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Influence of Temperature and Duration of Heat Treatment Used for Anaerobic Seed Sludge on Biohydrogen Fermentation

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Abstract

This study focused for the influence of temperature and duration of heat treatment applied to seed sludge on anaerobic biohydrogen fermentation. Temperature for the treatment varied from 90ºC to 100ºC, and duration from 15 and 180 min. The observed hydrogen production from the batch hydrogen fermentation was explained by the difference equation between cumulative hydrogen production and consumption, both of which are expressed by the modified Gompertz equations. The hydrogen production potential was effected by temperature and duration of heat treatment used for the anaerobic seed sludge. The maximum value of hydrogen production potential was 63.1 mL H_2/g glucose, which was obtained from seed sludge treated at 100ºC for 30 min. The compositions of volatile fatty acids in the products and their levels, as well as carbon dioxide content in the biogas, were also effected by temperature and duration of the heat treatment used for the anaerobic seed sludge, and they were important factors for determined the hydrogen production potential.

Keywords: *biohydrogen, heat treatment, temperature, duration, production, consumption*

1. Introduction

Fossil fuels, such as petroleum, coal, and natural gases, play an important role in the advancement of human civilization. However, these fossil fuels are becoming increasingly scarce as a form of natural resource, and their by-products, including carbon dioxide, are heavily contributing to the contamination of the environment (Mukhopadhya and Forssell, 2005; Kapdan and Kargi, 2006). There is no doubt that the most plentiful source of energy on our planet is sunlight. Green plants convert sunlight into chemical energy and save it in the form of organic matter through photosynthesis (Bolton, 1995). This organic matter is an important natural resource, which can be produced continuously on the planet, and represents a good storage mechanism for chemical energy (Bolton, 1995). Hydrogen contains a high level of energy, which is clean in the sense that the use of hydrogen does not produce pollutants (Kapdan and Kargi, 2006). Anaerobic hydrogen fermentation, by which the chemical energy contained in organic matter is converted into hydrogen, appears to offer a great potential. However, research into hydrogen fermentation is at its early stages, and there are several unknown factors concerning the biological and physico-chemical conditions required for obtaining the optimal hydrogen yield (Hawkes *et al*., 2002; Levin *et al*., 2004; Lay *et al*., 2003). In previous studies, anaerobic fermentation for hydrogen production was carried out using a facultative *Enterobacter* or several kinds of obligately anaerobic *Chlostridium* species (Kapdan and Kargi, 2006; Hawkes *et al*., 2002; Levin *et al*., 2004; Lay *et al*., 2003). These hydrogen producers use organic pollutants as a substrate, indicating that hydrogen fermentation is an important environmentally benign technology, which allows for the stabilization of organic pollutants, as well as the production of hydrogen gas (Kapdan and Kargi, 2006; Hawkes *et al*., 2002). The hydrogen yield obtainable from organic matter is very important for the practical use of hydrogen fermentation. The yield of hydrogen from organic matter depends on the pathway of hydrogen fermentation (Hawkes *et al*., 2002; Levin *et al*., 2004). Theoretically, from the hydrogen fermentation of 1 mole of glucose, 2 moles of hydrogen could be produced along with 1 mole of butyrate, and 4 moles of hydrogen along with 2 moles of acetate (Kapdan and Kargi, 2006; Levin *et al*., 2004). However, the hydrogen yield reported in previous studies did not reach the theoretical value. This may be attributable to several metabolic pathways involved in hydrogen consumption (Hawkes *et al*., 2002; Levin *et al*., 2004; Lay *et al*., 2003; Oh *et al*., 2003; Park *et al*., 2005; Grommen *et al*., 2006; Jia *et al*., 1996). It is generally thought that the metabolic pathways involved in hydrogen fermentation are governed by the dominant microorganisms, and can sometimes be effected by environmental conditions (Hawkes *et al*., 2002; Levin *et al*., 2004; Lay *et al*., 2003). However, a limited number of studies

