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The Effect of pH on Continuous Biohydrogen Production from Swine Wastewater Supplemented with Glucose

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Abstract The effect of pH on hydrogen production from liquid swine manure supplemented with glucose by a mixed culture of fermentative bacteria in an anaerobic sequencing batch reactor was evaluated in this study. At 37±1 °C, five pH values ranging from 4.7 to 5.9 at an increment of 0.3 were tested at a hydraulic retention time (HRT) of 16 h. The results showed that at this HRT, the optimal pH for hydrogen production was 5.0, under which the biogas comprised 33.57±5.65% of hydrogen with a production rate of 8.88±2.94 L-H_2/day and a yield of 1.48±0.49 L-H_2/L liquid swine manure. The highest biomass concentration, highest butyric acid to acetic acid ratio, lowest propionic acid concentration, and the best stability were all found at pH 5.0, while the highest CH_4 productivity was found at pH 5.9. For efficient hydrogen production, oxygen content should be controlled under 2%, beyond which an inverse linear relationship (R^2=0.986) was observed.

Keywords Hydrogen production · Swine manure · pH effect

Introduction

Hydrogen has been attracting attention for decades as an energy carrier due to its friendliness to environment and the highest gravimetric energy density among all known fuels [1]. Currently, hydrogen is commercially produced mainly by energy-intensive processes dependent on fossil fuel such as electrolysis of water, and steam reforming of methane or other hydrocarbons [2]. As a sustainable and renewable alternative to these conventional processes, hydrogen production by biological methods provides a cost-effective and pollution-free route [3].

Among all the biological hydrogen production approaches [3], dark fermentation, where hydrogen is produced from organic wastes by anaerobic bacteria, has been deemed a more economically feasible pathway because fermentative bacteria is technically simpler to operate than photosynthetic bacteria, and has a wider range of organic substrate [4, 5]. The feasibility

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of fermentative hydrogen production from organic wastes by dark fermentation has been extensively verified by various researchers [2, 6–18], but few are related to animal wastes.

Hydrogen production from anaerobic fermentation using swine manure as substrate has tremendous potential to provide clean energy from renewable biomass. It can not only produce bioenergy from an untapped sustainable resource but also help ameliorate the burden of animal wastes disposal on cropland.

Hydrogen production by dark fermentation is highly dependent on process conditions, including pH, which has a strong effect on hydrogenase activity [19], the metabolic pathway [20], as well as the suppression of the hydrogen-consuming methanogenic activities [21]. Many researchers have investigated the pH effect over a certain range, but the optima pH varies with different substrates used [17].

The objective of this research is to examine the pH effect on hydrogen production using swine manure as a major substrate and to determine optimum operating pH for the laboratory-scale anaerobic sequencing batch reactor (ASBR).

Materials and Methods

Bioreactor Configuration and Setup

A lab-scale ASBR system was constructed as shown in Fig. 1. The bioreactor was a polyethylene jar, 8 L in total volume with 4 L in working volume, and placed on a hot plate

