Effect of operational pH on biohydrogen production from food waste using anaerobic batch reactors

Chaeyoung Lee, Sewook Lee, Sun-Kee Han and Sunjin Hwang

ABSTRACT

This study was performed to investigate the influence of operational pH on dark H_2 fermentation of food waste by employing anaerobic batch reactors. The highest maximum H_2 yield was 1.63 mol H_2/mol $hexose_{added}$ at operational pH 5.3, whereas the lowest maximum H_2 yield was 0.88 mol H_2/mol $hexose_{added}$ at operational pH 7.0. With decreasing operational pH values, the *n*-butyrate concentration tended to increase and the acetate concentration tended to decrease. The highest hydrogen conversion efficiency of 11.3% was obtained at operational pH 5.3, which was higher than that (8.3%) reported by a previous study (Kim *et al.* (2011) 'Effect of initial pH independent of operational pH on hydrogen fermentation of food waste', *Bioresource Technology* 102 (18), 8646–8652). The new result indicates that the dark fermentation of food waste was stable and efficient in this study. Fluorescence *in situ* hybridization (FISH) analysis showed that *Clostridium* species Cluster I accounted for 84.7 and 13.3% of total bacteria at operational pH 5.3 and pH 7.0, respectively, after 48 h operation.

Key words | dark H_2 fermentation, FISH, food waste, pH, volatile fatty acids (VFAs)

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INTRODUCTION

As a sustainable energy source, hydrogen is a promising alternative to fossil fuels. It is a clean and environmentally friendly fuel, which produces water instead of greenhouse gases when combusted. Furthermore, it has a high energy content of 122 kJ/g, which is about 2.1 times greater than methane and about 2.8 times greater than gasoline (Kim *et al.* 2008; Khanal 2008).

Organic waste has been considered as an abundant resource for energy production (Benemann 1996). Hydrogen could be generated by dark H_2 fermentation of carbohydrate-rich waste (Khanal 2008). Thus, biohydrogen production from food waste is an ideal and reasonable approach that could achieve waste treatment and energy generation simultaneously. It has considerable potential to enhance the economic feasibility of waste treatment (Ike *et al.* 2010).

The understanding of the characteristics of dark H_2 fermentation lies in an appreciation of the response to environmental changes that influence the activity of *Clostridium* species and metabolism pathways. *Clostridium* sp. showed a higher hydrogen yield (1.61–2.36 mol/mol glucose) than other anaerobic bacteria like *Enterobacter* and *Bacillus* (Hawkes *et al.* 2002). Metabolism pathways were affected by several factors such as pH, temperature, carbon source, nutrients, retention time, etc. (Wang & Wan 2009).

Proper pH control is a key factor to improve the germination of *Clostridium* sp. as well as to initiate and operate a hydrogen-producing bioprocess (Han & Shin 2004). When food waste is degraded in fermentation, the pH decreases due to the production of volatile fatty acids (VFAs). Thus, maintenance of proper pH levels over the whole operation is crucial for stable fermentation (Hawkes *et al.* 2002).

Zhang *et al.* (2003) reported that the maximum H_2 yield of 92 mL/g starch was obtained at an initial pH of 6.0 in batch experiments treating sucrose-containing wastewater under thermophilic conditions (55 °C). According to Zhao & Yu (2008), the maximum yield of 1.61 mol H_2/mol hexose was found at an operational pH of 7.0 in an upflow anaerobic sludge blanket operation treating sucrose-rich synthetic wastewater under mesophilic conditions (39 °C). However, Chen *et al.* (2009) reported that the maximum yield of 2.53 mol H_2/mol sucrose was obtained at an operational pH of 4.9 in an anaerobic sequencing batch reactor operation treating sucrose-rich synthetic wastewater under mesophilic conditions (35 °C). Recent studies on the effect of pH on dark H_2 fermentation have reported various optimal pH values depending on different experimental conditions such as initial pH values,

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operational pH values, substrates, reactors, temperature and seed sludge (Wang & Wan 2009).

This study was, therefore, conducted to investigate the effect of operational pH on dark H_2 fermentation of food waste. The composition of metabolites was examined as an indicator to evaluate the efficiency of the fermentation. The microbial community was also analyzed by fluorescence *in situ* hybridization (FISH).

