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The hydraulic retention time influences the abundance of *Enterobacter*, *Clostridium*, and *Lactobacillus* during the hydrogen production from food waste

Headline: HRT on microbes in H_2 production

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SIGNIFICANCE AND IMPACT OF THE STUDY

It was demonstrated that hydrogen production using food waste was influenced by the hydraulic retention time (HRT), and closely related to changes in microbial communities together with differences in metabolic patterns (*e.g.* volatile fatty acids, lactate, etc.). The decrease of the HRT led to the dominance of lactic acid bacteria within the microbial community whereas the increase of HRT favored the emergence of *Clostridium* bacteria and

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the increase of acetic and butyric acids. Statistical data analysis revealed a direct relationship existing between the HRT and the microbial community composition in fermentative bacteria. This study provides new insight into the relationship between the bioprocess operation and the microbial community to understand better and control the biohydrogen production.

ABSTRACT

The influence of hydraulic retention time (HRT) on the microbial communities was evaluated in an anaerobic sequencing batch reactor (AnSBR) using organic waste from a restaurant as the substrate. The relationship between *Lactobacillus, Clostridium,* and *Bacillus* as key-microorganisms on hydrogen production from organic solid waste was studied. The effect of the HRT (8 to 48 h) on the hydrogen production and the microbial community was evaluated. Quantitative PCR was applied to determine the abundance of bacteria (in particular, *Enterobacter, Clostridium,* and *Lactobacillus* genera). An AnSBR fermentative reactor was operated for 111 cycles, with carbohydrate and organic matter removal efficiencies of 80 ± 15.42% and 22.1 ± 4.49%, respectively. The highest percentage of hydrogen in the biogas (23.2 ± 11.1 %), and the specific production rate (0.42 ± 0.16 mmol H_2 gVS_{added}^{-1} d^{-1}) were obtained at an HRT of 48 h. The decrease of the HRT generated an increase of the hydrogen production rate but decreasing the content of the hydrogen in the gas. HRT significantly influence the abundance of *Enterobacter, Clostridium, and Lactobacillus* during the hydrogen production from food waste leading the hydrogen production as well as the metabolic pathways. The microbial analysis revealed a direct relationship between the HRT and the presence of fermentative bacteria (*Enterobacter, Clostridium,* and *Lactobacillus* genera). *Clostridium* sp. predominated at an HRT of 48 h, while *Enterobacter* and *Lactobacillus* predominated at HRTs between 8 and 24 h.

Keywords: Biohydrogen; Dark fermentation; food waste; HRT; SBR.

ABREVIATION LIST

COD	Chemical Oxygen Demand
CO_2	Carbon Dioxide
H_2	Hydrogen
HRT	Hydraulic Retention Time
OLR	Organic Loading Rate
PCA	Principal Components Analysis
PCR	Polymerase Chain Reaction
RT-PCR	Real Time Polymerase Chain Reaction
SBR	Sequencing Batch Reactors
SRT	Solids Retention Time
TS	Total Solids
VFA	Volatile Fatty Acid
VS	Volatile Solids

INTRODUCTION

Dark fermentation is performed by anaerobic bacteria that grow in the absence of light and use substrates rich in carbohydrates, to produce hydrogen (H_2), carbon dioxide (CO_2), and volatile fatty acids. The microbial community plays a crucial role during the fermentation of organic waste and directly affects the hydrogen production efficiency (Zahedi et al. 2014). Microorganisms of the genera *Clostridium*, *Escherichia*, *Enterobacter*, and *Bacillus* have been well documented as biological producers of hydrogen (Levin et al. 2004).

During the fermentation of organic solid waste, several factors are involved in the stability of the process and influence efficient hydrogen production, such as the pH, temperature, hydraulic retention time (HRT), inoculum origin and the type of organic substrate (Akuzawa et al. 2011; Kim et al. 2013). In the operation of continuous and discontinuous reactors, the reactor size can be reduced by using short HRTs. At short HRTs (< 48 h), hydrogen-producing biomass are favored, since a growth rate of 0.172 h^{-1} has been reported for fermentative microorganisms, while methanogenic archaea have a growth rate ranging between 0.0167 and 0.02 h^{-1}. Several studies have already focused on reducing the HRT without causing problems related to biomass wash-out, and providing adequate time to hydrolyze the particulate substrates, such as organic waste (Kim et al. 2008; Badiei et al. 2011; Moreno-Andrade et al. 2015).

