



Biological hydrogen production from sterilized sewage sludge by anaerobic self-fermentation

Benyi Xiao, Junxin Liu*

Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, P.O. Box 2871, Beijing 100085, PR China

ARTICLE INFO

Article history:

Received 29 July 2008

Received in revised form 17 December 2008

Accepted 4 February 2009

Available online 12 February 2009

Keywords:

Anaerobic self-fermentation

Hydrogen production

Sterilization

Sewage sludge

ABSTRACT

Due to richness in proteins and carbohydrates, the sewage sludge produced from the wastewater treatment processes is becoming a potential substrate for biological hydrogen production. In this study, sterilized sludge was employed to produce hydrogen by batch anaerobic self-fermentation without any extra-feeds and extra-seeds. Sterilization can screen hydrogen-producing microorganisms from sludge microflora and release organic materials from microbial cells of sludge. Experimental results suggested that sterilization could accelerate and increase the hydrogen production of sewage sludge in the anaerobic self-fermentation, and the biogas did not contain methane. The hydrogen yield was increased from 0.35 mL H₂/g VS (raw sludge) to 16.26 mL H₂/g VS (sterilized sludge). Although sterilization could fully inhibit the activity of methanogens in the sludge, the hydrogen consumption still occurred in the anaerobic self-fermentation of sterilized sludge due to the existence of other hydrogen-consuming actions. The decrease of pH in the anaerobic self-fermentation of sterilized sludge was very lower (from 6.81 to 6.56) because NH₄⁺ produced by degradation of proteins could neutralize organic acids produced in the process. The soluble chemical oxygen demand (SCOD) increase of sterilized sludge was higher than that of raw sludge. Volatile fatty acids (VFA) were the important by-products and acetate was the major composition. The hydrogen fermentation of carbohydrates was the major source of hydrogen production.

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1. Introduction

The application of biological processes in wastewater treatment causes increased production of sludge. The sludge is composed of largely organic matters (59–88%) that can decompose and produce offensive odors [1]. These organics are mainly the microbial matters and the microorganisms include hydrogen-producing ones and hydrogen-consuming ones. The treatment and disposal of the excess sludge has become an important problem and a great challenge for many plants [1]. Anaerobic digestion is an appropriate technique for reduction in the volume and weight of excess sludge before final disposal, and it is employed worldwide as the oldest and most important process for sludge stabilization. Additionally, anaerobic digestion can recover partly the bioenergy of sludge through producing methane [2]. Hydrogen is an intermediary metabolite of anaerobic digestion, which is rapidly taken up and converted to other products by the hydrogen-consuming microorganisms in the third stage of anaerobic digestion [2–4]. However, hydrogen has been regarded as an ideal fuel since H₂O is its only product as it burns [5]. On the other hand, the use of hydrogen

is more extensive than that of methane. So, it is beneficial to get hydrogen in the anaerobic digestion of sludge. In order to harvest hydrogen, the anaerobic digestion of sludge must be blocked at the hydrogen and acetic acid formation stage, namely, the second stage of anaerobic digestion.

Many hydrogen-producing microorganisms can form endospores, which can be considered “survival structures” developed by these organisms when unfavorable environmental conditions are encountered, e.g., high temperature, and harmful chemicals including acid and alkaline [6–8]. When favorable conditions return, the spores germinate and become vegetative cells [9,10]. However, most of hydrogen-consuming microorganisms, e.g., methanogens, do not have this characteristic. So, thermal pretreatment (including sterilization), acid and alkaline pretreatments can be used to screen hydrogen-producing species [9–13]. Additionally, the growth rate of most hydrogen-producing microorganisms is faster than that of hydrogen-consuming ones.

Thermal sludge treatment was originally used to improve sludge dewaterability [1]. Thermal treatment can result in the breakdown of the sludge gel structure and the release of intracellular bound water [14]. The corresponding release of intracellular organic materials was seen as an important drawback before, but is now becoming interesting as an enhancing pretreatment prior to anaerobic digestion and as a means of producing internal carbon sources

* Corresponding author. Tel.: +86 10 62849133; fax: +86 10 62849133.
E-mail address: jxliu@mail.rcees.ac.cn (J. Liu).

for nutrient removal [14,15]. Haug et al. [16] proposed a thermal pretreatment process prior to anaerobic digestion of sludge. They found that methane production from the digestion of waste activated sludge could be increased by about 60% as a result of thermal pretreatment. Sterilized sludge (121 °C and 1.2 kgf/cm² for 30 min) was used to produce hydrogen by *Clostridium bifermentans* [13] and *Pseudomonas* sp. GZ1 [17].

