

Article

Biohydrogen Fermentation from Sucrose and Piggery Waste with High Levels of Bicarbonate Alkalinity

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Abstract: This study examined the influence of biohydrogen fermentation under the high bicarbonate alkalinity (BA) and pH to optimize these critical parameters. When sucrose was used as a substrate, hydrogen was produced over a wide range of pH values (5–9) under no BA supplementation; however, BA affected hydrogen yield significantly under different initial pHs (5–10). The actual effect of high BA using raw piggery waste (pH 8.7 and BA 8.9 g CaCO₃/L) showed no biogas production or propionate/acetate accumulation. The maximum hydrogen production rate (0.32 L H₂/g volatile suspended solids (VSS)-d) was observed at pH 8.95 and 3.18 g CaCO₃/L. BA greater than 4 g CaCO₃/L also triggered lactate-type fermentation, leading to propionate accumulation, butyrate reduction and homoacetogenesis, potentially halting the hydrogen production rate. These results highlight that the substrate with high BA need to amend adequately to maximize hydrogen production.

Keywords: bicarbonate alkalinity; biohydrogen fermentation; sucrose; piggery waste; pH; homoacetogenesis

1. Introduction

Hydrogen is a future sustainable and clean renewable energy carrier because it has a high energy yield and produces only water as the final product. A hydrogen society for sustainable development

has been already started and well-established with a growth rate of 5%–10% per year overall [1]. Hydrogen is produced commercially by thermocatalytic gasification or electrolytic process, which is quite energy intensive, as well as by microbial hydrogen fermentation. Microbial hydrogen production through the direct fermentation of organic wastes is one of the potential technologies for producing renewable hydrogen that couples the need for waste reduction and byproduct recovery, simultaneously. On the other hand, it is unclear if the technology can be developed to provide a high hydrogen yield and become economically competitive with gasoline or with alternative hydrogen production.

In fermentation approach to biohydrogen production, the main technical barriers include the low molar yield of hydrogen, byproduct acid accumulation and feed stock cost, *etc.* Among them, the relatively low molar yield of hydrogen from organics is the most undesirable barrier, resulting from the simultaneous production of waste organic acids and solvents. Although 1 mol of glucose can theoretically produce 12 mol of H₂ (1.4 L H₂/g chemical oxygen demand (COD) @ standard temperature and pressure (STP), as the theoretical maximum), as shown in Equation (1), the biological pathways maximally yield only 4 mol of H₂ per mole of glucose (0.467 L H₂/g COD, 0.333 g COD_{H₂}/g COD) @STP [2,3]. Most studies have reported a molar yield of 2 (0.233 L H₂/g COD, 0.167 g COD_{H₂}/g COD) @ STP or less [4]:



Laboratory studies were performed for fermentative hydrogen production using actual wastewater, and the results showed a much lower yield compared to that of a synthetic carbohydrate type substrate [5]. Compared to the biological maximum potential, the reason for the lower hydrogen recovery is unclear. Hydrogen production can be affected by the substrate characteristics, operating conditions, some inhibition by chemicals, and homoacetogenesis reactions, *etc.* [3–5]. Four fermentation pathways have been widely accepted to occur in the anaerobic acidogenesis of organic matter, namely acetate-type, butyrate-type, ethanol-type and lactate-type fermentation (Table S1). The primary byproducts of acetate-type fermentation can be acetate and butyrate for butyrate-type fermentation, whereas propionate (with acetate and butyrate) can be produced by lactate-type fermentation. Ethanol and butyrate can be the main byproducts of ethanol-type fermentation. Based on the fermentation types, the major and minor products are summarized with microorganisms involved in each fermentation pathway (Table S2).

In fermentative hydrogen production, pH is an important factor that affects the hydrogenase activity and metabolism pathway [5]. In general, the optimal initial pH of fermentative hydrogen production is believed to be between 5.5 and 6.7 [2]. On the other hand, many studies on the initial pH effect in biohydrogen fermentation reported a slightly different conclusion. For example, in batch experiments using sucrose-rich synthetic wastewater, the initial pH (4.5–7.5) conditions did not show any significant effects on the hydrogen production rate [4]. In a series of batch tests using sucrose and starch, Khanal *et al.* [6] concluded that the initial pH had profound effects on both the hydrogen production potential and hydrogen production rate under an initial pH of 4.5–6.5.

Previous studies reported a significant increase in hydrogen production under alkaline pH conditions when sucrose or sewage sludge was used as the substrate. Lee *et al.* [7] reported that the optimal initial pH for the biohydrogen fermentation of sucrose was 9.0. Cai *et al.* [8] showed that the maximal hydrogen yield from sewage sludge occurred at an initial pH of 11.0. In an acid elutriation

fermenter treating municipal primary sludge and slurry-type piggery waste, the maximal activity of hydrolysis and acidogenesis was pH 9 under both mesophilic (35 °C) and thermophilic (55 °C) conditions [9,10]. Under these conditions, the main reaction byproducts in the semi-continuous experiments were acetate, propionate and butyrate, and the ratio was 1:0.35–0.37:0.28–0.31 for mesophilic conditions and 1:0.23–0.36:0.16–0.28 for thermophilic conditions. These results suggest that alkaline pH conditions have a broader application potential for biohydrogen fermentation and the acidogenesis of actual waste.

