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ARTICLE *in* ENVIRONMENTAL SCIENCE AND TECHNOLOGY · JULY 2004

Impact Factor: 5.33 · DOI: 10.1021/es0349204 · Source: PubMed

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Environ. Sci. Technol. **2004**, *38,* 3195−3202

Enhanced Biohydrogen Production from Sewage Sludge with Alkaline Pretreatment

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Batch tests were carried out to analyze influences of the alkaline pretreatment and initial pH value on biohydrogen production from sewage sludge. Experimental results of the impact of different initial pH on biohydrogen production showed that both the maximal hydrogen yield occurred and that no methane was detected in the tests of at the initial pH of 11.0. The final pH decreased at the initial pH of 7.0−12.5 but increased at the initial pH of 3.0−6.0, probably due to the combination of solubilized protein from sludge and the formation of volatile fatty acids (VFAs) and ammonia during biohydrogen fermentation. The performance of biohydrogen production using the raw sludge and the alkaline pretreated sludge was then compared in batch fermentation tests at the initial pH of 11.0. The hydrogen yield was increased from 9.1 mL of H_2/g of dry solids (DS) of the raw sludge to 16.6 mL of H_2/g of DS of the alkaline pretreated sludge. No methane and less carbon dioxide (0.8% of control) were present in the biohydrogen production from the alkaline pretreated sludge. These results clearly showed that biohydrogen production could be enhanced and maintained stable by the combination of the high initial pH and alkaline pretreatment. The mechanism of biohydrogen production from sewage sludge at high initial pH was therefore investigated because the results of this study were different from previous studies of biohydrogen production. Results showed that protein was the major substrate for biohydrogen production from sewage sludge and that *Eubacterium multiforme* and *Paenibacillus polymyxa* were the dominant bacteria in biohydrogen production from alkaline pretreated sludge at an initial pH of 11.0. The combination of alkaline pretreatment and high initial pH could not only maintain a suitable pH range for the growth of dominant hydrogen-producing anaerobes but also inhibit the growth of hydrogen-consuming anaerobes. In addition, the changes in pH value, oxidation−reduction potential, VFAs and soluble COD during hydrogen fermentation were also discussed.

Introduction

Hydrogen is known as a clean renewable energy source because its product of reaction with oxygen is only H_2O. Hydrogen has a high-energy yield of 142.35 kJ/g, 2.75 times than that of any hydrocarbons (*1*, *2*). Hydrogen generation can be classified into two ways: chemical−physical and biological methods. The chemical−physical methods (e.g., through fossil fuel processing, water electrolysis using solar power) are energy-intensive and expensive (*3*). In contrast, the biological methods are environmentally favorable and consume less energy.

The biological wastewater treatment processes are used worldwide. However, large amounts of sewage sludge are produced from these biological processes. In 2001, about 4.22 billion t of municipal wastewater was treated in more than 150 municipal wastewater treatment plants in China, producing about 0.55−1.06 million t of dry sludge (*4*). Generally, the wasted sludge is treated by the anaerobic digestion process to produce methane. Hydrogen is found as an important intermediate product in the anaerobic digestion (*5*). Many investigations have shown that various wastes containing high organic matter have been used to produce hydrogen gas by anaerobic fermentation process (*6*−*10*). The sewage sludge is rich in polysaccharides and proteins and thus is a potential substrate for hydrogen production. Recently, some studuies are focusing on using the sludge to produce hydrogen by anaerobic fermentation. Due to low hydrogen yield from the raw sewage sludge (i.e., 0.16 mg of H_2/g of dried solids (DS)), several methods of sludge pretreatment such as ultrasonication, acidification, sterilization, and freezing-and-thawing have been introduced to enhance the hydrogen yield. Hydrogen yields were improved to 1.4 mg of H_2/g of DS using the boiled sludge and to 1.5∼2.1 mmol of H_2/g of COD with sludge pretreated by sterilization or freezing-and-thawing (*11*). However, pure culture method was used in most studies for biohydrogen production (*11*−*15*) and thus made the biohydrogen production process more complicated because of necessary sterilization. Therefore, microflora enriched from a natural population of bacteria by various methods such as heat-shocking and acid−base treatment are currently used in biohydrogen production studies (*2*, *5*−*7*, *17*).

It is generally thought that the anaerobic digestion process of organic wastewater or waste biosolids goes through three stages: hydrolysis, acidification, and methane production. Hydrogen production occurs in the acidification stage, and the pH value is one of important factors affecting the biohydrogen fermentation. In general, the optimal initial pH of biohydrogen fermentation is thought to be between 5.0 and 6.0 (*2*, *16*, *17*). However, Lee et al. reported an unusual result in which the optimal pH was 9.0 for the batch biohydrogen fermentation of sucrose (*18*). Further study is therefore needed on the impact of the initial pH of sewage sludge on biohydrogen fermentation from organic wastes. It is well-known that hydrogen producers and hydrogen consumers inhabit together in the sewage sludge during biohydrogen production. Hydrogen produced can be consumed when hydrogen consumers grow well during the anaerobic digestion. Although many methods such as controlling low pH, short sludge retention time (SRT), and heat-shocking treatment have been investigated to inhibit the hydrogen consumption during biohydrogen production (*2*, *6*, *7*, *17*), a problem of quick consumption of hydrogen produced still exists in biohydrogen production from sewage sludge (*11*, *12*). This phenomenon results in difficulty in maintaining high and stable hydrogen production and then limits the application of biohydrogen production from sewage sludge.