However, there are few reports on hydrogen bio-production from sterilized sludge by anaerobic self-fermentation without extra-seeds up to the present. So, the purpose of this study was to investigate the possibility of hydrogen production from the sterilized sludge by anaerobic self-fermentation without extra-seeds and extra-nutrition. The detailed process of hydrogen production from sterilized sludge and the possible mechanism were also discussed.

2. Materials and methods

2.1. Sewage sludge

The sewage sludge (raw sludge, RS) was obtained from a municipal wastewater treatment plant in Beijing (China), which handled 30,000 t wastewaters daily by activated sludge process. The pH value of the sludge samples was about 7.1 (± 0.1). The total chemical oxygen demand (TCOD) of the samples was 11,500 mg/L. The soluble chemical oxygen demand (SCOD) was 177.3 mg/L. The collected sludge samples were gravitationally settled for about 1 h and the sediments were stored at 4 °C before being used. The total solid (TS) and volatile solid (VS) of the sludge used in the test were 14,300 mg/L and 10,400 mg/L, respectively.

2.2. Sterilization of sludge

The sewage sludge sample was sterilized in an autoclave (VAR-IOKLAV steam sterilizer, 300/400/500 EP). The sludge was put into a 1 L conical flask ($D \times h = 10 \text{ cm} \times 20 \text{ cm}$) with stopper in the sterilization. Though the optimal temperature of thermal hydrolysis of sewage sludge was 170–175 °C [1,18], higher temperature would consume more energy. So, the conditions of thermal pretreatment were selected as 121 °C, 1.5 atm and 30 min of treatment time accordingly to the references [13,17,19]. After sterilizing, the pretreated sample (sterilized sludge, SS) was cooled to ambient temperature.

2.3. Hydrogen production by anaerobic self-fermentation

The sterilized sludge samples (150 mL) were added into 300 mL plexiglass reactors which were equipped with two ports for sampling gas and sludge, respectively. No extra-seeds and extra-feeds were added into these reactors. Both the headspaces of the reactors and the liquid phases were flushed with nitrogen in order to provide an anaerobic environment. The reactors were quickly sealed and agitated on a shaker (HKZ-C, China) with 140–150 rpm at 37 ± 1 °C. The sludge without sterilization was used as control of hydrogen production. Tests of each sludge samples were carried out in triplicate and all results were the average of replicate analysis.

2.4. Analysis

Biogas production was measured periodically by displacement of saturated salt solution making corrections for atmospheric pressure and temperature [20]. The biogas in the headspace of reactors was sampled with a 1 mL gastight syringe. Hydrogen and methane production was calculated from measurements of the biogas composition and the total volumes of biogas produced. The contents of hydrogen in the biogas were analyzed by a gas chromatograph (GC122, 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 a 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 the carrier gas with a flow rate of 30 mL/min.

The sludge was sampled with a 5 mL gastight syringe. The concentrations of VFA in the liquid phase were analyzed using another gas chromatograph (Shimadzu GC-9A, Japan) equipped with a flame ionization detector (FID) and a 1 m glass column packed with Chromosorb 101 (60–80 mesh). The carrier gas used nitrogen at 50 mL/min. Soluble proteins in the liquid phase were measured by Lowry's method using bovine serum albumin as a standard solution [21], soluble carbohydrates by the phenol-sulfuric acid method using glucose as a standard solution [22], lipids by Soxhlet extraction method with ether as extraction solvent, and $\text{NH}_4^+ - \text{N}$ by standard method issued by APHA [23]. The pH was measured by a pH meter (PHS-3C, China). The SCOD and TCOD were analyzed by a COD meter (CTL-12, China). The samples were filtered through a 0.45 μm membrane before determining the concentrations of SCOD. The biomass concentration was measured by total solid (TS, namely dry solid, DS) and volatile solid (VS) according to standard methods [23].

3. Results and discussion

3.1. Effect of sterilization on sludge characteristics

Sterilization could partly break down the gel structure and the microbial cell structure of sludge and result in the changes of sludge characteristics (Table 1). Due to some matters released from the microbial cells, the DS and VS of sludge were decreased from 14.3 mg/L and 10.4 mg/L to 11.2 mg/L and 7.7 mg/L, respectively. Once the microbial matters released from the cells, these matters would convert into soluble ones, which resulted in the increase of the sludge SCOD [1]. In the sterilization, some released organics would degrade into low-molecular weight organics, for example VFA [1]. Table 1 shows that the main compositions of SCOD in sterilized sludge were proteins, carbohydrates and VFA. After sterilization, the pH value of sludge decreased from 7.1 to 6.8 due to the increase in VFA.

