Effects of various pretreatments on biohydrogen production from sewage sludge

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The sewage sludge of wastewater treatment plant is a kind of biomass which contains many organics, mainly carbohydrates and proteins. Four pretreatments, acid pretreatment, alkaline pretreatment, thermal pretreatment and ultrasonic pretreatment, were used to enhance biohydrogen production from sewage sludge. The experimental results showed that the four pretreatments could all increase the soluble chemical oxygen demand (SCOD) of sludge and decrease the dry solid (DS) and volatile solid (VS) because the pretreatments could disrupt the floc structure and even the microbial cells of sludge. The results of batch anaerobic fermentation experiments demonstrated that all of the four pretreatments could select hydrogen-producing microorganisms from the microflora of sludge and enhance the hydrogen production. The hydrogen yield of the alkaline pretreated sludge at initial pH of 11.5 was the maximal (11.68 mL H₂/g VS) and that of the thermal pretreated sludge was the next (8.62 mL H₂/g VS). The result showed that the hydrogen yield of pretreated sludge was correlative with its SCOD. The hydrogen yields of acid pretreated sludge and alkaline pretreated sludge were also influenced by their initial pH. No methane could be detected in the anaerobic fermentation of alkaline pretreated sludge and thermal pretreated sludge, which suggested that these pretreatments could fully inhibit the activity of methanogens. The volatile fatty acids (VFA) production in anaerobic fermentation of alkaline pretreated sludge was the maximum and the next is that of thermal pretreated sludge.

biohydrogen production, effects, pretreatment, sewage sludge

The sewage sludge of wastewater treatment plants is composed largely of organic matters (for example, carbohydrates and proteins)^[1]. Anaerobic digestion technique has been traditionally employed to treat sludge and obtain methane^[2,3]. Hydrogen is an intermediate product of the anaerobic sludge digestion, but unstable, because it will be quickly consumed by hydrogen-consuming bacteria, such as methanogens and sulfate-reducing bacteria^[2].

In order to harvest hydrogen from anaerobic sludge digestion, the activity of consuming-hydrogen bacteria must be inhibited^[3,4]. In other words, the anaerobic process must be stopped at the hydrogen and acetic acid forming stage and the consumption of hydrogen must be blocked. At laboratory scale, the following methods for limiting hydrogen consumption are used: (1) Operation at a short HRT to wash out hydrogen-consuming bacte-

ria; (2) operation at low pH to inhibit them and (3) inoculation with pretreated sludge^[4]. Some hydrogen-producing microorganisms could form endospores, which are very resistant to heating or harmful chemicals including acid and alkaline and cannot be killed easily, while most of the hydrogen-consuming species do not have this characteristic. Once favorable conditions return, those spores germinate and become vegetative cells^[5,6]. So, it is possible to use some pretreatments to screen hydrogen-producing species. The methods of pretreatment include thermal pretreatment^[4,6,7], acid pretreatment, alkaline pretreatment^[4], and so on.

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On the other hand, most of the organics presented in sewage sludge are enclosed within the microbial cell wall^[1] and the hydrolysis step is considered as the rate-limiting step in the overall anaerobic digestion process^[2]. Some pretreatment methods, such as thermal pretreatment and alkaline pretreatment, have been used to disrupt the microbial cells and release the organics to liquid so as to enhance the anaerobic digestion of sludge^[1,8,9] and biohydrogen production of sludge^[10–14]. For example, Cai et al.^[10] utilized alkaline pretreatment to enhance hydrogen production from sewage sludge.

As mentioned above, sewage sludge has been used to produce hydrogen by anaerobic fermentation. But most studies dealt with a single pretreatment in comparison with non-pretreatment. Although Wang et al.^[13,14] had used four kinds of pretreated (ultrasonic, acidified, sterilized, frozen/thawed) sludge to produce hydrogen by *Clostridium* sp., no report has been published about various pretreatment on hydrogen production from sewage sludge by anaerobic fermentation without extra-seeds. Accordingly, the objective of this study was to investigate the effects of four pretreatments (acid, alkaline, thermal and ultrasonic pretreatments) on hydrogen production from sewage sludge by anaerobic fermentation without extra-seeds.

