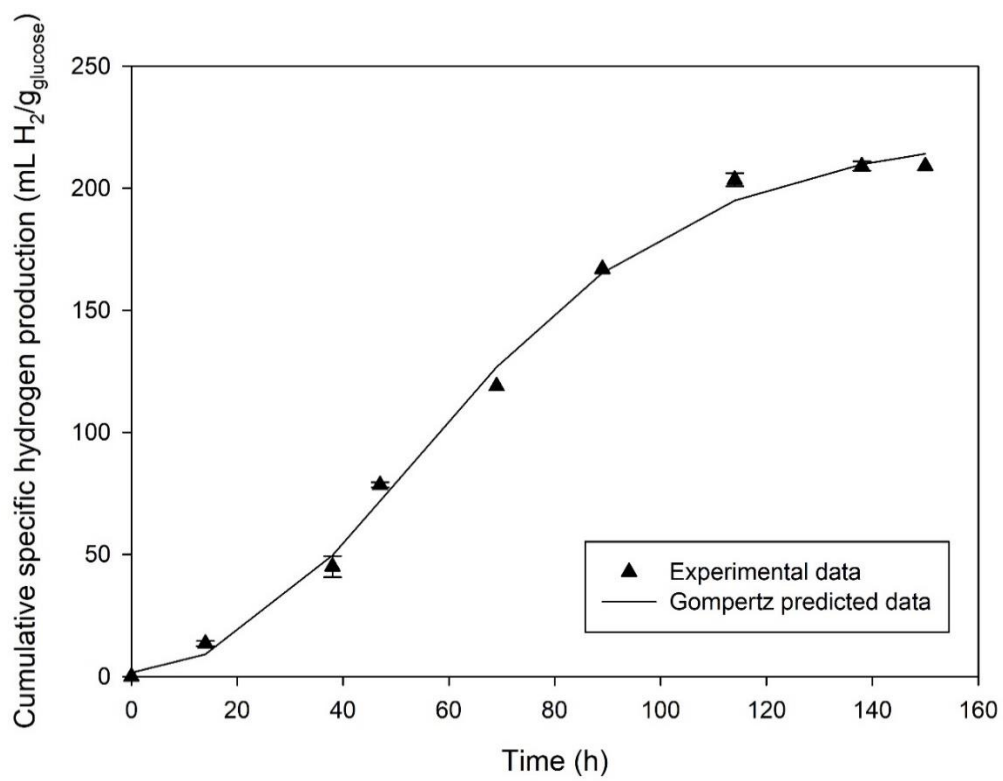
**Fig. 1** The *h-H-M* biorefinery concept

**Fig. 2** Cumulative specific hydrogen production using LH as substrate

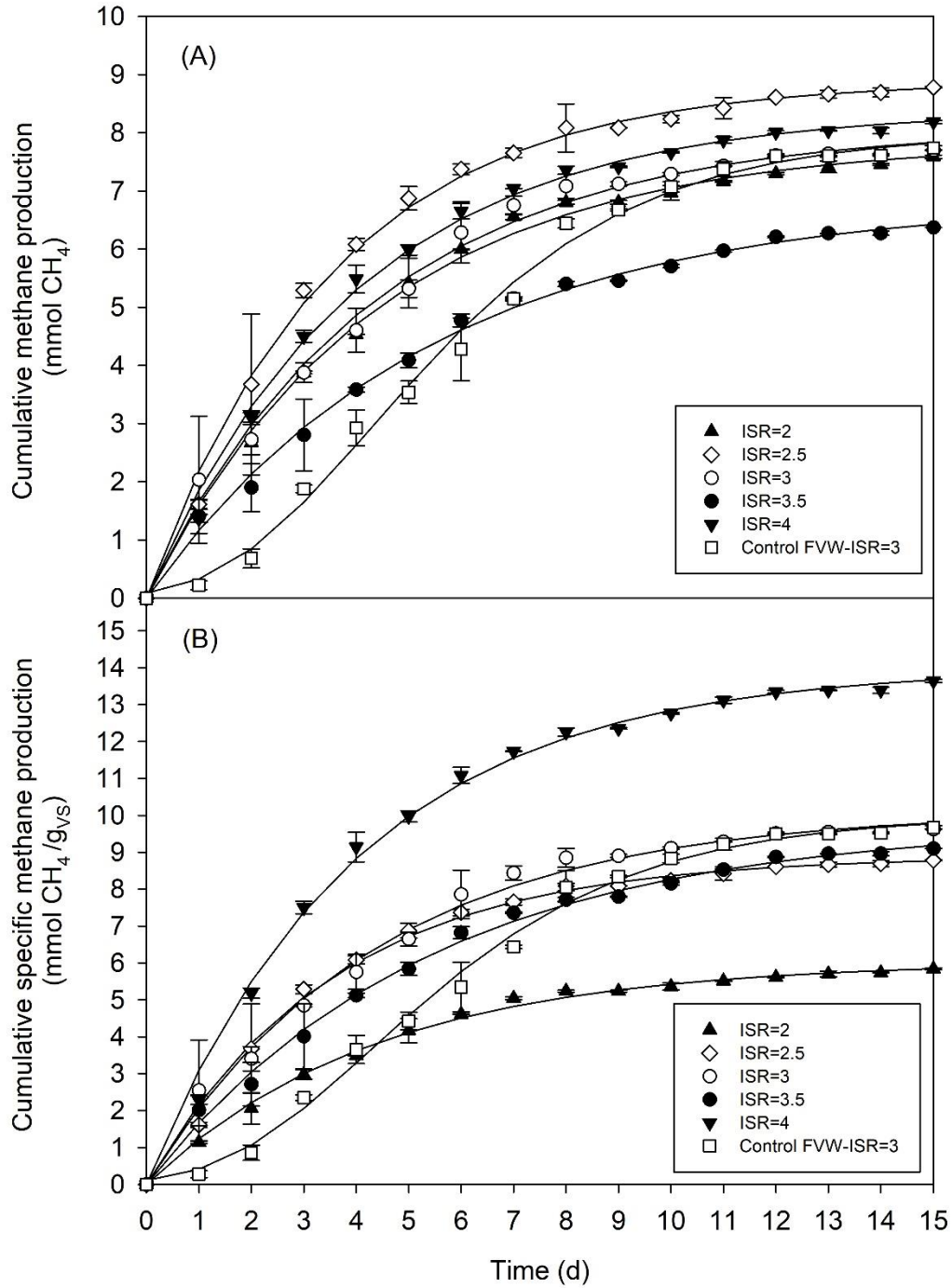
**Fig. 3** Anaerobic digestion performance using HS or FVW as substrate in terms of (A) cumulative methane production and (B) cumulative specific methane production



**Fig. 1** The *h-H-M* biorefinery concept



**Fig. 2** Cumulative specific hydrogen production using LH as substrate



**Fig. 3** Anaerobic digestion performance using HS or FVW as substrate in terms of (A) cumulative methane production and (B) cumulative specific methane production

The prediction lines for ISR 2 to 4 correspond to the first order model;

The prediction line for control corresponds to the Gompertz model.