

# Influence of the pH control strategy and reactor volume on batch fermentative hydrogen production from the organic fraction of municipal solid waste

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## Abstract

Three different experimental sets of runs involving batch fermentation assays were performed to evaluate the influence of the experimental conditions on biological hydrogen production from the source-separated organic fraction of municipal solid waste collected through a door-to-door system. The fermentation process was operated with and without automatic pH control, at a pH of 5.5 and 6.5, food-to-microorganism ratios of 1/3 and 1/1 (wet weight basis) and with different working volumes (0.5 and 3 L). The experimental results showed that the pH control strategy and the reactor volume did not affect the final hydrogen production yield but played an important role in determining the time evolution of the process. Indeed, although the different experimental conditions tested yielded comparable hydrogen productions (with maximum average values ranging from 68.5 to 88.5 NLH<sub>2</sub> (kgTVS<sub>OF</sub>)<sup>-1</sup>), the automatic pH control strategy improved the process from the kinetic viewpoint resulting in a  $t_{95}$  reduction from an average of 34.9 h without automatic pH control to an average of 19.5 h.

## Keywords

Batch fermentation assays, biochemical hydrogen production, food-to-microorganism ratio, organic fraction of municipal solid waste, pH

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## Introduction

Fermentative bio-hydrogen production is currently regarded as a key topic due to its potential benefits on both the energy balance and the environmental profile of the whole process (Zumar Bundhoo and Mohee, 2016). Such potential benefits are further enhanced if hydrogen is produced from biodegradable wastes (Cappai et al., 2014). Among the substrates tested for hydrogen production (Ghimire et al., 2015), the organic fraction of municipal solid waste (OFMSW) appears to be a promising feedstock due to its biodegradability characteristics as well as wide availability (Cappai et al., 2014; De Gioannis et al., 2013).

In order to get a preliminary indication about the viability of fermentative hydrogen production, batch tests are widely used in the literature, thanks to their easiness as well as reduced duration and cost compared to continuous experiments (Akhlaghi et al., 2017; Alibardi and Cossu, 2015, 2016; Cappai et al., 2014; Chinellato et al., 2013; Giordano et al., 2011; Lavagnolo et al., 2018). Despite this, biochemical hydrogen potential (BHP) tests still lack a standard reference procedure, leading to a wide variety of set-up conditions (pH control strategy, operating pH, inoculum/substrate ratio, reactor volume, etc.) having been adopted

so far. Heterogeneities in the testing conditions can, however, impair the comparability of results.

pH is recognized to be a crucial parameter for the fermentation process. Hydrogen production is maximized at operating pH values from 5 up to 6.5 (Cappai et al., 2014; De Gioannis et al., 2014; Moon et al., 2015). Under these conditions the acetate and butyrate pathways, which are commonly associated with high hydrogen production yields, are predominant. Conversely, strongly acidic or basic pHs negatively affect the activity of hydrogen-producing bacteria, since ATP would be used to ensure cell neutrality rather than to produce hydrogen (Nazlina et al., 2011). At values below 5, the hydrogenase activity is inhibited

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and non-hydrogen producing pathways, including solventogenesis and lactate production, take over (Micolucci et al., 2014; Nazlina et al., 2011). As a result, maintaining pH within the suitable range for hydrogen production is crucial. Several studies have focused on the effect of the initial pH of the feeding mixture, and in most cases pH is adjusted using NaOH or HCl with no further control during the test (Giordano et al., 2011; Ramos et al., 2012; Zhou et al., 2013). The major drawback is the pH decrease caused by the acidogenic reactions that may lead to the inhibition of the hydrogenase activity (Bao et al., 2013; Giordano et al., 2011; Ramos et al., 2012; Xiao et al., 2013; Zhou et al., 2013). Bao et al. (2013) and Xiao et al. (2013), who adopted initial pHs of 7 and 8, respectively, observed an inhibition of hydrogen production due to an excessive acidification of the system. Similar constraints were observed by Argun et al. (2008), who adopted a pH adjustment strategy through the intermittent addition of NaOH. Recent studies have adopted a pH control method based on the addition of buffers or alkaline solutions, including 2-(N-morpholino)ethanesulfonic acid (MES) (Alibardi and Cossu, 2015, 2016; Favaro et al., 2013; Lavagnolo et al., 2018), and phosphate (Favaro et al., 2013) or carbonate (Lavagnolo et al., 2018) solutions. Other investigators (Akhlaghi et al., 2017; Cappai et al., 2014; De Gioannis et al., 2017) adopted a continuous pH control strategy through the automatic addition of an alkaline (NaOH) solution. The pH control method adopted is expected to affect the hydrogen production yield, as even relatively small pH fluctuations during the process are recognized to influence the activity of the hydrogenogenic biomass.

