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Roles of pH in Biologic Production of Hydrogen and Volatile Fatty Acids From Glucose by Enriched Anaerobic Cultures

XIAN-JUN ZHENG AND HAN-QING YU*

Laboratory of Clean Bioenergy, School of Chemistry, The University of Science & Technology of China, Hefei, Anhui, 230026, China, E-mail: hqyu@ustc.edu.cn

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Abstract

Batch experiments were carried out to study the roles of pH in the biologic production of hydrogen and volatile fatty acids from glucose by enriched anaerobic cultures. The results showed that 95–99% of glucose in wastewater was acidified at 30°C and pH 4.0–8.5. Hydrogen yield fluctuated between 1.30 and 1.57 mol of H_2/mol of glucose when the reactor was operated at pH 4.0–5.0. However, a further increase in pH led to a considerable decrease in hydrogen yield, especially for the cases at pH 7.5 and 8.0. Acetate, propionate, butyrate, and ethanol were the key products of acidogenesis. Production of butyrate was favored at pH 4.0–5.0, whereas production of acetate was favored at pH 6.0–8.0. A modified Gompertz equation is able to properly describe the batch production of hydrogen from glucose. The optimum pH for the specific hydrogen production was found to be 5.5, close to 5.7, the optimum pH calculated using a semiempirical model.

Index Entries: Anaerobic fermentation; glucose; hydrogen production; pH; volatile fatty acids.

Introduction

Hydrogen is a promising candidate as a clean energy carrier in the future. Microbial hydrogen production using fermentative, photosynthetic bacteria or algae is an environmentally friendly and energy-saving process, and it has recently attracted considerable attention as a way of effectively converting organic wastes to hydrogen *(1)*. During the anaerobic acidogenesis of organic wastes, hydrogen, carbon dioxide, volatile fatty acids

*Author to whom all correspondence and reprint requests should be addressed.

(VFA), and sometimes alcohols are simultaneously produced *(2)*. The feasibility of applying acidogenesis of organic wastes to produce hydrogen has been widely demonstrated at various laboratories *(1,3–7)*. Product formation by a mixed acidogenic population is a very complex process and is greatly influenced by many factors. These factors include wastewater specificity, reactor configuration, hydraulic retention time, influent organic concentration, organic loading rate, pH, temperature, oxidation-reduction potential, and nutritional requirements *(8)*.

Since pH affects growth rate, changes in pH may cause drastic shifts in the relative numbers of different species in a heterogeneous population such as is present in the acidogenic reactor *(9)*. Many aspects of microbial metabolism are greatly influenced by variations in pH over the range within which the microorganisms can grow. These aspects include utilization of carbon and energy sources, efficiency of substrate degradation, synthesis of proteins and various types of storage material, and release of metabolic products from cells *(10)*. Moreover, variation in pH can affect cell morphology and structure and, therefore, flocculation and adhesion phenomena *(11)*. A substantial number of studies have been carried out on the effect of pH on acidogenesis of various wastes *(11–14)*. These studies have demonstrated that proper control of pH is crucial to the production of acidogenic products, owing to the effects of pH on the hydrogenase activity and on the metabolism pathways. So far, however, no relationship between hydrogen production and distribution of VFA has been established. Furthermore, the reported optimum pH values for hydrogen production always conflicted with each other, from pH 5.0 *(14,15)* to 9.0 *(6,13)*.

Therefore, this study was conducted to explore the roles of pH in the production of hydrogen and VFA in an acidogenic reactor, and to establish the relationship between hydrogen production and fermentation type. Glucose was used as the sole substrate, and heat-treated anaerobic sludge was employed as the seed sludge.

Materials and Methods

Seed Sludge

The anaerobic cultures used were heat-treated anaerobic granular sludge that originally came from a full-scale upflow anaerobic sludge blanket reactor treating citric acid-producing wastewater. Prior to use, the granular sludge was first washed with tap water five times and then was sieved to remove stone, sand, and other coarse matter. Thereafter, the sludge was heated at 85°C for 1 h to inactivate hydrogentrophic methanogens and to enrich hydrogen-producing bacteria.