METHODS

Seed sludge and substrate

The seed sludge was taken from an anaerobic digester in a local wastewater treatment plant. The pH, alkalinity and volatile suspended solids (VSS) concentration of the sludge were 6.9, 2.3 g/L as $CaCO_3$ and 19.0 g/L, respectively. It was heat-treated at 90 °C for 20 min to inactivate hydrogen consumers and to harvest spore-forming anaerobic bacteria (Kim *et al.* 2011).

Food waste, collected from apartment blocks, was used as the substrate after separating out bones and shells. Food waste contained grains, vegetables, fruits and meats, whose composition was 35, 35, 25 and 5%, respectively, as total solids (TS). The characteristics of the food waste are shown in Table 1. The total chemical oxygen demand (COD) of food waste was 36.1 g/L and the carbohydrate concentration of the waste was fixed as 30 g COD/L by dilution with distilled water. The substrate was heat treated for 60 min at 90 °C to inactivate hydrogenotrophic bacteria.

Table 1 | Characteristics of food waste

Parameters	Unit	Value
TS	g/L	43.3 ± 19.2
VS	g/L	40.3 ± 19.9
Total COD	g/L	36.1 ± 1.9
Soluble COD	g/L	20.3 ± 2.2
TKN	mg/L	578.2 ± 78.4
Ammonia	mg/L	71.6 ± 11.5
C	%	39.8 ± 1.6
H	%	6.2 ± 0.2
O	%	48.0 ± 0.0
N	%	2.8 ± 0.3
S	%	0.1 ± 0.0

Analysis method

Hydrogen production was measured using a 50 mL glass syringe. Hydrogen content in biogas was analyzed by gas chromatography (GC, Gow Mac series 580, USA) using a thermal conductivity detector and a 1.8 m × 3.2 mm stainless-steel column packed with molecular sieve 5A (80/100 mesh). The temperatures of column, injector and detector were kept at 50, 80 and 90 °C, respectively. High-purity nitrogen (99.999%) was used as a carrier gas. The hydrogen production curve was fitted to the modified Gompertz Equation (1), which was used as a suitable model for describing the hydrogen production in batch tests (Kim *et al.* 2011)

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

where H = Cumulative hydrogen production (mol H_2/mol hexose), P = Maximum hydrogen production (mol H_2/mol hexose), λ = Lag phase (h), R = Hydrogen production rate (mol H_2/mol hexose/h), $e = 2.71828$.

The parameters such as pH, TS, volatile solids (VS), VSS, COD, total Kjeldahl nitrogen (TKN), ammonia, and alkalinity concentration of the samples were measured according to *Standard Methods* (2005). The carbohydrate concentration was analyzed according to the method proposed by Dubois *et al.* (1956). The chemical composition of food waste was determined by an elemental analyzer (EA1110, Italy) after the waste was dried for 24 hours at 40 °C. VFAs were analyzed by a high-performance liquid chromatograph (LC, YL9100, Young-Lin Instrument Co. Korea) equipped with an ultraviolet (210 nm) detector and a 100 mm × 7.8 mm fast acid analysis column (Bio-Rad Laboratories) using 0.005 M H_2SO_4 for the mobile phase. Alcohols were determined by another high-performance LC (DX-600, Dionex) equipped with an electrochemical detector (ED50A) and a 250 mm × 4 mm Dionex CarboPac PA10 column using 0.01 M NaOH for the mobile phase. The liquid samples were pretreated with a 0.45 μm membrane filter before injection to both LCs.

FISH analysis

In the analysis, 16S rRNA-targeted oligonucleotide probes were used to examine changes in cultivated microbial communities. Probes for FISH analysis used in this study are shown in Table 2. A sample of 0.3 mL was obtained and mixed with 4% paraformaldehyde at a ratio of 1:3. The mixture was maintained for 3 hours at 4 °C. After centrifugal

Table 2 | Probes applied for FISH analysis

Probe	Sequence(5′–3′)	Dye	FA (%)	Target
EUB338I	GCTGCCTCCCGTAGGAGT	FAM	20	Eubacteria
EUB338II	GCAGCCACCCGTAGGTGT	FAM	20	Eubacteria
EUB338III	GCTGCCACCCGTAGGTGT	FAM	20	Eubacteria
Chis150	TTATGCGGTATTAATCTYCCTTT	Cy3	20	*Clostridium* sp. Cluster I & II
P932	GATYYGCGATTACTAGYAACTC	Cy5	20	*Clostridium* sp. Cluster I & XI