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involved in hydrogen fermentation concerning the production or consumption of hydrogen (Lay *et al*., 2003; Oh *et al*., 2003). The environmental conditions required for microbial growth, such as temperature, nutrients, pH, and hydraulic and solid retention, have been studied to select hydrogen-producing microorganism species (Hawkes *et al*., 2002; Levin *et al*., 2004; Lay *et al*., 2003; Oh *et al*., 2003). Those species that have previously been selected for hydrogen production include the *Chlostridium* species, which can form spores under unfavorable conditions, resulting in their having greater tolerance against changes in environmental conditions than non-spore-forming species such as propionate-producing, methanogenic, and lactate-producing bacteria (Hawkes *et al*., 2002; Levin *et al*., 2004; Noike *et al*., 2002). In previous studies, heat treatment was a popular method for obtaining the inocula required for hydrogen fermentation, by which the hydrogen-producing bacteria could be harvested from activated sludge, anaerobic sludge, compost, and soil (Hawkes *et al*., 2002; Lay *et al*., 2003; Oh *et al*., 2003; Fan *et al*., 2004; Iyer *et al*., 2004). Generally, microbial activity is effected by the intensity and duration of the heat treatment (Aymard and Belarbi, 2000). This indicates that the dominant species in the microbial consortium could be governed by the temperature of heat treatment as well as its duration. The heat treatment conditions may therefore influence the activity of hydrogen consumption, as well as the production, and could be the key to improving the hydrogen yield in anaerobic hydrogen fermentation. The hydrogen production potential from batch experiments is usually estimated by using the modified Gompertz equation to calculate the cumulative hydrogen production (Fan *et al*., 2004; Kim *et al*., 2004). However, the activity of hydrogen consumption, as well as hydrogen production, has to be considered when estimating the observed hydrogen production potential obtainable from a batch experiment (Oh *et al*., 2003; Park *et al*., 2005).

In this study, the activity of hydrogen production and consumption, as well as the hydrogen production potential, obtainable in batch fermentation, were estimated, and the influence of the temperature and duration of the heat treatment used for anaerobic seed sludge on biohydrogen fermentation was investigated.

2. Materials and Methods

2.1 Heat Treatment of Anaerobic Sludge

Anaerobic sludge was collected from an anaerobic digester used for sewage sludge in B metrocity in Korea. The sludge was screened with a 2-mm sieve, and then thickened at 4°C in a refrigerator for one day. In a water bath, the thickened sludge was treated by heat at 90°C and 100°C for predetermined time periods ranging from 0 to 180 min, and then used as the seed sludge. The concentration of volatile suspended solids in the seed sludge was approximately 10,600 mg/L.

2.2 Batch Hydrogen Fermentation

The effects of the temperature and duration of the heat treatment of the seed sludge on the hydrogen production activity were evaluated with a batch hydrogen fermentation experiment using 125-mL serum bottles. The medium used for the batch hydrogen fermentation was prepared by adding 20 g of glucose and 3 g of sodium bicarbonate to 1 L of distilled water, and the ratios of COD to N, P, and Fe were adjusted to 5.0, 1.0, and 0.33 by adding NH_4Cl, KH_2PO_4, and $FeCl_24H_2O$, respectively, as described in a previous study (Hawkes *et al*., 2002). The amounts of trace elements in the medium were adjusted by adding the following chemicals to 1 L of the medium; 0.32 g of $MgSO_47H_2O$, 0.032 g of $NiSO_46H_2O$, 0.05 g of $CaCl_2$, 0.007 g of $Na_2B_4O_710H_2O$, 0.014 g of $(NH_4)_6Mo_7O_{24}4H_2O$, 0.023 g of $ZnCl_2$, 0.021 g of $CoCl6H_2O$, and 0.01 g of $CuCl_22H_2O$ (Han and Shin, 2004).

For the experiments, 40 mL of the seed sludge and 40 mL of the medium were placed in the serum bottles, and the initial pH was adjusted to 5.5±0.2 using 1 N HCl and NaOH. The prepared serum bottles were flushed with nitrogen gas, sealed with n-butyl rubber stoppers and aluminum caps, and then incubated at 35°C in a darkened rotary shaker at 180 rpm. All of the experiments were performed in triplicate, along with two controls and they were seeded with non-heat treated sludge and with no added glucose, and were the experiments performed under the same conditions.