The purposes of this study were to obtain a stable and high biohydrogen production from sewage sludge without addition of any pure hydrogen-producing seed bacteria or

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10.1021/es0349204 CCC: $27.50
Published on Web 04/28/2004

TABLE 1. Characteristics of the Sewage Sludge

items	range	average ± SD
pH	7.01−7.61	7.25 ± 0.25
TSS (g/L)	10.36−13.33	12.11 ± 1.22
VSS (g/L)	5.83−8.47	7.05 ± 0.97
VSS/TSS	0.56−0.64	0.58 ± 0.03
SCOD (mg/L)	44.23−128.30	99.13 ± 33.81
TCOD (mg/L)	9482−12885	11517 ± 1255
SCOD/TCOD (%)	0.5−1.04	1.10 ± 0.26

nutrient sources. Therefore, this study first investigated the impact of the initial pH value of sewage sludge on biohydrogen production and then compared the performances of biohydrogen production using the raw sludge and the alkaline pretreated sludge. In addition, the mechanisms of biohydrogen production in this case were also discussed.

Materials and Methods

Sewage Sludge. The sewage sludge was obtained from the aeration tank of a municipal wastewater treatment plant in Beijing. The design capacity of the aeration tank is 30 000 m^3/d. The sludge was first concentrated by settling for about 24 h. Its characteristics are listed in Table 1.

Alkaline Pretreatment. The concentrated sludge was pretreated with the slow addition of alkali of 4 M sodium hydroxide, and the pH value was controlled at 12.0. After alkaline addition, the sludge was further stirred for 30 min and then placed in a temperature-controlled cabin at 25 °C for 24 h.

Batch Fermentation Tests. Batch experiments of biohydrogen production from sewage sludge by fermentation were carried out using serum vials with a working volume of 125 mL. The tested sludge, with or without alkaline pretreatment, was sampled at 50 mL and then put in the vial. The test using the raw sludge was set as the control. The initial pH values of sludge in other tests were adjusted from 3.0 to 12.5, respectively, by slowly adding 3 M hydrochloric acid or 2 M sodium hydroxide. No extra nutrients were added into the tested sludge. Oxygen in the vials was removed from the headspace by nitrogen gas sparging for 20 s. The vials were then capped with rubber stoppers and placed in a air-bath shaker (150 rpm) at 36 ± 1 °C. Tests of each initial pH value were carried out in triplicate. At each time interval, the total gas volume was measured by releasing the pressure in the bottles using a glass syringe (5−50 mL) to equilibrate with the room pressure as in the Owen method (*19*), and then the hydrogen concentration was determined.

The biohydrogen fermentation using the sludge with or without alkaline pretreatment was compared at the same initial pH value of 11.0. To avoid possible error during sampling, three serum bottles were chosen randomly for analyzing the gas and liquid components and then discarded after analysis at each time interval. In addition, tests were conducted to understand the changes of organic matter during biohydrogen fermentation using the raw sludge and the alkaline pretreated sludge at initial pH values of 5.0 and 11.0, respectively.

Analysis. Hydrogen concentration was measured by a gas chromatograph (GC122, China) equipped with a thermal conductivity detector (TCD) and a 2-m stainless column (activated carbon, 60−80 mesh). The temperatures of the injection, column, and detector were set at 70, 140, and 140 °C, respectively. Nitrogen was used as the carrier gas at the flow rate of 30 mL/min. Methane and carbon dioxide were also determined with the same GC-TCD and a 2-m stainless column filled with Porapak T (80−100 mesh). The operational temperatures of the injection port, the oven, and the detector were set at 110, 60, and 200 °C, respectively. H_2 was used as the carrier gas at a flow rate of 40 mL/min. The gas in the headspace of serum bottles was sampled with a 0.1-mL gastight syringe and measured by comparing the sample biogas with standard hydrogen or standard CH_4/CO_2 gas. The concentrations of the volatile fatty acids (VFAs) were analyzed with the filtrate samples through a 0.45-mm membrane using a Shimadzu GC-9A (Japan) gas chromatograph equipped with a flame ionization detector (FID) and a 2-m glass column packed with Chromosorb 101 (60−80 mesh). The temperatures of the injection port, the detector, and the column were set at 250, 250, and 170 °C, and nitrogen was the carrier gas at the flow rate of 50 mL/min.

The oxidation−reduction potential (ORP) value was determined by a pH meter pH330i (WTW, Germany) equipped with a SenTix-ORP electrode. The pH value was measured by a pH meter (PHS-3C, China). The soluble COD (SCOD) and total COD (TCOD) were analyzed by a CTL-12 COD meter (Huatong Company, China). The SCOD, protein, and carbohydrate concentrations were measured with the filtrate samples through a 0.45-mm membrane. Protein was determined by the Lowry method using bovine albumin as the standard (*21*), while carbohydrate was measured by the phenol sulfuric acid method using glucose as the standard (*22*), and lipid was analyzed after ether extraction of the supernatant. The concentrations of total suspended solids (TSS) and volatile suspended solids (VSS) were determined by a 10-mL sample at 105 °C (4 h) and 600 °C (2 h), respectively.

Dominant bacteria screening and identification were carried out using two culture media according to methods described in Zhu et al. (*20*). Photomicrographs of dominant bacteria were taken by a scanning electron microscopy (SEM) (Philips Feiquanta-200) and a transition electron microscopy (TEM) (Hitachi S-600), respectively.

The accumulative volume of hydrogen produced (*H*) over the time course during the batch tests was fitted with the Gompertz equation (*7*):

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

where P is the hydrogen potential (mL), R_m is the maximum hydrogen production rate (mL/h), λ is the lag phase time (h), and e is 2.718 281 828. In this paper, R_m is expressed as mL of H_2/(g of DS h^{-1}), and the specific hydrogen production potential (P_s) is defined as mL of H_2/g of DS. Both Gompertz equation fit and Pearson correlationship analysis were carried out using SPSS 10.0 (SPSS Inc., USA). In this paper, the data before the peak point of the hydrogen profiles were fitted by the Gompertz equation because of its suitability for the profile of no gas consumption.