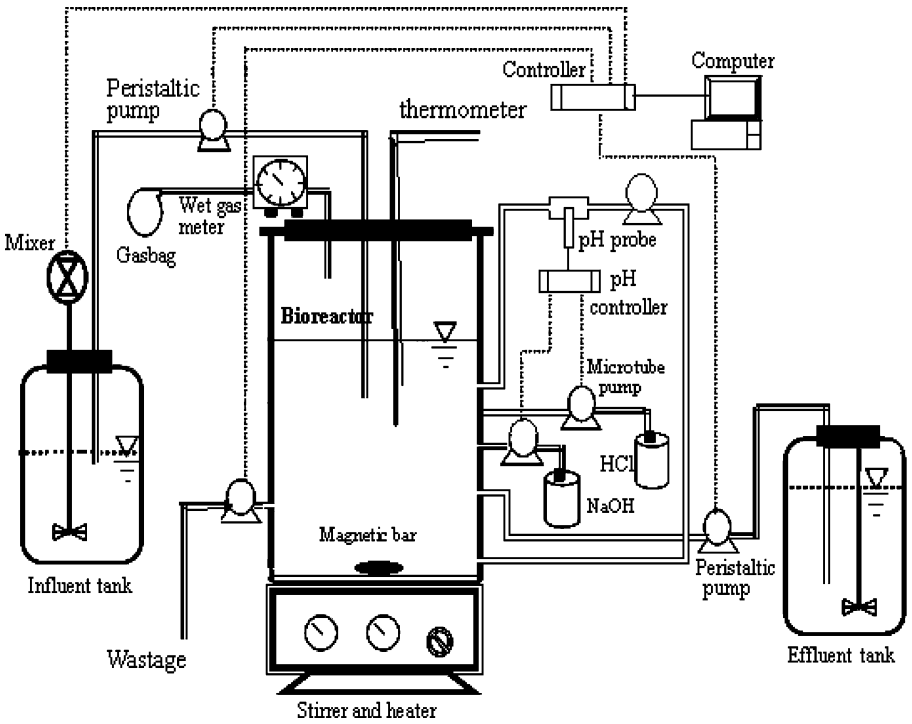

Fig. 1 The schematic diagram of the anaerobic sequencing batch reactor for biohydrogen production from liquid swine manure

stirrer that maintained the temperature of the reactant at 37±1 °C. Complete-mix conditions were achieved by a centrifugal water pump circulating the liquid through a T connector holding a pH probe at one end to simultaneously monitor the real-time pH. The pH probe was connected to a pH controller, which maintained the pH in the fermenter by turning on and off two separate peristaltic pumps that added either hydroxide (0.5 M NaOH) or acid (0.5 M HCl) to the reactor. Manure as substrate was reserved in a 20-L influent tank with a mixer during feeding and pumped into the reactor at preset time intervals, and the treated manure was discharged into an effluent tank. The influent and effluent flows were regulated by two separate peristaltic pumps according to the preset HRT. The system was operated by a programmable control module (Campsci CR1000) with its software (Campsci PC400) capable of carrying out all the needed programs. The amount of biogas produced was recorded daily using a wet-gas meter. In order to avoid washout of bacteria from the bioreactor, 1-h settling time was applied to the reactor before discharging the effluent.

Inoculum Pretreatment and Enrichment

The inoculum used in this project consisted of two parts. One was the anaerobic digester sludge obtained from a local wastewater treatment plant located at Waseca, MN, USA, and the other was obtained from a wastewater treatment lagoon of a local cattle farm (St. Peter, MN, USA). The ratio of the two sludges was 1:1, and the volume of the seed sludge was 400 mL, which was 10% of the working volume. Raw seed sludge was filtered through a screen with pore size of 2 mm to remove fibrous undigested materials before use. The seed sludge was first acclimated in a batch reactor for 30 h at pH 5.0 using a CT medium with the following ingredients (g/L): KH_2PO_4 1.5, $Na_2HPO_4.12H_2O$ 4.2, NH_4Cl 0.5, $MgCl_2.6H_2O$ 0.18, yeast extract 2.0, peptone 5.0, and glucose powder 10.0; and the ratio of the sludge to the media was 1:1. Then the content in the batch reactor was thermally treated at 100 °C for 15 min to inactivate thermal-susceptible methanogenic bacteria and other non-hydrogen-producing flora present in the sludge. After being cooled down to room temperature, the enriched seed sludge was transferred into the reactor, and the reactor was ready to start.

Manure Source and Preparation

The manure used in this project was collected from a swine barn at the University of Minnesota Southern Research and Outreach Center at Waseca. The manure was diluted with tap water from a solids content of about 8% to around 1%. In order to provide sufficient nutrients to hydrogen-producing bacteria, each liter of liquid swine manure was supplemented with (mg/L) $CoCl_2.5H_2O$ 0.125, $FeSO_4$ 55, glucose 10,000, K_2HPO_4 125, $MgCl_2.6H_2O$ 100, $NaHCO_3$ 600, and NH_4HCO_3 500. After placing the prepared liquid swine manure in the influent tank, the manure pH was adjusted to that in the reactor.

Experimental Design and Operation

When the construction of the ASBR system was completed, the pretreated seed sludge was transferred into the reactor with additional liquid swine manure to fill up the working volume of 4 L. The reactor was then closed airtight and purged with argon gas for about 8 L to remove any air left in the headspace. The system was run at HRT 16 h according to the schedule shown in Table 1, which covered a range of pH from 4.7 to 5.9 at an increment of 0.3. The temperature of the reactor was maintained at 37±1 °C, and the pH was controlled

Table 1 Experimental design.

