pH dependency of hydrogen fermentation from alkali-pretreated sludge

XIAO Benyi & LIU Junxin

Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, Beijing 100085, China

Correspondence should be addressed to Liu Junxin (jxliu@mail.rcees.ac.cn)

Abstract Batch tests were carried out to study the possibility of hydrogen production from alkali-pretreated sludge without seed and the pH dependency of hydrogen fermentation from alkali-pretreated sludge. Experimental results showed that the sewage sludge with alkali-pretreatment could be successfully applied to biologically producing hydrogen without seed and extra-feed. The results also showed that the initial pH value of sewage sludge was an important factor throughout the hydrogen fermentation of alkali-pretreated sludge. The maximum hydrogen yield was obtained at initial pH value of 11.0 (14.4 mL·g VS⁻¹). The hydrogen yield of alkali-pretreated sludge at alkaline initial pH value was much higher than that of acidic or neutral initial pH value. The optimal pH value of hydrogen production from alkali-pretreated sludge was approximately 9.5. The consumption of hydrogen could be inhibited when the pH value of sludge was above 8.5. The change of hydrogen yield at various initial pH values was similar to that of sludge SCOD. The change of sludge pH value was slow and acetate was the major component of volatile fatty acids produced in the process of hydrogen production. The yield and the constitution of volatile fatty acids were sensitive to the initial pH value.

Keywords: alkali-pretreatment, anaerobic fermentation, hydrogen production, sewage sludge, pH value.

Large amount of excess sludge is produced in conventional activated sludge processes for wastewater treatment. The processing and disposal of the excess sludge is currently becoming an important problem for

many wastewater treatment plants. Anaerobic digestion techniques have traditionally been employed to reduce the volume and weight of sludge^[1,2]. Methane is the major fuel produced from the anaerobic digestion of sludge. In the anaerobic digestion of sludge, hydrogen is an intermediary product, which is rapidly taken up and converted into CH₄ by methanogens or H₂S by sulfate-reducing bacteria^[1,3]. If the converting process of hydrogen can be stopped, it is possible to harvest the intermediate-hydrogen, which is considered as a promising alternative of fossil fuels as an ideal and clean source of energy in the future. Some methods have been used to realize the above object, such as operations at low pH value to inhibit the hydrogen-consuming microorganisms, or operations at a short hydraulic retention time (HRT) to wash them out^[4]. Some pretreatments, like heat-shock^[5], heat treatment^[1] and acid-base treatment^[6], are other effective methods that have also been employed since many hydrogen-producing microorganisms can form endospores, which are very resistant to unfavorable environmental conditions^[5]. These pretreatments can inhibit or kill hydrogen-consuming microorganisms and screen the hydrogen-producing species from sludge microflora.

Most of organics in the sludge are compartmentalized within the microbial cell membranes. The hydrolysis of the microbial cell limits the speed of the anaerobic digestion of sludge and results in a long HRT^[2,7]. To improve anaerobic digestion, the most logical approach is to disrupt the microbial cells of sludge. Pretreatments, like mechanical disintegrations, thermal hydrolysis, and chemical or thermo-chemical hydrolysis, can disrupt the cells effectively and have been used to promote the anaerobic digestion of sludge^[2,7–9] and the hydrogen production from anaerobic fermentation of sludge^[10–12]. After pretreatment (ultrasonication, acidification, sterilization, or freezing/thawing), the sludge was applied to producing hydrogen by *Clostridium bifermentans*^[11–13]. Lin *et al.*^[2] had succeeded in using alkaline-hydrolyzed waste activated sludge to produce hydrogen with heated sludge as seed, and the hydrogen yield reached 1.7 g·kg TCOD⁻¹. Although the sewage sludge has been used to biologically produce hydrogen, seed was used in all previous studies.

1) Sung, S., Rakin, L., Duangmanee, T. *et al.*, Hydrogen production by anaerobic microbial communities exposed to repeated heat treatments, Proceeding of the 2002 U.S. DOE hydrogen program review, 2002.

