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Effect of Sodium Polyacrylate on the Fermentative Production of Biohydrogen

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ABSTRACT. This research evaluated the inhibitory or toxic effects of sodium polyacrylate (*SAP*, a moisture absorbent used in diapers) on hydrogenogenic dark fermentation of a model that resembles waste diapers. Three types of substrate bond paper and filter paper (*P*, substrate control), only sodium polyacrylate (*SAP* control or hydrogel, *HG*) and paper-hydrogel (*P-HG)* were tested in hydrogenogenic batch bioreactors at 37 °C. The bioreactors were operated using the anaerobic hydrogenogenic fermentation with intermittent venting and headspace flushing (*SSAHF-IV*) process. The reactors were loaded with the substrate at *ca.* 25 % of total solids and 10% w/w inoculum. It was found that the *SAP* hydrogel did not produce hydrogen. Units loaded with *P-HG* showed H_2 productions ca. 25% lower than that of bioreactors loaded with only paper. Our results suggest that diaper manufacturers could consider *SAP* replacement in diapers taking into account the optimization of H_2 yields during the treatment of waste diapers. For sustainability sake, *SAP* could be replaced by special starches such as those used by the food industry, that are known to absorb important amounts of water and are still degradable by dark fermentation. Alternatively, inoculum acclimation to acrylate degradation could be explored by using low amounts of acrylic acid or low molecular weight acrylates in the cellulosic substrate.

To the best of our knowledge, this is the first time that there is a documented evidence of deleterious effect of *SAP* on the hydrogenogenic dark fermentation of cellulosic wastes.

INTRODUCTION

Disposable diapers used in Mexico constitute about 6.5% of urban solid waste, and are currently sent to landfills (SEMARNAT, 2012). However, the disposable diaper contains about 35% of degradable cellulose fiber; the remainder is constituted by inert, innocuous and non-biodegradable synthetic materials such as polyethylene, polypropylene and a synthetic super absorbent polymer (*SAP*) (EDANA, 2011). Among the main components that make diaper degradation difficult is *SAP*; diapers can contain up to 30% of *SAP* (on dry basis). *SAP* consists of sodium polyacrylate, which some authors have evaluated for their use in agriculture to retain soil moisture. There are no studies so far on the possible inhibitory or toxic effects of *SAP* on biological treatment of wastes. The objective of this work was to determine the inhibitory effect of *SAP* in the hydrogen fermentation production system from diaper-like (cellulosic) wastes.

MATERIALS AND METHODS

Experimental design and reactors. Assays were performed with a combination of a mixture of bond paper and filter paper (*P*, substrate control), only sodium polyacrylate (*SAP* control or hydrogel, *HG*) and paper-hydrogel (*P-HG*) as substrates.

A 30% hydrogel *SAP* was added to the *P-HG* bioreactors, trying to simulate the amount present in the disposable diapers. All bioreactors were seeded with an anaerobic inoculum previously subjected to a heat-shock procedure. The test was carried out using the anaerobic hydrogenogenic fermentation with intermittent venting and headspace flushing (*SSAHF-IV*) process. All treatments were performed in triplicate.

The main response variable was the yield or cumulative H_2 production per unit of

substrate mass, P_{H2}, along with concentrations of volatile organic acids (acetic acid, *Hac*; propionic acid, *HPr*; butyric acid, *HBu*), lactic acid (*HLac*) and solvents (*Acetone, MeOH, EtOH, BuOH*) in the fermented solids.

Bioreactors were glass serum bottles of 120 mL capacity. There were loaded with each substrate, a mineral medium, and inoculum to give a final concentration of ca. 25% total solids (*TS*). Reactors were seeded with 10% w/w of digestates (25% *TS*) from methanogenic solid substrate anaerobic digesters degrading a mixture of organic solid waste. Methanogenic digestates were subjected to heat-shock at 90 °C for 1 h before loading into the serum bottles (Escamilla-Alvarado et al., 2012). They were filled with 37.5 mL of medium per bioreactor and contained 12.5 g_{db} of substrate (either *P, P-HG* or *HG*) to give a final concentration of ca. 25% *TS*. The mineral medium consisted of 31.6 g $NaHCO_3$ and 63.3 g K_2HPO_4 per liter with pH 7.3. Initial pH of the overall contents of bioreactors was in the range 5.6-6.2 (Sotelo-Navarro, Ph D Thesis, 2017). To maintain anoxic conditions, the bioreactor headspaces were flushed with N_2. Then, the bioreactors were incubated in static and dark conditions in an incubator Milipore 632 kept at 37 °C.