2.3 Analysis and Calculations

To monitor the production of hydrogen, a glass syringe was intermittently used to measure the biogas production from each serum bottle. The composition of the biogas was analyzed with a gas chromatograph (GowMac Series 580) equipped with a thermal conductivity detector (Kim *et al*., 2004). The columns used for the analysis were a 1.8 m × 3.2 mm stainless steel column with a molecular sieve for hydrogen and a 1.8 m × 3.2 mm stainless steel column with Porapak *Q* (6 ft × 1/8" SS) for methane and carbon dioxide. Nitrogen gas at a flow rate of 40 mL/min was used as a carrier gas for all of the gas analyses. Temperatures of injector, detector, and oven were 80°C, 90°C, and 50°C, respectively. The biogas produced from the serum bottles was converted into the STP state using Eq. (1).

$$V_g(L)_{at\ STP} = V_{g,at\ T} \times \frac{273}{273+T} \times \frac{(760-W)}{760} \quad (1)$$

Where T is the incubation temperature (°C) of the serum bottle, and W is the water vapor pressure at each temperature (mm Hg). The hydrogen production at each time interval was calculated from the measurements of the biogas composition in the headspace and the total volume of biogas produced using the mass balance equation.

$$V_{H,i} = C_{H,i}(V_{G,i} + V_H) - V_H C_{H,i-1} \quad (2)$$

Where $V_{H,i}$ is the hydrogen gas volume at the current (i) time interval, $V_{G,i}$ is the total biogas volume in the current time interval, $C_{H,i}$ and $C_{H,i\text{-}1}$ are the fractions of hydrogen gas in the headspace of the bottle measured using gas chromatography in the current and previous intervals, respectively, and V_H is the

total volume of the headspace in the bottle. The liquid content of the serum bottle was measured before the incubation for the batch experiment and after the completion, and characteristics, such as pH, TSS, VSS, SCOD and VFA, were measured. The VFAs, including C1-C6, were analyzed with an HPLC (DX-500) equipped with an ultraviolet detector and an Aminex HPX-87H column, and the solvent was 0.005 M of sulfuric acid. The SCOD was measured according to the Standard Methods (1995) (APHA, 1995). To estimate the cumulative hydrogen production and consumption, the observed cumulative hydrogen production (H_{pobs}) was assumed to be equal to the difference between the cumulative hydrogen production (H_p) and the consumption (H_c) as described in Eq. (3).

$$H_{pobs} = H_p - H_c \tag{3}$$

Where the modified Gompertz equations were adopted for the description of the cumulative hydrogen production and the consumption, as described by Eqs. (4) and (5), respectively.

$$H_p = H_{pu} \cdot \exp(-\exp[\alpha(\lambda - t) + 1]) \tag{4}$$
$$H_c = H_{cu} \cdot \exp(-\exp[\beta(\lambda_c - t) + 1]) \tag{5}$$

Where α is equal to $R_m \cdot \exp(1)/H_{pu}$, β is equal to $R_{cm} \cdot \exp(1)/H_{cu}$. λ and R_m are the lag phase time for hydrogen production and the maximum rate of hydrogen production, and λ_c and R_{cm} are the lag phase time for hydrogen consumption and the maximum rate of hydrogen consumption, respectively. The parameters used in the above equations were estimated by fitting the observed cumulative hydrogen production from the batch fermentation into Eq. (3) using Sigma plot 8.0.

3. Results and Discussion

3.1 Observed Biogas Production

In the batch fermentations, the observed cumulative hydrogen production increased sharply after the short lag phases, as shown in Fig. 1, and approached the maximum values between 25 and 64 hr. The maximum values were significantly affected by the heat treatment conditions, including the temperature and duration of the heat treatment used for the anaerobic seed sludge. The differences in the observed maximum hydrogen productions might be attributed to the variation in the dominant species of hydrogen-producing bacteria with the heat treatment conditions, and their metabolic pathways (Hawkes *et al*., 2002; Levin *et al*., 2004; Lay *et al*., 2003; Iyer *et al*., 2004; Kim *et al*., 2004; Fang and Liu, 2002). However, the observed cumulative hydrogen productions gradually decreased after reaching their maximum values, indicating that the rate of hydrogen consumption was higher than the production. The decreases in the observed cumulative hydrogen productions were also affected by the heat treatment conditions used for the seed sludge. The hydrogen consumption in the anaerobic dark fermentation might be related to the activity of the non-spore formers, including i) the formation of reduced acids, such as propionic acid and lactic acid (Hawkes *et al*., 2002; Levin *et al*., 2004; Noike *et al*., 2002), ii) hydrogenotrophic methanogenesis (Hawkes *et al*., 2002; Oh *et al*., 2003; Park *et al*., 2005), and iii) the reduction of a variety of compounds, such as the sulfates, nitrates, iron, and dissolved oxygen contained in the raw material (Oh *et al*., 2003; Grommen *et al*., 2006; Jia 1996), but the activity of the non-spore-formers could be prevented by the heat treatment (Hawkes *et al*., 2002; Lay *et al*., 2003; Oh *et al*., 2003; Park *et al*., 2005; Fan *et al*., 2004).