Experiments

Hydrogen production experiments were conducted in a 5-L fermentor (Baoxin Biotech, Shanghai). One hundred milliliters of heat-treated granular sludge and a certain volume of nutrients solution were added to the

fermentor. The working volume of the fermentor was adjusted to 2.0 L with distilled water. The volatile suspended solids (VSS) concentration of biomass was 0.8 g/L. The solution in the fermentor was composed as follows: 5000 mg/L of glucose, 500 mg/L of NH_4Cl, 250 mg/L of KH_2PO_4, 250 mg/L of K_2HPO_4, 15 mg/L of $FeSO_4 \cdot 7H_2O$, 125 mg/L of $MgCl_2 \cdot 6H_2O$, 0.5 mg/L of $NiCl_2 \cdot 6H_2O$, 1.0 mg/L of $CaCl_2$, 20 mg/L of KCl, 0.5 mg/L of $(NH_4)_6Mo_7O_{24}$, 0.5 mg/L of $CoCl_2 \cdot 6H_2O$, 0.5 mg/L of $MnCl_2 \cdot 4H_2O$, 0.5 mg/L of $ZnSO_4 \cdot 7H_2O$, 0.5 mg/L of $CuSO_4 \cdot 5H_2O$. Prior to operation, the fermentor was purged with N_2 for 10 min to ensure anaerobic condition.

The temperature in the fermentor was controlled at 30°C. The fermentor was stirred at a constant rate of 150 rpm to ensure thorough mixing and to facilitate rapid diffusion of the hydrogen. The pH of the mixed liquor was controlled automatically by feeding NaOH (4 *M*) and HCl (2 *M*) solutions via respective peristaltic pumps. The pH in the fermentor was stepwise changed from 4.0 to 8.0 with 0.5 increments. The amount of biogas produced was recorded using a water-displacement method. At each pH level, the volume and composition of biogas, degradation of glucose, and production of VFA were monitored.

Analytical Methods

The composition of biogas was analyzed by a gas chromatograph (Lunan, Model SP-6800A) equipped with a thermal conductivity detector and a 2-m stainless column packed with a 5-Å molecular sieve. The temperatures of the injector, column, and detector were kept at 100, 50, and 105°C, respectively. Argon was used as carrier gas at a flow rate of 30 mL/min. The concentrations of the VFA and alcohols in the liquor were determined by a second gas chromatograph (Angilent, Model 6890NT) equipped with a flame ionization detector and a 30 m × 0.25 mm × 0.25 µm fused-silica capillary column (DB-FFAP). The VFA and alcohols analyzed were acetate, propionate, butyrate, i-butyrate, valerate, caproate, ethanol, and propanol. The liquor samples were first centrifuged at 12,000 rpm for 5 min, then acidified by formic acid and filtrated through a 0.2-µm membrane, and finally measured for free acids. The temperatures of the injector and detector were 250 and 300°C, respectively. The initial oven temperature was 70°C for 3 min followed by a ramp of 20°C/min for 5.5 min and a final temperature of 180°C for 3 min. Nitrogen was used as carrier gas with a flow rate of 2.6 mL/min. The detectable levels were 5 mg/L for individual VFA and alcohols.

Glucose was measured by using an anthrone–sulfuric acid method *(16)*, while the VSS and chemical oxygen demand were measured according to standard methods *(17)*.