3.2. Hydrogen production and hydrogen consumption in the anaerobic self-fermentation

Sterilized sludge was used to produce hydrogen by anaerobic self-fermentation and raw sludge was used as control. The cumulative hydrogen yields of sterilized sludge and raw sludge are shown in Fig. 1. Owing to the co-existence of hydrogen-producing microorganisms and hydrogen-consuming ones and their different growth rates [24], the hydrogen production and hydrogen consumption (methane production) could be detected in the anaerobic self-fermentation of the raw sludge (Fig. 1). However, most

Table 1
Change of sludge characteristics after sterilization.

Item	RS	SS
pH	7.1	6.8
DS (g/L)	14.3	11.2
VS (g/L)	10.4	7.7
SCOD (mg/L)	177.3	2824.8
Proteins ^a (mg/L)	32.1	1431.2
Carbohydrates ^a (mg/L)	8.1	583.1
VFA (mg/L)	113.5	194.1

^a Soluble matters.

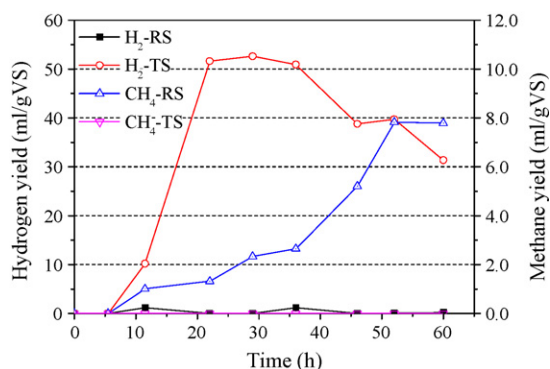


Fig. 1. Cumulative hydrogen and methane yields in the anaerobic fermentation.

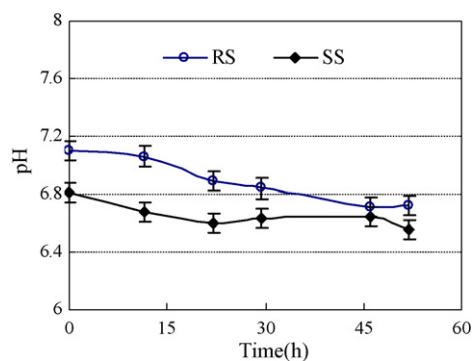


Fig. 2. Changes of pH in the anaerobic fermentation.

of the organics presented in the raw sludge were of microbial cells, which were enwrapped by cell walls, and such few available organics in raw sludge for hydrogen-producing microorganisms and hydrogen-consuming ones resulted in their slow growth and metabolism. As a result, there was little hydrogen detected in the anaerobic self-fermentation of raw sludge (Fig. 1). Apart from raw sludge, the sterilization had disrupted some microbial cells and released some intracellular organics from cell, and thus the thermally screened hydrogen-producing microorganisms in the sterilized sludge could quickly use those organics and produce hydrogen (Fig. 1). Fig. 1 shows the maximal hydrogen yield of the sterilized sludge (16.26 mLH₂/g VS) was much higher than that of the raw sludge (0.35 mLH₂/g VS). Although most hydrogen-consuming microorganisms (mainly methanogens) died during sterilization, hydrogen consumption still occurred in the later stage (after 36 h) of anaerobic self-fermentation of the sterilized sludge. Interestingly, no methane was detected in the anaerobic self-fermentation of sterilized sludge (Fig. 1). There were three reasons for the hydrogen consumption: (1) there are a few hydrogen-consuming microorganisms (for example, Homoacetogenic bacteria) in the sludge that can also form spores and resist autoclaving. In the initial stage of anaerobic fermentation, the hydrogen-consuming action was weak and unobvious because of fewer hydrogen-consuming microorganisms and their lower growth rate compared with hydrogen-producing ones. In the later stage of anaerobic fermentation, the hydrogen-consuming action became obvious along with the increase in hydrogen-consuming microorganisms [6–8,24]. (2) The increase of VFA, particularly, acetic acid could inhibit further growth of hydrogen-producing microorganisms and then result in the decrease of the hydrogen production rate [25]. (3) The anaerobic fermentation of proteins could not only produce hydrogen but also

consume hydrogen, and high content of proteins in the sterilized sludge may also result in the consumption of hydrogen [26].