separation at about 10,000 rpm, the upper liquid was poured away. The remainder was cleaned with 1* PBS (NaCl 8 g, Na_2HPO_4 1.1 g, KCl 0.2 g, KH_2PO_4 0.2 g, H_2O 1,000 mL), had 1* PBS 0.3 mL added and was then cooled with ethanol 0.3 mL to form a ratio of 1:1:1. A 3 μL of the sample was poured on the slide glass and air-dried. After complete drying, it was deposited in 50, 80 and 95% ethylalcohol in order, and then dried and dehydrated. Then, oligonucleotide probes and hybridization buffer (0.9 M NaCl, 20 mM Tris hydrochloride (pH = 7.2), 0.01% sodium dodecyl sulfate (SDS), 20% Formamide) were mixed at a ratio of 1:8 and maintained for about 3 h at 46 °C for hybridization. Next, the mixture was cleaned with several mL of washing buffer (10 g/L NaCl, 20 mM Tris hybridization (pH = 7.2), 0.01% SDS), deposited in about 50 mL of washing buffer for 20 min at 48 °C, and cleaned with ultrapure water to complete hybridization. Moreover, the hybridized sample was mounted with antifade solution and covered with a glass cover for observation purposes using CLSM (Confocal Laser Scanning Microscopy; LSM 700 Meta, Zeiss).

Apparatus and procedure

An anaerobic hydrogen batch reactor with a working volume of 300 mL was operated at 35 °C, as shown in Figure 1. The F/M ratio of food waste and seed sludge was set as 6.0 (Pan *et al.* 2008). The head space of the reactor was purged using high-purity N_2 gas for 5 min. The pH values of the reactor were fixed at 4.7, 5.0, 5.3, 5.7, 6.0, 6.5 and 7.0, respectively, using 2 N HCl and 5 N KOH. The reactor was agitated at 200 rpm.

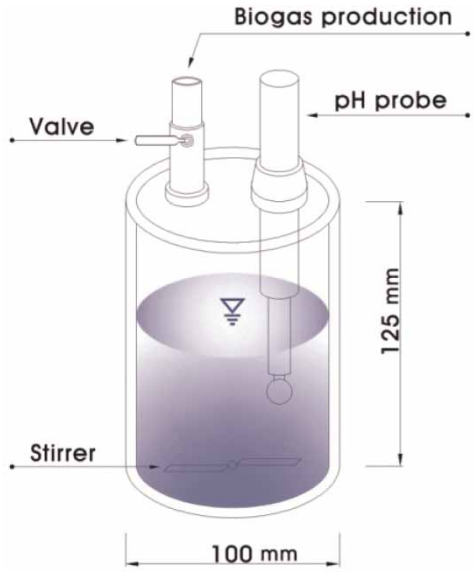

Figure 1 | Schematic diagram of anaerobic hydrogen batch reactor.

RESULTS AND DISCUSSION

Effect of operational pH on hydrogen production

Figure 2 illustrates the effect of an operational pH on cumulative hydrogen production during dark H_2 fermentation of food waste. Cumulative hydrogen production increased, as operational pH increased from 4.7 to 5.3. However, cumulative hydrogen production decreased, as operational pH increased from 5.3 to 7.0. Table 3 shows the effect of operational pH on lag phase, H_2 production rate and maximum H_2 yield during fermentation. The lag phase was in the range of 4.6–6.2 h, depending on operational pH values, which was lower than those (11–12 h) of other studies using non-heat treated substrates (Zong *et al.* 2009). The highest H_2 production rate was 0.199 mol H_2/mol $hexose_{added}$/h at operational pH 5.7, while the lowest H_2 production rate was 0.090 mol H_2/mol $hexose_{added}$/h at operational pH 4.7. The highest maximum H_2 yield was 1.63 mol H_2/mol $hexose_{added}$ at operational pH 5.3, whereas the lowest maximum H_2 yield was 0.88 mol H_2/mol $hexose_{added}$ at operational pH 7.0. It was reported that hydrogen evolution by *Clostridium* sp. was inhibited at pH 4.0–5.0 (Chen *et al.* 2006). According to Li *et al.* (2008), excessive