The relative amount of substrate and inoculum is another key parameter in batch fermentative assays, which is commonly expressed through the food-to-microorganisms (F/M) ratio. A microbial culture can shift from substrate-limited to substrate-sufficient growth depending on the relative availability of substrate and biomass, thus affecting the production of hydrogen. Differently, operation at high substrate loads involves an accumulation of volatile fatty acids (VFAs) that can lead to the abovementioned inhibition of hydrogenase activity (Micolucci et al., 2014).

As far as the system volume is concerned, the size of the reaction system and the working volume/total volume ratio are operationally relevant parameters, due to the need to ensure sample representativeness in the case of heterogeneous substrates, to guarantee thorough and uniform mixing, as well as to set the required headspace inside the reactor. The suggested reactor volumes for biogas production potential estimation range from 100 mL to 2 L (Angelidaki et al., 2009), with a recommended headspace volume of ~10–30% (depending on the biogas withdrawal frequency) of the total volume (Pagga and Beimborn, 1993). BHP tests are usually performed at the laboratory scale, commonly adopting total volumes of 1 to 3 L (Akhlaghi et al., 2017; Alibardi and Cossu, 2015, 2016; Argun et al., 2008; Cappai et al., 2014; Chinellato et al., 2013; De Gioannis et al., 2017; Ghimire et al., 2015; Giordano et al., 2011; Lavagnolo et al., 2018) with working volumes typically as large as a half of the total volume. Some authors (Angelidaki

et al., 2009; Raposo et al., 2011) suggested that the required working volume is a function of the nature of the substrate, with more homogeneous materials in principle requiring smaller reactor volumes to derive an accurate estimation of the biogas production potential. The biogas production potential is reported not to be affected by the working volume (Raposo et al., 2011) provided that homogeneity is adequately guaranteed inside the reactor. Some authors, however, (Pagga and Beimborn, 1993; Qamaruz Zaman, 2010) also documented that the reproducibility and repeatability of results improve for larger working volumes and smaller headspace volumes.

In the present study, biological hydrogen production from OFMSW was evaluated using three different experimental setups based on BHP methods adopted in previous research (Akhlaghi et al., 2017; Alibardi and Cossu, 2015, 2016; Cappai et al., 2014; De Gioannis et al., 2017; Favaro et al., 2013; Lavagnolo et al., 2018). To the authors' knowledge, this is the first time that different biochemical hydrogen potential test setups are compared in terms of hydrogen yields and kinetics.

## Materials and methods

### *Substrate and inoculum*

Food wastes (300 kg) were manually sorted and homogenized from source-separated OFMSW collected in Tuscany (Italy) by means of a door-to-door system. The total solids (TS) content of the homogeneous sample, hereinafter referred to as OF, was then adjusted by adding tap water to a TS content of approximately 5% by weight.

Activated sludge (AS) collected from the aerobic unit of a municipal wastewater treatment plant was used as the inoculum. The use of the aerobic inoculum was preferred over the anaerobic one in order to prevent the presence of a potentially methanogenic biomass in the system. In accordance with previous studies (Alibardi and Cossu, 2016; Cappai et al., 2014; Li and Fang, 2007), in order to harvest the hydrogen-producing biomass, AS was heat-shocked at 105°C for 30 minutes before the start of each experiment.

The characteristics of OF and AS in terms of TS, total volatile solids (TVS), total organic carbon (TOC) and pH (see Table 1) were determined according to standard methods (American Public Health Association (APHA), 2006).

### *Experimental set-up*

The experimental design was planned in order to study the influence of the set-up on hydrogen production by varying the operating pH values, the pH control strategy, the F/M ratios and the reactor volume. For the sake of comparison of the test results, the working volume/total volume ratio was maintained the same throughout all of the experiments.

The selected set-ups were operated using different pH control systems and different volumes as follows:

- (i) Laboratory-scale 1 L reactors operated by setting the initial pH with a MES buffer solution described by Alibardi and Cossu (2015, 2016), Favaro et al. (2013) and Lavagnolo et al. (2018);
- (ii) Laboratory-scale 1 L reactors equipped with an automatic NaOH dosing system as described by Akhlaghi et al. (2017), Cappai et al. (2014) and De Gioannis et al. (2017);
- (iii) Pilot-scale 6 L reactors equipped with an automatic NaOH dosing system based on the method described by Pecorini et al. (2018).