Table 2 summarizes the maximal hydrogen yield in some similar research works. Although the maximal hydrogen yield of the sterilized sludge in this study was not the highest among these works, there were some advantages of using sterilized sludge to produce hydrogen: (1) without any extra-seeds and any extra-feeds, it is simple and convenient to produce hydrogen by anaerobic self-fermentation; (2) without any extra-added materials (such as alkaline and acid used in alkaline or acid pretreatment), the sterilized sludge was easy to be treated after hydrogen production; (3) no methane was produced in the process of anaerobic self-fermentation because most of the methanogens were killed by autoclave (Fig. 1).

3.3. Changes of liquid phase in the anaerobic self-fermentation

Fig. 2 summarizes the change of pH during the process of anaerobic self-fermentation of the raw sludge and the sterilized sludge. In the batch anaerobic fermentation of carbohydrates, with degradation of carbohydrates and production of organic acids, the pH value rapidly drops to the range 4.5–5.0 and leads to the inhibition of hydrogen production [30]. While in the anaerobic self-fermentation of sterilized sludge, the change of pH was similar to that of the anaerobic digestion of sludge, i.e., it decreased a little from 6.81 to 6.56 because NH₄⁺-N produced by degradation of proteins could neutralize organic acids produced in the process (Fig. 3). Wang et al. [13] reported the similar results.

In sludge sterilization, sludge was partially solubilized, some microbial cells of sludge were disintegrated, and some organics (mainly proteins and carbohydrates) were released into water (Table 1). These released organics were degraded in the anaer-

Table 2
Maximal hydrogen yield in some similar works.

Authors	Substrate	Seed	Reactor type	Maximal hydrogen yield (mLH ₂ /g DS)
This study	Sterilized sludge	Consortiums in sterilized sludge	Batch reactor	11.77
	Raw sludge	Consortiums in raw sludge	Batch reactor	0.25
Huang et al. [27]	WAS	Consortiums in digested sludge	Batch reactor	1.79
Cheng et al. [28]	Boiling treated sludge	Consortiums in boiled treated sludge	Batch reactor	11.54
Wang et al. [16]	Freezing and thawing WAS WAS	<i>Clostridium bifermentans</i>	Serum bottles	131.35
			Serum bottles	36.65
Cai et al. [11]	Alkaline pretreated sludge	Consortiums in raw sludge	Serum bottles	16.59
Massanet-Nicolau et al. [29]	Heat and enzymatic pretreated primary sewage sludge	Consortiums in heated treated anaerobic sludge	Batch reactor	18.14
Guo et al. [17]	Sterilization pretreated sludge	<i>Pseudomonas</i> sp. GZ1	Batch reactor	30.38

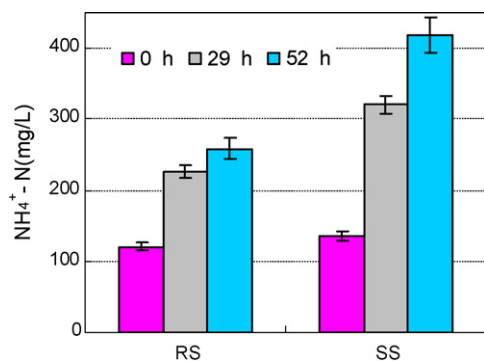


Fig. 3. Changes of NH₄⁺ in anaerobic fermentation.

obic self-fermentation (Fig. 4). As shown in Fig. 4, the content of soluble proteins continuously decreased in the anaerobic self-fermentation, while that of soluble carbohydrates decreased in 0~30 h and then slightly increased in the later stage. Little change of lipids (including the soluble and the insoluble) was found in the anaerobic self-fermentation of the sterilized sludge (data not shown). As far as the raw sludge was concerned, the disruption of cell wall led to release of intracellular organics (including proteins and carbohydrates) and resulted in a little increase in these organics (Fig. 4). With the consumption of hydrogen, the decrease rate of soluble proteins was reduced and the soluble carbohydrates began increasing. This might be caused by the hydrogen-consuming microorganisms using little soluble proteins and soluble carbohydrates which were still produced from the hydrolysis of insoluble organic materials.

Comparing Fig. 4 and Fig. 1, it could be found that the changes of soluble proteins and soluble carbohydrates were correlative with the production of hydrogen, and the stage of hydrogen production corresponded with that of carbohydrate decrease. In the anaerobic self-fermentation of the sterilized sludge, if the hydrolysis of sludge was not taken into account, the decrease of soluble carbohydrates was 58.54 mg (counted with glucose) (Fig. 4). The theoretical hydrogen yield of sterilized sludge should be 12.61–25.23 mL H₂/g VS on account of the decrease in carbohydrates. Since the factual hydrogen yield of the sterilized sludge was 16.26 mL H₂/g VS, it could be proposed that the hydrogen was mainly produced by the fermentation of carbohydrates. In other words, carbohydrates were the main substances of hydrogen production in the anaerobic self-fermentation of the sterilized sludge. Massanet-Nicolau et al. [29] reported that hydrogen fermentation of carbohydrates was the resource of hydrogen production in the anaerobic fermentation of sewage sludge. This result was different from that of Cai et al. [11] and Guo et al. [17] who thought that hydrogen was produced by the fermentation of proteins. The reason may be that both of the