Results and Discussion

Impacts of Initial pH on Hydrogen Production. In this study, the influence of initial pH on hydrogen production from the raw sludge by anaerobic fermentation was first investigated (Figure 1). After about 6 h, the system started to produce hydrogen in all the tests except those at the initial pH of 3.0, 4.0 (data not shown in Figure 1), 5.0, and 6.0 and the control. A significant increase of hydrogen production was observed at the initial pH values from 10.0 to 11.5, and the maximal yield of 8.1 mL/g of DS occurred at the initial pH value of 11.0 after 24-h fermentation. However, hydrogen accumulated in the headspace of bottles was then consumed by hydrogen consumers in the following fermentation time. The consumption of hydrogen gas was as the following order of initial pH values: 12.0 < 11.5 < 11.0 < 10.5 < 10.0 < 9.0. Although the hydrogen at the initial pH of 12.0 was consumed slower than that at other pH values, it had longer lag time (about 22 h) than other tests. These results clearly showed

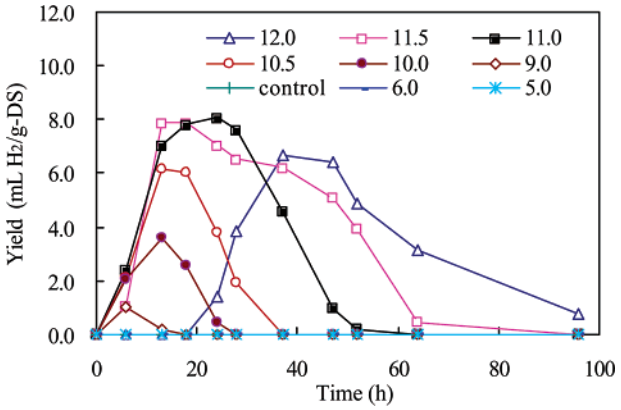


FIGURE 1. Profiles of hydrogen yield at different initial pH values using the raw sludge (TSS = 10.36 g/L).

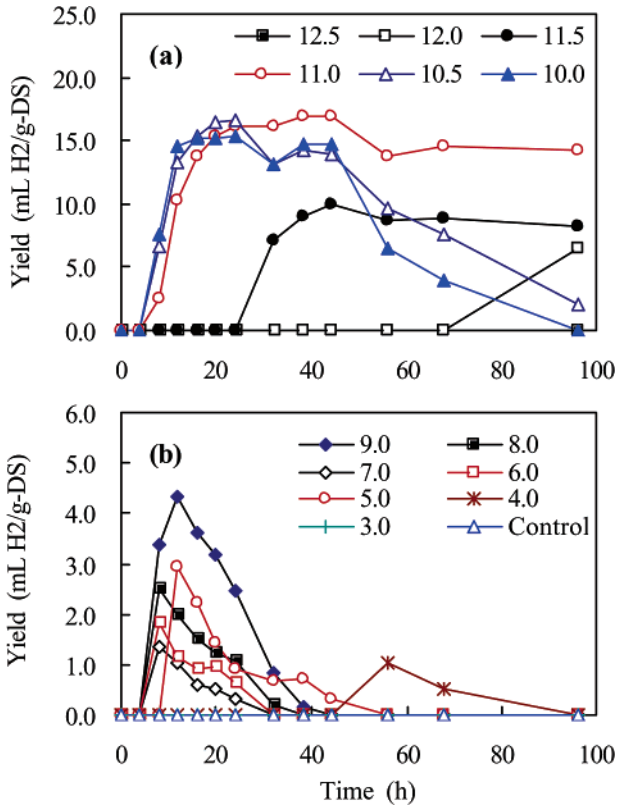


FIGURE 2. Hydrogen yield at different initial pH values (a) from 10.0 to 12.5 and (b) from 3.0 to 9.0 using the alkaline pretreated sludge (TSS = 10.97 g/L).

that the high initial pH value of the tested sludge was effective for biohydrogen production and that the hydrogen-producing bacteria might resist a high pH condition.

The alkaline pretreated sludge was then used for biohydrogen production at different initial pH values from 3.0 to 12.5 (Figure 2). Compared with hydrogen production from the raw sludge (Figure 1), hydrogen production in the tests of initial pH values at 10.0, 10.5, and 11.0 markedly increased and stayed longer time (e.g., hydrogen in the tests of initial pH at 10.0 and 10.5 began to decrease after about 44 h) (Figure 2). The maximal hydrogen yield (16.9 mL/g of DS) in the tests using the alkaline pretreated sludge occurred at initial pH of 11.0 after 40-h fermentation. It is noted that few hydrogen were consumed in the test at initial pH of 11.0. Hydrogen production also happened in the tests of pH at 11.5 and 12.0, respectively, but the lag phases were too long, about 25 and 77 h, respectively. Biohydrogen production

TABLE 2. Estimated Values of Parameters in the Modified Gompertz Equation

initial pH	P_s	R_m	λ	R^2
Raw Sludge				
control				
10.0	3.629	1.364	4.468	1.000
10.5	6.063	6.208	5.658	0.999
11.0	8.082	0.906	3.341	1.000
11.5	7.886	2.486	5.656	1.000
12.0	6.601	0.687	22.041	0.998
Alkaline Pretreated Sludge				
control				
4.0	1.266	1.119	54.947	1.000
5.0	4.429	1.484	9.921	1.000
6.0	1.840	1.258	4.953	1.000
7.0	1.341	1.078	4.838	1.000
8.0	3.811	1.393	6.104	1.000
9.0	4.363	1.791	5.870	1.000
10.0	15.298	3.678	5.902	1.000
10.5	16.477	2.214	5.041	0.999
11.0	16.428	1.854	6.588	0.997
11.5	9.805	1.156	25.394	0.999
12.0	6.880	0.518	77.144	1.000
Comparison Test				
11.0[a]	15.579	1.136	11.326	0.991
11.0[b]	9.138	0.696	0.609	0.994