Experimental runs									
1		2		3		4		5	
pH	HRT	pH	HRT	pH	HRT	pH	HRT	pH	HRT
4.7	16 h	5.0	16 h	5.3	16 h	5.6	16 h	5.9	16 h

at the designed value. To eliminate the interactions between different pH, each run started with new inoculum. After inoculation, the reactor was operated in a batch mode, and the startup period was considered complete after 24 h of inoculation. The reactor then entered a continuous operating mode and was fed every 4 h during one HRT (16 h). Before feeding, 1-h settling time was allowed. For each feeding, 1 L of the reactor content was removed followed by the same amount of influent added. The reactor was stirred constantly by the circulation system, except during the settling and feeding period, to ensure a thorough mixing and to facilitate rapid diffusion of H_2. The steady state was considered established after the gas volume produced reached a relatively constant value (with a variation of within 5–10%) for five consecutive HRTs, and the length of test for each condition was 12 to 24 HRTs according to the practical situation. The samples were taken every other HRT, totalizing around six samples for each condition. The biogas was released continuously to keep a low H_2 partial pressure in the headspace, and the gas volume was recorded daily by a wet-gas meter.

Sampling and Analysis

Biogas was collected at the discharge port and sampled every other HRT after steady state, and the composition, including hydrogen (H_2), oxygen (O_2), methane (CH_4), and carbon dioxide (CO_2), was analyzed using a micro-gas chromatography [(GC)-(CP-4900 QUAD MICRO-GC, Varian Inc., Palo Alto, CA, USA)].

The liquid samples, including influent, effluent, and content in the fermenter, were taken at the same time with the gas sample. Effluent and fermenter content were collected at the effluent discharge port, while the influent sample was obtained from the influent tank under mixing, and each sample (50 mL) was taken in triplicate. The total volatile suspended solids (TVSS), which represents biomass concentration, was determined according to the standard method [22]. Volatile fatty acids (VFAs), including acetic acid, propionic acid and butyric acid, and alcohols, including ethanol, propanol, and butanol, were analyzed by a gas chromatography equipped with a flame ionization detector (Varian Star 3900,Varian Inc., Palo Alto, CA, USA). The temperature used for the injector and the flame ionization detector was 250 °C. Nitrogen was used as a carrier gas with a 25 mL/min flow rate (25 psi). The oven temperature was programmed as follows: 60 °C for 2 min, increased to 140 °C at 5 °C/min, and then kept constant at 140 °C for another 10 min. A 50-m× 0.32-mm internal diameter fused silica capillary column was used, coated with 0.2 μm CP-Wax 57 CB. The correlation coefficients (R^2) for the standard calibration curves of all VFAs and alcohols were larger than 0.998.

All the data were analyzed by software SPSS. The effect of pH on biogas productivity and biogas content, microbial growth, soluble metabolites distribution, and the stability of the reactor was determined according to the p value in one-way ANOVA analysis.

Results and Discussion

The p values for the effect of pH on biogas productivity and biogas content, microbial growth, soluble metabolites distribution, and the stability of the reactor in one-way ANOVA analysis were all much smaller than 0.05, suggesting that the pH had a significant effect on these variables.

The Effect of pH on Hydrogen Production

Table 2 presents information about the biogas production under each pH condition. Generally speaking, at HRT 16 h, the total volume of biogas produced per day as well as hydrogen percentage in the biogas increased with the decrease of pH, and peaked at pH 5.0, after which both of them declined. The hydrogen content increased from 0.14% at pH 5.6 to 33.57% at pH 5.0, and then decreased to 13.66% at pH 4.7. The total biogas productivity increased from 11.38 L/day at pH 5.9 to 26.09 L/day at pH 5.0, and then dropped to 10.10 L/day at pH 4.7

At pH 5.3, both the gas productivity and hydrogen percentage were lower than that at pH 5.6, which might be due to the higher biomass synthesis rate under the operation condition. Past research shows that under certain conditions, bacteria will divert their metabolic pathway from hydrogen production, which is a natural consequence of the truth that fermentation is optimized by evolution to produce cell biomass rather than hydrogen, which is only an intermediate product [4]. At pH 5.9, the total gas volume and the hydrogen content were very low, which suggested that hydrogen-producing organisms could not function well. It also indicated that the pretreatment of the sludge could effectively suppress non-spore-forming microbes.