Alkalinity requirements for methanogenic reactors are well known, however, the requirements for biohydrogen production are not well established. Mohammadi *et al.* [11] studied the effect of bicarbonate concentration ranging from 0.2 to 2 g CaCO₃/L and achieved the maximum hydrogen yield at bicarbonate alkalinity (BA) of 1.1 g CaCO₃/L. Valdez-Vazquez and Poggi-Varaldo [12] showed that biohydrogen production was influenced by alkalinity and total solids concentrations. Although the effect of alkalinity was previously studied, the detailed information regarding the effect of high alkalinity feed such as raw piggery waste is still scarce. The characteristics of biohydrogen production from piggery waste that contains high alkalinity (~9 g CaCO₃/L) were studied at the pH of 6 and 9 [13], however, the effect of high alkalinity was excluded.

In this study, the effects of the initial BA in biohydrogen fermentation were examined by performing a series of batch experiments with a sole organic substrate (sucrose for synthetic) and piggery waste (for actual waste). The optimal initial alkalinity conditions for improving biohydrogen production were estimated based on the specific hydrogen production rate and its effects on the intermediate products were also tested.

2. Materials and Methods

2.1. Seed Sludge

Anaerobic granular sludge, which was obtained from a full scale upflow anaerobic sludge bed (UASB) reactor treating brewery wastewater in Kwangju City, Korea, was used as the seed inocula. The hydrogen producing bacteria of the anaerobic sludge were screened by adopting heat treatment, which is the most common method [5,13]. The granular sludge was pretreated (baked) in an oven at 105 °C for 2 h and cooled to room temperature in a desiccator. The heat-treated sludge was homogenized by a blender before the experiment.

2.2. Experimental Procedure

This experiment was divided into three categories: (1) effect of the initial pH (Run I); (2) effect of the BA (Run II to V); and (3) using real waste with high pH and alkalinity. Therefore, the first experiment (Run I) was performed under several designated pH conditions without the supplementation of BA using a simple carbohydrate (sucrose) as the substrate. The initial pH ranged from 5 to 10 using HCl (1 M) and NaOH (1–2 M). For the second experiment (Run II to V), NaHCO₃ was added to the designated levels from zero to 13.1 g CaCO₃/L (or 17.5 g CaCO₃/L of total alkalinity) under the designated initial pH levels (from pH 5 to 10). Table 1 summarizes the overall experimental conditions for the serum bottle activity test for batch mode biohydrogen fermentation. The serum

bottles with a working volume of 100 mL (total reactor volume of 156 mL) were used for the experiment. 30 mL (25 g) of the heat-treated biomass (fraction of volatile suspended solids (VSS)/total suspended fraction (TSS) = 0.72) was placed into each serum bottle. Run I was operated with 16 g/L of the initial COD and 20 mL/L of a salt stock solution. In Runs II to V, 4 mL of a concentrated organic substrate stock solution (255 g/L of sucrose) and 66 mL of a diluted nutrient solution (contained with 20 mL/L of salt stock solution) were added to the reactor. The organic substrate was not added to the control reactor. Each liter of salt stock solution contained KH_2PO_4 13.61 g/L, NH_4Cl 49.20 g/L, CaCl_2 4.44 g/L, $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$ 8.13 g/L and 10 mL/L of a trace element solution containing FeCl_3 19.44 g/L, $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$ 4.74 g/L, ZnCl_2 3.27 g/L, $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$ 2.05 g/L, $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$ 2.86 g/L, $\text{Na}_2\text{B}_4\text{O}_7 \cdot 10\text{H}_2\text{O}$ 1.15 g/L, $\text{Na}_3\text{C}_6\text{H}_5\text{O}_7$ 176.5 g/L, $(\text{NH}_4)_6\text{Mo}_6\text{O}_{24} \cdot 4\text{H}_2\text{O}$ 2.08 g/L, and $\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$ 3.00 g/L.

Table 1. Operational conditions for serum bottle activity test.

Run	Bicarbonate Alkalinity Control	Initial Bicarbonate Alkalinity (mg CaCO_3/L)					
		pH 5	pH 6	pH 7	pH 8	pH 9	pH 10
I	No	0	13	79	214	379	663
II	Yes	0	21	113	221	506	1,023
III	Yes	0	450	2,525	3,038	3,175	4,663
IV	Yes	0	978	3,129	4,201	4,488	6,551
V	Yes	0	575	5,050	6,113	6,363	13,100

Notes: pHs were amended with HCl and NaOH for run I, and HCl and NaHCO_3 for run II–V. The amount of BA varied (~320, 3,200, 4,200 and 6,500 mg CaCO_3/L for phase II–V, respectively) before making the designated pHs. Sucrose was used for a substrate.