Analyses. Hydrogen concentration of biogas in the headspace was monitoring daily. Volatile organics acids and solvents were measured at the end of the test. The H_2, CO_2 and CH_4 in biogas were determinate by gas chromatography in a Gow-Mac chromatograph model 350, fitted with a thermal conductivity detector and a column 91 cm length packed with Molecular Sieve 5A. The injector, detector, and column temperatures were 25, 100 and 25 °C, respectively. Argon was the carrier gas (Sotelo-Navarro et al., 2017).

Cumulative hydrogen production (P_{H2}) was calculated from the values of biogas hydrogen concentration (taking into account the headspace volumes of bioreactors), and afterward, the effect of type of substrate on P_{H2} were evaluated with an analysis of variance (*ANOVA*).

Volatile organic acids (*VOA*), lactic acid, and solvents were analyzed as reported elsewhere (Váldez-Vázquez and Poggi-Varaldo, 2009, Muñoz-Páez *et al*., 2014). An aliquot of the filtrate was injected into a gas chromatography Varian Star 3400 equipped with a flame ionization detector for metabolite determination. The injector and detector temperatures were set at 250 °C. N_2 was used as a carrier gas with a 20 mL min^{-1} flowrate. The oven temperature was programmed as 60 °C for 2 min, increasing 140 °C at 5 °C min^{-1}, and then kept constant at 140 °C for another 6 min. A 50 m 0.32 mm internal diameter fused silica capillary column coated with 0.2 mm CP-Wax 57 CB was used.

Statistical analysis was performed using Statgraphics v. 15.2, Statpoint Technologies, Inc., Warrenton, VA, USA.

RESULTS AND DISCUSSION

The main characteristics of the mixtures substrate plus inoculum of the treatments are shown in Tables 1 and 2. *HG* treatment showed the lowest content of organic matter and higher level of ash (49% *VS* and 51% ash) probably due to the sodium and impurities present in the commercial *SAP*. This might have influenced the production of hydrogen by this substrate. The nitrogen contents in all bioreactors were low. that was contributed by the inoculum shows low results for all three cases, which significantly affected the carbon-nitrogen (C / N) ratio which was high for all cases. This, in turn, could have affected the degradation of the substrates, so it is recommended to adjust C / N to an average ratio of 30 to 40 in future tests (Li *et al*., 2014; Yadvika *et al*., 2004; Lin and Lay, 2004).

TABLE 1. Initial composition of mixtures substrate plus inoculum in the bioreactors.

Parameter	*P* [a]	*P-HG* [b]	*HG* [c]
pH	5.58 ± 0.09	6.19 ± 0.07	6.17 ± 0.11
Alkalinity (mg $CaCO_3$/kg)	2939 ± 161	1897 ± 130	564 ± 122
TS (%) initial in bioreactor	23.52 ± 7.08	29.37 ± 0.51	25.79 ± 0.81
VS (% TS)	69.72 ± 14.52	71.51 ± 0.64	48.91 ± 0.64
Ash (% TS)	30.28 ± 14.52	28.49 ± 0.64	51.09 ± 0.05
TKN (% TS)	0.36 ± 0.01	0.57 ± 0.06	0.65 ± 0.01
C/N	112.3 ± 23.4	73.6 ± 7.5	43.6 ± 0.3
Cellulose (% TS)	46.66 ± 1.69	41.37 ± 1.33	25.97 ± 3.08
Soluble COD (mg/L)	5453 ± 742	1566 ± 356	2900 ± 766
Protein (% TS)	2.25 ± 0.01	3.54 ± 0.39	4.07 ± 0.04

Notes: [a] paper; [b] paper-hydrogel; [c] hydrogel

TABLE 2. Initial profile of organic acids and selected solvents in the contents of bioreactors.