Sequencing batch reactors (SBR) are based on the periodic repetition of a well-defined sequence of phases: fill, react, settle, draw and idle (Tchobanoglous et al. 2002). SBR provides different advantages such as the decoupling of the solids retention time (SRT) and the HRT, decreasing the instabilities over long-term operational periods and the biomass washout at short HRTs as has been reported in a continuous process (Castelló et al. 2009). SBR also offers process flexibility and do not require an additional unit for the settling step. In SBR, the microbial community not only depends on the HRT but also on the SRT used in

this type of reactor. Understanding the diversity of the microbial community with respect to the different factors that affect dark fermentation will aid in designing an efficient hydrogen production system.

In a previous study, Moreno-Andrade et al. (2015) reported the characterization of a system producing H_2 from food waste. These authors reported a stable microbial composition (low diversity and low evenness) at two different HRT (24 and 72 h), with *Megasphaera* spp. as dominant species that maintained in the system despite the differences in hydrogen production. In order to analyze the effect of HRT on hydrogen production, it is necessary to understand the correlation of the bacterial populations with H_2 production. *Clostridium* and *Enterobacter* genera have been widely reported as hydrogen-producers in dark fermentation systems (Lin, 2018; Hung et al. 2011; Vardar-Schara et al. 2008). It has been reported that *Enterobacter* strains are a suitable microorganism for hydrogen production in industrial applications due to their rapid growth rates, the capacity to transform different substrates, the robustness to the presence of dissolved oxygen, and variation on pH (Zhang et al., 2011).

The enrichment of hydrogen-producing microorganisms is necessary when a nonspecific inoculum is used. For instance, microorganisms of *Clostridia* and *Bacilli* classes generate spores when exposed to hostile environmental conditions such as thermal shocks, changes in the concentration of the nutrient, the presence of chemicals, and other cellular stresses (Setlow 2003). The syntrophic co-culture of *Bacillus* and *Clostridium* may play the key role for developing the industrialized bio-fuels and bio-hydrogen production due to their high metabolic ability (Chang et al., 2008). Some species of Bacillus (e.g., *B. thermoamylovorans*) were exhibited phenotypic characteristics similar to another probiotic as microorganisms of *Lactobacillus* genus (Combet-Blanc et al., 1995). *Bacillus* sp. also showed the capacity of simultaneous saccharification and co-fermentation of the hydrolyzed sugars to produce lactate (Patel et al., 2005). For this reason, not only the relation between *Bacillus* and

Clostridium are important, but the presence and interaction of *Lactobacillus* need to be considered in the dark fermentation process for hydrogen production.

Moreover, when using complex substrates such as organic solid waste, microbial community composition may change during operation of the reactor due to the coexistence of bacterial hydrogen producers and bacterial hydrogen consumers (Noike et al. 2002). For instance, lactic acid bacteria, eg. *Lactobacillus* spp. that are fermentative bacteria but not producing H_2, were already found in systems producing hydrogen from complex substrates (Castelló et al. 2009), since they are part of the indigenous microflora in organic waste. *Lactobacillus* sp. can co-exist with hydrogen-producing *Clostridium* species but could have an adverse effect on hydrogen production (Kawagoshi et al. 2005). The association between *Lactobacillus, Clostridium,* and *Bacillus* as key-microorganisms upon the hydrogen production from organic solid waste has not been studied profoundly and can reveal new insight about bioprocess operation to better control of the microbial community producing biohydrogen.

The objective of this study is to investigate the effect of HRT on three key microbial populations during hydrogen production *(Clostridium, Lactobacillus,* and *Enterobacter)* from organic solid waste and using the RT-PCR technique for their quantification.

RESULTS AND DISCUSSION

Start-up and operational data

An SBR was operated over 111 cycles with a total carbohydrate removal efficiency of 80 ± 15%, and organic matter removal efficiency of 29.6 ± 4.49%. Table 1 shows the operational parameters and metabolite generation at the different HRT tested. A direct relationship was observed between HRT and organic matter removal. The initial HRT was fixed at 48 h during 41 cycles of operation. At this HRT, the highest hydrogen percentage (23.2 ± 11.1%) and specific production rate (0.42 ± 0.16 mmol H_2 L^{-1} gVS_{added}^{-1} d^{-1}) were obtained (Figure 1A