Each experimental configuration was tested at a pH of 5.5 and 6.5 and F/M ratios of 1/3 and 1/1 (wet weight basis), corresponding to 1.33 and 4.00 gVS<sub>(OF)</sub> gVS<sub>(AS)</sub><sup>-1</sup>. Before the onset of the experiments, the reactors were flushed with N<sub>2</sub> gas to drive off air from the reactor headspace. All of the experiments were performed in duplicate under mesophilic conditions (38.0°C ± 1.0°C) and were stopped once biogas production was no longer detected.

A summary of the experimental runs performed is provided in Table 2.

*BHP tests without automatic pH control – 1 l (BHP1).* The first set of experiments, BHP1, involved pH control by initially adding 2.5 M HCl to set the initial pH at the desired value, along with 50 mL of 0.5 M MES (VWR, Italy) buffer solution. The same test conducted with larger additions of the buffer solution

**Table 1.** Organic fraction of municipal waste and inoculum characteristics. Values are expressed as average values and related standard deviation.

	TS (%)	TVS/TS (%)	pH	TOC (gC l <sup>-1</sup> )
Organic fraction	5.4 ± 0.3	91.5 ± 0.3	3.8 ± 0.0	23.4 ± 0.8
Activated sludge	1.7 ± 0.4	76.6 ± 3.2	7.8 ± 0.0	-

resulted in an inhibition of the hydrogenogenic process (data not shown), so that 50 mL was considered to be the threshold for practicable buffer application to the system. The tests were conducted using 1 L (0.5 L working volume) stainless-steel batch reactors tightly closed by a lid provided with a ball valve to enable gas sampling (Pecorini et al., 2016). The vessels were placed on a hotplate magnetic stirrer and incubated in a water jacket. Gas production was periodically estimated by measuring the pressure evolution in the headspace of each reactor and then converting it to a gas volume by means of the ideal gas law. Pressure was measured using a membrane pressure gauge (HD 2304.0, Delta Ohm S.r.l., Italy).

The hydrogen content of the gas was measured by using a gas micro-chromatograph equipped with thermal conductivity detectors (3000 Micro GC, INFICON, Switzerland). A Molsieve column (30 µm/20 µm/10 m) was used for the analysis of hydrogen, oxygen, nitrogen and methane. Argon was used as the carrier gas at a temperature of 50°C. Carbon dioxide and hydrogen sulphide passed through a PLOTQ column (INFICON, Switzerland) (10 µm/20 µm/8 m) using helium as the carrier gas at a temperature of 55°C.

*BHP tests with automatic pH control – 1 L (BHP2).* The second set of tests, BHP2, was carried in 1 L (working volume = 0.5 L) glass reactors equipped with magnetic stirring and connected to eudiometers for gas measurement on the basis of the volume displacement principle. The eudiometers were filled with a NaCl-saturated solution, acidified with HCl to pH = 2 to prevent gas dissolution and connected to an electronic balance that periodically weighed the volume of solution displaced from the eudiometers. The electronic balance was interfaced with an automatic control system that recorded the total biogas volume produced over time. The reactors were connected to an automatic system for data acquisition and continuous pH control through NaOH addition.

During the tests, gas samples were periodically collected through an air-tight syringe connected to the eudiometer sampling

**Table 2.** Design of the experiments.

Set	Run	Total volume (L)	Working volume (L)	pH set-point	Food to microorganisms (w/w)
BHP1	BHP1_5.5_1/3	1	0.5	5.5 <sup>a</sup>	1/3
	BHP1_5.5_1/1			5.5 <sup>a</sup>	1/1
	BHP1_6.5_1/3			6.5 <sup>a</sup>	1/3
	BHP1_6.5_1/1			6.5 <sup>a</sup>	1/1
BHP2	BHP2_5.5_1/3	1	0.5	5.5	1/3
	BHP2_5.5_1/1			5.5	1/1
	BHP2_6.5_1/3			6.5	1/3
	BHP2_6.5_1/1			6.5	1/1
BHP3	BHP3_5.5_1/3	6	3	5.5	1/3
	BHP3_5.5_1/1			5.5	1/1
	BHP3_6.5_1/3			6.5	1/3
	BHP3_6.5_1/1			6.5	1/1

<sup>a</sup>Initial value.

port and analysed for hydrogen, nitrogen, carbon dioxide and methane using a Varian 3600 CX gas chromatograph (Agilent, California, USA) equipped with a thermal conductivity detector and a 2 m stainless column packed with Porapak Q (50/80 mesh) at operating temperatures of injector, oven and detector of 250°C, 80°C and 130°C respectively. Helium was used as the carrier gas.