Type	Concentration before fermentation (mg COD/kg_{db})									
	HAc	*HPr*	*HBu*	Σ*VOA*	HLac	Ace	MetOH	EtOH	BuOH	Σsolv
P	158	11569	866	12593	1320	3.53	14.6	432.6	4.01	451
HG	59	1049	415	1524	0	0.16	0.0	0.0	0.94	1
P-HG	114	1756	102	1973	1115	1.40	8.9	93.6	1.33	104

Notes: P: paper; P-HG: paper-hydrogel; HG: hydrogel, *HAc* : acetic acid; *HPr* : propionic acid; *HBu* : butyric acid; HLac: lactic acid; Σ*VOA* : sum of volatic organic acids (it excludes lactic acid); Ace: acetone; MetOH: methanol; EtOH: ethanol; BuOH: butanol; ΣSolv: sum of solvents

It was found that the *SAP* hydrogel did not produce hydrogen. Units loaded with *P-HG* showed H_2 productions *ca.* 25% lower than that of bioreactors loaded with only paper (Table 3). Two cycles of H_2 production were achieved for the P-bioreactors whereas a third cycle was observed for *P-HG* units (Fig. 1). Yet, the H_2 production in the 3rd cycle of *P-HG* bioreactors could not offset the high H_2 production obtained in 2 cycles of P-units (Fig. 1). Not only *SAP* alone did not produce H_2 under *SSAHF-IV* process, rather there was a moderate toxic or inhibitory effect of *SAP* on the bioH_2 production from cellulosic wastes. Likely, this effect could also occur with diaper dark fermentation since diapers contain up to 35% of cellulose and 30 % of SAP.

Table 4 shows the *ANOVA* analysis where a single factor (substrate type) was considered. The effects of types of substrate (and implicitly the effect of *SAP* presence) on cumulative bio-H2 production were significant ($p < 0.05$). A test of means (not shown here) indicates that H_2 production of the treatment *HG* was significantly lower than the other two H_2 productions (*P* and *P-HG*). Acetic, propionic, butyric and lactic acids, as well as acetone, methanol and ethanol, were the main soluble microbial products detected in this work. Interestingly, butanol was not found in the fermentation products of this experiment (Tables 2 and 5, before and end of the fermentation respectively).

Interestingly, the units loaded with only *HG* exhibited the highest concentrations of *HPr* and *HBu* in our work (approx. 68000 and 6600 mg COD/kg_{db}, respectively, Table 5). Likely, these

TABLE 3. BioH_2 in each cycle of incubation and total biohydrogen production.

Reactor	P_{H2} [a] per incubation cycle (mmol H_2/g_{TS})			Total P_{H2} ($\sum_{j=1}^{3} P_{H2,j}$)
	1	2	3	
P[b]	5.25	0.14	0.11	5.50 ± 0.89
P-HG[c]	2.82	0.41	0.99	4.22 ± 0.66
HG[d]	0.01	0.01	0.01	0.02 ± 0.005

Notes: [a] production of hydrogen; [b] paper; [c] paper-hydrogel; [d] hydrogel.

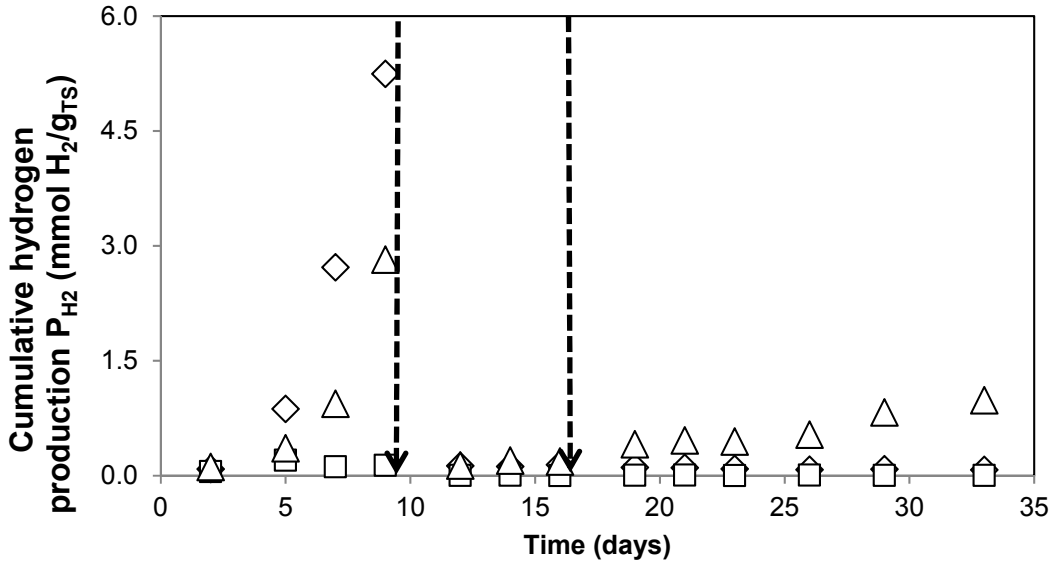

FIGURE 1. Time course of bioH_2 production.
Keys: paper (rhombus), paper-hydrogel (triangles), hydrogel (squares).