1 Materials and methods

1.1 Pretreatments of sludge

The raw sludge (RS) used in the study was obtained from the aeration tank of a municipal wastewater treatment plant of Beijing, China, whose design capacity is 30000 m³/d. The pH value of the sludge was about 7.16 (± 0.2). The chemical oxygen demand (COD) of the sludge was 11,500 mg/L and the soluble chemical oxygen demand (SCOD) was 113.7 mg/L. The dry solid (DS) and volatile solid (VS) of the sludge were 8,960 mg/L and 6190 mg/L, respectively. The sludge was stored at 4°C before being used.

Four methods (acid, alkaline, thermal and ultrasonic pretreatments) were used to treat the sludge before they are used to anaerobic fermentation.

Acid pretreatment. The pH of the sludge sample were adjusted to 2.0 ± 0.1 and stabilized for 5 min under stirring with 6 mol/L hydrochloric acid. The pretreated sample was acid pretreated sludge (AS).

Alkaline pretreatment. The pH of sludge sample was adjusted to 12.0 ± 0.1 and also stabilized for 5 min

under stirring with 6 mol/L sodium hydroxide. The pretreated sample was alkaline pretreated sludge or basified sludge (BS).

Thermal pretreatment. The sludge sample was autoclaved at 121°C and 1.2 kgf/cm² (VARIOKLAV steam sterilizer, 300/400/500 EP) for 30 min. The pretreated sample was thermal pretreated sludge (TS).

Ultrasonic pretreatment. The ultrasonic pretreatment was performed with the help of a cell-breaker (SONICATOR-JY92-2D2, Xinzhi Inc., China) at a frequency of 20 kHz^[11,12]. The sludge sample (250 mL) was placed in a 300 mL graduated flask with the ultrasonic probe positioned in 3 cm under the surface of sludge. The sonication time was 30 min and the sonication power was 200 W. During sonication, the temperature of sludge was not controlled and increased from 16°C to 41°C. The pretreated sludge was ultrasonic sludge (US).

AS and BS were stored at ambient temperature (15°C–24°C) for 12 h to enhance the effect of pretreatments, while TS and US were stored at ambient temperature (15°C–24°C) for 3 h to cool the pretreated sludge.

1.2 Anaerobic fermentation of the pretreated sludge

Before the pretreated sludge samples were used to anaerobic fermentation, the pH of parts of AS and BS was adjusted to 7.0 ± 0.1 with 6 mol/L sodium hydroxide or 6 mol/L hydrochloric acid. The pretreated sludge samples (150 mL) were added into 300 mL plexiglass digesters, respectively. No other material was added in the digesters. The digesters were equipped with two ports for gas and sludge sampling. The headspaces and the liquid phases of the digesters were flushed with nitrogen to provide an anaerobic environment. The digesters were quickly sealed and placed on a shaker (HKZ-C, China) with 140–150 rpm at $37 \pm 1^\circ\text{C}$. The pH of the tested sludge samples is shown in Table 1. As control, the RS samples with pH being 2.5, 7.0 and 11.5 (± 0.1), were also anaerobically fermented (Table 1). Tests of the same sludge were carried out in triplicate and all the results were the mean value of the replicated analyses.

1.3 Analyses

Biogas production was measured periodically by displacement of saturated aqueous NaCl with 5% H₂SO₄ in a graduated cylinder^[15]. The biogas in the headspace of digesters was sampled with a 1 mL gastight syringe. The

Table 1 The tested sludge samples and their SCOD in the experiment

No	1	2	3	4	5	6	7	8	9
Sludge sample	RS	RS	RS	AS	AS	BS	BS	TS	US
Initial pH	2.5	7.0	11.5	2.5	7.0	7.0	11.5	6.8	6.9
SCOD (mg/L)	354.5	113.7	899.4	765.6	486.8	1085.4	3048.6	2442.2	1484.8

hydrogen contents of biogas were analyzed by a gas chromatograph (GC-122, China) equipped with a thermal conductivity detector (TCD) and a 2 m stainless column packed with activated carbon (60–80 mesh). Nitrogen was used as the carrier gas with a flow rate of 30 mL/min. The content of methane in the biogas was analyzed by another gas chromatograph (Agilent 6890, America) equipped with a flame ionization detector (FID) and a 30 m capillary column (HP-5). Nitrogen was also used as carrier gas with a flow rate of 30 mL/min.