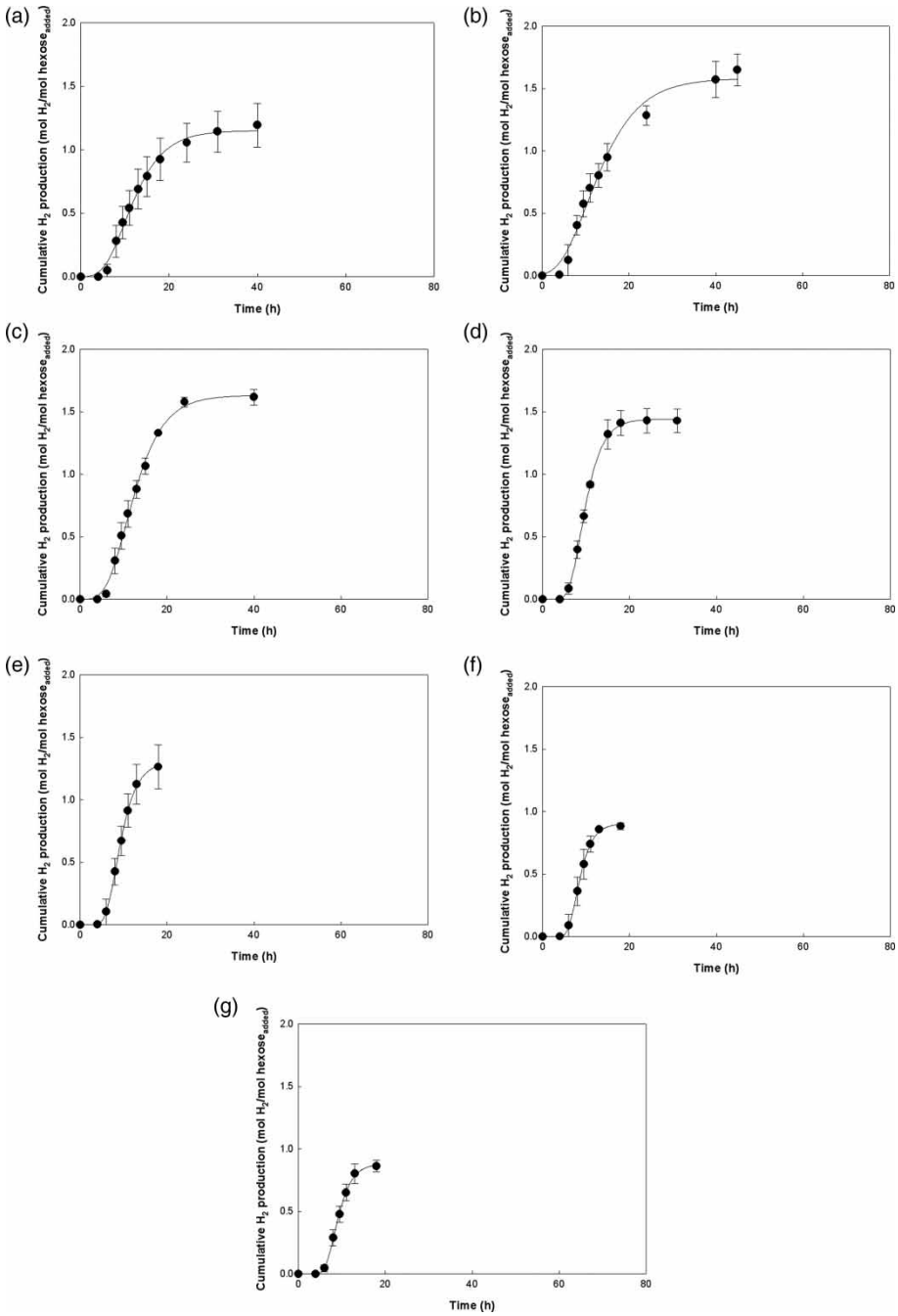

Figure 2 | The effect of operational pH values on cumulative hydrogen production during dark H_2 fermentation of food waste. (a) pH 4.7, (b) pH 5.0, (c) pH 5.3, (d) pH 5.7, (e) pH 6.0, (f) pH 6.5, (g) pH 7.0.

injection of alkali for pH maintenance led to a rapid neutralization of VFAs generated in dark fermentation and to the reduced activity of hydrogen-producing bacteria. Lin *et al*. (2008) also reported that, in batch experiments at different pH values, the pH range of 6.0–7.0 was detrimental to dark fermentation, while the pH range of 5.3–5.7 was beneficial to fermentation. Moreover, as non-hydrogen producers, such as propionic acid bacteria, were inactivated by the heat-treatment of food waste (Noike *et al*. 2002), the maximum H_2 yield of this study was higher than those (0.04–0.05 mol H_2/mol hexose) of other research using non-heat treated substrates (Shin *et al*. 2004).