2) Lin, H. Y., Tsai, Y. Y., Wu, R. H., Study of hydrogen production from the anaerobic digestion of hydrolyzed biological sludge, The Twenty-sixth Wastewater Technology Conference, Kanhsiung, Taiwan, 2001.

ARTICLES

pH value is an important factor that affects the biological hydrogen production. The effect of pH value on the hydrogen production of carbohydrates has been studied by many researchers^[13–15]. However, little work discussed the effect of pH even though it is really a critical factor ruling over the anaerobic reactions. So, the purposes of this study were to investigate the possibility of biohydrogen production from the sewage sludge without seed and feed by alkali-pretreatment and the pH dependency of hydrogen fermentation from alkali-pretreated sludge.

1 Materials and methods

1.1 Sewage sludge

The sludge was collected from aeration basin of a municipal wastewater treatment plant in Beijing, which handled 30000 t wastewater daily. The pH value of the sludge samples was about 6.9 (± 0.2). The chemical oxygen demand (COD) of the samples was 11500 mg·L⁻¹. The soluble chemical oxygen demand (SCOD) was 158 mg·L⁻¹. The total solid (TS) and volatile solid (VS) of the samples were 15200 and 10900 mg·L⁻¹, respectively. The collected sludge samples were gravitationally settled for 1 h and the sediments were stored at 4°C before use.

1.2 Alkali-pretreatment of sludge

The pH value of sludge was adjusted to 12.0 (± 0.1) using 6 mol/L sodium hydroxide and stabilized for about 5 min under stirring. The sample was stored at ambient temperature (17–24°C) for 12 h to enhance the efficiency of the alkali-pretreatment and this process was called alkali-pretreatment.

1.3 Experiments of hydrogen production

Fermentative hydrogen production of the sludge was carried out in passel plexiglas reactors with volume of 300 mL. 200 mL sludge at certain pH values was added to these reactors, and no further nutrients were added. The headspaces of these reactors were flushed with nitrogen gas and the liquid phases were also bubbled with nitrogen gas to remove the oxygen in the system and provided an anaerobic environment. Then these reactors were quickly sealed with rubber stoppers and placed in an air bath shaker (HKZ-C, China) with 140–150 rpm at 37 $\pm$ 1°C. Tests of each sludge samples were carried out in triplicate and all results were mean of replicate analyses.

1.4 Analytical methods

Biogas production was measured periodically by displacement of saturated aqueous NaCl in a graduated cylinder^[16]. The biogas in the headspace of the reactors was sampled with a 1 mL gastight syringe. Hydrogen production was calculated from headspace measurements of gas composition and the total volumes of biogas produced. The contents of hydrogen in the gas phase of the reactors were analyzed by a gas chromatograph (GC-122, China) equipped with a thermal conductivity detector and a 2 m stainless column packed with activated carbon (60–80 mesh). Injection, column and detector temperatures were kept at 70, 140 and 140°C, respectively. Nitrogen gas was used as the carrier gas with a flow rate of 30 mL·min⁻¹.

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 flame ionization detector and a 1 m glass column packed with chromosorb 101 (60–80 mesh). The temperatures of the injection port, the detector and the column were 250, 250 and 170°C, respectively. The carrier gas also was nitrogen at 50 mL·min⁻¹.

The pH value was measured by a pH meter (PHS-3C, China). The COD and SCOD were analyzed by a CTL-12 COD meter (Huatong Company, China). The samples were filtered through a 0.45 μ m membrane before determining the concentrations of SCOD. The biomass was measured by TS and VS according to standard methods^[17].

1.5 Kinetic analysis

The modified Gompertz equation (eq. (1)) was used to fit the experimental data of accumulative hydrogen production^[18,19].