and 1B). A decrease in the hydrogen production rate from cycle 33 to 48 was related to an increase in the percentage of methane (observing an average of 15.3 ± 7.7%) (Figure 1A). Thus, the long HRT was favorable to the emergence of hydrogen-consuming microorganisms, such as methanogenic and non-producing acidogenic microorganisms, which is in agreement with the results reported by Wang and Zao (2009). When the HRT was changed to 24 h, the hydrogen production was stable, and the methane production reached only 5.9±3.3% in two cycles (64 and 65). When the HRT was lowered to 8 h, the highest hydrogen production rate of 1.72 mmol H_2 L^{-1} d^{-1} was achieved, with a production rate of yield of 0.02 mmol H_2 L^{-1} gVS_{added}^{-1} d^{-1} (Figure 1C). The hydrogen production stability was observed when no methane was present in the biogas at HRT of 16h.

In our study, a relation between the hydrogen yield and the solid retention time (SRT) was observed (Figure 2A). Statistical analysis (ANOVA), demonstrated significant differences between the different SRT, except for the comparison between the 42 and 38 h, where the values did not show significant differences (t student, P=0.49). When the SRT increased, the biomass retention also increased, providing sufficient time for hydrolysis of the particulate substrate. However, at high SRT (42 h), methane production was also observed, most likely because of the development of indigenous hydrogen-consuming microbial populations in the non-sterilized substrate (Li et al. 2014). Therefore, determining appropriate values for SRT and HRT is crucial and closely related to the applied substrates, since the optimal conditions will vary according to the origin of the waste. Both parameters can affect the hydrogen production as well as the substrate consumption, microbial population activity and, thus, the possibility to obtain the desired metabolic pathways (Kim et al. 2008).

The volatile fatty acid (VFA), ethanol and lactate reaction products generated during reactor operation are shown in Table 1. Acetate and butyrate significantly accumulated with the increase of the HRT. Propionate production decreased at an HRT of 8 h. Lactate was the

main metabolite produced during the reactor operation, except for an HRT of 48 h where acetic and butyric acids were the main fermentation end-products. The best H_2 production found at an HRT of 48 h was related to the highest acetic acid (the stoichiometric yield of H_2 is 4 mol for each mole of glucose, i.e. 544 mL H_2 g hexose^{-1} at 25 °C) and butyric acid (2 mol for a mole of glucose, i.e. 272 mL H_2 g hexose^{-1} at 25 °C) accumulation, whereas H_2 production decreased when lactic acid was produced since no hydrogen production or failure in the H_2 production has been reported due to accumulation of lactic acid (Ghimire et al. 2015; Wu et al. 2012). Besides, the utilization of lactate and acetate for the production of butyrate and hydrogen by several microorganisms including *Clostridium acetobutylicum, C. tyrobutyricum, C. beijerinckii,* and *Butyribacterium methylotrophicum* has been reported, but only for batch experiments (Ghimire et al. 2015).

The increase of the HRT can result in the accumulation of subproducts as VFA resulting in a decrease in the pH. In our study, the pH was controlled since this is an important parameter affecting the pH production and microbial community composition. Reduction of pH (>4.8) is related to the increase of undissociated acids inhibiting the H_2 producers. The pH is related with methanogen growth limitation and regulation of shift to solventogenesis (Ziara et al. 2019; Valdez-Vazquez et al. 2009), for this reason, the control of pH is important to maintain stable H_2 production.

Microbial community analysis

In Figure 2B, the quantitative PCR results show correlations above 86% between the HRT and the presence of bacteria from the *Enterobacter* (R^2=0.9598), *Clostridium* (R^2=0.8702) and *Lactobacillus* (R^2=0.8560) genera. Interestingly, an increase of the HRTs stimulated *Clostridium* bacteria more than *Enterobacter* and *Lactobacillus*, which were more related to a decrease of the HRT. *Enterobacter* and *Lactobacillus* showed a negative relation with not

only the HRT, but also the HPR and acetate and butyrate production. Since a reduction of the HRT consequently increases the organic loading rate (OLR) and decreases the rate of substrate hydrolysis; this may be the main reason for the decrease of the volumetric hydrogen production and the unassociated metabolic products during hydrogen production (Wang et al. 2009).

A Principal Components Analysis (PCA) was applied to examine fewer variables by forming a smaller set of correlated components that represent the highest amount of information within the original variables (Figure 3). The results are presented in two dimensions, Principal Component 1 (PC1) and Principal Component 2 (PC2), which represent about 78% of the data variance (Figure 2B). The points in the plot indicate the samples and vectors. The PCA shows a positive relationship among higher values of HRT, HPR, butyrate production and *Clostridium* bacteria abundance. These variables were mainly related to samples 1 and 2 (HRT = 48 h). When the HRT was decreased from 24 to 16 h, an increase in ethanol and propionate production was observed.