Table 3 | Variation of lag phase, H_2 production rate and maximum H_2 yield at different operational pH values

Operational pH	Lag phase (h)	H_2 production rate (mol H_2/mol $hexose_{added}$/h)	Maximum H_2 yield (mol H_2/mol $hexose_{added}$)
4.7	5.2 ± 0.6	0.090 ± 0.018	1.15 ± 0.16
5.0	4.6 ± 0.4	0.092 ± 0.016	1.58 ± 0.12
5.3	6.0 ± 0.7	0.131 ± 0.002	1.63 ± 0.07
5.7	6.1 ± 0.6	0.199 ± 0.021	1.44 ± 0.11
6.0	5.8 ± 0.6	0.191 ± 0.011	1.30 ± 0.20
6.5	5.9 ± 0.8	0.172 ± 0.007	0.90 ± 0.03
7.0	6.2 ± 0.4	0.150 ± 0.020	0.88 ± 0.05

Effect of operational pH on hydrogen conversion and carbohydrate removal efficiency

As shown in Figure 3, the carbohydrate removal efficiencies of the reactors were similar to each other, which were over 90% at different operational pH values. The carbohydrate content of food waste was 83.1% (that is, the carbohydrate COD of food waste/the total COD of food waste × 100 = 30.0 g COD/L/36.1 g COD/L × 100). Theoretically, as shown in Equation (2), 12 mol of hydrogen is generated from 1 mol of glucose. The highest H_2 conversion efficiency was 11.3% at an operational pH of 5.3 (that is, the highest maximum H_2 yield of this study/theoretical H_2 yield of glucose × the carbohydrate content of food waste = 1.63 mol H_2/mol $hexose_{added}$/12 mol H_2/mol $hexose_{added}$ × 0.831). The highest H_2 conversion efficiency of 11.3% obtained in this study indicated that this experiment was stable and efficient, compared to that (8.3%) reported by Kim *et al.* (2011). As shown in Equations (3)–(7), the production of propionate, valerate, and lactate was not related to H_2-producing reactions, while the production of acetate and butyrate was accompanied by H_2 production (Kim *et al.* 2010)

$$\text{Glucose} + 12H_2O \Rightarrow 12H_2 + 6CO_2 \quad (2)$$

$$\text{Glucose} + 2H_2O \Rightarrow 2\text{Acetate} + 2CO_2 + 4H_2 \quad (3)$$

$$\text{Glucose} \Rightarrow \text{Butyrate} + 2CO_2 + 2H_2 \quad (4)$$

$$\text{Glucose} + 2H_2 \Rightarrow 2\text{Propionate} + 2H_2O \quad (5)$$

$$\text{Propionate} + 6H_2 + 2CO_2 \Rightarrow \text{Valerate} + 4H_2O \quad (6)$$

$$\text{Glucose} \Rightarrow 2\text{Lactate} \quad (7)$$

Effect of operational pH on VFA production

Figure 4 illustrates the variation of VFA production in fermentation. As operational pH values decreased, *n*-butyrate concentration tended to increase and acetate concentration tended to decrease. During the fermentation, *n*-butyrate production was more favorable to hydrogen evolution, compared to acetate production (Kim *et al.* 2011). As an operational pH gets closer to neutrality, hydrogen consumers are predominant instead of hydrogen producers during substrate consumption. It was reported that 2 mol

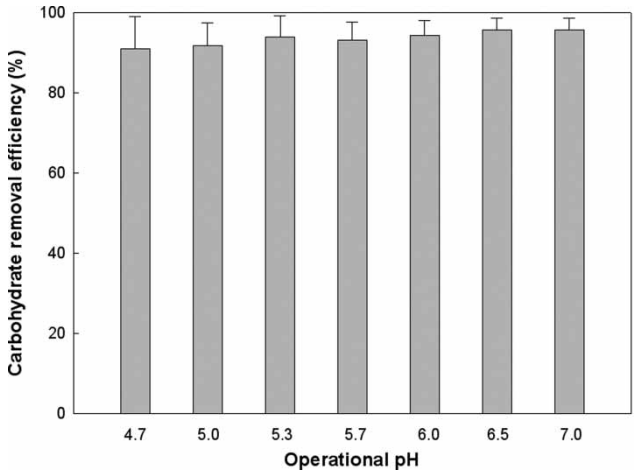

Figure 3 | The effect of operational pH values on carbohydrate removal efficiency during dark H_2 fermentation of food waste.