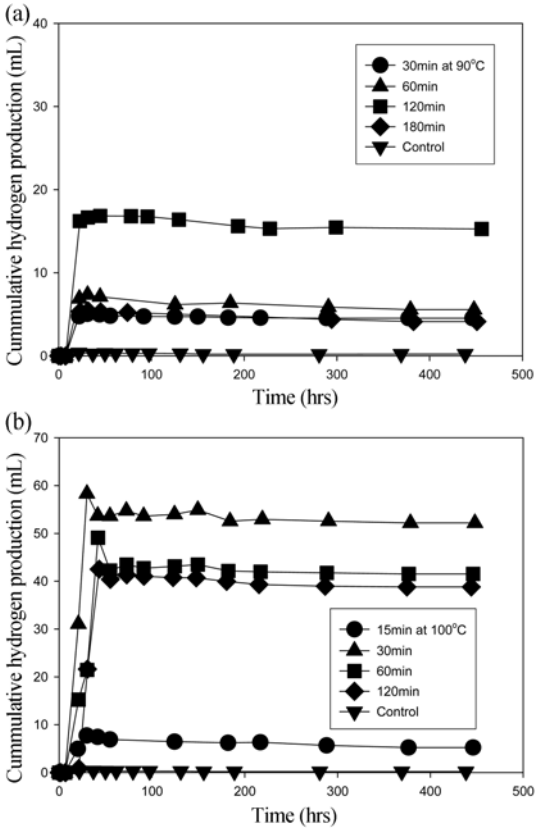

Fig. 1. Observed Cumulative Hydrogen Production from the Seed Sludge Treated with Heat at 90°C (a) and 100°C (b)

It is more likely that the hydrogen loss in the batch fermentation is due to homoacetogenesis and solventogenesis (Hawkes *et al*., 2002; Oh *et al*., 2003; Park *et al*., 2005). In the case of homoacetogenesis, the hydrogen could be utilized to form acetate, to an extent that would depend on the hydrogen partial pressure, according to Eq. (6), and which is mediated by *C. aceticum* and *C. thermoautotrophicum* (Oh *et al*., 2003; Park *et al*., 2005).

$$2HCO_3^- + 4H_2 \rightarrow H^+ + CH_3COO^- + 4H_2O,$$
$$\Delta G^{0'} = 105 \text{ kJ/mol} \tag{6}$$

The process of solventogenesis could also consume the hydrogen to form alcohol or other solvents, and this is more likely at low pH (Oh *et al*., 2003; Park *et al*., 2005). The above results indicate that the hydrogen consumption, as well as its production, could be considerably effected by the heat treatment conditions, including the temperature and duration.

In anaerobic hydrogen fermentation, hydrogen and carbon

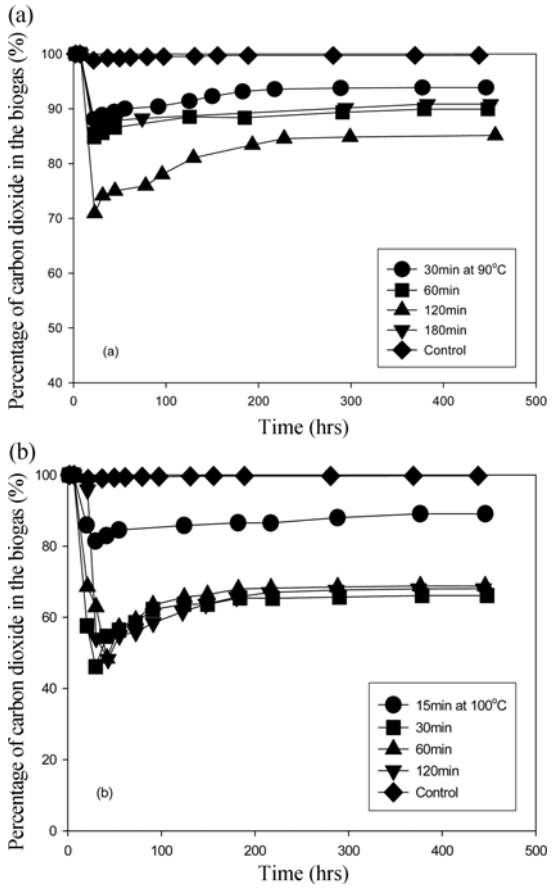

Fig. 2. Change in Carbon Dioxide Content in the Cumulative Total Biogas Production from the Seed Sludge Treated with Heat at 90°C (a) and 100°C (b)