Results and Discussion

Concentrations of glucose and acidogenic products in the mixed liquor were monitored in all batches at predetermined time intervals. In all

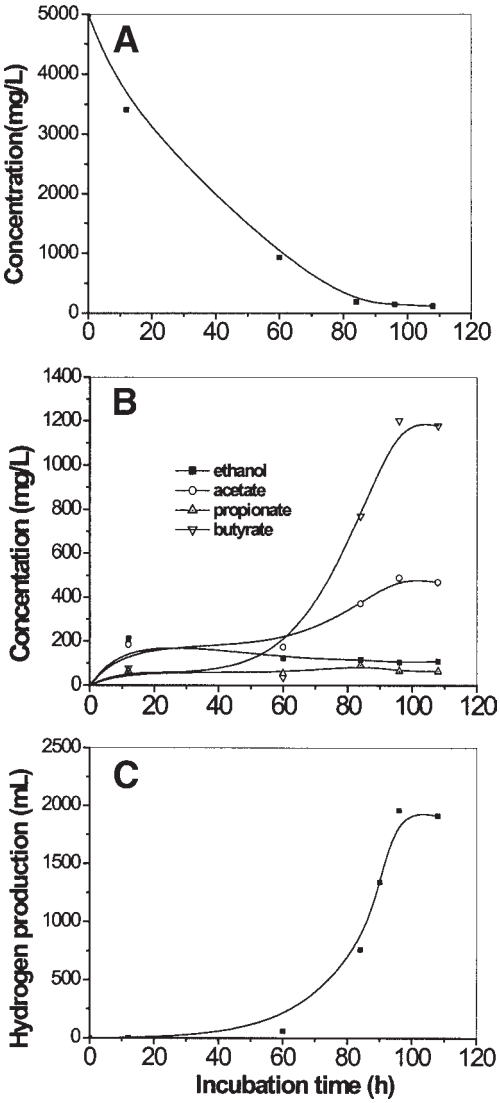

Fig. 1. Experimental results at pH 4.0: **(A)** glucose degradation; **(B)** VFA production; **(C)** hydrogen production.

batches, the biogas was mainly composed of hydrogen and carbon dioxide, and the mixed liquor was composed of VFA and ethanol. The VFA were mostly acetate, propionate, and butyrate, plus smaller quantities of i-butyrate, valerate, i-valerate, and caproate.

Figure 1 illustrates the concentration changes of glucose (Fig. 1A); acetate, propionate, butyrate, and ethanol (Fig. 1B); and cumulative H_2 production (Fig. 1C) at lower pH levels, using pH 4.0 as an example. Figure 2 illustrates the corresponding results at pH 8.0, for comparison.

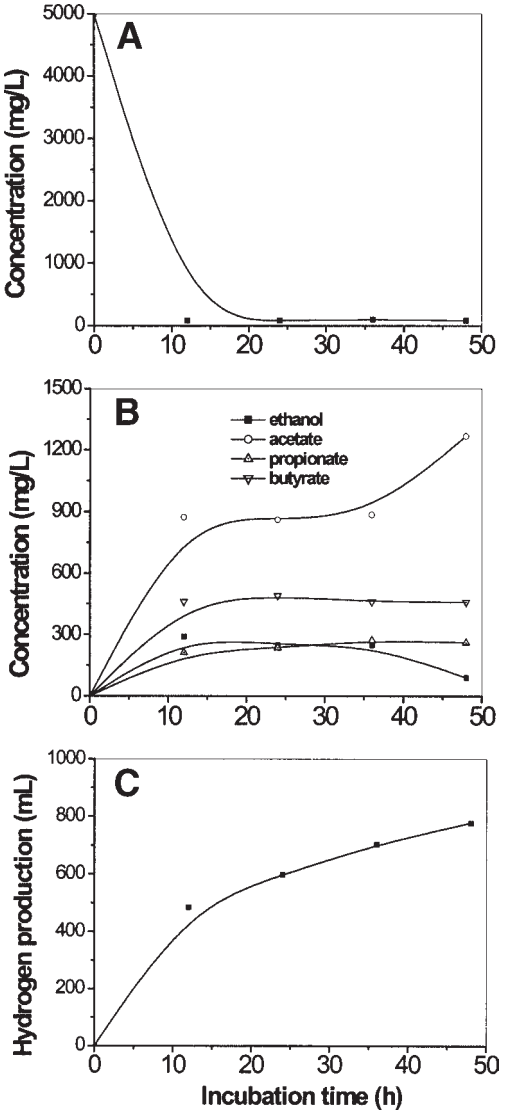

Fig. 2. Experimental results at pH 8.0: **(A)** glucose degradation; **(B)** VFA production; and **(C)** hydrogen production.