For the third experiment examining real waste, raw piggery waste was obtained from the inlet of a livestock waste treatment plant (Sangju, Korea) and stored in a temperature-controlled room (4 °C). The characteristics of the piggery waste were as follows: 35 g COD/L, 24.4 g/L of total solids (TS), 0.63% volatile solids fraction (VS/TS), pH 8.7 and 9.0 g CaCO_3/L of BA (12 g of total alkalinity, TA). The serum bottle reactors were operated with 90 mL of the pre-treated (screened for coarse particle) slurry-type piggery waste and 10 mL of the homogenized anaerobic granular sludge (10 mL) pretreated with heat (104 °C for 2 h).

The serum bottles were capped with a Wheaton rubber septum stopper and flushed with oxygen-free N_2 gas (99.99%) for 30 s to remove the oxygen from the solution. The bottles were operated in an orbital shaker (SI-600R, Lab Companion, Daejeon, Korea) running at 200 rpm in a temperature-controlled room (35 ± 1 °C). Each experiment was triplicated and operated with three cycles under constant operating conditions. Control bottles (blank) were also operated without a substrate. The biogas produced was measured using the displacement method with appropriately sized wetted glass syringes. All experiments were performed according to the biochemical methane potential (BMP) protocol [14,15]. The mean values from batch experiments were used for plotting graphs.

2.3. Analysis

The hydrogen gas fraction (percentage of H_2 and CO_2) in the headspace of the serum bottle was determined using a 500 μL gastight syringe, and the collected biogas was compared with a standard of

pure H₂ (99.99%) using a gas chromatograph (GC, Shimadzu GC-14B, Kyoto, Japan) equipped with a thermal conductivity detector (TCD). The column was 6 ft × 1/8 in stainless steel (SS) 350A Molecular Sieve 13 × 80/100 (Alltech, State College, PA, USA). The operational temperatures of the injection port, oven and detector were 100, 50 and 100 °C, respectively. N₂ was used as the carrier gas at a flow rate of 75 mL/min. The minimum amount of H₂ detectable was 1%.

N₂, CO₂ and CH₄ were determined by comparing the collected biogas with a standard of pure (99.99%) N₂, CO₂, and CH₄ using a gas chromatograph (Shimadzu GC-8A) with a 6 ft × 1/8 in SS Hayasep Q 80/100 column (Alltech). The column temperature program was ramped to 200 °C at 10 °C/min. The injector and detector temperatures were 120 °C. Helium was used as the carrier gas with a flow rate of 140 mL/min. The minimum detectable amount of CH₄ was 1%.

The concentrations of volatile organic acids, such as lactate, acetate, propionate, and butyrate, were analyzed using filtrate samples through a 0.45 μm membrane by high performance liquid chromatography (HPLC, Shimadzu) equipped with an auto-injector, under isocratic elution conditions with UV-Vis detection (Shimadzu SPD-10A) at 210 nm and an Aminex HPX-87H column (300 mm × 7.8 mm, Bio-Rad, Hercules, CA, USA). The flow rate and pressure of the HPLC pump were 0.6 mL/min and 88–89 kgf/cm², respectively. The column oven temperature was 35 °C, and 0.008 M H₂SO₄ used as the eluent. The pH was measured using a pH meter (Orion 470, Waltham, MA, USA). The chemical oxygen demand (COD) was measured using a colorimetric method according to Standard Methods [16]. The BA and TA were analyzed using the Kappe methods [17].

The accumulated volume of the hydrogen production potential over time during the tests was fitted with the modified Gompertz equation [18,19]:

$$H = P \exp \left\{ -\exp \left[\frac{R_m e}{P} (\lambda - t) + 1 \right] \right\} \quad (2)$$

where H is the cumulative hydrogen production (mL), P is the hydrogen production potential (mL), λ is the lag phase time (h), R_m is the maximum hydrogen production rate (mL/h), t is the incubation time (h), and e is the $\exp(1) = 2.718$. The parameters were estimated using the solver function in Microsoft Excel version 5.0 (Microsoft, Inc., New York, NY, USA). Subsequently, contour plots were constructed using the SigmaPlot Ver. 10 software (Systat Software Inc., San Jose, CA, USA) to show the effect of BA and pH on biohydrogen production.