dioxide are the main components of the biogas, while small amounts of methane, nitrogen, and hydrogen sulfide are also observed (Levin *et al*., 2004; Oh *et al*., 2003; Park *et al*., 2005; Kim *et al*., 2004; Fang and Liu, 2002). The minor components of biogases in hydrogen fermentation are generally produced from the hydrogen consumption activities, such as hydrogenotrophic methanogenesis, denitrification, and sulfate reduction. However, the carbon dioxide content is also effected by various hydrogen consumption activities, such as hydrogenotrophic methanogenesis, homoacetogenesis, and solventogenesis, as well as hydrogen production activities (Kapdan and Kargi, 2006; Hawkes *et al*., 2002; Oh *et al*., 2003; Park *et al*., 2005; Iyer *et al*., 2004; Kim *et al*., 2004). Therefore, the carbon dioxide content in the biogas and the amount produced give useful information for the study of the metabolic pathway of hydrogen fermentation. In this study, hydrogen and carbon dioxide together accounted for more than 99% of the biogas, and so the methane content could be ignored, indicating that the methanogenic activity was effectively suppressed by the heat treatment (Hawkes *et al*., 2002; Lay *et al*., 2003; Grommen *et al*., 2006; Kim *et al*., 2004).

Fig. 2 shows the carbon dioxide content in the cumulative biogas productions. In the control, no hydrogen or methane was observed; instead, only carbon dioxide was present in the biogas, indicating that hydrogen consumption was a major pathway during the batch fermentation. However, following the heat treatment of the seed sludge, the carbon dioxide content in the biogas rapidly decreased to its minimum value, and then slightly increased as the incubation time increased. However, the heat treatment at 90°C was not effective in controlling the hydrogen consumption reactions. The biogas production from the seed sludge treated at 90°C was very low, and the carbon dioxide content was more than 85%. The order of the carbon dioxide content according to the duration of the heat treatment was 120, 60, 180, and 30 min, which was the opposite of that observed for the hydrogen production, as shown in Fig. 1. For the seed sludge treated at 100°C, the carbon dioxide content observed for a heat treatment duration of 30 min was about 66%, which was similar to those observed for the heat treatment durations of 60 and 120 min. However, the observed cumulative hydrogen production for the heat treatment duration of 30 min was considerably higher than those observed for the heat treatment durations of 60 and 120 min, as shown in Fig. 1. This seems to indicate that the pathways consuming hydrogen and carbon dioxide, such as homoacetogenesis, could be effectively blocked by heat treatment at 100°C for 30 min.

3.2 Estimation of Hydrogen Production and Consumption

The observed cumulative hydrogen production obtained from the batch reactors was fitted to Eq. (3). The adjusted determination coefficients (adjusted R^2) for all of the seed sludges subjected to heat treatment were higher than 0.99, as shown in Table 1. This indicates that the observed cumulative hydrogen production from the batch fermentation was well described by the difference between the cumulative hydrogen production and the consumption, which are expressed by the modified Gompertz type equations. The estimated results for the cumulative hydrogen production and the consumption, as well as the hydrogen production potential, are summarized in Table 1. Generally, some of the proteins constituting the bacterial body, enzymes, and physiologically active substances are likely to be denatured by heat, and so their metabolic pathway could also be significantly shifted (Aymard and Belarbi, 2000). In this study, the lag phase times (λ_p) for the hydrogen productions from the seed sludge subjected to heat treatment were from 9 to 24 hr, which were shorter than the values reported (about 1-4 days) in previous experiments with heat-treated inocula (Hawkes *et al*., 2002; Noike *et al*., 2002; Kim *et al*., 2004). This suggests that the spore-forming hydrogen-producing bacteria were not damaged by the heat treatment, and were quickly germinated into active vegetative cells appropriate for fermentation (Hawkes *et al*., 2002; Lay *et al*., 2003; Noike *et al*., 2002; Kim *et al*., 2004). However, the lag phase times (λ_p) from the seed sludge treated at 100°C were slightly longer than those from the seed sludge treated at 90°C, and the lag phase times (λ_c) for the hydrogen consumptions were shorter than those for the production. The longer lag times were likely due to the increased requirements for the microorganisms to adapt their physiological state to the