[a] Alkaline pretreated sludge. [b] Raw sludge.

from the sludge has an optimal range suitable for hydrogen-producing bacteria. Long lags in this study could be due to toxic effects of alkaline pretreatment, and it also could be due to time to lower the pH into a range suitable for hydrogen production. In addition, the lower hydrogen yields and quick hydrogen consumption in the tests of initial pH from 4.0 to 9.0 were observed as compared with those in the tests of pH between 10.0 and 11.5 (Figure 2). No hydrogen was detected in the tests of initial pH at 3.0, 12.5, and the control test, respectively. Compared with hydrogen production from the raw sludge (Figure 1), the maximal hydrogen yield was enhanced from 8.1 to 16.9 mL/g of DS.

A common phenomenon, higher hydrogen yields and slower hydrogen consumption occurring at high initial pH values, was observed in the tests using both the raw sludge and the alkaline pretreated sludge. However, hydrogen produced was consumed more quickly, and less hydrogen remained in the end of biohydrogen fermentation from the raw sludge as compared with that from the alkaline pretreated sludge at the same initial pH between 11.0 and 12.0 (Figures 1 and 2). These results showed that the biohydrogen production could be enhanced and maintained stable from sewage sludge with alkaline pretreatment. In addition, the estimated parameters of P_s, R_m, and λ are quite consistent with these experimental data (Table 2). These batch experiments were repeated for several times, and the same results were found. In this case, the initial pH value of 11.0 was thus considered optimal for biohydrogen batch fermentation from the waste activated sludge.

Hydrogen Production of Comparison Tests. There was an increase in hydrogen production at the same initial pH of 11.0 due to alkaline pretreatment of the raw sludge (Figure 3). In the test using the raw sludge, the hydrogen produced early (0.6 h lag time), and the maximal hydrogen yield was 9.1 ± 0.15 mL of H_2/g of DS, but the hydrogen was consumed quickly. In contrast, the hydrogen production sharply increased after a longer lag period (11.3 h) and reached to the maximal yield of 16.6 ± 0.4 mL of H_2/g of DS at about 44 h, and little hydrogen consumption occurred in the test using alkaline pretreated sludge (Figure 3). It was interestingly found that no methane was detected and that few carbon

TABLE 3. Comparison of the Literature Data on Biohydrogen Production Using Different Substrates

ref	seed inocula	substrate	reactor	maximal hydrogen yield: mL of H_2/g of substrate	maximal hydrogen yield: mg of H_2/g of substrate	maximal hydrogen yield: mg of H_2/g of COD
this study	raw sludge	raw sludge	serum bottles	9.13[a], 9.14[b]	0.81[a], 0.82[b]	0.90[a], 0.91[b]
this study	alkaline treated sludge	alkaline treated sludge	serum bottles	16.59[a], 15.58[b]	1.48[a], 1.39[b]	1.65[a], 1.55[b]
11	digester sludge	waste activated sludge	batch reactor	na[c]	0.16	na
12	*C. bifermentans*	waste activated sludge	serum bottles	na	1.8	1.2
11	C. *bifermentans*	freezing and thawing and sterilization pretreatment activated sludge	serum bottles	na	na	3.0−4.2
11	boiling treated sludge	concentrate of boiling treated sludge	batch reactor	na	na	1.42
11	boiling treated sludge	boiling treated sludge	batch reactor	na	1.03	0.40
24	digester sludge	peptone	batch reactor	15.23	1.36	na
15	*Clostridium* pure culture	glucose	CSTR	293.7	26.4	na
3	soybean soil	glucose	CSTR	177.9	16.0	na

[a] Experimental data. [b] Estimated data using Gompertz equation. [c] na, not analyzed.

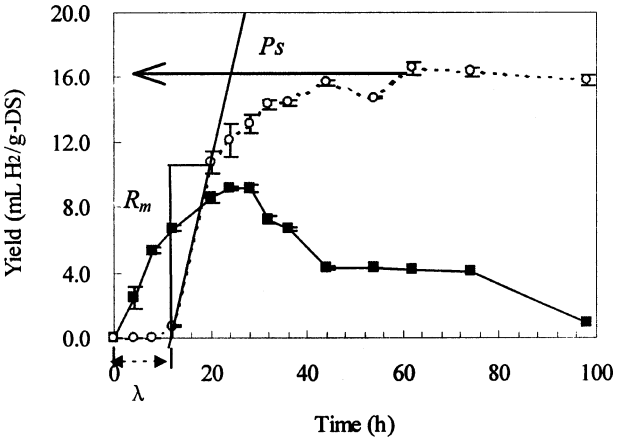


FIGURE 3. Hydrogen yields of comparison tests using the raw sludge and the alkaline pretreated sludge at the same initial pH of 11.0. Solid square: raw sludge. Open circle: alkaline pretreated sludge (TSS = 12.60 g/L).

dioxide (about 0.8% of the control) were present in both tests. Results of biohydrogen production in this study are compared with other results in the literature as shown in Table 3.

Methane and Carbon Dioxide Production. Methane and carbon dioxide production from the raw sludge and the alkaline pretreated sludge at different initial pH values were also compared after 96-h anaerobic fermentation. For biohydrogen fermentation from the raw sludge, the methane yields ranged from 12.7 to 26.0 mL of CH_4/g of DS in the tests of initial pH ranging from 5.0 to 10.0, but no methane gas was detected in the other tests (Figure 4a). However, no methane gas was present in biohydrogen production from the alkaline pretreated sludge except that in the control test (Figure 4b). It is well-known that H_2 is ubiquitous in the anaerobic environment but usually exhibits a fast turnover at very low hydrogen concentration (*23*). The hydrogen-consuming methanogenic bacteria can convert the formed hydrogen to methane if the methanogenesis step goes smoothly.