3. Results and Discussion

3.1. Hydrogen Yield under Various pHs Condition

In the first step of the experiment excluding the effects of BA in the substrate, most reactors designed for a range of initial pH levels required approximately 5–6 h of lag time for hydrogen production except for those at pH 9 and 10 (approximately 8–15 h of lag time). Figure 1a presents the hydrogen yield, showing that there was no significant difference in the hydrogen yield (0.12 g COD H₂/g COD, 268–272 mL H₂/g hexose) at the initial pH from 5 to 7. The fraction of hydrogen was in a range of between 80.2% and 84.1% under pH 5–8 and 92.1% at pH 9 conditions. Hydrogen production was possible in a range of pH 5 to 9. The finding suggests that various organic wastes covering a wide range of pH values can be used for hydrogen fermentation without adjusting the

initial pH. Lee *et al.* [7] reported that hydrogen production yields were higher at high alkaline pH than lower pH conditions (pH from 5 to 8), suggesting that hydrogenase enzyme systems are functioned at high alkaline pH conditions. Hydrogen production yield in the initial pH 10 decreased with a long lag time, which could be due to the toxic effects of a high pH. When pH condition increases from 9 to 10, the distribution of bicarbonate and carbonate is switched in the system, leading to the reduction of bicarbonate (Figure S1). Accordingly, high pH condition caused the decrease of H₂ production yield, suggesting the inhibition of growth rate and metabolic activity of bacteria [20].

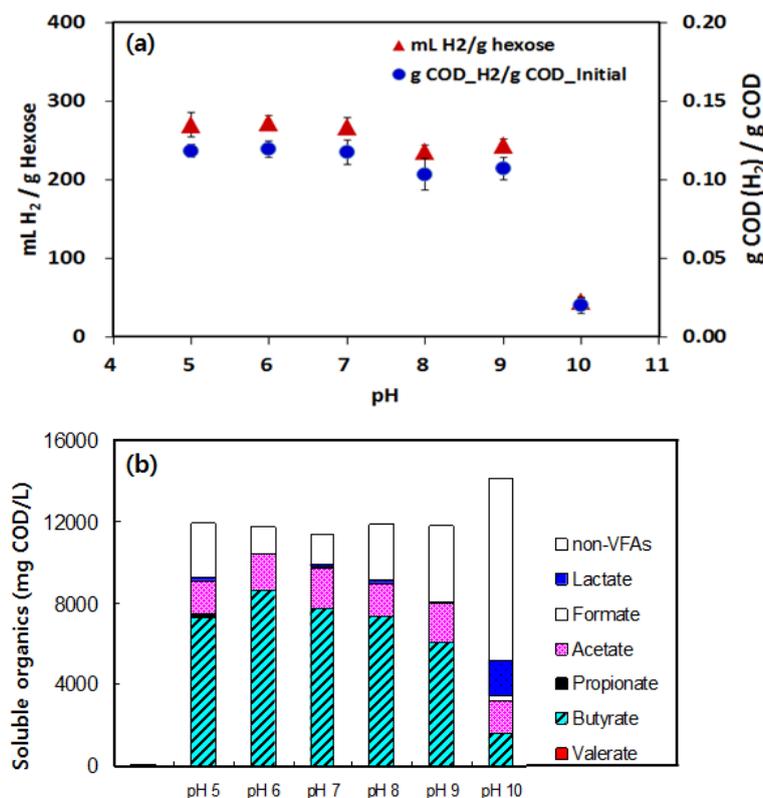


Figure 1. Hydrogen yield (a) and organic acids production (b) under designated pHs without BA supplementation (substrate: sucrose, initial COD = 16 g/L).

Figure 1b represents the profile of soluble organics produced in hydrogen fermentation under a range of pHs. At pH 5 to 9, butyrate was the most dominant byproduct, followed by acetate, whereas relatively lower butyrate and higher lactate concentrations were observed at pH 10. The distribution of a byproduct in hydrogen fermentation provides an important indication that the butyrate/acetate ratio can be the indicator evaluating the effectiveness of hydrogen production [21]. At an initial pH 5 to 9, the butyrate and acetate concentrations were 6.02–8.51 g COD/L and 1.51–2.23 g COD/L, respectively. The butyrate/acetate ratios were 3.5–5.0 at pH 5 to 9, and 1.06 at pH 10. These ratios were comparable to the previous data (3.7–5.1) obtained in a hydrogen fermenter fed with sucrose [22]. A greater than 2.6 of butyrate/acetate ratio also showed efficient hydrogen production from a wheat starch co-product by anaerobic microflora [23]. Therefore, a high hydrogen yield is typically associated with the production of butyrate, and low yields with propionate production and reduced products (e.g., lactate or alcohol). This is correspondent to the increase in lactate and the decrease in butyrate in the pH 10 reactor.

The pHs (7, 8, 9) in reactors were dropped immediately except at pH 10 when hydrogen fermenter was started (Figure 2). A drop of pH in the reactors is possibly due to volatile fatty acid (VFA) accumulation and weak buffering capacity. From the initial pH 10 test, the pH was slightly reduced from 10 to 9.1 during the initial 12 h possibly due to the low acidogenesis, compared to other pH conditions. Regardless of what the initial pH was (4–9) in the experiments, the pH changed with time, and the final pH was between 4.5 and 5.9.