pH 4.0

Figure 1A illustrates that, after incubation, glucose was degraded rapidly. About 81% of glucose was degraded within 60 h. Thereafter, the degradation of glucose slowed down, leveling off by 96 h. About 98% of glucose was degraded after 108 h of incubation. These results demonstrate that glucose was readily acidified even at pH 4.0.

Figure 1B shows that with the degradation of glucose, acetate and butyrate first increased slightly, then remained constant for 48 h; afterward, the concentrations of the two acids increased rapidly, reaching 470 and

1178 mg/L, respectively, at h 108. By contrast, ethanol and propionate were produced in the 12 h of incubation; thereafter, their concentrations did not change significantly in the following incubation period. Acetate and butyrate were the two most abundant species in the final aqueous products, respectively accounting for 26 and 65% of the final acidogenic products.

As illustrated in Fig. 1C, almost no hydrogen was produced in the first 60 h; afterward hydrogen was produced sharply, peaking at h 108, then decreasing slightly. The rapid production of hydrogen coincided with the sharp increase in acetate and butyrate from h 60 to h 108, suggesting that the evolution of hydrogen was greatly associated with the generation of both acids.

pH 8.0

The experimental results at high pH levels, using pH 8.0 as an example, are illustrated in Fig. 2. Most of the results of acidogenesis of glucose-rich wastewater were independent of pH, but there were a few exceptions. Figure 2A shows that glucose was degraded rapidly and became nearly depleted within 12 h. The glucose removal efficiency exceeded 98% at pH 8.0, comparable with that at pH 4.0.

Figure 2B illustrates that the distribution of acetate, propionate, butyrate, and ethanol at pH 4.0 was significantly different from that at pH 8.0. The concentration of acetate increased rapidly in the first 12 h, then kept constant at 860 mg/L for the following 24 h, but increased sharply after h 36. Propionate and butyrate had similar changing patterns over the incubation period, but the concentration of butyrate was higher than the corresponding propionate concentration. Ethanol was produced in the first 12 h, and thereafter its concentration decreased slightly. Among the aqueous products, acetate and butyrate were the two most abundant species, each respectively accounting for 68 and 24% of total VFA and alcohol.

As illustrated in Fig. 2C, hydrogen production increased rapidly in the first 12 h and then increased gradually in the subsequent 36 h. The maximum hydrogen production was 776 mL, much less than that at pH 4.0. This suggests that hydrogen production was not favorable under weak alkali conditions.

Effects of pH

Table 1 summarizes the effects of pH on specific hydrogen production and specific VFA production of the acidogenic reactor. The specific hydrogen production rate increased from 30 mL/(g of VSS·h) at pH 4.0 to 45 mL/(g of VSS·h) at pH 5.0 and peaked (104 mL/[g of VSS·h]) at pH 5.5; a further increase in pH resulted in a decrease in specific hydrogen production rate.

As shown in Table 1, the hydrogen yield fluctuated in a narrow range between 1.30 and 1.57 mol of H_2/mol of glucose when the reactor was operated at pH 4.0–5.0. However, a further increase in pH led to a considerable decrease in hydrogen yield, especially for the cases at pH 7.5 and 8.0. In terms of hydrogen yield, pH 5.5 was the optimum value for hydrogen production.