Table 1. Summary of Estimated Hydrogen Production and Consumption for Seed Sludge subjected to Heat Treatment

Content		H_2 production			H_2 consumption			Observed H_2 production		
		H_{Pu} (mL H_2/g glucose)	R_{Pm} (mL H_2/g glucose/d)	λ_P (hr)	H_{Cu} (mL H_2/g glucose)	R_{cm} (mL H_2/g glucose/d)	λ_C (hr)	$H_{pu,obs}$	Correlation (AR^2)	H_2 yield (mol H_2/mol glucose)
100°C	15	7.574	5.554	19.232	2.476	0.008	23.862	7.404	0.992	0.07
	30	70.32	22.315	19.204	17.004	1.426	20.885	63.07	0.998	0.63
	60	69.884	1.906	16.061	27.155	1.882	45.336	49.54	0.977	0.50
	120	57.183	3.356	24.02	17.244	1.064	35.799	42.46	0.997	0.43
90°C	30	5.059	0.585	9.346	0.528	0.003	10.946	4.97	0.999	0.05
	60	7.457	0.807	9.112	1.83	0.009	14.993	7.22	0.995	0.07
	120	16.778	2.137	10.271	1.453	0.017	107.433	16.78	0.999	0.17
	180	5.437	0.819	9.249	1.423	0.005	45.591	5.37	0.999	0.05

new environment (Hawkes *et al*., 2002; Lay *et al*., 2003; Fan *et al*., 2004; Kim *et al*., 2004).

For the seed sludge treated at 100°C, the maximum value of the cumulative hydrogen production was around 70 mL H_2/g glucose, which was obtained for heat treatment durations ranging from 30 to 60 min. However, for the seed sludge treated at 90°C, the maximum value of the cumulative hydrogen production was obtained with a heat treatment duration of 120 min, but was much less than that obtained for the seed sludge treated at 100°C. This probably reflects the biasing of the fermentation pathway into non-hydrogen production, due to the insufficient deactivation of the non-spore forming species at 90°C, compared to that at 100°C. For the seed sludge treated at 100°C, the cumulative hydrogen consumption was higher than that observed for the seed sludge treated at 90°C. However, the cumulative hydrogen consumption for the sludge treated at 100°C for 60 min was higher than that of the sludge treated for 30 min, even though the cumulative hydrogen production was similar for both heat treatment durations. This indicates that the hydrogen consumption activity, as well as the production, could be optimized by adjusting the duration of the heat treatment. In the study, the maximum value of the hydrogen production potential was about 63 mL H_2/g glucose, which was obtained from the seed sludge treated at 100°C for 30 min.

3.3 Volatile Fatty Acids

The end products of hydrogen fermentation are generally hydrogen, carbon dioxide, volatile fatty acids, and alcohols (Kapdan and Kargi, 2006; Lay *et al*., 2003; Oh *et al*., 2003; Fan *et al*., 2004; Kim *et al*., 2004). The hydrogen yield from the hydrogen fermentation and the contents of acetic acid and butyric acid in the final products increased as suppression of the hydrogen consumption activities increased (Hawkes *et al*., 2002; Levin *et al*., 2004; Fang and Liu, 2002). The hydrogen consumption activity is related to the presence of methane, alcohols, and some reduced volatile fatty acids, including lactic acid and propionic acid in the hydrogen fermentation product (Hawkes *et al*., 2002; Lay *et al*., 2003; Oh *et al*., 2003; Noike *et al*., 2002; Fan *et al*., 2004). In particular, the ratio of butyric acid to acetic acid (the B/A ratio) is often of crucial value in assessing the performance and stability of the hydrogen fermentation process (Hawkes *et al*., 2002; Han and Shin, 2004). Fig. 3 shows the compositions of volatile fatty acids and their levels in the fermentation metabolites for the seed sludge treated by heat.