Although no methane gas was produced in the tests of initial pH ranging from 10.5 to 12.0 (Figure 4a, raw sludge) and from 4.0 to 10.5 (Figure 4b, alkaline pretreated sludge), hydrogen consumption still occurred in these tests (Figures 1 and 2). In addition, it was also found that high hydrogen yield, no methane detection, and less hydrogen consumption occurred in the test of using the alkaline pretreated sludge at the initial pH of 11.0. In general, hydrogen-producing anaerobes coexist with hydrogen-consuming bacteria in sewage sludge. Various types of hydrogen-consuming anaerobes, including methanogens, acetogens, and sulfate-reducing bacteria can obtain energy by utilizing molecular hydrogen (*24*) as in eq 2. Hence it was no doubt that either

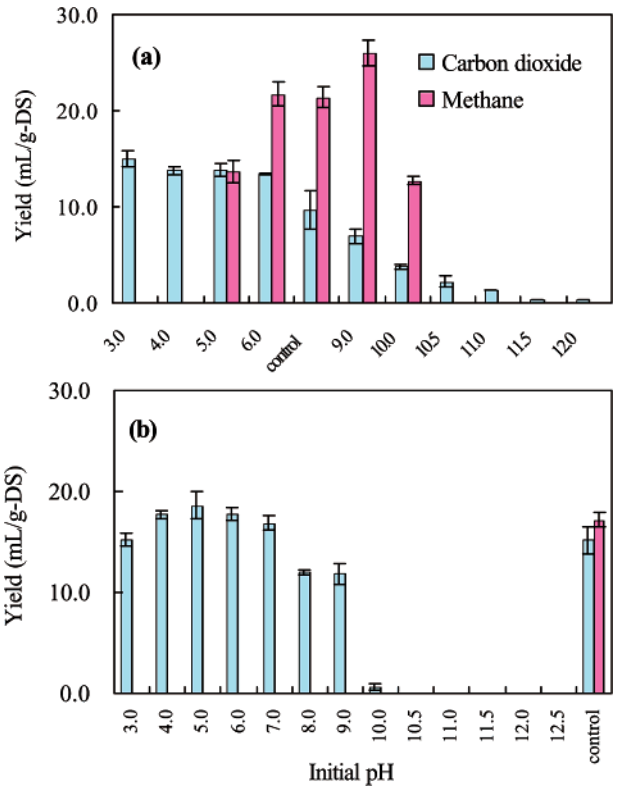
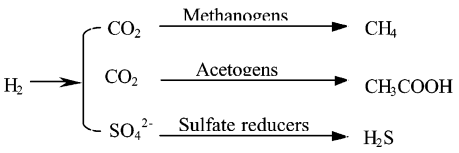


FIGURE 4. Profiles of methane and carbon dioxide yield using (a) the raw sludge and (b) the alkaline pretreated sludge.



(2)

the alkaline pretreatment of sludge or the high initial pH heavily inhibited the growth of methanogenic bacteria but had little impact on the other hydrogen-consuming anaerobes.

As Figure 4 shows, the concentrations of CO_2 decreased with the increasing of initial pH values, and almost no CO_2 was detected in the tests of pH between 10.5 and 12.0. These results can be explained that the CO_2 produced during biohydrogen fermentation is adsorbed by the sludge at high

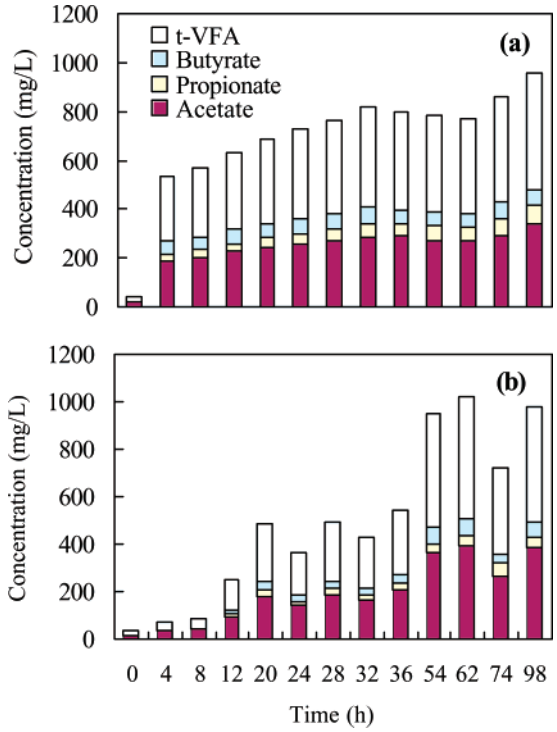


FIGURE 5. VFAs formation during hydrogen fermentation using (a) the raw sludge and (b) the alkaline pretreated sludge.

pH. According to the carbonate equilibrium equation (eq 3):

$$CO_2 + H_2O \leftrightarrows HCO_3^- + H^+ \quad (3)$$

the higher the pH, the more carbon dioxide is dissolved into the sludge. However, such phenomenon is helpful for the subsequent hydrogen gas purification process and improving the buffering potential capacity of the sludge.