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

where $H(t)$ is the accumulative hydrogen production (mL) during the fermentative time t (h), P the hydrogen production potential (mL), R_m the maximum production rate (mL·h⁻¹), λ the lag time (h), and e 2.718281828. The values of P , R_m and λ were determined by best fitting the hydrogen-producing data to eq. (1) using the Matlab 6.5 (Math Works. Inc. Copyright 2001–2002)^[14,20]. The maximum specific hydrogen production rate (mL·g VS⁻¹·h⁻¹) was calculated by dividing R_m by the initial sludge VS. The hydrogen yield (mL·g

VS^{-1}) was calculated by dividing P by the quantity of sludge (VS) added in the reactors.

2 Results and discussion

2.1 Biohydrogen production without seed

The alkali-pretreated sludge was directly used to produce hydrogen by anaerobic fermentation without seed and pH value adjustment (initial pH value about 11.3 ± 0.1). As control, the sludge without alkali-pretreatment was also used to the experiment of hydrogen production. The concentration of both sludge samples was 10.3 g VS/L . The changes of hydrogen yield of the sludge are shown in Fig. 1. It shows that little hydrogen was produced in the anaerobic fermentation of raw sludge, while the hydrogen yield of the alkali-pretreated sludge could reach $11.9 \text{ mL} \cdot \text{g VS}^{-1}$. The result indicated that it is possible to produce hydrogen from alkali-pretreated sludge without seed by anaerobic fermentation. Although Wang *et al.*^[10,11] got a higher hydrogen yield (about $46.7\text{--}65.3 \text{ mL} \cdot \text{g VS}^{-1}$) from frozen/thawed sterilized sludge by *Clostridium bifermentans*, the culture of seed contained many organic matters and the hydrogen was not really produced from sludge. Additionally, both the culture of *Clostridium bifermentans* and the addition of seeds were complicated. The method applied here (i.e. fermentation without seed) could cut down those procedures and make the process of hydrogen production simpler and more convenient.

2.2 Effect of initial pH on hydrogen production

In the experiments, the effect of initial pH value on

Fig. 1. Hydrogen production from sewage sludge.

hydrogen production from alkali-pretreated sludge was investigated. The pH values of the pretreated sludge were adjusted to 2.5, 4.0, 6.0, 8.0, 10.5, 11.0 and 12.0 (± 0.1), respectively, with 6 mol/L sodium hydroxide or 6 mol/L hydrochloric acid. The concentration of all sludge samples was $8.1 \text{ g VS} \cdot \text{L}^{-1}$.

Fig. 2 shows the changes of accumulative hydrogen production (AHP) from alkali-pretreated sludge at various initial pH values. Table 1 summarizes the kinetic parameters for hydrogen production from the sludge at various initial pH values. The results showed that no hydrogen could be detected in the reactors of initial pH 2.5, in which pH value only increased to 4.2 (data are not showed) after fermentation. It indicated that hydrogen production of the sludge was inhibited at low pH value ($\text{pH} < 4.2$). The maximal values of P (43.1 mL) and hydrogen yield ($14.4 \text{ mL} \cdot \text{g VS}^{-1}$) occurred at initial pH 11.0, the maximum special hydrogen production rate ($0.21 \text{ mL} \cdot \text{g VS}^{-1} \cdot \text{h}^{-1}$) and R_m ($3.2 \text{ mL} \cdot \text{h}^{-1}$) at initial pH 10.5 and λ (14.3 h) at initial pH 12.0. The results also showed that the hydrogen yield of the alkali-pretreated sludge at alkaline initial pH values was much higher than that at acidic and neutral initial pH values. The duration of hydrogen production at alkaline pH values and at acidic pH values was longer than that at neutral initial pH values. In fact, the complete degradation of organic materials into hydrogen and carbon dioxide was subjected to thermodynamic constraints (Table 2). Table 2 suggests that the biological hydrogen production becomes thermodynamically more attractive if performed under alkaline pH values¹⁾.

The difference of hydrogen production from the alkali-pretreated sludge at various initial pH values may

Fig. 2. Changes of accumulative hydrogen production from alkali-pretreated sludge at various initial pH values.

1) Keltjens, J. T., Geerts, W. J., Fermentative hydrogen production under alkaline conditions, <http://www.biohydrogen.nl/publicitem.m?pgid=20602&ctxid=1012>.