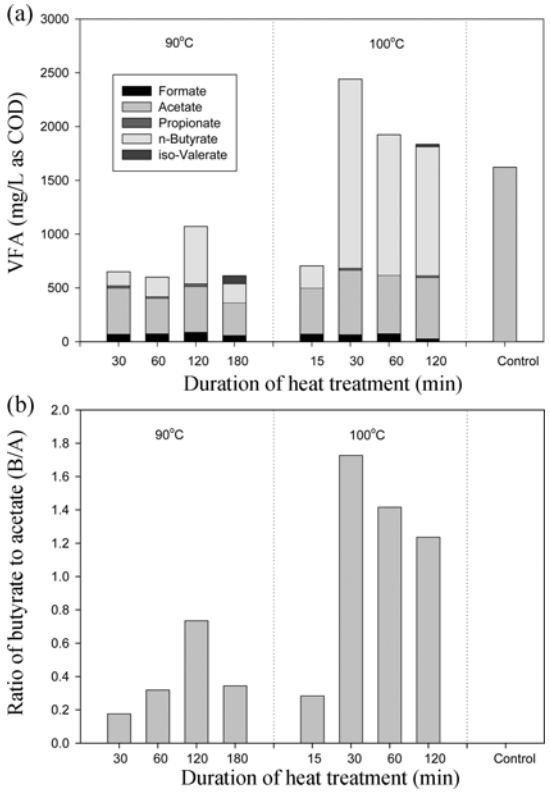

Fig. 3. Composition of VFA (a) and B/A Ratio (b) in the End Products of the Batch Hydrogen Fermentation with the Seed Sludge subjected to Heat Treatment

The levels of total volatile fatty acids were higher at higher hydrogen productions from the seed sludge treated by heat, as shown in Fig. 1, probably due to the higher fermentation efficiency or lower production of alcohols (data not presented) (Oh *et al*.,

2003; Fan *et al*., 2004; Kim *et al*., 2004; Han and Shin, 2004). Acetic acid and butyric acid were abundant in the final product obtained from the seed sludge subjected to heat treatment, and some formic acid was also observed, but hardly any propionic acid was observed in the products, suggesting that the activity of the non-spore-forming propionate producing bacteria could be effectively inactivated by the heat treatment (Hawkes *et al*., 2002). The pattern observed for the ratio of butyric acid to acetic acid was similar to that of the total volatile fatty acids. This implies that the formation of butyrate was a major pathway in the hydrogen fermentation from the seed sludge subjected to heat treatment (Kapdan and Kargi, 2006; Han and Shin, 2004).

For the seed sludge treated at 100°C, the total volatile fatty acids were maximized at a heat treatment duration of 30 min. However, for the heat treatment durations of 60 and 120 min, the total volatile fatty acids, especially the butyric acid levels, were decreased, and so the ratios of butyrate to acetate were lower than that observed in the case of the 30-min duration. This was probably due to some undesirable hydrogen-consuming pathways, such as solventogenesis, coming into play, thus forming alcohols instead of butyrate (Hawkes *et al*., 2002, Oh *et al*., 2003; Park *et al*., 2005; Kim *et al*., 2004), and thereby contributing to the increase in the hydrogen consumption activities in the case of the durations of 60 and 120 min, compared to the duration of 30 min, as shown in Table 1. In the case where a heat treatment temperature of 90°C was used, the compositions of the volatile fatty acids were similar to those at 100°C. However, the levels of total volatile fatty acids were lower than those observed at 100°C, and the ratio of butyric acid to acetic acid was also smaller than that observed at 100°C, thus contributing to the lowering of the hydrogen production potentials from the seed sludge treated at 90°C, as shown in Table 1. These results show that the hydrogen production activity could be effectively enhanced by using a heat treatment temperature of 100°C and a duration of 30 min for the anaerobic seed sludge.

4. Conclusions

1. The hydrogen production observed in the batch hydrogen fermentation can be described by the difference between the cumulative hydrogen production and the consumption, which can be described by the modified Gompertz equations.
2. The hydrogen production potential, combined with the hydrogen production and the consumption, was effected by the temperature and duration of the heat treatment used for the anaerobic seed sludge.
3. The maximum value of the hydrogen production potential was 63 mL H_2/g glucose, which was obtained from the anaerobic seed sludge subjected to heat treatment at 100°C for 30 min.
4. The compositions of the volatile fatty acids in the end products and their levels, as well as the amount of carbon dioxide in the biogas, were dependent on the temperature and duration of the heat treatment used for the anaerobic seed sludge, and together constituted an important factor that determined the hydrogen production potential.