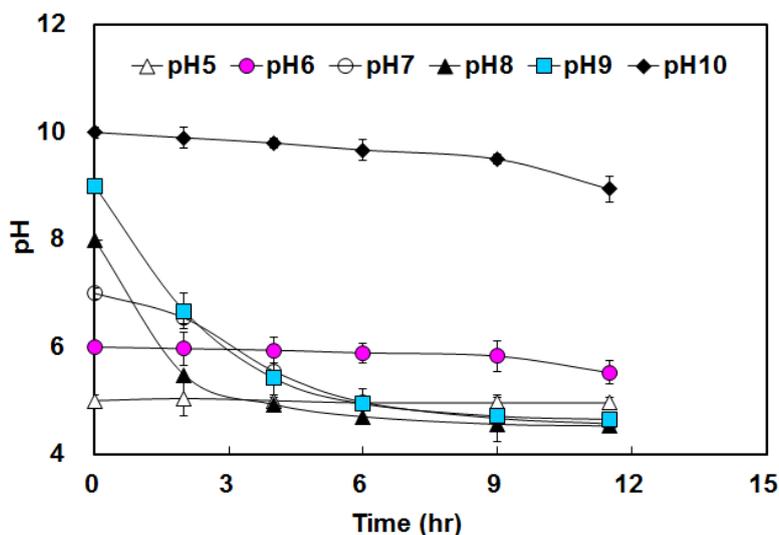


Figure 2. pH profiles during the batch biohydrogen fermentation (substrate: sucrose). Bicarbonate alkalinity was not amended.

3.2. Effects of BA

BA has buffering capacity to reduce the large pH fluctuations during hydrogen fermentation accompanied by VFA or solvents. A failure of pH control from alkalinity and the imbalance between the organic acids may result in interfering hydrogen production. Furthermore, bicarbonate correlates with pH and carbon dioxide, which is the other end product in anaerobic fermentation [15]. High BA in the feed can increase the CO_2 fraction in the reactor, suggesting the possibility of hydrogen consumption (*i.e.*, acetate production) by homoacetogenesis [21]. Zhang and Wang [20] reported that suitable addition of alkali-rich materials to food waste synergistically improved hydrogen fermentation performance and lag time, which attributed to buffering effect and nutrient characteristics of hydrogen metabolism.

The initial BA and pH significantly affected the hydrogen yield, as shown in Table 2 and Figure 3. When low alkalinity (<0.1 g CaCO_3/L) was present, the maximum hydrogen yield was ~ 1.5 mol H_2/mol glucose at pH 6 and 7, whereas ~ 1.08 mol H_2/mol glucose in pH 8 and 9 conditions, where the initial BA of ~ 2.5 g CaCO_3/L were present. When the initial BA increased to more than 3 g CaCO_3/L , the hydrogen yield was decreased in all reactors. At an initial pH 10, 0.4 mol H_2/mol glucose was produced in low BA, whereas no hydrogen production was observed in high BA (>7 g CaCO_3/L). Despite the buffering capacity of BA, higher concentration of BA could be a cause of toxicity in the fermentation process. Moreover, excess addition of alkali-rich materials may be harmful to hydrogen-producing microbes and reduce H_2 production from food waste [20]. Figure 4 shows a

hydrogen production rate at the initial BA doses and pH. The specific hydrogen production rates were 0.26 and 0.29 L H₂/g VSS-d at an initial pH of 7 and 8 (with initial BA dose of 2.5 and 3.0 g CaCO₃, respectively), and the highest hydrogen production rate of 0.32 L H₂/g VSS-d was observed at pH 8.95 with an initial BA of 3.18 g CaCO₃/L. Zhu *et al.* [24] reported that the addition 1.0 M K₂HPO₄ could increase hydrogen production, whereas less hydrogen production was confirmed under high buffer addition. Based on these results, the initial BA in the substrates can significantly affect hydrogen production, indicating a strong effect on major metabolites of H₂-producing bacteria. BA greater than 4 g CaCO₃/L led to a decrease in the specific hydrogen production rates (<0.13 L H₂/g VSS-d). The performance of hydrogen fermenter can be affected by level of TA and BA [11,13,21].

Table 2. Specific hydrogen production rate in various pHs and BA level.

Run	pH	Bicarbonate Alkalinity (mg CaCO ₃ /L)	H ₂ Yield (mol H ₂ /mol glucose)	R (mL H ₂ /g VSS-d)	r/r_{\max}
II	4.95	0	1.38	63.9	0.2
	6.00	21	1.51	71.8	0.23
	7.04	113	1.60	62.9	0.20
	8.05	221	1.28	49.3	0.15
	9.07	379	0.82	49.1	0.15
	9.98	663	0.43	18.1	0.06
III	5.00	0	1.15	139.1	0.44
	6.10	450	1.23	154.9	0.49
	7.10	2,525	1.36	258.3	0.81
	8.10	3,038	1.46	296.4	0.93
	8.95	3,175	1.48	318.5	1.00
	10.10	4,663	0.01	15.6	0.05
IV	5.02	0	0.52	56.6	0.18
	6.17	978	1.01	109.6	0.34
	7.13	3,129	1.00	143.9	0.45
	8.12	4,201	1.00	133.8	0.42
	9.01	4,488	1.02	121.5	0.38
	9.96	6,551	0.12	11.0	0.03
V	5.14	0	0.52	40.3	0.13
	6.27	575	0.88	65.4	0.21
	7.36	5,050	0.76	81.9	0.26
	8.10	6,113	0.85	101.6	0.32
	8.99	6,363	0.40	61.8	0.19
	10.10	13,100	0.00	0.00	0.00

Note: “ r ” and “ r/r_{\max} ” represent H₂ production activity and normalized activity by maximum H₂ production activity, respectively.