Figure 2 illustrates the hydrogen productivity under different pH. At HRT 16 h, hydrogen productivity increased with the decrease of pH and peaked at pH 5.0. Under this optimum pH, the highest hydrogen productivity observed was 8.88±2.94 L-H_2/day with a yield of 1.48±0.49 L-H_2/(L liquid swine manure), which demonstrated a product to substrate ratio of 1.48. This means that for every liter of manure fermented, 1.48 L of hydrogen were produced, which was significant and encouraging.

The results in this section indicated that low pH can facilitate the growth of hydrogen-producing bacteria as well as the activity of hydrogenase, which is responsible for the increase of hydrogen content in the offgas, but too low a pH would inhibit hydrogen production since pH affects the activity of iron containing hydrogenase enzymes [19]. The results observed from this project are in accordance with the previous research reports. Dabrock et al. [19] also reported that at pH above 5, higher production rate was observed,

Table 2 Summary of biogas production under different pH.

Experimental conditions		Biogas volume (L/day)	H_2%	CH_4%	CO_2%
HRT (h)	pH				
16	4.7	10.10±2.99	13.66±12.32	3.85±1.75	75.74±9.30
16	5.0	26.09±6.73	33.57±5.65	0.92±1.54	59.06±0.72
16	5.3	10.33±2.61	23.24±11.09	0.00±0.00	66.05±11.39
16	5.6	15.52±1.03	0.14±0.05	17.96±0.89	62.89±0.85
16	5.9	11.38±0.87	4.19±1.49	32.57±25.41	29.45±8.00

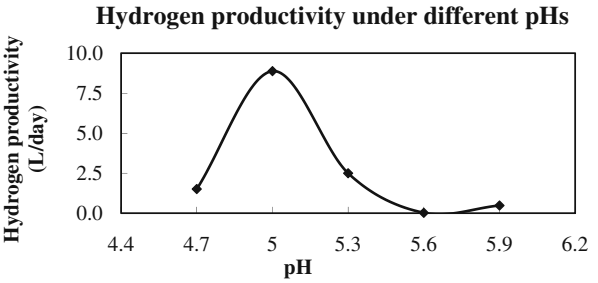

Fig. 2 Hydrogen productivity under different pH

whereas at pH below 5, the production rate dropped distinctly, and at pH below 4.8, steady-state conditions could not be obtained anymore.

The Effect of pH on CO_2 Production

As shown in Table 2, in all cases, CO_2 is the main byproduct in the biogas produced during dark fermentation except at pH 5.9. Regardless of the type of the end-product, CO_2 is always simultaneously produced during metabolic process with the formation of hydrogen, methane, or other metabolites. In general, at HRT 16 h, CO_2 percentage was relatively low at pH 5.0, which indicated that at 5.0, hydrogen-producing bacteria could process a higher hydrogen production rate with a lower metabolic rate and provide better hydrogen productivity. The lowest CO_2 percentage was observed at pH 5.9 due to the low activity and survival rate of hydrogen-producing bacteria at a high pH.

The Effect of pH on CH_4 Production

One of the many problems associated with the use of dark fermentation to produce hydrogen is the presence of methanogens in the environment that compete with the hydrogen-producing bacteria for carbon and hydrogen. From the results shown in Table 2, methane produced in all conditions was relatively low, which indicated that the heat treatment of seed sludge and the operational conditions in the fermenter could effectively inactivate hydrogen-consuming methanogens and homoacitogens. Overall, methane percentage increased with the increase of pH at HRT 16 h. The highest methane percentage and productivity were obtained at pH 5.9, since it is close to the pH range considered necessary to maintain adequate activity of methanogens [23].

The Effect of pH on Microbial Growth

Hydrogen-producing bacteria enriched from the natural source are mainly *Clostridium* that is gram-positive rod-shaped anaerobes, and for which pH is a key factor during the germination and growth process [24]. The *Clostridium* species capable of producing hydrogen includes *Clostridium acetobutylicum* [25], *Clostridium butylicum* [26], *Clostridium butyricum* [27], *Clostridium kluyveri* [28], and *Clostridium pasteurianum* [29], which are known acidophilic species and can grow at pH lower than 6 [30, 31].