The sludge was sampled with a 5 mL gastight syringe. The concentrations of volatile fatty acids (VFA) in the sludge were analyzed using another gas chromatograph (Shimadzu GC-9A, Japan) equipped with a FID and a 1 m glass column packed with chromosorb 101 (60–80 mesh). The carrier gas was nitrogen at flow rate of 50 mL/min. The pH of sludge was measured by a pH meter (PHS-3C, China). The COD and SCOD of sludge were analyzed by COD detector (HACH 2800 America). The samples were filtered through a 0.45 µm membrane before determining the SCOD. Soluble proteins in the liquid phase were measured by the Lowry's method using bovine serum albumin as a standard solution^[16], and soluble carbohydrates by the phenol-sulfuric acid method using glucose as a standard solution^[17]. The DS and VS of sludge samples were determined according to APHA standard methods^[18].

1.4 Kinetic analysis

The modified Gompertz equation (eq. (1)) was used to fit the experimental data of accumulative hydrogen production^[19].

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

where $H(t)$ is the accumulative hydrogen production (mL) during the fermentation time $t(h)$, P the hydrogen production potential (mL), R_m the maximum production rate (mL/h), λ the lag-phase time (h), and e is 2.718281828. The values of P , R_m and λ were determined by best fitting the hydrogen producing data for Eq. 1 using the Matlab 6.5^[20]. The maximum specific hydrogen production rate (mL H₂/g VS·h) was calculated by dividing R_m by the initial sludge VS. The hydrogen yield (mL H₂/g VS) was calculated by dividing P by the quantity of sludge (VS) added in the digesters.

2 Results and discussion

2.1 Effects of various pretreatments on sludge

Because the mechanisms of the four pretreatments are different^[21], the impacts of these pretreatments on the sludge are also different. Table 2 summarizes the effects of the four pretreatments on the sludge. Because these pretreatments could disrupt part of the floc structures and the microbial cells of sludge^[1], some microbial matters were released into water and converted from insoluble ones to soluble ones after pretreatment. Table 2 shows that all of the four pretreatments could decrease the DS and VS of sludge and increase the SCOD concentration. But their effects were different. The SCOD of BS was the maximal (3048.6 mg/L) and that of AS was the minimal (only 765.6 mg/L). The results of DS and VS were reverse to those of SCOD. Since the content of soluble proteins and soluble carbohydrates could reach 70%–80% of SCOD, they were the major com-

Table 2 Effects of four pretreatments on some characteristics of sludge

	pH	DS (g/L)	VS (g/L)	SCOD (mg/L)	Car ^{a)} (mg/L)	Pro ^{b)} (mg/L)
RS	7.16	8.96	6.19	113.72	24.23	26.76
AS	3.09	8.15	6.08	765.61	93.42	347.93
BS	11.8	6.70	3.79	3048.63	270.31	2085.36
TS	6.80	7.55	4.74	2442.05	377.45	1367.19
US	6.90	7.92	5.84	1484.80	135.25	569.26

a) Soluble carbohydrates; b) soluble proteins.

ponents of the soluble matters in the pretreated sludge. In addition, the maximal content of soluble carbohydrates and soluble proteins was achieved by TS and BS, respectively.

2.2 Production of hydrogen

The hydrogen production of the four kinds of pretreated sludge in anaerobic fermentation is shown in Figure 1. The hydrogen yield of BS at initial pH of 11.5 was the maximal (11.68 mL H₂/g VS) and the next was that of TS (8.62 mL H₂/g VS), while the anaerobic fermentation of some sludge samples did not produce hydrogen, i.e., AS and RS at initial pH of 2.5. According to Figure 1, hydrogen was produced in the first stage of fermentation and consumed in the sequent stage, which was correlative with the growth of the hydrogen-producing bacteria and hydrogen-consuming ones in the sludge, respectively^[22]. The differences of the experimental conditions, such as sewage sludge, initial pH and reactor form, were the reasons causing the differences of the hydrogen yields between this study and the literatures. Figure 1 also shows that the effect of initial pH on the hydrogen yield of four sludge samples was significant because high pH could further disrupt the microbial cell of sludge^[10,12]. For example, the hydrogen yield of BS at

initial pH of 11.5 was 11.68 mL H₂/g VS, while that at initial pH of 7.0 was only 1.46 mL H₂/g VS. These experimental results suggested that all of the four pretreatments could enhance hydrogen production from sewage sludge by anaerobic fermentation.