A correlation between lactic acid production and *Lactobacillus* bacteria was confirmed (Figure 4A). The presence of lactic acid bacteria, decreased H_2 production likely due to substrate competition but also to the production of antimicrobial peptides by some members of the lactic acid bacteria family (Gomes et al. 2015). Lactic acid bacteria are Gram-positive and can produce lactic acid as the main product of carbohydrate fermentation (Rosa et al. 2015). The presence of *Lactobacillus* has been observed in H_2-producing reactors operated under stable and unstable conditions, since the fermentation of lactate played a dual role in the reactor, as both bioH_2-producing (acetate + lactate → butyrate + H_2) and non-H_2-producing (lactate → propionate + acetate) (Fuess et al. 2018). The correlation observed in our study between lactic acid and *Lactobacillus*, and the other subproducts (e.g., butyric and ethanol) agree with the results reported by Fuess et al. (2018), concluding that the availability

of lactic acid is also most likely played a determining metabolic role. It is possible to confirm the idea that *Lactobacillus* is a key-genus in the hydrogen production process independently of the operation condition in the system (e.g., operated under different HRT).

Therefore, by changing the HRT, it was found possible to control the process towards a desired fermentative route. Although it is well accepted that lactic acid bacteria are part of the indigenous microbiota found in organic waste, this study shows that it is still possible to decrease the concentration of lactic acid bacteria by increasing the HRT (Figure 3). Consistently, Gomes et al. (2015), studied the relation existing between hydrogen production and HRT in two anaerobic fluidized bed reactors, using cheese whey waste. They found that an HRT of 14 h stimulated the hydrogen production compared to 6 h. In this last case, a bacteriocin activity was suspected. Indeed, two strains belonging to the *Lactococcus* and *Lactobacillus sp.* genera were responsible for the production of antimicrobial peptides resulting in interactions with the Gram-negative bacteria through lipopolysaccharides and lipoproteins, producing areas of instability within the external membrane. Once located in the membrane, they can change conformation and cause irreversible damages in the membrane or internally (Téllez and Castaño 2010). From a practical point of view, a short HRT represent a high volume of waste treated, but also the dominance of lactic acid leading the reduction in hydrogen percentage, butyric production, and increase in propionic production. For this reason, a compromise between the HRT and the hydrogen production need to be reached, to maintain the emergence of *Clostridium* spp. (HRT 24 h) obtaining the hydrolysis of the substrate (58%), the increase of VFA and hydrogen percentage.

In the present study, the microbial community *Enterobacter spp*. was favored at low HRT and SRT. Previous studies reported the relation between *Enterobacter* spp. abundance and hydrogen production, which can produce between 0.9 to 1.8 mol H_2 mol glucose^{-1} in suspended cultures at mesophilic conditions (Harun et al. 2012; Kumar and Das 2000),

enhancing this value to 2.56 1.8 mol H_2 mol glucose^{-1} when immobilization of the strain is applied (Fatemeh et al. 2019). A strong correlation was observed between the hydrogen percentage and the amount of *Enterobacter spp*. bacteria at a certain HRT. The increase in these values leads to low hydrogen production during operation of the SBR (Figures 1 and 4B). Moreover, *Enterobacteria* are facultative anaerobic bacteria that can remove oxygen that is present during the fermentation process and live symbiotically with *Clostridium* bacteria (Harun et al. 2012). Previous studies reported the presence *Enterobacter aerogenes* and *Enterobacter cloacae* during dark fermentation of different carbon sources, such as sugar, glycerol and wastewater (Harun et al. 2012; Reungsang et al. 2013; Sun et al. 2015).

Reungsang et al. (2013) studied hydrogen production via *E. aerogenes* using glycerol as the substrate in an anaerobic upflow reactor (UASB), and obtained hydrogen production percentages between the 37% and 24%. They reported ethanol, 1,3-propanediol, formic acid and acetic acid as the main soluble products. These products were obtained from the fermentation of sucrose by *E. cloacae*, in which the main fermentative route to the hydrogen production was through 2,3-butanediol (Sun et al. 2015). However, in this study, a weak relationship was found between hydrogen production, solvent production and *Enterobacter* bacteria abundance. Hydrogen production can also be attributed to acetic and lactic acid production by *E. cloacae* (Sun et al. 2015).