*BHP tests with automatic pH control – 6 L (BHP3).* The third set of tests, BHP3, was performed using pilot-scale stainless-steel reactors (6 L total volume, 3 L working volume).

Continuous mixing inside the reactors was ensured by mixing blades, while reactor heating was performed through circulation of water heated by a thermostatic bath (FA90, Falc Instruments s.r.l., Italy) into the reactor jacket. A pH probe (InPro4260i, Mettler Toledo, Italy) was placed inside the reactor and was connected to a transmitter (MT M300, Mettler Toledo, Italy). The volume of the gas produced during the test was measured through a volumetric counter. A pressure transducer (HD 9908T Baro, Delta Ohm S.r.l., Italy) and a T-type thermocouple (PT100, Delta Ohm S.r.l., Italy) were used to measure ambient pressure and temperature, respectively. All electric signals from the reactors were acquired by a cRIO 9030 system (National Instruments, Italy) and were processed by software specifically developed in Labview® (National Instruments, Italy). The acquisition system and the software were also used to control a peristaltic pump (Reglo ICC, Ismatec, Germany) dedicated to the dosage of 1 M NaOH for pH control. In particular, 3 mL of solution were automatically added when the pH decreased to below the set value in order to constantly keep the pH in the range  $\pm 0.1$  throughout the tests.

The hydrogen content of the gas was measured by gas chromatography with the same instruments and methods described for the BHP1 tests.

### Kinetic analysis

The kinetics of the hydrogen production process were evaluated by fitting the experimental cumulative hydrogen production data with a two-stage model derived from the Gompertz equation (see equation (1)) to take into account the presence of substrate constituents having different degradation kinetics (Akhlaghi et al., 2017; De Gioannis et al., 2014)

$$H(t) = H_{\max,1} \exp \left\{ -\exp \left[ \frac{R_1 \cdot e}{H_{\max,1}} (\lambda_1 - t) + 1 \right] \right\} + H_{\max,2} \exp \left\{ -\exp \left[ \frac{R_2 \cdot e}{H_{\max,2}} (\lambda_2 - t) + 1 \right] \right\} \quad (1)$$

where:

- $H(t)$ : cumulative hydrogen production at time  $t$ ;
- $H_{\max,1}$  and  $H_{\max,2}$ : maximum hydrogen production of the first and second stage;
- $R_1$ ,  $R_2$ : maximum hydrogen production rate of the first and second stage;

- $\lambda_1$  and  $\lambda_2$ : lag phase duration of the first and second stage;
- $t$ : time.

The total maximum hydrogen production,  $H_{\max}$ , was obtained as the sum of  $H_{\max,1}$  and  $H_{\max,2}$ . The experimental data were fitted with equation (1) by means of least-square linear regression using Table Curve2D® (Sigmaplot, London, UK). As proposed in our previous studies (Akhlaghi et al., 2017; Cappai et al., 2014; De Gioannis et al., 2017), in order to evaluate the overall duration of the process, the time required for hydrogen production to attain 95% of the maximum yield was also calculated ( $t_{95}$ ).

### Statistical analysis

In order to evaluate differences between the experimental set-ups, an analysis of variance (ANOVA) test and Tukey's test in pairwise comparison were performed using XLStat2018 software (Addinsoft, New York, US), assuming a confidence level of 95%.