Figure 3 shows the effect of pH on TVSS, which represents biomass concentration at HRT 16 h under different pH. The biomass concentration increased obviously as the pH decreased from 5.9 to 5.0, and stayed stable as the pH dropped from 5.0 to 4.7, indicating that low pH can facilitate the growth of hydrogen-producing bacteria, but further decreases

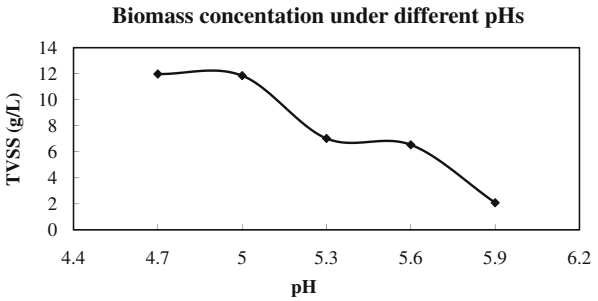

Fig. 3 The effect of pH on biomass concentration

in pH inhibit their growth, which is consistent with those from previous researchers reporting that at pH above 5, higher growth rate were observed, whereas at pH below 4.8, the growth rate decreased distinctly [19].

The Effect of pH on Soluble Metabolites Distribution

In most studies, the type of end-product is highly dependent on the media pH [17]. Table 3 lists the concentration of acetic acid, butyric acid, propionic acid, ethanol, butanol, and propanol under all operating conditions, based on which several observations can be summarized below.

First, the main end products from fermentation were acetic acid, butyric aicd, propionic acid, and alcohol, but their distribution was highly dependent on pH. Second, at HRT 16 h, the highest butyric acid to acetic acid ratio was observed at pH 5.0, which was consistent with the hydrogen productivity, indicating that higher B/A could result in higher hydrogen productivity, but no linear relationship was observed between these two parameters. Third, lower pH seems to favor butyric acid production, while higher pH favors acetic acid production. These findings were in close agreement with the results obtained by previous researchers [20, 32–34].

$$C_6H_{12}O_6 + 2H_2O \rightarrow 2CH_3COOH + 2CO_2 + 4H_2 \quad (1)$$

$$C_6H_{12}O_6 \rightarrow CH_3CH_2CH_2COOH + 2CO_2 + 2H_2 \quad (2)$$

Equations 1 and 2 show the major metabolic pathway involved in hydrogen production. According to the equations, it might be explained as that at a lower pH range, the reaction is driven predominantly to acetate and butyrate fermentation, which results in higher hydrogen

Table 3 The concentration of specific VFA and alcohol at different pH.

Experimental conditions		Alcohol (mg/L)			Volatile fatty acid (mg/L)			B/A
HRT (h)	pH	Ethanol	Propanol	Butanol	Acetic acid	Propionic acid	Butyric acid	
16	4.7	1,041.3	0.0	23.9	329.3	220.9	899.3	2.7
16	5.0	454.3	0.0	0.0	88.8	21.6	906.5	10.2
16	5.3	384.8	15.0	0.0	339.5	198.3	524.7	1.5
16	5.6	75.1	7.8	0.0	281.5	257.2	835.2	3.4
16	5.9	51.0	1.6	0.0	598.6	630.0	1,187.1	1.98

Note: B/A=butyric acid concentration (mg/L)/acetic acid concentration (mg/L)

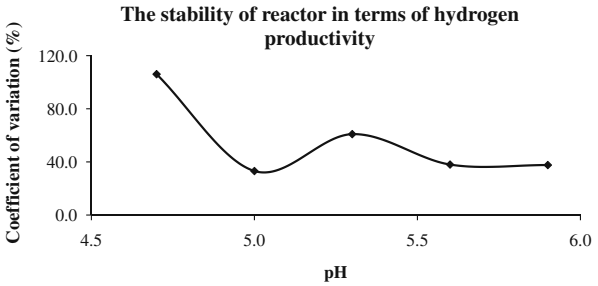

Fig. 4 The effect of pH on reactor stability

productivity, and the alteration of the distribution of the end-product indicated a metabolic shift due to environmental change, i.e., pH increase, change in biogas composition, and intermediate accumulation. Fourthly, the concentration of ethanol was higher at pH 4.7 and 5.0, indicating that the reaction shown in Eq. 3 was preferred at lower pH.