Table 1
Specific Hydrogen and Specific VFA Production Rates at Various pH Levels

pH	Specific H_2 production rate (mL/[g VSS·h])	H_2 yield (mol H_2/mol glucose)	Specific VFA production rate (mg/[g VSS·h])	Final P_{H2} (atm)
4.0	30	1.57	28	0.34
4.5	33	1.46	103	0.34
5.0	45	1.52	87	0.34
5.5	104	1.30	103	0.30
6.0	69	1.06	155	0.26
6.5	51	1.10	221	0.28
7.0	44	0.89	227	0.27
7.5	17	0.69	119	0.23
8.0	13	0.62	98	0.21

Table 2
Distribution of Final Aqueous Products

pH	Acetate (%)	Propionate (%)	Butyrate (%)	Ethanol (%)	VFA (mg/L)	Alcohols (mg/L)
4.0	25.8	3.5	64.7	6.0	1821	109
4.5	30.4	11.2	49.6	4.7	2294	107
5.0	35.3	11.7	48.1	1.0	2020	20
5.5	37.6	8.8	16.7	4.3	1910	82
6.0	60.9	8.3	23.7	3.9	2361	92
6.5	68.0	17.2	12.8	ND[a]	3655	ND[a]
7.0	84.4	10.6	4.4	ND[a]	2923	ND[a]
7.5	55.4	14.0	27.4	ND[a]	2074	ND[a]
8.0	67.5	13.9	24.5	4.9	1875	91

[a]ND, under detection limit (5 mg/L).

In addition to the gaseous products, VFA and ethanol were produced from the acidogenesis of glucose. Specific VFA production rate is an important parameter to evaluate the efficiency of an acidogenic reactor. Table 1 shows that the changing pattern of specific VFA production rate was different from that of specific hydrogen production rate. Generally, the specific VFA production rate increased with pH when pH varied between 4.0 and 7.0. However, a further increase in pH from 7.0 to 7.5 and later 8.0 resulted in a substantial decrease in specific VFA production rate.

The final partial pressures of hydrogen in all the batches are also given in Table 1. At pH 4.0–7.0, the final H_2 partial pressures fluctuated from 0.27 to 0.34 atm. However, when pH increased to 7.5 and 8.0, the final H_2 partial pressures fell to 0.23 and 0.21 atm, respectively.

Table 2 summarizes the concentrations of total VFA and alcohols as well as the distribution of the four main aqueous products. pH had a considerable effect on such a distribution. With the increase in pH, the

percentage of acetate generally increased, whereas the percentage of butyrate had an opposite change pattern. On the other hand, the percentages of ethanol and propionate did not change significantly with pH, except for the case when ethanol was not detected at pH 6.5–7.5. At pH 4.0–5.5, butyrate was the predominant VFA, whereas acetate was the major acidogenic product at pH 6.0–8.0. These results demonstrate that the metabolic pathway of glucose during acidogenesis was greatly influenced by pH.

Modeling

The cumulative hydrogen production (H) data were fitted to a modified Gompertz equation *(3)*, which has been found to be an appropriate model for describing the progress of cumulative biogas production in a batch experiment *(4)*:

$$H = P \cdot \exp\{-\exp[\frac{r_m \cdot e}{P}(\lambda - t) + 1]\} \quad (1)$$

in which P is the hydrogen production potential (mL), r_m is the maximum hydrogen production rate (mL/h), λ is the lag-phase time (h), and $e = 2.718$. The three parameters P, r_m, an λ were nonlinearly evaluated using the function of Microsoft Origin 6.1 by converting the residual sum of squares between the experiment and the estimation to a minimum value. Table 3 summarizes the values of the parameters.

According to Table 3, hydrogen production should have a maximum potential of 2002 mL at pH 4.0, while the biomass would reach its highest hydrogen production rate of 718 mL/h at pH 6.0. By contrast, the lag-phase time decreased with the increase in pH. The very high values of the correlation coefficients given in Table 3 suggest that the modified Gompertz equation is able to properly describe the batch production of hydrogen from glucose by enriched cultures.