Table 3 summarizes the three kinetic parameters (P , R_m and λ), hydrogen yield and maximum specific hydrogen production rate (MSHPR), for biohydrogen production from all sludge samples at various initial pH. The results showed that those parameters were influenced by the pretreatments and the initial pH of sludge samples. For example, the hydrogen production potential (P) of BS at initial pH of 11.5 was 10.84 mL (the maximum) while that of US was 3.56 mL and that of BS at initial pH of 7.0 was only 1.36 mL. It was similar for the other parameters. The difference of the lag-phase time suggested that the effects of the four pretreatments on the growth of the selected hydrogen-producing bacteria were different^[19].

For biohydrogen production, the soluble organics are very important since the hydrogen-producing microorganisms need use them to grow and produce hydrogen. The soluble organics in the sludge can be presented by the initial SCOD (Table 1). Table 1 shows that the initial SCOD of the pretreated sludge samples was affected by the pretreatment methods. For example, the initial SCOD of BS at initial pH of 11.5 was the highest (3048.63 mg/L) and that of TS was the second highest (2442.05 mg/L). It also suggested that the initial SCODs of AS and BS were affected by the initial pH. It was reasonable since the organics of AS and BS were solubilized at acid and alkaline conditions, respectively. The statistical analysis (made using SPSS 13.0) showed that the hydrogen yield of pretreated sludge had significant correlation with its SCOD and the Pearson correlation coefficient was 0.846.

2.3 Production of methane

The hydrogen accumulated in the headspace of digesters

Figure 1 Hydrogen yield of various pretreated sludge samples at various initial pH.

Table 3 Kinetic parameters of hydrogen production from sludge samples

Sludge sample	RS			AS		BS		TS	US
Initial pH	2.5	7.0	11.5	2.5	7.0	7.0	11.5	6.8	6.9
P (mL)	— ^{b)}	1.12	7.03	—	3.02	1.36	10.84	8.00	3.56
R_m (mL/h)	—	0.21	0.62	—	0.21	0.37	0.44	1.84	0.64
λ (h)	—	12.32	9.76	—	8.39	6.94	16.22	9.55	4.00
MSHPR ^{a)} (mL H ₂ /g VS · h)	—	0.15	0.67	—	0.23	0.40	0.47	1.98	0.69
Hydrogen yield (mL H ₂ /g VS)	—	1.21	7.57	—	3.25	1.46	11.68	8.62	3.83

a) Maximum specific hydrogen production rate; b) not analyzed.

was consumed in the last stage of anaerobic fermentation (Figure 1). Many researchers have reported similar results^[10–12] and methanogenesis is believed to be the main hydrogen consuming action in the anaerobic digestion of sludge^[2]. Figure 2 summarizes the changes of methane yields in the anaerobic fermentation of the four pretreated sludge samples. No methane could be detected in the anaerobic fermentations of BS and TS, which suggests that the alkaline pretreatment and the thermal pretreatment could kill methanogens or inhibited their activities which could not recover with the change of environment condition. Methane was also not detected in the systems added with sludge at initial pH of 2.5 and 11.5. Since the methanogenesis decreases or ceases when ambient pH is lower than 6.3 or higher than 7.8^[23], it was expectable that the activity of methanogens was inhibited under the pH of 2.5 and 11.5. Methane was produced from the AS and RS at initial pH of 7.0 and US. Obviously, the acid pretreatment could inactivate methanogens, but the activity of methanogens would recover with the change of environmental pH. Ultrasonic pretreatment could not inhibit the activity of methanogens. The experimental results suggested that the influences of the four pretreatments on the methanogens were different. This was reasonable since the function mechanisms of the four pretreatments on bacteria are different: acid pretreatment are depended on the free H^+ , alkaline pretreatment depended on the free OH^- , thermal pretreatment depended on the high temperature and ultrasonic pretreatment depended on the temperature and the shear produced in the sonication^[1,9]. Although there was no methane production in anaerobic fermentation of some sludge samples, the hydrogen consumption also occurred in the last stage of the fermentation because there were other hydrogen-consum-

Figure 2 Methane yields of various pretreated sludge samples at various initial pH.

ing functions like homoacetogenesis, sulfate reduction, propionic acid production, and so on.