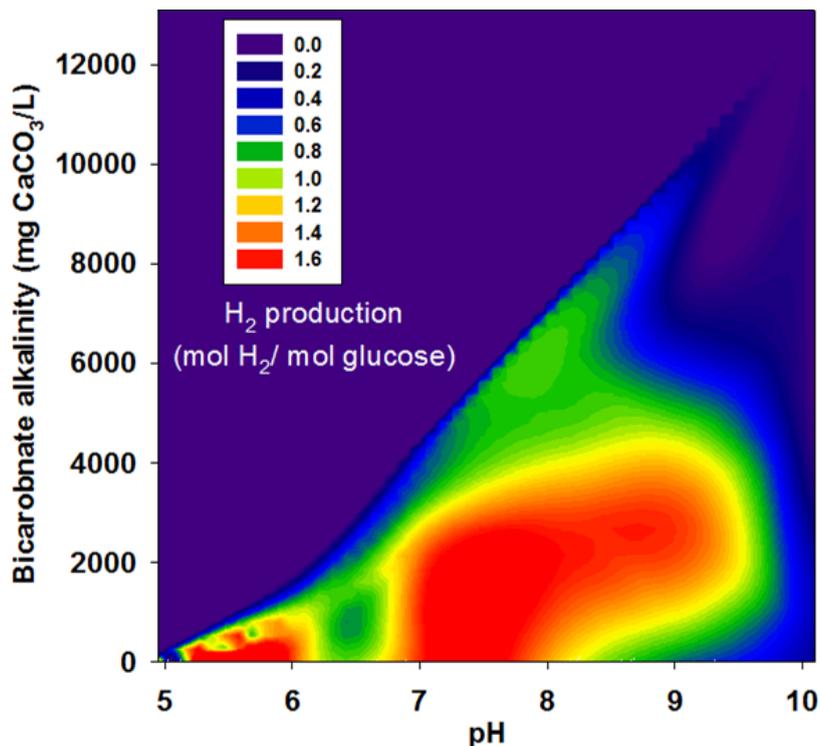


Figure 3. Hydrogen yield (mol H₂/mol glucose) with variation of BA and pHs (substrate: sucrose).

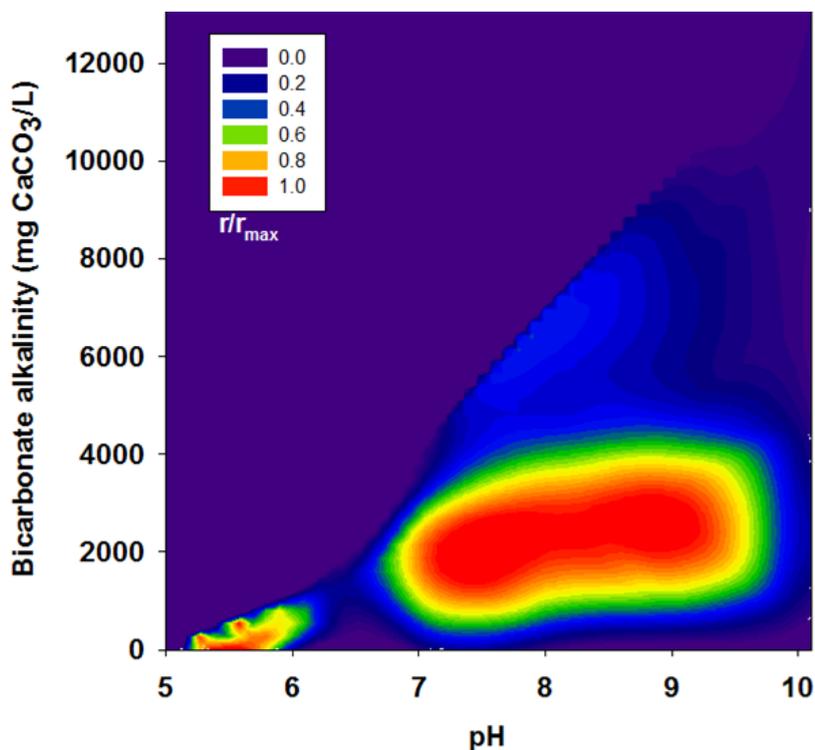


Figure 4. Hydrogen production rate of H₂ producing mixed culture with BA (substrate: sucrose). The r/r_{max} represents that specific hydrogen production rate (mL H₂/g VSS-d) is divided by maximum hydrogen production activity.