TABLE 4. Analysis of variance one single factor (type of substrate). Effect on cumulative bio-H_2 production

Groups	*Count*	*Sum*	*Average*	*Variance*		
P [a]	3	16.49	5.50	0.79		
P-HG [b]	3	12.65	4.22	13.42		
HG [c]	3	0.04	0.01	0.00		
Source of Variation	*SS*[d]	*df* [e]	*MSS* [f]	*F* [g]	*p-value* [h]	F_c [j]
Between Groups	49.39	2	24.69	5.21	0.049	5.14
Error	28.42	6	4.74			
Total	77.81	8				

Notes: [a] paper; [b] paper-hydrogel; [c] hydrogel; [d] sum of squares; [e] degrees of freedom; [f] mean of the sum of squares; [g] Fisher statistics estimated from experimental results; [h] probability value of the Fisher statistics; [j] critical Fisher statistics for alpha = 0.05 and (2, 6) degrees of freedom.

TABLE 2. Profile of metabolites at the end of the fermentation.

Type	Metabolites (mg COD/kg_{db}) and (mmol acid/kg_{db})										Ratio A/B[k]	ρ[l]
	HAc[a]	*HPr*[b]	*HBu*[c]	Σ*VOA*[d]	HLac[e]	Ace[f]	MeOH[g]	EtOH[h]	BuOH[i]	ΣSolv[j]		
P [m]	980 13.4	37216 314.0	3652 24.8	41848 352.2	1053 13.4	1.71 0.01	38.2 0.85	8508 96.2	< DL[n]	8548 97.1	0.27 0.54	4.90 3.63
P-HG[o]	781 10.5	23397 197.4	2675 18.2	26852 226.1	931 11.9	0.90 0.007	53.0 1.17	2169 24.5	< DL	2223 25.7	0.29 0.58	12.08 8.80
HG [p]	1289 17.7	68056 574.8	6629 45.1	75973 637.6	305 3.9	1.58 0.01	34.9 0.77	9880 111.7	< DL	9917 112.5	0.19 0.39	7.66 5.66

Notes: [a] acetic acid; [b] propionic acid; [c] butyric acid; [d] sum of volatic organic acids (it excludes lactic acid); [e] lactic acid; [f] acetone; [g] methanol; [h] ethanol; [i] butanol; [j] sum of solvents; [k] ratio: acetic acid/butyric acid; [l] factor ρ: Σ*VOA* /Σsolvents; [m] paper; [n] detection limit; [o] paper-hydrogel; [p] hydrogel.

acids were generated either by the fermentation of the cellulosic material of the inoculum itself or possible non-hydrogenogenic fermentation of acrylic acid (Ladd and Walker, 1958) probably
generated by hydrolysis of *SAP*. However, there is no evidence that accumulation of *HBu* and *HPr* in *P-HG* units was higher than in *P* units (Table 5).

Works by Ladd and Walker (1958) and Lewis and Elsden (1955) indicated that some microorganisms can degrade the acrylate by fermentation pathways. Indeed, Ladd and Walker (1958) observed that there is little production of hydrogen from the acrylate fermentation, which can be explained in terms of the consumption of the hydrogen by the substrate to achieve the reduction of the substrate to propionic acid. On the other hand, Lewis and Elsden (1955) reported the fermentation of acrylate to hydrogen, CO_2 and high amounts of propionic acid from a Gram-negative coccus bacteria is possible.

In our work *HG* reactors did not produce H_2, but exhibited high concentration of propionic acid compared to *P* and *P-HG*, almost double in both cases. This is likely the result of a vigorous non hydrogenogenic fermentation in *HG* reactors, which is consistent with the findings of Ladd and Walker (1958).