2.4 Production of VFA

The volatile fatty acids (VFA) were produced in the anaerobic fermentation of sludge and the results are summarized in Table 4 (sampling time was 76.5 h). The VFA were mainly composed of acetate (45%–76%) and butyrate (12%–22%), being very different from most studies of carbohydrates. The compositions of VFA are similar to the former researches^[10–12]. The complicated organic components of the four kinds of pretreated sludge, which were degraded in the anaerobic fermentation, were the reason for the different compositions of VFA. The results in Table 4 suggest that the production of VFA and their composition in the process of hydrogen fermentation were affected by the pretreatment methods. For example, the production of VFA from BS was the maximum at initial pH of 11.5 and that from raw sludge was the minimum at initial pH of 7.0 (excluding those undetermined). Table 4 also shows that the production

Table 4 VFA production at various initial pH (76.5 h)

Sludge	Initial pH	VFA (mg/L)	HAc ^{a)} (%)	HP ^{b)} (%)	HBu ^{c)} (%)	HPe ^{d)} (%)	A/B ^{e)}
RS	2.5	–	–	–	–	–	–
	7.0	287.13	76.20	8.76	6.64	8.38	11.5
	11.5	929.55	53.61	19.59	15.96	10.85	3.4
AS	2.5	–	–	–	–	–	–
	7.0	797.82	66.67	13.16	13.18	6.97	5.1
BS	7.0	1666.14	45.51	21.56	21.85	1.12	2.1
	11.5	2117.75	63.00	18.19	12.01	6.81	5.3
TS	6.8	1142.25	66.23	11.10	13.46	9.21	4.9
US	6.9	820.02	60.71	18.18	12.39	8.72	4.9

a) Acetate; b) propionate; c) butyrate; d) pentanoate; e) acetate/butyrate; –, not determined.

and the composition of VFA were sensitive to the initial pH. For instance, the production of VFA from BS at initial pH of 11.5 was higher than that at initial pH of 7.0. VFA could be used to produce methane or hydrogen in the post-treatment^[2,23,24]. The more the VFA are, the more methane or hydrogen will be produced. So, the remainder of BS after biohydrogen production could produce the most methane or hydrogen in the post-treatment and that of TS is the next. By the way, for the sludge pretreated by acid and alkaline, namely AS and BS, it is necessary to consider the ion, which would increase the cost of the post-treatment, while this problem does not exist for the TS and US.

2.5 Change of pH

The changes of the four pretreated sludge pH during the anaerobic fermentation are summarized in Figure 3. The change of pH is caused by the production of VFA and $\text{NH}_4^+\text{-N}$. For raw sludge at initial pH of 11.5, the decreases of pH were rapid and the pH decrease rate was faster than that of BS at the same initial pH, while the

Figure 3 Changes of pH in the anaerobic fermentation for various pretreated sludge

end pH was above 9.0. For neutral initial pH, the changes of pH were very for all pretreated sludge samples. The pH of sludge samples at initial pH of 2.5 increased. The terminal pH of raw sludge at initial pH of 11.5 and 2.5 was 9.05 and 3.85, respectively, which could also explain why there was no methane production under such initial pH. The differences in the change of pH were concerned with the SCOD of the sludge samples since the productions of VFA and $\text{NH}_4^+\text{-N}$ is the result of organics (SCOD) degradation.

3 Conclusions

(1) All of the four pretreatments, acid, alkaline, thermal and ultrasonic pretreatments, could select hydrogen-producing microorganisms from the microflora of sludge and enhance hydrogen yield of sludge by anaerobic fermentation without extra-seeds. The hydrogen yield of alkaline pretreated sludge at initial pH of 11.5 was the maximal (11.68 mL $\text{H}_2/\text{g VS}$) and that of thermal pretreated sludge was the next (8.62 mL $\text{H}_2/\text{g VS}$).

(2) All of the four pretreatments could increase the SCOD of sludge and the hydrogen yield of pretreated sludge had a significant correlation with its SCOD.

(3) Both alkaline pretreatments and thermal pretreatment could fully inhibit the activity of methanogens, while acid pretreatment and ultrasonic pretreatment could not.

(4) The VFA production of alkaline pretreated sludge (at initial pH of 11.5) was the maximum and that of thermal pretreated sludge is the next. The remainder of alkaline pretreated sludge (at initial pH of 11.5) after biohydrogen production could produce the most methane or hydrogen in the post-treatment and that of thermal pretreated sludge was the next.

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