Even though the level of alkalinity range was relatively high, critical hydrogen production at high alkalinity ranges was observed in this and previous studies [7] and effect of high alkalinity was required to

apply with a real waste including high alkalinity itself, such as piggery waste (alkalinity = 8.9 g CaCO₃/L, pH 8.7) [13].

3.3. Acetate and Butyrate as the Final Byproducts

Each type of hydrogen fermentation could be characterized by hydrogen producing and consuming thermodynamical reaction under different pH and microbial properties, determining the main byproducts (Table S1). One of the widely studied hydrogen-producing microorganisms is *Clostridium butyricum*, which produces butyric acids as the major byproduct with CO₂, acetic acid and H₂. This pathway was found in approximately 50% of experimental studies that isolated *Clostridia* to date [6]. Other fermentation pathways include the production of propionate by *Clostridium arcticum* [25], succinate by *Clostridium coccides* [26] and lactate by *Clostridium barkeri* [27].

Figure 5a shows the ratios of acetate and butyrate in the total organic acids. When the bicarbonate concentration was higher than 4 g CaCO₃/L, butyrate was reduced significantly, meaning that butyrate type fermentation was reduced, whereas the acetate concentration was increased. The increase in acetate by acetate type fermentation could be due to the decrease in hydrogen production up to 4 g CaCO₃/L (Figures 3 and 4). On the other hand, when the bicarbonate concentration was sufficient in the liquid phase, the hydrogen produced can be consumed by homoacetogenesis, resulting in the acetate production (see the Equations (3) and (4)). Lin and Lay [21] reported that a high NH₄HCO₃ concentration resulted in low hydrogen production due to bicarbonate dissolution and the toxicity of a high ammonium concentration:



3.4. Lactate and Propionate as Final Byproducts

In general, propionate is not a major byproduct of the fermentative biohydrogen production process. According to thermodynamically potential pathway of hydrogen fermentation, propionate production can be observed only in the pathway of lactate-type fermentation, resulting in the consumption of the hydrogen produced (Table S1). Therefore, propionate accumulation in the reactor can also be a barrier for hydrogen and lactate production [2].

Figure 5b shows the effects of BA (0–1.31 g CaCO₃/L) in the ratio of the acids per total organic acids. Almost no relationship was observed between BA and the acid/total organic acids ratios under 4 g CaCO₃/L. On the other hand, the lactate ratio increased to 0.16 g/g at 6 g CaCO₃/L and the propionate ratio reached 0.16 g/g at 6.5 g CaCO₃/L. With further increases in BA from 6.5 g CaCO₃/L, the propionate ratio increased and the lactate ratio decreased, possibly due to lactate-type fermentation, which involves the accumulation of propionate as a byproduct.

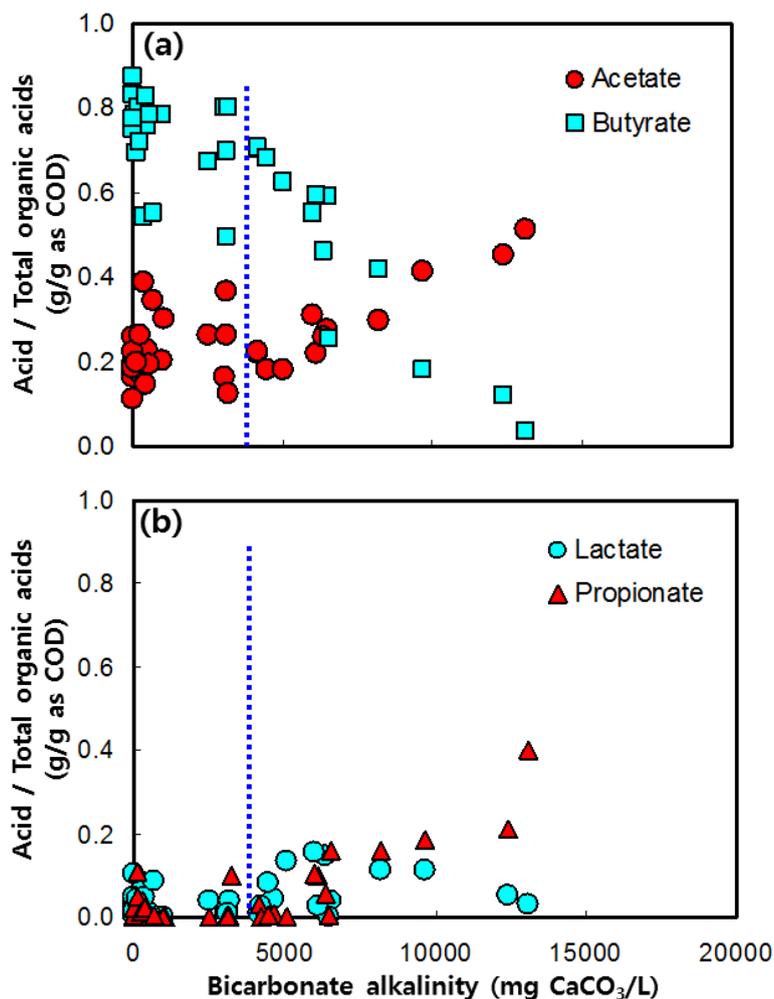


Figure 5. (a) Acetate and butyrate, (b) lactate and propionate in the batch hydrogen fermentation (substrate: sucrose) as a function of the BA.