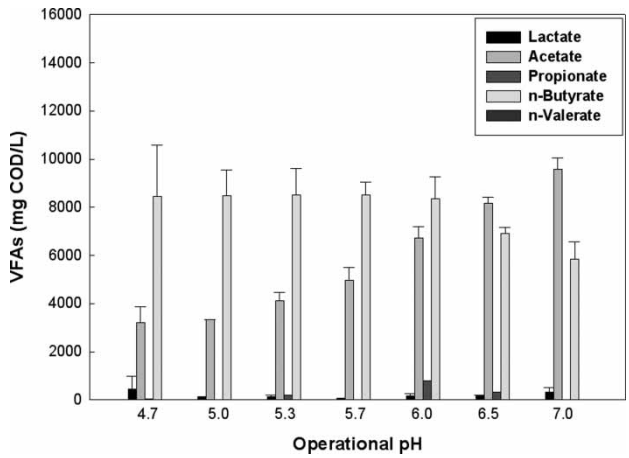

Figure 4 | The effect of operational pH values on VFA production during dark H_2 fermentation of food waste.

of propionate could be produced from 1 mol of glucose and 2 mol of hydrogen as shown in Equation (5) or from 3 mol of lactate as shown in Equation (8) (Kim *et al.* 2008). However, due to the use of heat-treated waste, there was almost no propionate production. Propionic acid bacteria could be inhibited by heat treatment (Noike *et al.* 2002). Thus, the highest maximum H_2 yield (1.63 mol H_2/mol $hexose_{added}$ at operational pH 5.3) of this study was higher than that (0.05 mol H_2/mol hexose) of fermentation with no heat treatment (Shin *et al.* 2004)

$$3\text{Lactate} \rightarrow \text{Acetate} + 2\text{Propionate} \quad (8)$$

Effect of operational pH on microorganisms

Figure 5 illustrates FISH analysis of microorganisms at operational pH 5.3 and 7.0. Table 4 shows the calculated results of the ratio of *Clostridium* sp. clusters I, II and XI to total bacteria. After 48 h reaction, *Clostridium* sp. cluster I accounted for 84.7 and 13.3% at operational pH 5.3 and pH 7.0, respectively. According to Lee *et al.* (2009), it corresponded to hydrogen production by *Clostridium* sp. during dark fermentation using mixed culture. However, no *Clostridium* sp. cluster II was identified.

pH change profile and alkali requirement

As shown in Figure 6, operational pH decreased from initial pH 8.0 to pH 7.0 after about 5 h and finally to pH 6.5, 6.0, 5.7, 5.3, 5.0 and 4.7 in order. Figure 7 illustrates that alkali addition increased with increasing operational pH values. The pH values of the reactors were fixed using 2 N HCl and 5 N KOH. As operational costs are one of the key considerations in continuous reactor operation, alkali

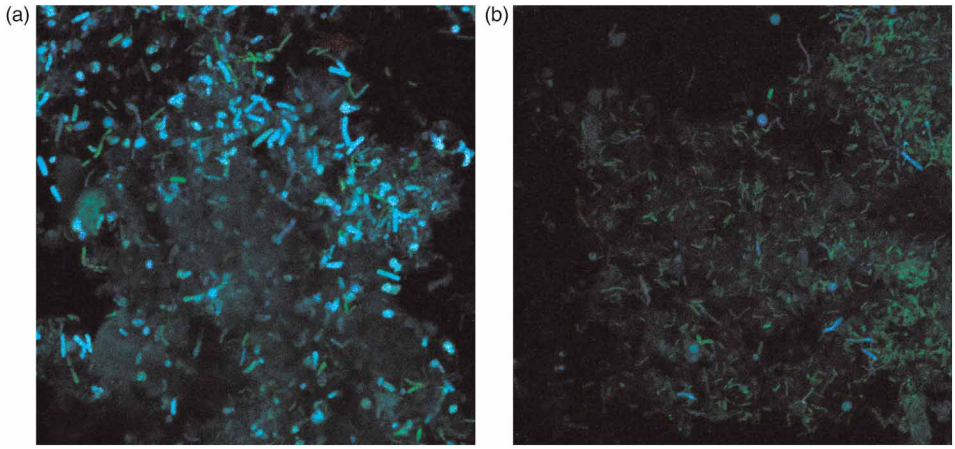

Figure 5 | FISH analysis of microorganisms at operational pH 5.3 (a) and 7.0 (b). Eubacteria were detected by FAM-labeled EUB338 (green), *Clostridium* species of Cluster I and XI were detected by Cy5-labeled P932 (blue), and *Clostridium* species of Cluster I and II were detected by Cy3-labeled Chis 150 (red).