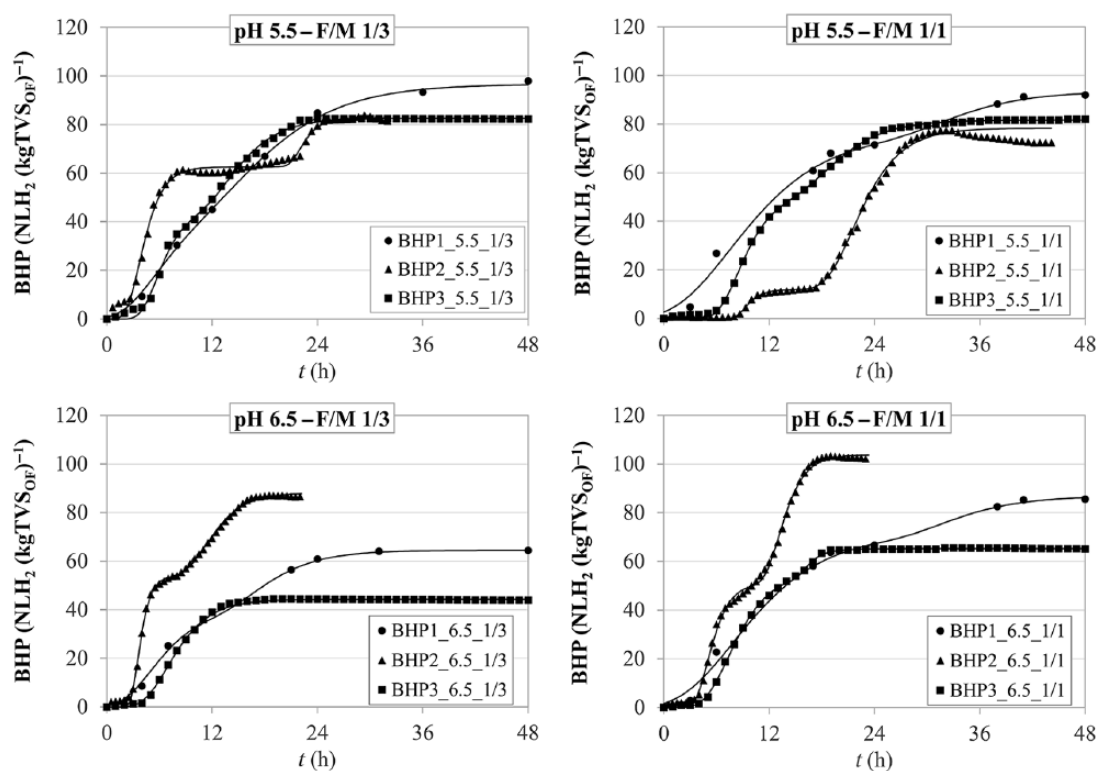
## Results and discussion

Heating of the inoculum prior to the fermentative process proved effective since no methane was detected in the biogas over the entire duration of any of the experiments, and the major components were only hydrogen and carbon dioxide.

When pH was controlled through automatic addition of the NaOH solution, pH was rather stable over all the tests, with fluctuations within  $\pm 0.1$  units. Conversely, the initial addition of the buffer solution in the BHP1 set was not suitable for adequate pH control, so that the final pH was significantly lower than the desired set-up value. Nevertheless, the pH was in all cases found to lie above the commonly recognized threshold for potential inhibition of hydrogenase activity (Micolucci et al., 2014). The pH decrease was found to be slightly larger for the tests performed at higher F/M ratios. More specifically, the final pH was found to be  $5.3 \pm 0.1$ ,  $5.0 \pm 0.0$  for BHP1\_5.5\_1/3, and BHP1\_5.5\_1/1, while  $5.9 \pm 0.1$  and  $5.7 \pm 0.0$  for BHP1\_6.5\_1/3 and BHP1\_6.5\_1/1. This may be ascribed to the higher production of VFAs in the experiments. Further investigations are planned to investigate the individual and overall VFA evolution over time.

For the BHP2 and BHP3 sets of experiments, the dosage of the alkaline solution used for pH control was higher when the pH set-point was adjusted at 6.5, and also at the higher F/M ratio. Nevertheless, the specific consumption ( $\text{mL NaOH (L of reactor)}^{-1}$ ) was comparable for the BHP2 and BHP3 tests at pH 5.5 alone; under these conditions, the measured dosages were  $38.8 \text{ mL L}_r^{-1}$  for BHP2 and  $32.0 \text{ mL L}_r^{-1}$  for BHP3 at F/M 1/3; and  $53.4 \text{ mL L}_r^{-1}$  for BHP2 and  $50.0 \text{ mL L}_r^{-1}$  for BHP3 at F/M 1/1. On the other hand, at pH 6.5 the NaOH dosages measured in BHP3 were almost twice those in BHP2: more specifically,  $50.5 \text{ mL L}_r^{-1}$  for BHP2 and  $99.0 \text{ mL L}_r^{-1}$  for BHP3 at F/M 1/3; and  $97.4 \text{ mL L}_r^{-1}$  for BHP2 and  $168.0 \text{ mL L}_r^{-1}$  for BHP3 at F/M 1/1.