3.5. BA in High Strength Piggery Waste

BA is produced in fermentative hydrogen production processes and contributes to maintain the pH at favorable range for hydrogen producing activity. Limited studies, however, were performed on the effect of BA (Lin and Lay [21]; Mohammadi *et al.* [11]). In addition, a few results showing successful biohydrogen fermentation using real waste have been reported, and the hydrogen yield was much lower than with the pure feed [5]. In particular, biohydrogen fermentation from high strength piggery waste showed low energy recovery [13,28,29], even though piggery waste is considered as an attractive source for bioenergy harvesting [9].

Figure 6 shows the batch mode experimental results of the effect of high BA using raw piggery waste (8.9 g CaCO₃/L, pH 8.7). No butyrate and biogas (hydrogen and methane) production was observed, whereas the acetate and propionate levels increased with time, indicating that the accumulation of acetate seems to imply the inhibition of acetate-consuming activity. On the other hand, the presence of homoacetogenic activity, that hydrogen and carbon dioxide are utilized, is possible due to the characteristics of raw piggery waste containing a high percentage of complex organic and inorganic molecules. The high BA is likely to affect the production of biohydrogen and organic acids by the consumption of BA and hydrogen produced. This can be explained by the distribution of the

bicarbonate fractions, as previously described (Figure S1). Compared to the different pH condition (6 to 8.7) at 25 °C and zero salinity conditions, the bicarbonate percentage increases to 95% at pH 8.7, driving to more acetate production in the fermenter, potentially by activating homoacetogenesis. Therefore, the activity of the acetogenic bacteria can be favorable at high BA. Regarding the reaction converting hydrogen to acetate under anaerobic conditions (Equations (3) and (4)), the presence of acetogenic bacteria has been proposed to be responsible for the consumption of hydrogen produced during biohydrogen fermentation from organic wastewater or waste in the case of no methanogens [30]. This result showed that a proper amendment of the substrate characteristics (in levels of pH and bicarbonate contents) will be required for successful biohydrogen harvesting from organic waste with pH and BA.

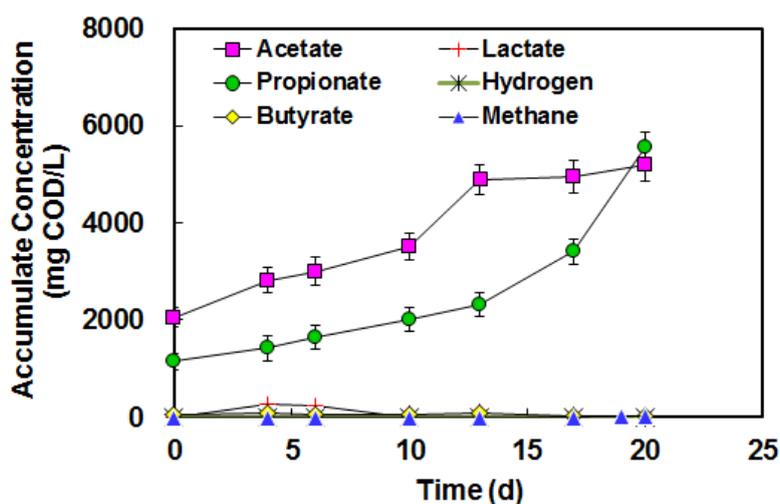


Figure 6. Profiles of organic acids and biogas in a batch mode fermenter using raw piggery waste and H₂ producing mixed culture (BA: 8.9 CaCO₃/L, pH 8.7).

4. Conclusions

The initial BA and pH of the feed substrates are very important parameters for fermentative biohydrogen production. Hydrogen production was observed over a wide range of pH values from 5 to 9 under the exclusion of alkalinity. The maximum hydrogen production rates were obtained at pH 9 > 8 > 7 with ~3 g CaCO₃/L of BA. The significant decrease in hydrogen production over BA of ~3 g CaCO₃/L was attributed to the effect of bicarbonate, which could be used as a carbon source for hydrogen consumers. The higher BA in the substrate resulted in higher acetate production, in which homoacetogens may be activated. Therefore these results demonstrate that the substrate with high BA should be amended to maximize hydrogen production adequately.

Supplementary Materials

Supplementary materials can be accessed at: <http://www.mdpi.com/1996-1073/8/3/1716/s1>.

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Author Contributions

Jeongdong Choi carried out the experiments and prepared the first draft, and Youngho Ahn managed this study and analyzed data deeply. Both authors contributed to the interpretation of the results.

Conflicts of Interest

The authors declare no conflict of interest.

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