Table 1 Kinetic parameters for hydrogen production from sludge at various initial pH values

Initial pH values	<i>P</i> (mL)	<i>R_m</i> (mL·h ⁻¹)	<i>λ</i> (h)	Maximum specific hydrogen production rate (mL·g VS ⁻¹ ·h ⁻¹)	Hydrogen yield (mL·g VS ⁻¹)
2.5	—	—	—	—	—
4.0	7.0	0.5	12.9	0.04	2.3
6.0	3.7	1.0	6.4	0.07	1.3
8.0	5.0	1.4	7.5	0.09	1.7
10.5	28.9	3.2	6.5	0.21	9.7
11.0	43.1	2.2	12.6	0.14	14.4
12.0	42.2	1.5	14.3	0.10	14.1

Table 2 pH dependence of Gibbs free energy changes of hydrogen production

Reaction	ΔG_o (kJ·mol ⁻¹)				
	pH 7	pH 8	pH 9	pH 10	pH 11
$C_6H_{12}O_6 + 6H_2O \rightarrow 12H_2 + 6CO_2$	-26.2				
$C_6H_{12}O_6 + 6H_2O + 6OH^- \rightarrow 6HCO_3^- + 12H_2$		-23.8	-59.3		
$C_6H_{12}O_6 + 12OH^- \rightarrow 6CO_3^{2-} + 12H_2$				-78.2	-149.2
$C_6H_{12}O_6 + 2H_2O \rightarrow 2CH_3COO^- + 2H^+ + 2CO_2 + 4H_2$	-215.7				
$C_6H_{12}O_6 + 4OH^- \rightarrow 2CH_3COO^- + 2HCO_3^- + 4H_2$		-223.9	-247.6		
$C_6H_{12}O_6 + 6OH^- \rightarrow 2CH_3COO^- + 2H_2O + 2CO_3^{2-} + 4H_2$				-265.7	-301.2
$CH_3COO^- + 2H_2O + H^+ \rightarrow 2CO_2 + 4H_2$	+94.8				
$CH_3COO^- + 3H_2O + OH^- \rightarrow 2HCO_3^- + 4H_2$		+100.1	+94.1		
$CH_3COO^- + H_2O + 3OH^- \rightarrow 2CO_3^{2-} + 4H_2$				+93.7	+76.0

be related with the SCOD of the sludge. Fig. 3 shows the SCOD of the sludge at various pH values. Although all sludge samples were pretreated by alkaline, the SCOD of the sludge samples was influenced by the pH value of sludge samples, because some alkali-solubilized organics would precipitate and convert into insoluble ones when the pH value changed. The higher the SCOD of the pretreated sludge is, the more the substrates available to hydrogen-producing microorganisms are. From Figs. 2, 3 and Table 1, it can be found that the change of hydrogen yield at various initial pH values was similar to that of the sludge SCOD.

Fig. 3. SCOD of alkali-pretreated sludge at various pH values.

The change of sludge pH value during the process of producing hydrogen was determined (Fig. 4). In the first stage, the pH value of sludge was rapidly changed, i.e. it increased under acid initial pH values and decreased under alkaline pH values, while little hydrogen was produced. This stage was the lag stage, in which the microorganisms in the sludge were acclimated. The change of sludge pH values was caused by production of VFA and NH₄⁺-N^[21]. Unlike the hydrogen fermentation of carbohydrates^[15], the change of sludge pH value was unobvious.

Fig. 4. Change of pH values in the process of hydrogen production.

The maximal hydrogen production and hydrogen consumption occurred at certain pH value, which were different at different initial pH values. The optimal pH values were reported for hydrogen production from sludge, i.e. pH 5.0–5.5 and pH 7.0 for batch fermentation of wastewater sludge^[10–13] and waste activated sludge¹⁾, respectively. However, in this study, the optimal pH value of hydrogen production from alkali-pretreated sludge was 9.5. It was much higher than the values reported above. The difference may be caused by two reasons: (1) the hydrogen-producing microorganisms screened by alkali-pretreatment were basophilic, and (2) the organic materials in the sludge samples solubilized by alkali-pretreatment were mainly alkaline-soluble ones. Hydrogen production was not observed when the pH value of sludge was at highly alkaline pH value (>10.5), which showed that the activity of hydrogen producing microbes was inhibited at these pH values.