The bacterial activities may be controlled by the overall enzymatic activity *(11)*. Since enzymes are made of amino acids, their activities are thus pH dependent, as shown in the following equations:

$$E^+ \leftrightarrow E + H^+ \quad (2)$$

$$E \leftrightarrow E^- + H^+ \quad (3)$$

in which E is the active enzyme, and E^+ and E^- are the less active forms of charge-carrying enzyme *(10)*. Assuming K_H and K_{OH} are the respective equilibrium constants of reactions 2 and 3, the enzymatic activity can be expressed as follows:

$$R = \frac{R_{max}}{1 + \frac{[H^+]}{K_H} + \frac{K_{OH}}{[H^+]}} \quad (4)$$

Table 3
Parameters for Hydrogen Production Calculated From Nonlinear Regression of Eq. 1

pH	P (mL H_2)[a]	r_m (mL/h)[b]	λ (h)[c]	r^2[d]
4.0	2002	140	78.8	0.990
4.5	1820	499	34.9	0.998
5.0	1901	229	13.5	0.999
5.5	1840	449	7.4	0.999
6.0	1324	718	7.4	0.999
6.5	1377	286	5.8	0.999
7.0	1109	186	6.0	0.999
7.5	864	184	8.1	0.999
8.0	698	72	5.0	0.975

[a]P, hydrogen production potential (mL).
[b]r_m, maximum hydrogen production rate (mL/h).
[c]λ, lag-phase time (h).
[d]r^2, regression coefficient.

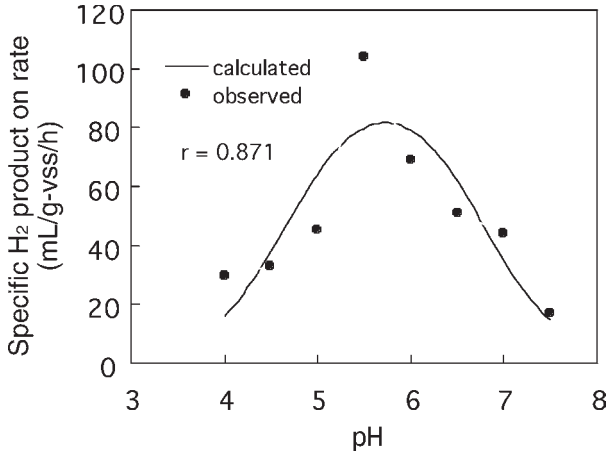

Fig. 3. Relationship between specific hydrogen production rate and pH value.

in which R is the specific enzymatic reaction rate, and R_{max} is the maximum value of R, assuming all enzymes are present in charge-neutral form of E. Figure 3 illustrates that the measured specific hydrogen production rate at various pH values matched the best-fit curve of Eq. 4. The best-fit curve was plotted based on the following parameters obtained from regression analysis: R_{max} of 97 mL/(g of VSS·h), K_H of 2.00×10^{-5} M, and K_{OH} of 1.77×10^{-7} M. Based on Eq. 4, the maximum enzymatic activity occurred at $(pK_H + pK_{OH})/2$, i.e., pH 5.7. These calculated results match satisfactorily with the observed results, i.e., maximum bioactivity of 104 mL/(g of VSS·h) at the optimum pH of 5.5.