In conclusion, it was found that the HRT significantly influenced the hydrogen production as well as the metabolic pathways due to the changes in abundance of *Enterobacter, Clostridium, and Lactobacillus* when from food waste is used as substate. The highest H_2 percentages and specific hydrogen productions were obtained with an HRT of 48 h. At that high HRT, acetic acid and butyric acid were produced as main metabolites, while the highest concentration of lactic acid was observed at a low HRT of 8 h. The microbial analysis revealed a direct relationship between the HRT and the microbial community composition in

fermentative bacteria. A long HRT (48 h) favored the growth of *Clostridium* bacteria, while lower HRTs were more favorable to the emergence of lactic acid bacteria.

MATERIALS AND METHODS

Inoculum

Anaerobic granular sludge from an anaerobic sludge blanket reactor treating brewery wastewater was used as the inoculum. The sludge was thermally treated at 100°C for 24 h, to inhibit methanogenic microorganisms (archaea methanogenic) (Buitrón and Carvajal, 2010). The physicochemical characteristics of the inoculum were as follows: 40.68 ± 4.5 g TS L^{-1}, 29.05 ± 3.06 g VS L^{-1} and 28.4 ± 3.4 g COD L^{-1}.

Substrate

The organic solid waste was obtained from a buffet-type restaurant located in Queretaro City, Mexico. The food waste was collected over three weeks, during which 50 kg of food waste was obtained. Components such as paper, paperboard, plastic, bones, and metal were discarded. The collected waste was mashed with a mill and blended to homogenize the particle size (<0.5 mm); then, the organic waste was refrigerated at -20°C for preservation. This homogenized residue was used as the feedstock. The total chemical oxygen demand (COD), total and volatile solids (TS, VS), ammonia nitrogen (NH_3) and moisture content were determined according to standard methods (APHA. 2005). The total carbohydrates were measured by the phenol-sulfuric acid method, as described by Dubois et al. (1956). Protein quantification was performed according to Lowry et al. (1951). Volatile fatty acids (acetic, propionic, butyric, isobutyric and isovaleric acids), ethanol and acetone were determined by gas chromatography (FID, flame ionization detector), according to Ramos et al. (2012).

Lactate was determined by ion chromatography using a Dionex ICS-1500 system with an REIC IonPac AS23 250 × 4 mm column, according to Ramos et al. (2012).

Food waste characterization

The characterization of the collected food waste was as follows: 63 ± 4% fruit and vegetable waste, 14 ± 3% flour-derived waste (including bread, corn tortilla, cookies, etc.), 8 ± 2% meat and 15 ± 3% other materials (including non-separated materials). The sample was homogenized and crushed, and the physicochemical analysis showed the following values: density 1090 kg m^{-3}, moisture 79 ± 2%, total solids 155 ± 13.6 g kg^{-1}, fixed solids 12.2 ± 1.6 g kg^{-1}, volatile total solids 142.8 ± 12.6 g kg^{-1}, COD_{total} 32.8 ± 2.4 g kg^{-1}, ammonia nitrogen 9.7 ± 1.3 g kg^{-1}, and total carbohydrates 71.63±5.68 g kg^{-1}.

Reactor setup and operation

An acrylic SBR with a total volume of 1.5 L, working volume of 1 L, headspace of 0.5 L and exchange volume of 50% was used. The temperature was controlled to 35 ± 1°C using a recirculation water jacket. The reactor was operated under the following operating conditions: filling time (5 min), reaction time (variable depending on the HRT), settling time (30 min), and idle time (5 min). The initial pH was adjusted to 5.5 and controlled by an automatic pH controller (Black Stone BL931700). It was coupled to a low flow peristaltic pump (Marlow Watson 120U) that added sodium hydroxide (NaOH) 2 N when the pH dropped below 5.4 and stopped when the pH reached a 5.5 value. The reactor was operated at four HRTs (48, 24, 16 and 8 h). The solid retention time was calculated by dividing the VSS in the reactor by the VSS in the effluent multiplied by the HRT. The solid retention time (SRT) during reactor operation was 42, 37, 17 and 16 h for HRT of 48, 24, 16 and 8 h, respectively. The SRT was controlled by varying the settling time and purging a certain amount of sludge.

Analysis of the microbial communities

DNA extraction from each HRT sample was performed with an Ultra Clean TM Soil DNA isolation kit from MoBio. The total bacteria, *Lactobacillus, Enterobacter,* and *Clostridium* were determined by RT-PCR.