The hydrogen accumulated in the headspace of the reactors was consumed in the sequent fermentation when the initial pH values of sludge samples were acidic and neutral pH values (Fig. 2). While little hydrogen was consumed under alkaline pH values (such as 10.5, 11 and 12). The consumption of hydrogen could be inhibited when the pH value of sludge sample was above 8.5. It suggested that the activity of hydrogen consuming microbes was inhibited at alkaline pH values. Though alkali-pretreatment can inhibit or kill some hydrogen-consuming microorganisms, some spore-form ones, for example *Clostridium* and *Desulfotomaculum* (not methanogens since no methane was detected in the biogas and data are not showed), can also survive after alkali-pretreatment^[22]. Once the circumambient condition is favorable they would grow and consume hydrogen.

VFA are important by-products of hydrogen production by anaerobic fermentation. The sludge was sampled in 76.5 h in order to determine the production of VFA from fermentation of pretreated sludge at various initial pH values, and the results are summarized in Table 3. The major component of VFA was acetate (about 68%–78%), and butyrate was little (about 6%–11%). This result was different from the results of hydrogen production from carbohydrates by anaerobic

Table 3 VFA production at various initial pH values (Sampling time: 76.5 h)

Initial pH values	VFA (mg·L ⁻¹)	Acetate (%)	Propionate (%)	Butyrate (%)	Pentanoate (%)
4.0	291.3	69.5	12.3	6.4	11.9
6.0	329.9	74.8	10.1	10.3	4.8
8.0	515.3	68.5	12.1	10.4	9.0
10.5	787.8	68.4	11.8	8.3	11.5
11.0	914.2	68.5	13.2	9.6	8.7
12.0	765.1	77.8	12.2	6.1	3.8

fermentation^[6] in which butyrate was the major component. The reason may be the complicated substrate of hydrogen production in the alkali-pretreated sludge, which contains many components, such as proteins, carbohydrates and lipids. Table 3 also shows that the production and the constitution of VFA were sensitive to the initial pH value.

3 Conclusions

The sewage sludge was successfully applied to biologically producing hydrogen without seed and extra-feed. Alkali-pretreatment of the sludge possibly can not only screen hydrogen-producing microorganisms and inhibit or kill some hydrogen-consuming microorganisms, but also disrupt the cells of sludge microorganisms to release organic matter into water.

The initial pH value played an important role in the hydrogen production of alkali-pretreated sludge. The initial pH value of 11.0 was the optimal for hydrogen production from the alkali-pretreated sludge and the hydrogen yield reached 14.4 mL·g VS⁻¹. The optimal pH value of hydrogen production from the sludge was approximately 9.5. The hydrogen production of alkali-pretreated sludge at alkaline initial pH value was much higher than that of acidic or neutral initial pH value. Hydrogen consumption could be inhibited at alkaline pH value, especially above 8.5.

The change of hydrogen yield at various initial pH values was similar to that of sludge SCOD. In the process of hydrogen production, the change of sludge pH value was tardy and acetate was the major component of VFA. The yield and the constitution of VFA were sensitive to the initial pH value.

1) See footnote 1) on page 401.

ARTICLES

Acknowledgements The authors would like to thank Wei Yuansong and Li Lin for help in the paper writing. This work was supported by the National Natural Science Foundation of China (Grant No. 20277043).