$$C_6H_{12}O_6 + 2H_2O \rightarrow CH_3CH_2OH + CH_3COOH + 2H_2 + 2CO_2 \quad (3)$$

The finding was different with that stated in the research done by Lay [20], in which there was an inverse relation between alcohols and hydrogen/VFAs productions. The controversy may be due to the fact that the feedstock used in Lay's study was starch, which was less complex than swine manure and led to fewer side reactions. Finally, propionic acid was present at higher concentrations than previous studies [20, 32–34], indicating that not only *C. butyricum* but also other propionate-producing species such as *Clostridium arcticum* [30], *Clostridium novyi* [31], and *Clostridium propionicum* [35] were present in the system. This fact verified that a mixed culture was involved in the ASBR system developed. This adds significance to the engineering prospect for this project since a mixed culture is more feasible than a pure one in hydrogen fermentation from organic wastes.

The Effect of pH on Reactor Stability in Terms of Hydrogen Productivity

The operating pH was found to have a tremendous influence on many aspects of the fermenter system, including the stability of the reactor. As shown in Table 2, during the pseudo-steady state, there was some variation in the system in terms of hydrogen content. Figure 4 shows the relationship between the coefficient of variation (CV) and the pH, with higher CV indicating lower repeatability. There was no linear relationship between the two

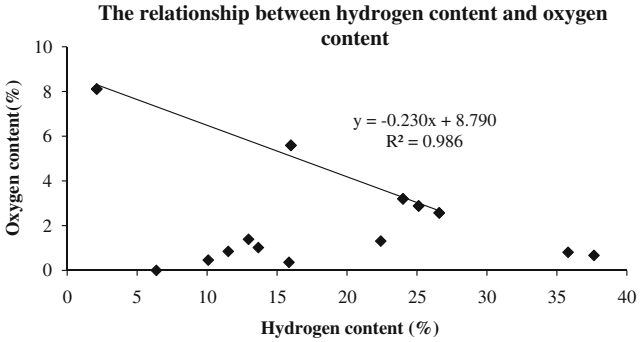

Fig. 5 Effect of oxygen content on hydrogen production

parameters. The lowest point was found at pH 5.0, and further decrease of pH below 5.0 would significantly increase the instability of the reactor, which is in accordance with the observation from a previous study that steady state was hard to maintain at pH lower than 4.8 [19].

The Effect of Oxygen on Hydrogen Production

Among a large number of microbial species, facultative anaerobic and strict anaerobes, which are sensitive to oxygen inhibition, are efficient producers of hydrogen. Figure 5 shows the relationship between oxygen and hydrogen content in the biogas produced. When oxygen content was kept below 2.0%, it had an insignificant effect on hydrogen productivity. But when its composition increased to around 2% and above, the production of hydrogen decreased linearly with the increase of oxygen content, indicating that this process was very sensitive to oxygen and could be inhibited by very low concentration of oxygen in the reactor. The hydrogen bacteria enriched from the anaerobic digester sludge are mainly *Clostridium*, which are strict anaerobes and endospore formers [5]; thus, in order to provide an anaerobic environment, the reactor must be sealed tightly and flushed with argon after air exposure. From the point of practical use, facultative anaerobes are considered a better microorganism than strict anaerobes because they are less sensitive to oxygen, and are sometimes able to recover hydrogen production activity after accidental oxygen damage by rapidly depleting oxygen present in the broth [36]. The source of oxygen in this study could largely be attributed to the mixing in the influent tank, which was not sealed, causing some air entrapped in the manure prior to feeding the reactor.

Conclusions

The operating pH has profound impact on biogas production, biogas composition, microbial growth, soluble metabolites distribution, and reactor stability. The optimum pH under HRT 16 h is found at 5.0, under which the hydrogen productivity is 8.88±2.94 L-H_2/day with a yield of 1.48±0.49 L-H_2/(L liquid swine manure). The highest biomass concentration, highest butyric acid to acetic acid ratio, lowest propionic acid concentration, and the best stability were all found at pH 5.0, while the highest CH_4 productivity was found at pH 5.9. For efficient hydrogen production, oxygen content in the offgas should be controlled under 2%; otherwise, an inverse linear relationship (R^2=0.986) was observed.

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