At pH 5.5, the comparable NaOH dosages between the BHP2 and BHP3 experiments were mirrored by similar hydrogen yields



**Figure 1.** Specific cumulative hydrogen production yields as a function of the experimental conditions. Data points indicate experimental results, while solid lines represent Gompertz model curves.

and thus suggest similar metabolic pathways occurring in the two systems. Conversely, at pH 6.5 the higher hydrogen yields displayed by the BHP2 tests as opposed to BHP3 were not mirrored by an increased NaOH demand. It is tempting to hypothesize that in BHP3, the fermentation process was accompanied by a larger production of acidic metabolites deriving from non-hydrogenogenic pathways. Indeed, propionic, lactic, alcoholic fermentations and homoacetogenesis are hydrogen-consuming pathways that may occur in BHP tests using food waste as substrate (Cappai et al., 2014; De Gioannis et al., 2017; Ramos et al., 2012). More specifically, propionic and alcoholic pathways consume hydrogen as reducing equivalents ( $\text{NADH}_2$ , potential  $\text{H}_2$ ) to produce propionate and alcohols (ethanol and propanol). Conversely, homoacetogenic bacteria produce acetate by reducing carbon dioxide and organic compounds using molecular  $\text{H}_2$  as electron donor (Saady, 2013; Zumar Bundhoo and Mohee, 2016). Cappai et al. (2014) highlighted that at extreme pH values (4.5 and 8.5) the production of hydrogen was inhibited due to the onset of the alcoholic pathway. Conversely, for pH in the range 5.5–7.5, the process was affected by homoacetogenesis. Similarly, the tests performed by Ramos et al. (2012) and De Gioannis et al. (2017) also highlighted homoacetogenesis together with lactate and propionate formation at pH 7 and 6.5, respectively. This issue is believed to deserve further investigation to assess the hypothesis above, identify the potential reasons for the observed behaviour and provide a better understanding of the type of metabolic reactions involved.

As far as the hydrogen yield was concerned,  $H_{\text{max}}$  was found to range from 44.3 to 104.5  $\text{NLH}_2$  ( $\text{kgTVS}_{\text{OF}}^{-1}$ ). Previous studies

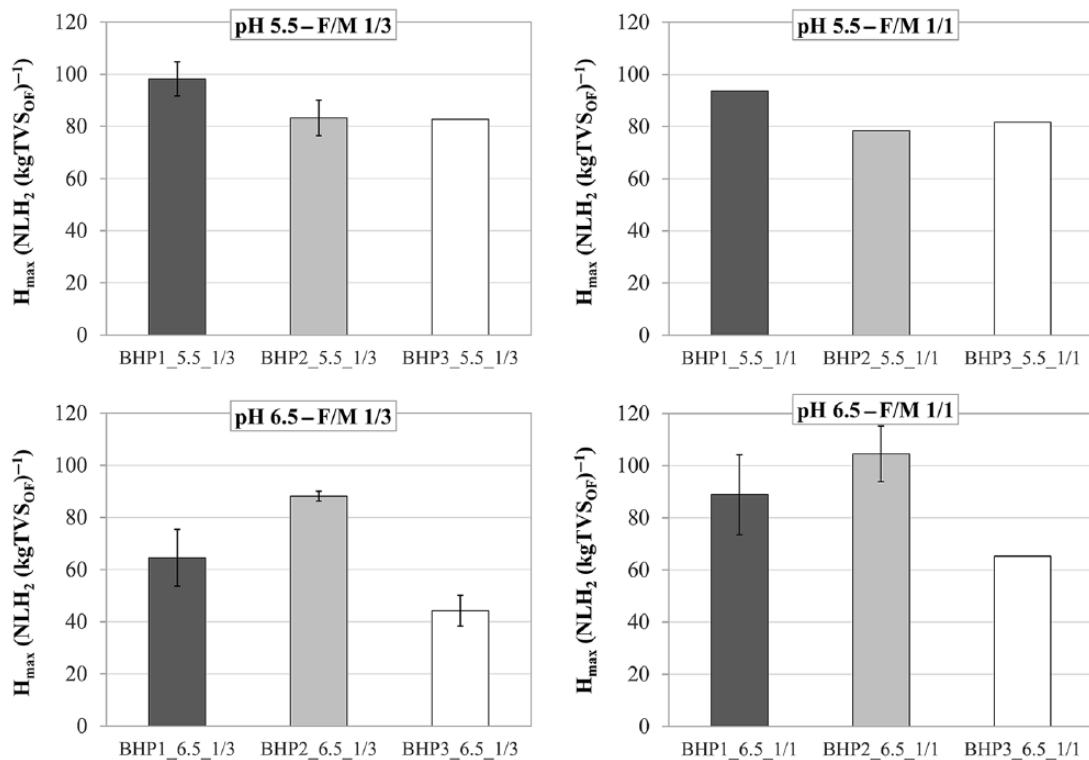
on similar substrates showed comparable results, with yields (in  $\text{NLH}_2$  ( $\text{kgTVS}_{\text{OF}}^{-1}$ )) of: 25–85 (Alibardi and Cossu, 2015), 55 (Pecorini et al., 2017), 59 (De Gioannis et al., 2017), 61 (Ghimire et al., 2016), 65 (Pan et al., 2008), 78–135 (Alibardi and Cossu, 2016), 89–97 (Kim et al., 2009), 90 (Cappai et al., 2018), 103 (Sreela-or et al., 2011), 110 (Kim et al., 2011) and 161 (Im et al., 2012). With the exception of the abovementioned results for BHP3 at pH 6.5, which displayed lower hydrogen yields, the other experimental conditions produced process performances in the same order of magnitude. Figure 1 presents the trend of the cumulative hydrogen production of the three experimental set-ups.