Quantitative PCR for bacteria was performed using the Taqman method with the two universal primers W208 (5'-ACTCC TACGG GAGGC AG-3') and W209 (5'-GACTA CCAGG GTATC TAATC C-3'). Additionally, the fluorescent probe W210 (5'-Yakima Yellow- TGCCA GCAGC CGCGG TAATA C -Tamra-3') was used. The 20 µL PCR tubes contained the following: 100 nM of the primers, 200 nM TaqMan, 4 mM $MgCl_2$ and 2 µL DNA. The reactions were performed in a Mastercycler thermocycler (Eppendorf). The PCR amplification conditions were as follows: 40 activation cycles for 2 min at 94°C (7 sec of denaturation at 95°C, 25 sec of annealing at 60°C and 50 sec of extension at 72°C); the PCR products were confirmed by electrophoresis with 1% (w/v) agarose gel (Yu et al. 2005).

In the quantitative PCR of *Enterobacter, Clostridium* and *Lactobacillus,* the genes of the different bacteria were amplified with the follows primers: W329 (CAGGTCGTCACGGTAACAAG) and W330 (GTGGTTCAGTTTCAGCATGTAC) for *Enterobacter,* W234 (ACCGGTGGAGTTATGGAAGC) and W233 (CATCCACCTGGGCATGCCAT) for *Clostridium*, and W394 (CGGTAATACGTAGGTGG) and W395 (CGATGCACTTCYYCGG) for *Lactobacillus*. The 25 µL PCR tubes contained the following components: 250 nM of the primers, 12.5 µL Sybr Green, 5.5 µL water and 5 µL genomic DNA. The reactions were performed in a Mastercycler thermocycler (Eppendorf). The PCR amplification conditions were as follows: 40 cycles of enzyme activation for 2 min at 85°C (15 sec of denaturation at 98°C and 1 min of extension at 60°C).

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CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

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Figure 1. A) hydrogen and methane percentage in biogas, B) hydrogen specific production rate and, C) hydrogen production rate, during the operation of the SBR. ●H_2; ○CH_4; dotted line HRT.

Figure 2. A) Relation between the hydrogen yield and solid retention time and, B) RT-PCR for *Enterobacter*, *Clostridium* and *Lactobacillus*. Data expressed as mean abundance of 16S rDNA gene (copies ml^{-1}). ●*Enterobacter*; ▲*Clostridium;* ■*Lactobacillus*.

Figure 3. PCA profiles obtained during the microbial community analysis. ●HRT 48 h; □HRT 24 h; ΔHRT 16 h; ◊HRT 8 h.

Figure 4. A) Relation between bacteria from the *Lactobacillus* spp. and lactate production, and B) Relation between *Enterobacter* spp. and the percentage of hydrogen in the biogas.

Table 1. Operational parameters and metabolite profile at different hydraulic retention times

Parameters	HRT			
	8 h	16 h	24 h	48 h
HPR (mmol H_2 $L_{reactor}^{-1}$ d^{-1})	1.7 ± 0.4	4.4 ± 0.7	6.1± 1.9	6.0 ± 2.3
SHPR (mmol H_2 gVS_{added}^{-1} d^{-1})	0.02 ± 0.01	0.1± 0.01	0.2 ± 0.07	0.4 ± 0.16
Hydrolysis (%)	40.2 ± 13.7	41.0 ± 14.5	58.2 ± 14.1	71.7 ± 5.7
Hydrogen (% v/v)	4.9 ± 0.8	7.7 ± 5.6	17.9 ± 7.9	23.2 ± 11.1
Acetic (g L^{-1})	0.17 ± 0.07	1.11± 0.44	0.87 ± 0.18	1.86 ± 0.5
Propionic (g L^{-1})	0.44 ± 0.12	1.03 ± 0.22	0.98 ± 0.21	1.02 ± 0.31
Butyric (g L^{-1})	0.45 ± 0.09	0.79 ± 0.15	0.67 ± 0.13	1.63 ± 0.36
Isovaleric (g L^{-1})	0.18 ± 0.03	0.06 ± 0.02	0.07 ± 0.02	0.16± 0.02
Lactic (g L^{-1})	4.62 ± 0.80	2.12 ± 0.22	3.10 ± 0.45	1.04 ± 0.38
Ethanol (g L^{-1})	Not detected	0.38 ± 0.01	0.27 ± 0.03	Not detected