Acknowledgements

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References

American Public Health Association (APHA) (1995). "Standard methods for the examination of waste and wastewater." 19th Ed., Washington D.C.: APHA, AWWA.

Aymard, C. and Belarbi, A. (2000). "Kinetics of thermal deactivation of enzymes: A simple three parameters phenomenological model can describe the decay of enzyme activity, irrespectively of the mechanism." *Enzyme Microb. Technol.*, Vol. 27, pp. 612-618.

Bolton, J. (1995). "The photochemical conversion and storage of solar energy: An historical perspective." *Solar Energy Materials and Solar Cells*, Vol. 38, pp. 543-554.

Fan, Y., Li, C., and Lay, J. J. (2004). "Hou H., Zhang G., optimization of initial substrate and pH levels for germination of sporing hydrogen producing anaerobes in cow dung compost." *Biores. Technol.*, Vol. 91, pp. 198-193.

Fang, H. H. P. and Liu, H. (2002). "Effect of pH on hydrogen production from glucose by a mixed culture." *Biores Technol.*, Vol. 82, pp. 87-93.

Grommen, R., Verhaege, M., and Verstraete, W. (2006). "Removal of nitrate in aquaria by means of electrochemically generated hydrogen gas as electron donor for biological denitrification." *Aquacultural Engineerign*, Vol. 34, pp. 33-39.

Han, S. K. and Shin, H. S. (2004). "Biohydrogen production by anaerobic fermentation of food waste." *Int. J. Hydrogen Energy*, Vol. 29, pp. 569-577.

Hawkes, F. R., Dinsdale, R., Hawkes, D. L., and Hussy, I. (2002). "Sustainable fermentative hydrogen production: challenges for process optimization." *Int. J. Hydrogen Energy*, Vol. 27, pp. 1339-1347.

Iyer, P., Bruns, M. A., Zhang, H., Ginkel, S. V., and Logan, B. E. (2004). "H_2 producing bacterial communities from a heat treated soil inoculum." *Appl. Microbiol. Biotechnol*., Vol. 66, pp. 166-173.

Jia, X. S., Furumai, H., and Fang, H. H. P. (1996). "Extracellular polymers of hydrogen utilizing methanogenic and sulfate reducing sludges." *Water Res*., Vol. 30, No. 6, pp. 1493-1444.

Kapdan, I. K. and Kargi, F. (2006). "Bio-hydrogen production from waste materials." *Enzyme and Microbial. Technology*, Vol. 38, pp. 569–582.

Kim, S. H., Han, S. K., and Shin, H. S. (2004). "Feasibility of biohydrogen production by anaerobic co-digestion of food waste and sewage sludge." *Int. J. Hydrogen Energy*, Vol. 29, pp. 1607-1616.

Lay, J. J., Fan, K. S., Chang, J. I., and Ku, C. H. (2003). "Influence of chemical nature of organic wastes on their conversion to hydrogen by heat shock digested sludge." *Int. J. hydrogen energy*, Vol. 28, pp. 1361-1367.

Levin, D. B., Pitt, L., and Love, M. (2004). "Biohydrogen production: prospects and limitations to practical application." *Int. J. Hydrogen Energy*, Vol. 29, pp. 173-185.

Mukhopadhyay, K. and Forssell, O. (2005). "An empirical investigation of air pollution from fossil fuel combustion and its impact on health

in India during 1973-1974 to 1996-1997." *Ecological Economics*, Vol. 55, pp. 235-250.

Noike, T., Takabatake, H., Mizuno, O., and Ohba, M. (2002) "Inhibition of hydrogen fermentation of organic wastes by lactic acid bacteria." *Int. J. Hydro. Energy*, Vol. 27, pp. 1367-1371.

Oh, S. E., Ginkel, S. V., and Logan, B. E. (2003) "The relative effectiveness of pH control and heat treatment for enhancing biohydrogen gas production." *Environ. Sci. Technol.*, Vol. 37, No. 22, pp. 5186-5190.

Park, W., Hyun, S. H., Logan, B. E., and Kim, I. S. (2005) "Removal of headspace CO_2 increases biological hydrogen production." *Environ. Sci. Technol.*, Vol. 39. No. 12, pp. 4416-4420.