Table 3 presents the kinetic parameters calculated using the two-stage model in equation (1). Figure 2 shows the values for  $H_{\text{max}}$  and the related standard deviations. All of the hydrogen production values are reported as standard volumes of hydrogen per unit of TVS mass of the OF. The two-stage Gompertz model adopted always displayed a good degree of fitting of the experimental data ( $R^2 > 0.989$ ). It was noted that, unlike  $H_{\text{max}}$ , a change in the experimental conditions affected the process kinetics. Indeed, by comparing the data in Table 3, it is evident that the automatic pH control significantly enhanced the degradation rate reducing the total process duration. In particular, the  $t_{95}$  values for the BHP1 runs were found to be 31–57% higher than the corresponding values for the other two sets of experiments at pH 5.5, and as much as 61–150% higher at pH 6.5. This suggests the pivotal role of accurate pH control in promoting the microbial activity of the hydrogenogenic biomass. Similar considerations

**Table 3.** Kinetic parameters of H<sub>2</sub> production according to equation (2).

Run	H <sub>max</sub> (NLH <sub>2</sub> (kgTVS <sub>OF</sub> ) <sup>-1</sup> )	R <sub>1</sub> (NLH <sub>2</sub> (kgTVS <sub>OF</sub> h) <sup>-1</sup> )	R <sub>2</sub> (NLH <sub>2</sub> (kgTVS <sub>OF</sub> h) <sup>-1</sup> )	λ <sub>1</sub> (h)	λ <sub>2</sub> (h)	t <sub>95</sub> (h)
BHP1_5.5_1/3	98.2 ± 6.5	6.0 ± 1.1	2.1 ± 1.6	3.1 ± 2.0	25.6 ± 12.5	32.7 ± 7.4
BHP2_5.5_1/3	83.2 ± 6.8	15.9 ± 1.1	4.9 ± 2.8	2.3 ± 0.0	19.7 ± 2.4	24.4 ± 0.0
BHP3_5.5_1/3	82.7	9.0	4.4	3.9	10.9	20.9
BHP1_5.5_1/1	93.7 ± 0.0	4.6 ± 0.1	1.1 ± 0.0	1.3 ± 0.0	26.5 ± 0.0	39.1 ± 0.0
BHP2_5.5_1/1	78.3	4.0	8.1	8.1	18.4	29.9
BHP3_5.5_1/1	81.6	8.2	3.0	6.1	15.2	25.9
BHP1_6.5_1/3	64.5 ± 10.9	5.3 ± 1.3	1.7 ± 0.3	2.6 ± 1.4	14.3 ± 0.4	25.1 ± 2.0
BHP2_6.5_1/3	88.1 ± 1.9	24.6 ± 1.3	6.0 ± 0.8	2.7 ± 0.2	9.3 ± 1.3	15.6 ± 2.4
BHP3_6.5_1/3	44.3 ± 5.9	5.9 ± 6.7	5.8 ± 0.3	2.4 ± 2.8	5.9 ± 1.3	13.9 ± 0.4
BHP1_6.5_1/1	88.9 ± 15.3	4.6 ± 1.1	1.1 ± 0.1	2.0 ± 0.5	23.3 ± 7.2	42.7 ± 7.4
BHP2_6.5_1/1	104.5 ± 0.7	13.7 ± 0.3	11.6 ± 3.9	3.6 ± 0.1	11.4 ± 0.2	17.1 ± 0.5
BHP3_6.5_1/1	65.3	7.6	3.2	4.6	15.0	17.7
BHP1 (average)	86.3 ± 15.0 <sup>a</sup>	5.1 ± 0.7 <sup>a</sup>	1.5 ± 0.5 <sup>a</sup>	2.3 ± 0.8 <sup>a</sup>	22.4 ± 5.6 <sup>a</sup>	34.9 ± 7.8 <sup>a</sup>
BHP2 (average)	88.5 ± 11.4 <sup>a</sup>	14.5 ± 8.4 <sup>a</sup>	7.7 ± 3.0 <sup>b</sup>	4.2 ± 2.7 <sup>a</sup>	14.7 ± 5.1 <sup>a,b</sup>	21.7 ± 6.7 <sup>b</sup>
BHP3 (average)	68.5 ± 18.0 <sup>a</sup>	7.7 ± 1.3 <sup>a</sup>	4.1 ± 1.3 <sup>b</sup>	4.3 ± 1.5 <sup>a</sup>	11.8 ± 4.4 <sup>b</sup>	19.5 ± 5.0 <sup>b</sup>