Volatile Fatty Acids (VFAs). Hydrogen production was accompanied with the formation of volatile fatty acids (VFAs) throughout the sludge fermentation (Figure 5). In this study, the amounts of butyrate and propionate were very low in total VFAs, but acetate accounted for 70−80% of total VFAs (t-VFAs). This result is quite different from those in biohydrogen fermentation from glucose, in which VFAs mainly consists of butyrate and acetate (*5*, *7*, *17*). Statistical analysis showed that the hydrogen yield was significantly correlated with acetate formation (Table 4). Cheng et al. demonstrated the similar result in the study of biohydrogen production by protein degradation (*25*). Therefore, these results might be due to the fact that the organic matter contained in the sewage sludge is mainly composed of protein (32−41%), which is different from glucose or sucrose (*26*). So the metabolic mechanism of biohydrogen fermentation from sewage sludge may be different from that from glucose or sucrose, which will be discussed in the following text.

SCOD and Ratio of SCOD/TCOD. In the comparison test, the soluble COD (SCOD) of the raw sludge was comprised of only 1.38% of total COD (TCOD). After 24-h fermentation, the SCOD increased to 12.9−18.1% of TCOD and maintained steady during the following 74 h (Figure 6). For the alkaline pretreated sludge, both the initial SCOD concentration (2434.0 mg/L) and the initial ratio of SCOD/TCOD (22.8%) were much higher than those in the raw sludge (Figure 6). In addition, the ratio of SCOD/TCOD during biohydrogen production from the alkaline pretreated sludge gradually decreased with time, different from changes in the ratio of SCOD/TCOD during the test using the raw sludge. A combination of these results and biohydrogen production revealed that the alkaline pretreatment was effective for solubilizing organic matter from sewage sludge and provided more bioavailable organic matter for hydrogen production from sewage sludge.

TABLE 4. Pearson Correlations of VFAs and Hydrogen Yield during Biohydrogen Fermentation Tests[a]

	propionate	butyrate	TVFA	butyrate/acetate	Y_{H_2}
Raw Sludge					
acetate ($n = 13$)	0.924**	0.912**	0.997**	0.626*	0.551
propionate ($n = 13$)		0.791**	0.940**	0.431	0.295
butyrate ($n = 13$)			0.926**	0.864**	0.652*
TVFA ($n = 13$)				0.643*	0.534
butyrate/acetate ($n = 13$)					0.578*
Alkaline Pretreated Sludge					
acetate ($n = 13$)	0.916**	0.982**	0.999**	0.693**	0.852**
propionate ($n = 13$)		0.876**	0.929**	0.704**	0.844**
butyrate ($n = 13$)			0.983**	0.752**	0.824**
TVFA ($n = 13$)				0.716**	0.858**
butyrate/acetate ($n = 13$)					0.750**

[a] Key: *, correlation is significant at the 0.05 level (2-tailed). **, correlation is significant at the 0.01 level (2-tailed).

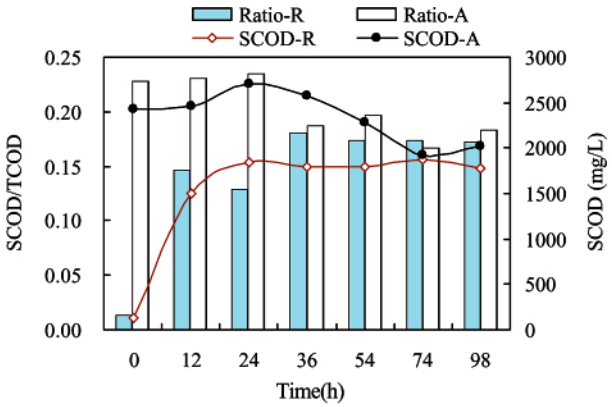


FIGURE 6. Changes of SCOD and SCOD/TCOD ratios during hydrogen fermentation using the raw sludge (R) and the alkaline pretreated sludge (A) at the initial pH of 11.0.

TABLE 5. pH Changes in the End of Biohydrogen Fermentation at Different Initial pH Values

raw sludge		alkaline pretreated sludge	
initial pH	final pH	initial pH	final pH
3.0	4.83 ± 0.67	3.0	4.08 ± 0.06
4.0	6.30 ± 0.02	4.0	5.45 ± 0.00
5.0	6.59 ± 0.02	5.0	5.84 ± 0.01
6.0	6.90 ± 0.02	6.0	6.24 ± 0.04
control (7.35)	7.13 ± 0.03	7.0	6.72 ± 0.04
9.0	7.62 ± 0.06	8.0	7.11 ± 0.08
10.0	8.12 ± 0.08	9.0	7.16 ± 0.06
10.5	8.53 ± 0.03	10.0	8.61 ± 0.04
11.0	8.80 ± 0.10	10.5	9.09 ± 0.06
11.5	9.38 ± 0.09	11.0	9.43 ± 0.07
12.0	10.12 ± 0.02	11.5	9.63 ± 0.12
		12.0	10.23 ± 0.05
		12.5	12.27 ± 0.05
		control (7.02)	6.79 ± 0.06

pH and ORP. The pH changes of biohydrogen fermentation from the raw sludge and the alkaline pretreated sludge are listed in Table 5. The final pH decreased at the initial pH of 7.0−12.5 but increased at the initial pH of 3.0−6.0. This can be explained by the combination of solubilized protein

TABLE 6. Concentrations of Organic Matters in Aqueous Phase of the Raw Sludge and the Alkaline Pretreated Sludge at Different Initial pH

	protein (mg/L)			carbohydrate (mg/L)			lipid (mg/L)	
	pH 3.0	pH 5.0	pH 11.0	pH 3.0	pH 5.0	pH 11.0	pH 5.0	pH 11.0
raw sludge[a]	94.2	71.5	412.5	72.2	50.1	160.9	46.0	68.0
alkaline pretreated sludge[b]		1037.5	1262.5		267.1	373.0	110.0	160.0

[a] Adjusting the initial pH at 3.0, 5.0, and 11.0 in 25 °C for 12 h. [b] Alkaline treatment at pH 12.0, 25 °C for 24 h, and then adjusting the pH at 5.0 and 11.0, respectively.