Another issue that could have contributed to low H_2 production in *HG* reactors is related to high concentrations of both *HPr* and *HBu* in those units at end of fermentation (Table 5). Jones et al. (2015) and Hawkes et al. (2007) have reported that *VOA*s can negatively affect H_2 production by two mechanisms, i.e., product (acid) inhibition (Jones et al, 2015; Hawkes et al., 2007), and cell lysis of the H_2-producing bacteria (Choudhari et al., 2014; Jones et al., 2015). Indeed, van Ginkel and Logan (2005) and Zhang et al. (2012) have reported that butyric acid is more toxic than acetic acid. Muñoz-Páez et al. (2012) have shown that removing organic acids by washing fermented solids in batch solid substrate hydrogenogenic fermentation of Organic Fraction of Municipal Solid Waste (*OFMSW*) removes the production inhibition effect and increased bioH_2 generation. Moreover, Zumar and Mohee (2016) observed a similar effect as Muñoz-Páez et al. (2012) by reducing organic acids from fermented liquors by bipolar membrane electrodialysis leading to an increase of hydrogen production in dark fermentation (*DF*).

Table 6 below compiles selected cases of H_2 *DF* that experienced inhibition by *HBu* and *HPr*. Typically, the range of *HBu* concentration tested was around 0-300 mM. *HBu* iInhibitory thresholds were generally between 20 to 60 mM for mild inhibition (20% or less inhibition) and around 200-300 mM for severe bioH_2 production inhibition (90% or higher). Our results show that *HBu* concentrations in *HG* reactors were moderate (Table 5) and fall in the range of mild inhibition of Table 6. Furthermore, since *HAc* has been reported as another inhibitor to bioH_2 production (Jones et al., 2015), synergistic inhibitory actions of *HAc* and *HBu* cannot be ruled out.

TABLE 3. Inhibition of H_2 production in dark fermentation by butyric and propionic acids.

Substrate and process conditions	Inoculum	Concentration range of *HBu* (mM) (inhibitory threshold)	Maximum H_2 yield [in mol H_2/mol glucose added] (and *HBu* concentrations at given inhibition degrees)	Ref.
Glucose, 35 °C, Batch 125 rpm Lab scale	*Clostridium bifermentans 3AT-ma*	0-250 mM (250 mM *HBu* , 83.47%)	33[a] [1.21] (1.21 at 0 mM *HBu*) (0.20 at 250 mM *HBu*)	1
Glucose, 35 °C, Batch No mixing Lab scale	Digested sludge	0-300 mM (87.91 % inhibition)	277.0[b] [2.22] (NR)[c]	2
Glucose, 37 °C, Batch 150 rpm Lab scale	Anaerobic microflora	0-25.08 g/L [0-285 mM] (285 mM *HBu* , 81.71%)	1.85[d] (1.75 at 0 mM *HBu*) (0.32 at 285 mM *HBu*)	3
Glucose, 30 °C, Semicontinuous No mixing Lab scale	Agricultural soil	0-60 (25 mM, 22% inhib.) (60 mM, 93%+ inhib.)	2.50[d] (2.0 at 25 mM *HBu*) (< 0.2 at 60 mM *HBu*)	4
HG reactors, 37 °C, Batch Lab scale	Heat-shocked methanogenic consortium	45.1 mM	0.0	This work
Substrate and process conditions	**Inoculum**	**Concentration range of *HPr* (mM) (inhibitory threshold)**	**Maximum H_2 yield [in mol H_2/mol glucose added] (and *HPr* concentrations at given inhibition degrees)**	**Ref.**
Glucose, 35 °C, Batch, No mixing Lab scale	Digested sludge	0-300 mM (74.5% inhibition)	21.5[a] [1.29] (NR)	2
Glucose, 37 °C, Continuous,	Enriched mixed culture	10-32 mM (38% inhibition)	1.68[d] (1.08 at 32 mM)	5
Cassava stillage, 37 °C,	Anaerobic Sludge	5-24 mM (82% inhibition)	3.10[d] (0.57 at 24 mM)	6
Cheese whey, 37 °C, Continuous	Anaerobic granular sludge	47-108 mM (64 % inhibition)	2.8[d] (1.01 at 108 mM)	7
Sucrose, 35 ° C, Batch to continuous	Anaerobic sludge	14-47 mM (92.5% inhibition)	0.8[d] (0.06 at 47 mM)	8
HG reactors, 37 °C, Batch Lab scale	Heat-shocked methanogenic consortium	575 mM (100% inhib)	0.0	This work