References

1. Parkin, B. G. F., Owen, W., Fundamentals of anaerobic digestion of wastewater sludges, J. Environ. Eng. ASCE, 1986, 112 (5): 867—920.
2. Weemaes, M. P. J., Verstraete, W. H., Evaluation of current wet sludge disintegration techniques, J. Chem. Technol. Biotechnol., 1998, 73: 83—92.
3. Reith, J. H., Wijffels, R. H., Barten, H., Bio-methane & bio-hydrogen. Status and perspectives of biological methane and hydrogen production, Dutch biological hydrogen foundation, Wageningen, 2003, 104—109.
4. Hawkes, F. R., Dinsdale, R., Hawkes, D. L. *et al.*, Sustainable fermentative hydrogen production: change for process optimization, Int. J. Hydrogen Energ., 2002, 27: 1339—1347.
5. Lay, J. J., Fan, K. S., Chang, J. L. *et al.*, Influence of chemical nature of organic wastes on their conversion to hydrogen by heat-shock digested sludge, Int. J. Hydrogen Energ., 2003, 28: 1361—1367.
6. Chen, C. C., Lin, Y. Y., Lin, M. C., Acid-base enrichment enhances anaerobic hydrogen production process, Appl. Microbiol. Biot., 2002, 58: 224—228.
7. Alvarez, J. M., Macé, S., Llabrés, P., Anaerobic digestion of organic solid wastes. An overview of research achievements and perspectives, Bioresource Technol., 2000, 74: 3—16.
8. Kim, J. S., Park, C. W., Kim, T. H. *et al.*, Effects of various pre-treatment for enhanced anaerobic digestion with waste activated sludge, J. Biosci. Bioeng., 2003, 95 (3): 271—275.
9. Müller, J. A., Prospects and problems of sludge pre-treatment processes, Wat. Sci. Tech., 2001, 44 (10): 121—128.
10. Wang, C. C., Chang, C. W., Chu, C. P. *et al.*, Hydrogen production from wastewater sludge using a *Clostridium* strain, J. Environ. Sic. Heal. Part A, 2003, 38(9): 1867—1875.
11. Wang, C. C., Chang, C. W., Chu, C. P. *et al.*, Producing hydrogen from wastewater sludge by *Clostridium bifermentans*, J. Biotechnol., 2003, 102: 83—92.
12. Wang, C. C., Chang, C. W., Chu, C. P. *et al.*, Using filtrate of waste biosolids to effectively produce biohydrogen by anaerobic fermentation, Wat. Res., 2003, 37(11): 2789—2793.
13. Lay, J. J., Modeling and optimization of anaerobic digested sludge converting starch to hydrogen, Biotechnol. Bioeng., 2000, 58(3): 269—278.
14. Fang, H. H. P., Liu, H., Effect of pH on hydrogen production from glucose by a mixed culture, Bioresource Technol., 2002, 82: 87—93.
15. Lee, Y. J., Miyahara, T., Noike, T., Effect of pH on microbial hydrogen fermentation, J. Chem. Technol. Biotechnol., 2002, 77: 694—698.
16. Owen, W. F., Stuckey, D. C., Jr Healy, J. B. *et al.*, Bioassay for monitoring biochemical methane potential and anaerobic toxicity, Wat. Res., 1979, 13: 485—493.
17. APHA, Standard methods for the examination of water and wastewater (20th), Washington, D.C., USA, 1998, 2-54-2-60.
18. Lay, J. J., Li, Y. Y., Noike, T., Mathematical model for methane production from landfill bioreactor, J. Environ. Eng. ASCE, 1998, 124 (8): 730—736.
19. Lay, J. J., Biohydrogen generation by mesophilic anaerobic fermentation of microcrystalline cellulose, Biotechnol. Bioeng., 2001, 74: 280—287.
20. Zhang, T., Liu, H., Fang, H. H. P., Biohydrogen production from starch in wastewater under thermophilic condition, J. Environ. Manage., 2003, 69: 149—156.
21. Sung, S. H., Liu, T., Ammonia inhibition on thermophilic anaerobic digestion, Chemosphere, 2003, 53: 43—52.
22. Hu, J. C., Theory and technology of anaerobic wastewater treatment (in Chinese), Beijing: China Architecture & Building Press, 2003, 19—37.

(Received May 11, 2005; accepted July 7, 2005)