Table 4 | FISH analysis of microorganisms at different operational pH 5.3 and 7.0

	Eubacteria					
	***Clostridium* species**					
	Cluster I	**Cluster II**	**Cluster XI**	**Other Eubacteria**	**Archaea**	**Total**
Color in FISH image	Blue-green	Yellow	Purple	Green	Red	–
Relative ratio calculation	$\frac{PC}{E+A}$	$\frac{C-(PC)}{E+A}$	$\frac{[P-(PC)]}{E+A}$	$\frac{[E-P-C]}{E+A}$	$\frac{A}{E+A}$	–
pH 5.3	84.7%	0%	5.9%	9.4%	0%	100%
pH 7.0	13.3%	0%	3.4%	83.3%	0%	100%

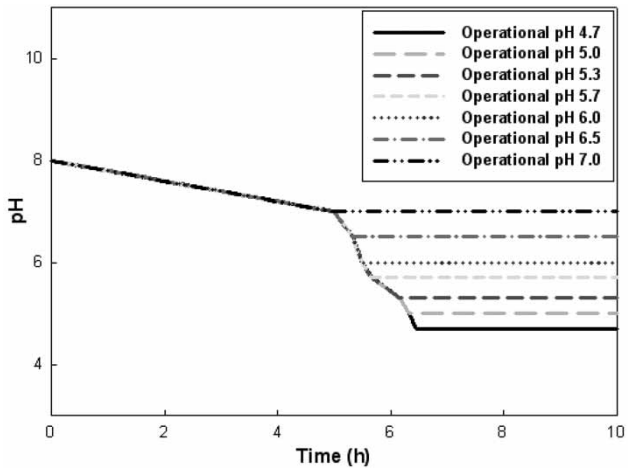

Figure 6 | pH change profile at initial stage depending on operational pH values.

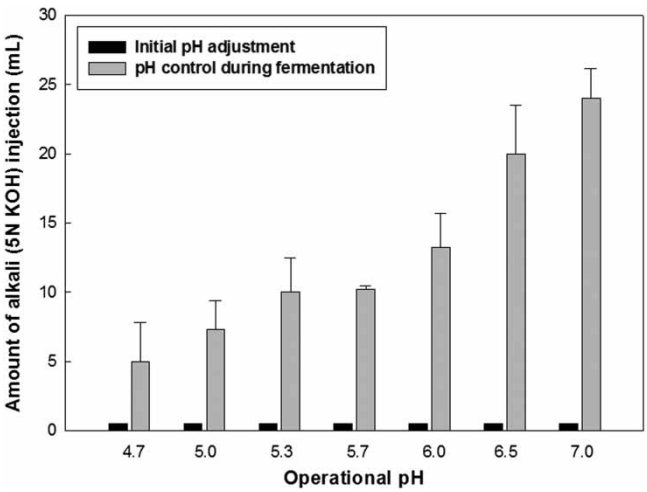

Figure 7 | The amount of alkali injection required at different operational pH values.

injection is an important factor for maintenance of a constant operational pH. Because the highest maximum H_2 yield was obtained at operational pH 5.3, less alkali was required compared to operational pH 6.0–7.0.

CONCLUSIONS

Dark fermentative H_2 production is affected by several factors such as pH, temperature, carbon source, retention time, etc. Among them, proper pH control is a key factor to initiate the germination of *Clostridium* sp. and to operate a hydrogen fermenter effectively. Although considerable research has studied the effect of an initial pH on dark fermentation, the results are not meaningful because the initial pH continues to change, depending on the state of degradation during fermentation. Thus, successful dark fermentation can be accomplished only when it is operated at a proper operational pH, which is not changeable during fermentation. FISH analysis demonstrated that the proper control of an operational pH could provide favorable conditions for the growth of H_2-producing bacteria and delay the shift of predominant metabolic flow from a hydrogen- and acid-forming pathway to a solvent-forming pathway. The strategy using the proper control of an operational pH was effective in improving the efficiency of dark fermentation.

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REFERENCES

Benemann, J. R. 1996 Hydrogen biotechnology: progress and prospects. *Nature Biotechnology* **4** (9), 1101–1103.

Chen, W. H., Chen, S. Y., Khanal, S. K. & Sung, S. 2006 Kinetic study of biological hydrogen production by anaerobic fermentation. *International Journal of Hydrogen Energy* **31** (15), 2170–2178.