<sup>a,b</sup>The same letters shows that the values are not significantly different,  $p \rightarrow 0.05$ .

**Figure 2.** Maximum hydrogen production H<sub>max</sub> (average values and standard deviations).

can also be made for the maximum production rate of each stage (R<sub>1</sub> and R<sub>2</sub>).

Regarding the biomass acclimation phase, no experimental evidence could be gained of any potential influence of the investigated conditions on the lag phase duration for the first stage of the fermentation process, while the second stage (associated with the degradation of more slowly fermentable substrate constituents) for the BHP1 set turned out to display 30–142% longer lag phase durations than the BHP2 and BHP3 experiments.

As for the statistical analyses, the kinetic parameters of the two-stage Gompertz model for the different experiments were assumed as samples of a unique statistical population and processed through the ANOVA followed by Tukey's test pairwise comparisons (Table 3).

In line with what is reported above, the statistical analysis underlined that the experimental set-up affected the kinetics of the fermentative process much more than the final production of hydrogen. The ANOVA carried out on maximum hydrogen

productions for the different experimental configurations indicated the finding of not statistically different results ( $p < 0.01$ ). Conversely, this result was not confirmed for the other kinetic parameters ( $R_2$  and  $\lambda_2$ ) and the time required for hydrogen production to attain 95% of the maximum yield ( $t_{95}$ ). The Tukey's test in pairwise comparisons performed on these parameters indicated a similarity only between results obtained from the experimental set-up using the automatic addition of alkaline solution (BHP2 and BHP3). Under the whole set of the experimental conditions tested, the  $t_{95}$  values for the experiments with automatic pH control were considerably lower than those of the corresponding test with no automatic control. The process kinetics appeared to be faster, with  $t_{95}$  values always below 26 hours, for BHP2 and BHP3. This may be ascribed to the fact that a more accurate pH control throughout the test was more favourable to the hydrogenogenic biomass, since even small pH fluctuations are recognized to influence the fermentative process (Ghimire et al., 2015). Conversely, maximum hydrogen production rates of the second stage ( $R_2$ ) were even twice than those found for BHP1. These findings allow us to conclude that the experimental set-up did not deeply influence the final production of hydrogen, while it played an important role in the kinetic evolution over time.

## Conclusions

Three different experimental set-ups of batch fermentative assays aimed at biological hydrogen production were carried out using source-separated organic fraction of municipal solid waste as the substrate and activated sludge as the microbial source. Hydrogen production was evaluated by means of biochemical hydrogen potential tests with and without automatic pH control and with different reactor volumes. The experiments were performed by varying the pH conditions and the food-to-microorganisms ratio.

When pH was controlled through automatic NaOH addition, the pH was rather stable throughout the tests; on the other hand, the initial addition of the buffer solution was not suitable for adequate pH control. The hydrogen yield appeared to be unaffected by the increase of added substrate or the change in pH. Although the different set-ups showed comparable final hydrogen productions (with maximum yields on average between 68.5 and 88.5 NLH<sub>2</sub> (kgTVS<sub>OF</sub>)<sup>-1</sup>), the automatic pH control system improved the fermentation process in terms of kinetics and pH stability. To this regard, the  $t_{95}$  was reduced by almost a half, being reduced from an average of 34.9 h for the tests performed with initial buffer addition to an average of 19.5 h for the tests with automatic pH control.

These findings demonstrate the crucial role of the pH control strategy during hydrogen production tests and suggest the use of an automatic control to set up future experiments.

## Abbreviations

AD, anaerobic digestion; AS, Activated sludge; BHP, biochemical hydrogen potential; F/M, food to microorganisms; OF, organic

fraction; OFMSW, organic fraction of municipal solid waste; TOC, total organic carbon; TS, total solids; TVS, total volatile solids; VFA, volatile fatty acids.

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