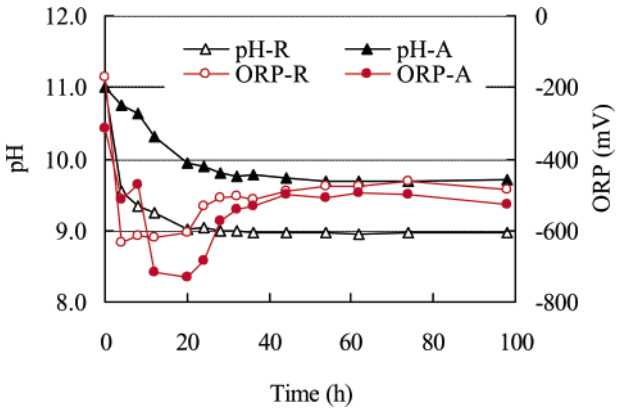


FIGURE 7. Changes of pH and ORP in the comparison tests using the raw sludge (R) and the alkaline pretreated sludge (A).

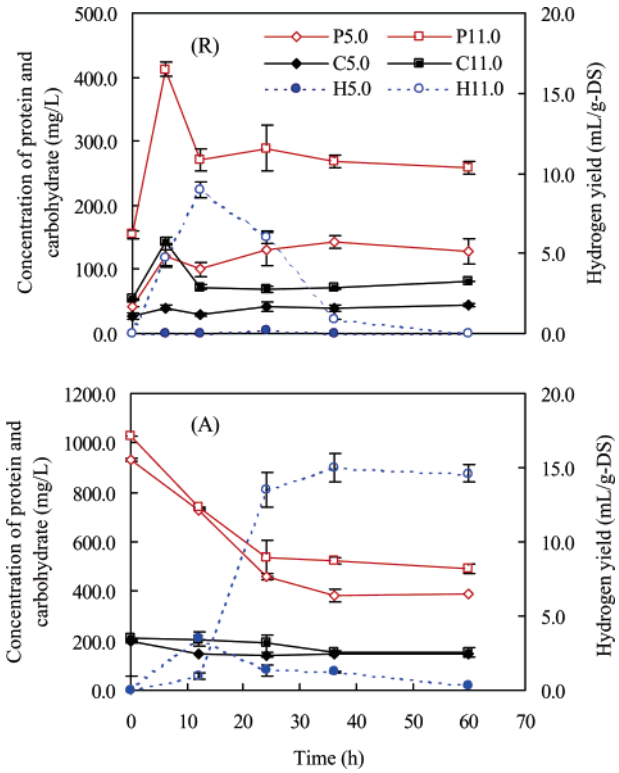


FIGURE 8. Changes of organic matters and hydrogen yield during biohydrogen fermentation from the raw sludge (R) and the alkaline pretreated sludge (A). P5.0, C5.0, H5.0, P11.0, C11.0, and H11.0 refer to protein, carbohydrate concentrations, and hydrogen yields in the tests of initial pH at 5.0 and 11.0, respectively.

from sludge and the formation of VFAs and ammonia during biohydrogen fermentation. Protein is an amphoteric substance and has a large buffering capacity, but changes of soluble protein in this study will be discussed in the following text.

pH drops were also found in the comparison tests. The pH values decreased sharply in the first 20 h and then kept steady at about 9.0 and 9.7, respectively, during tests using both the raw sludge and the alkaline pretreated sludge at the initial pH of 11.0 (Figure 7). However, the pH drop in the biohydrogen fermentation from the alkaline pretreated sludge was lower than that from the raw sludge. On the basis of profiles of hydrogen production and pH changes, optimal pH values were around 9.5 and 10.0 for biohydrogen production from the raw sludge and the alkaline pretreated sludge, respectively.

The ORP value generally reflects the amount and type of oxidative−reductive substrates containing in the liquid. The most common hydrogen-producing bacteria contained in the sludge are kinds of strict anaerobic microorganisms (e.g., *Clostridium* sp.), which require a relative low ORP level between about −200 and −250 mV. The ORP value was around −300 mV during the hydrogen fermentation from waste sludge by adding pure hydrogen-producing bacteria at neutral pH (*11*). Sung et al. (*17*) and Lin and Lay (*27*) also reported the ORP value of −320 to −340 mV and −311 to −368 mV, respectively, in the biohydrogen production using sucrose as the substrate (*17*, *27*). As shown in Figure 7, the ORP values in this study dropped to a very low level (−600 to −730 mV) during the first 24 h (this period corresponded with maximal hydrogen production) and then rose and stayed stable at around −500 mV, much lower than those in studies cited above. This difference might be due to the high initial pH adopted in this study.

Investigation of Mechanism of Hydrogen Production at High Initial pH. In our study, the higher hydrogen yield was obtained at the high initial pH of 10.0−11.0 as compared with those at the initial pH range of 3.0−8.0. These results are somehow different from all existing studies on biohydrogen production. It is therefore necessary to know the mechanism of hydrogen production from sludge at high initial pH.