Chen, W. H., Sung, S. & Chen, S. Y. 2009 Biological hydrogen production in an anaerobic sequencing batch reactor: pH and cyclic duration effects. *International Journal of Hydrogen Energy* **34** (1), 227–234.

Dubois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. A. & Smith, F. 1956 Colorimetric method for determination of sugars and related substances. *Analytical Chemistry* **28** (3), 350–356.

Han, S. H. & Shin, H. S. 2004 Biohydrogen production by anaerobic fermentation of food waste. *International Journal of Hydrogen Energy* **38** (29), 569–577.

Hawkes, F. R., Dinsdale, R., Hawkes, D. L. & Hussy, I. 2002 Sustainable fermentative hydrogen production: challenges for process optimisation. *International Journal of Hydrogen Energy* **27** (11–12), 1339–1347.

Ike, M., Inoue, D., Miyano, T. & Liu, T. T. 2010 Microbial population dynamics during start-up of a full-scale anaerobic digester treating industrial food waste in Kyoto eco-energy project. *Bioresource Technology* **101** (11), 3952–3957.

Khanal, S. K. 2008 *Anaerobic Biotechnology for Bioenergy Production*. 1st edn, Wiley-Blackwell, Oxford, UK, pp. 189–190.

Kim, D. H., Kim, S. H., Ko, I. B., Lee, C. Y. & Shin, H. S. 2008 Start-up strategy for continuous fermentative hydrogen production: Early switchover from batch to continuous

operation. *International Journal of Hydrogen Energy* **33** (5), 1532–1541.

Kim, D. H., Kim, S. H., Kim, K. Y. & Shin, H. S. 2010 Experience of a pilot-scale hydrogen-producing anaerobic sequencing batch reactor (ASBR) treating food waste. *International Journal of Hydrogen Energy* **35** (4), 1590–1594.

Kim, D. H., Kim, S. H., Jung, K. W., Kim, M. S. & Shin, H. S. 2011 Effect of initial pH independent of operational pH on hydrogen fermentation of food waste. *Bioresource Technology* **102** (18), 8646–8652.

Lee, M. J., Lee, J. H., Song, S. J. & Hwang, S. J. 2009 Effects of acid pre-treatment on bio-hydrogen production and microbial communities during dark fermentation. *Bioresource Technology* **100** (3), 1491–1493.

Li, Z., Wang, H., Tang, Z., Wang, X. & Bai, J. 2008 Effects of pH value and substrate concentration on hydrogen production from the anaerobic fermentation of glucose. *International Journal of Hydrogen Energy* **33** (24), 7413–7418.

Lin, C. Y., Chang, C. C. & Hung, C. H. 2008 Fermentative hydrogen production from starch using natural mixed cultures. *International Journal of Hydrogen Energy* **33** (10), 2445–2453.

Noike, T., Takabatake, H., Mizuno, O. & Ohba, M. 2002 Inhibition of hydrogen fermentation of organic wastes by lactic acid bacteria. *International Journal of Hydrogen Energy* **27** (11–12), 1367–1371.

Pan, J., Zhang, R., El-mashad, H. M., Sun, H. & Ying, Y. 2008 Effect of food to microorganism ratio on biohydrogen production from food waste via anaerobic fermentation. *International Journal of Hydrogen Energy* **33** (23), 6968–6975.

Shin, H. S., Youn, J. H. & Kim, S. H. 2004 Hydrogen production from food waste in anaerobic mesophilic and thermophilic acidogenesis. *International Journal of Hydrogen Energy* **29** (13), 1355–1363.

Standard Methods for the Examination of Water and Wastewater 2005 21st edn, American Public Health Association/ American Water Works Association/Water Environment Federation, Washington, DC, USA.

Wang, J. & Wan, W. 2009 Factors influencing fermentative hydrogen production: a review. *International Journal of Hydrogen Energy* **34** (2), 799–811.

Zhang, T., Liu, H. & Fang, H. H. P. 2003 Biohydrogen production form starch in wastewater under thermophilic condition. *Journal of Environmental Management* **69** (2), 149–156.

Zhao, Q. B. & Yu, H. Q. 2008 Fermentative H2 production in an upflow anaerobic sludge blanket reactor at various pH values. *Bioresource Technology* **99** (5), 1353–1358.

Zong, W., Yu, R., Zhang, P., Fan, M. & Zhou, Z. 2009 Efficient hydrogen gas production from cassava and food waste by a two-step process of dark fermentation and photo-fermentation. *Biomass and Bioenergy* **33** (10), 1458–1463.

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