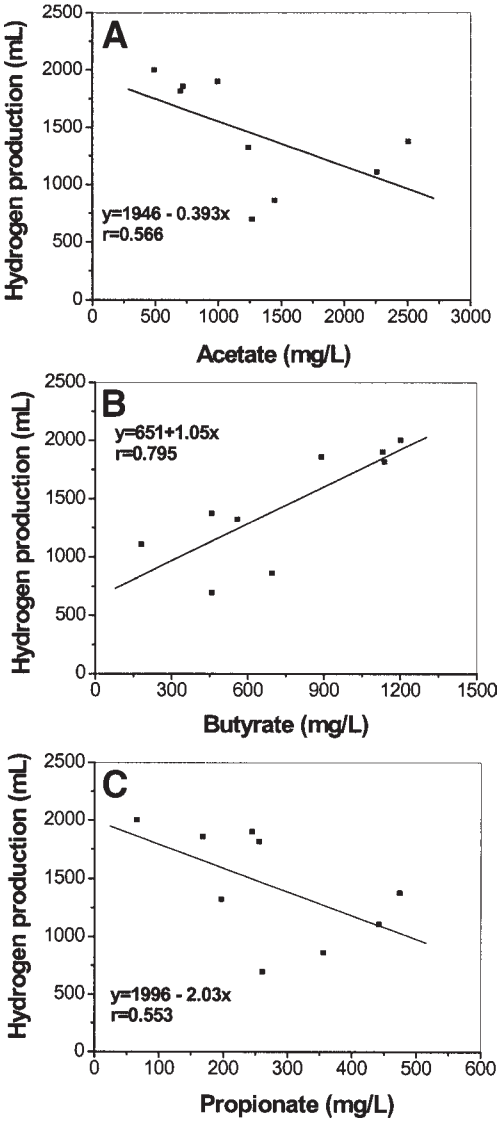

Fig. 4. Correlation of hydrogen production with concentration of **(A)** acetate, **(B)** butyrate, and **(C)** propionate.

Fermentation Type

Figure 4 illustrates the correlation of hydrogen production against the concentrations of acetate, butyrate, and propionate. As shown in Fig. 4A, hydrogen production did not correlate with the formation of acetate, as evidenced by the low value of correlation coefficient (r = 0.566).

On the other hand, Fig. 4B illustrates that hydrogen production correlated well with the formation of butyrate. This implies that the formation of butyrate was associated with hydrogen production. Figure 4C shows that the formation of propionate was not associated with hydrogen produc-

tion, the same case as for the formation of acetate. These results were in agreement with previous studies *(5,8)*.

There are two common types of acidogenesis: butyrate- and propionate-type fermentations. The former produces not only butyrate and acetate but also carbon dioxide and hydrogen, whereas the latter produces propionate and acetate, with no significant hydrogen production *(8)*. Many operational and environmental factors, such as substrate type, biomass type, substrate concentration, reactor configuration, hydraulic retention time, temperature, and pH, determine the fermentation types of an acidogenic reactor *(8,9)*. Based on the VFA distributions and gas production, it appears that in the present study butyrate-type fermentation was predominant, especially at low pH levels. However, as pH increased, it appears that both butyrate- and propionate-type fermentations were coexistent in the reactor. The change in fermentation type might be owing to either the metabolism of the same population or a change in the population itself or a combination of both these changes. Furthermore, since the significant changes in product distribution occurred at pH 4.0–8.0 and hydrogen production was highly accompanied by the formation of butyrate, pH control should be important for the stable production of hydrogen from an acidogenic reactor.

Conclusion

The results showed that 95–99% of glucose in wastewater was acidified at 30°C and pH 4.0–8.5 by enriched hydrogen-producing bacteria. Hydrogen yield fluctuated between 1.30 and 1.57 mol of H_2/mol of glucose when the reactor was operated at pH 4.0–5.0. However, a further increase in pH led to a considerable decrease in hydrogen yield, especially for the cases at pH 7.5 and 8.0. Acetate, propionate, butyrate, and ethanol are the key products of acidogenesis. Production of butyrate was favored at pH 4.0–5.0, whereas production of acetate was favored at pH 6.0–8.0. A modified Gompertz equation was able to properly describe the batch production of hydrogen from glucose. The optimum pH for the specific hydrogen production was found to be 5.5, close to 5.7, the optimum pH calculated using a semiempirical model.

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