Sewage sludge is mainly composed of microorganisms, and its organic substance composition is therefore different from carbohydrate-rich substrates such as glucose or starch. The domestic sludge consists of 41% protein, 25% lipid, 14% carbohydrate, and 20% unknown components on the basis of COD (*28*) or 32−41% protein and 5−12% fats on the basis of total dry solids (TDS) (*26*). Thus, protein is the largest constituent of the waste activated sludge. In this study, soluble organic matters including protein, carbohydrates, and lipids of the raw sludge and alkaline pretreated sludge were analyzed at different initial pH values (Table 6). Obviously, all concentrations of three soluble organic matters in the alkaline pretreated sludge were much higher than those in the raw sludge. The soluble protein was the major part of all three soluble organic matter. For the raw sludge, alkaline pretreatment was more effective for solubilizing organic matter than acidic pretreatment. For the alkaline pretreated sludge, the adjustment of pH had impact on soluble organic matter, and all three soluble organic matter at high initial pH of 11.0 were more than those at low initial pH of 5.0 (Table 6). So alkaline pretreatment of sewage sludge could provide more

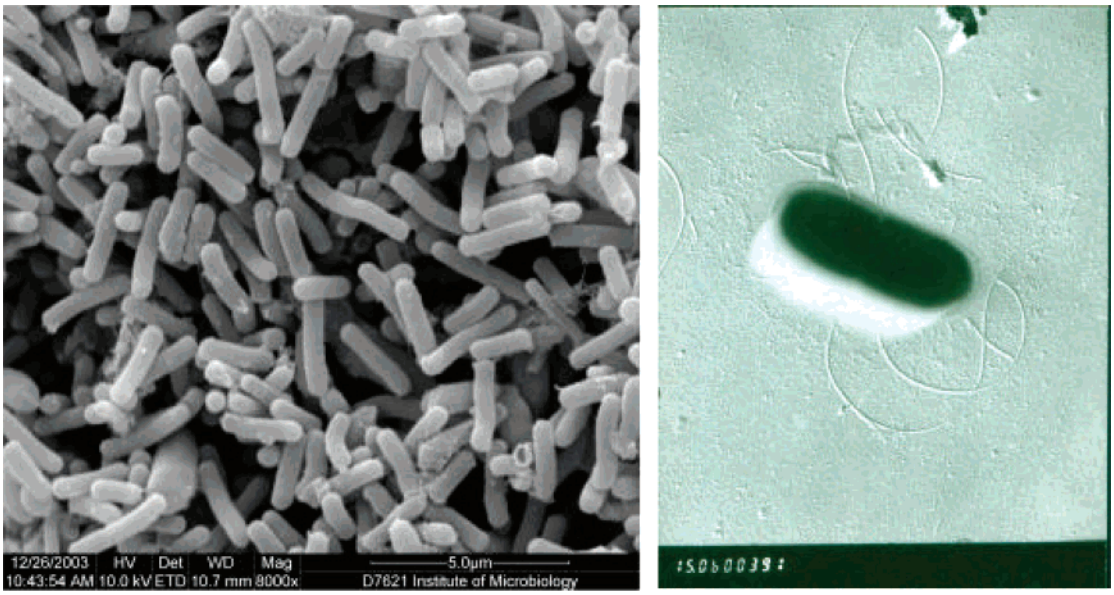


FIGURE 9. Scanning electron microscopy (SEM) and transition electron microscopy (TEM) photographs of *Eubacterium multiforme* (No. A1-1) and *Paenibacillus polymyxa* (No. B3-1).

soluble organic matter for biohydrogen production from sewage sludge.

Because of hydrolysis, soluble protein and carbohydrate in the tests using raw sludge first increased to their maximal concentrations at 6 h and then gradually decreased. However, their concentrations were still higher at the end of the tests than at the beginning of the tests (Figure 8R). As shown in Figure 8A, it was obvious that no rise in soluble organic matter occurred in biohydrogen production from the alkaline pretreated sludge at the initial pH values of 5.0 and 11.0, respectively. This phenomenon showed that the biohydrogen production from the raw sludge first experienced a hydrolysis process to solubilize organic matter but that using the alkaline pretreated sludge did not need such a process because of more existing bioavailable organic matter. Hydrogen gas was produced correspondingly with an increase of soluble organic matter and reached to its maximum at 12 h in the test using raw sludge at the initial pH of 11.0, but few hydrogen were detected in the test at an initial pH of 5.0. In the test of biohydrogen fermentation from alkaline pretreated sludge, the protein concentration sharply decreased in the first 24 h and then remained stable, but little change in carbohydrate concentration occurred throughout the period. Meanwhile, almost no changes in lipid were found in both tests (data not shown in Figure 8). Compared with biohydrogen production from the alkaline pretreated sludge at the initial pH of 11.0, both lower hydrogen yield and quicker hydrogen consumption were observed in the test of initial pH at 5.0, although a higher protein degradation rate (58.8%) happened (Figure 8). These results clearly showed that protein was the major substrate for biohydrogen fermentation from sewage sludge, and the combination of alkaline pretreatment and high initial pH was helpful to inhibit the growth of hydrogen-consuming anaerobes and thus was able to maintain stable and high biohydrogen production from sewage sludge.

In the primary investigation of microflora, 14 strains of bacteria were screened, purified, and identified after biohydrogen production from the alkaline pretreated sludge at an initial pH of 11.0. The dominant bacteria were identified as *Eubacterium multiforme* and *Penibacillus polymyxa* (Figure 9). It is reported that *E. multiforme* can biodegrade peptone or glucose to produce acids and simultaneously generate hydrogen gas (*20*, *29*). In our pure culture experiments, *E. multiforme* and *P. polymyxa* grew well in the pH range up to 9.0−9.5, but were inactive at pH less than 6.0. This result was consistent with results of biohydrogen production from the alkaline pretreated sludge at initial pH of 11.0. It also indicated that these bacteria could acclimatize themselves to the high pH condition during anaerobic fermentation. Compared with the results of pH changes mentioned in Table 5 and Figure 7, the microflora investigation showed that the combination of the high initial pH and the buffering capacity of sewage sludge could not only maintain a suitable pH range for the growth of dominant hydrogen-producing anaerobes but also inhibit the growth of hydrogen-consuming anaerobes. Further work is needed for the characterization of hydrogen fermentation of these dominant bacteria.

Acknowledgments

This study is financially supported by National Natural Science Foundation of China (No. 20277043).

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Received for review August 20, 2003. Revised manuscript received February 18, 2004. Accepted March 19, 2004.

ES0349204