Notes: [a] mL; [b] mL/g subtrate; [c] not reported; [d] mol/mol susbtarte. References: 1 Zhang et al. (2012); 2 Wang et al. (2008); 3 Zheng and Yu (2005); 4 Van Ginkel and Logan (2005); 5 Sivagurunathan et al., (2014); 6 Luo et al. (2010); Davila-Vazquez et al. (2009); 8 Kim et al. (2008)

On the other hand, the accumulation of propionic acid during dark fermentation has also been reported as a possible inhibition of fermentative hydrogen production (Table 6)

in concentrations between 0 and 300 mM, our case shows a concentration of 575 mM. It may indicate another possible cause of the non-presence of H_2 in the *HG* bioreactors.

Concentrations of *HLac* were moderate for all the treatments and at levels lower than the initial contents of lactic acid for treatment *P* and *P-HG* due to inoculum (Table 2). It has been reported that lactic acid fermentation could be a sink of bio hydrogen because the fermentation of hexose by homolactic and heterolactic pathways (Escamilla-Alvarado *et al.* 2012) does not produce H_2. Besides the loss of H_2 from lactic acid production, another issue is the possible toxic effect of lactic acid bacteria on other members of the H_2-producing consortium via bacteriocins (Muñoz-Paéz *et al.* 2008, Noike *et al.* 2002). Results in Table 5 do not provide evidence of a significant *HLac* deviation of the *DF* in our bioreactors, or that lower H_2 production in *P-HG* than in *P* bioreactors could be associated to lactic acid deviation.

CONCLUSION

- Units loaded with *P-HG* showed H_2 productions 25% lower than that of bioreactors loaded with only paper. So, there was an inhibitory effect of sodium polyacrylate (*SAP* or *HG*) on dark fermentation of paper, a typical cellulosic substrate.
- Two cycles of H_2 production were achieved for the P-bioreactors whereas a third cycle was observed for *P-HG* units. Yet, the H_2 production in the 3rd cycle of *P-HG* bioreactors could not offset the high H_2 production obtained in 2 cycles of *P* units.
- There was some evidence that *SAP* could undergo non-hydrogenogenic fermentation, based on metabolite profiles in the *HG* treatment and information in the open literature regarding acrylic acid fermentation by a variety of microorganisms.

Our results suggest that *SAP* could also negatively affect hydrogen production from the *DF* of waste diapers, considering the high content of both cellulose and *SAP* of this waste, similar to our model treatment *P-HG*.

Diaper manufacturers could consider *SAP* replacement in diapers taking into account the optimization of H_2 yields during the treatment of waste diapers. For sustainability sake, *SAP* could be replaced by special starches such as those used by the food industry, that are known to absorb important amounts of water and are still degradable by dark fermentation. Alternatively, inoculum acclimation to acrylate degradation could be explored by using low amounts of acrylic acid or low molecular weight acrylates in the cellulosic substrate in *ad hoc* acclimation procedures.

To the best of our knowledge, this is the first time that there is a documented evidence of deleterious effect of *SAP* on the hydrogenogenic dark fermentation of cellulosic wastes.

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NOTATION

ANOVA	analysis of variance
BuOH	butanol
COD	chemical oxygen demand
C/N	carbon nitrogen ratio
db	dry basis
df	degrees of freedom
DF	dark fermentation
EtOH	ethanol
F_{exp}	value of the Fisher statistic based on experimental results
F_c	critical value of Fisher statistic based on Fisher distribution ant the degrees of freedom of the source and the error, at 0.05 probability of significance
FID	flame ionization detector
HAc	acetic acid
HBu	butyric acid
HG	hydrogel
HLac	lactic acid
HPr	propionic acid
MeOH	methanol
MSS	mean of the sum of squares
MSE	mean of the sum of squares of the error
P	bond paper and filter paper

P_{H2}	production of H_2
P-HG	paper and hydrogel
p-value	probability of the Fisher statistic based on experimental results
SAP	sodium polyacrylate
Solv	solvents
SS	sums of squares
SSAHF-IV	solid substrate anaerobic hydrogen fermentation with intermittent venting
TKN	total Kjeldahl nitrogen
TS	total solids
VOA	volatile organic acids, *i.e.*, low molecular weight organic acids
VS	volatile solids