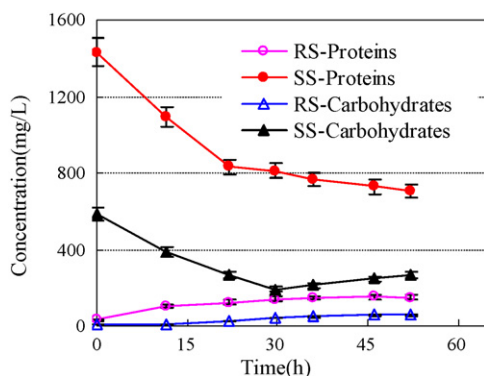


Fig. 4. Changes of soluble proteins and carbohydrates in the anaerobic fermentation.

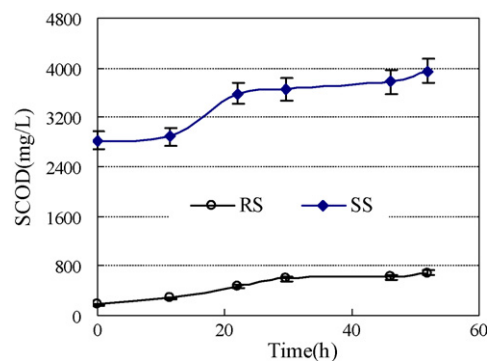


Fig. 5. Changes of SCOD in the anaerobic fermentation.

seeds and substrates of hydrogen production were different in these studies.

The changes of SCOD in the process of anaerobic self-fermentation of two sludge samples are shown in Fig. 5. The SCOD of both two sludge samples increased due to the hydrolysis of sludge, but the SCOD augment of the sterilized sludge was higher than that of the raw sludge. In the process of sludge sterilization, some disrupted cell walls of sludge made the organic matters more easily hydrolyzed and converted into more VFA than those of the raw sludge, and the VFA was not converted to methane and accumulated in the fermentation process, which resulted in a higher SCOD augment of the sterilized sludge.

In the anaerobic self-fermentation of two sludge samples, the organic matters (such as proteins and carbohydrates) were degraded and converted to low-molecular weight materials, e.g., H₂, CO₂, H₂O and VFA, and so on [2]. Table 3 summarizes the production of VFA in the anaerobic self-fermentation of two sludge samples (sampling at time 46 h). More VFA were produced in the self-fermentation of the sterilized sludge, which also proved a rapid acidification of organic materials. For the sterilized sludge, acetic acid was the major composition of VFA (55.12%) and the second one was butyric acid (16.06%). However, for the raw sludge, acetic acid accounted for 71.67% of VFA and propionic acid was the second (12.31%). The butyric acid/acetic acid ratio was about 0.29 for the sterilized sludge, while about 0.17 for the raw sludge. Although this result was different from those of hydrogen fermentation of carbohydrates in many studies [31,32], it was similar to those of various pretreated sludge [11,17,29]. Guo et al. [17] reported that no propionic acid and valeric acid were produced in the hydrogen fermentation of sterilized sludge by *Pseudomonas* sp. GZ1 (EF551040). Therefore, the high acetate content of VFA might be caused by the fact that the substances of hydrogen-producing microorganisms were complicated and the compositions of VFA were affected by the hydrogen-producing ones. By the way, after self-fermentation, the fermented sludge could be used to produce methane by anaerobic fermentation or hydrogen by photosynthesis fermentation and there are many such studies [8,33,34].

Table 3
Production of volatile fat acids (VFA) in the anaerobic fermentation (sampling time 46 h).

Sludge	VFA (mg/L)	Acetic acid (%)	Propionic acid (%)	Butyric acid (%)	Pentanoic acid (%)	B/A ^a
RS	427.34	71.67	12.31	11.89	4.13	0.17
SS	1789.16	55.12	14.82	16.06	7.63	0.29

^a Butyric acid/acetic acid.

4. Conclusions

The sterilized sludge was used to produce hydrogen by anaerobic self-fermentation without any extra-feeds and extra-seeds. The hydrogen yield of the sterilized sludge could reach 16.26 mLH₂/gVS. Methane was not detected in the biogas. The pH value in anaerobic self-fermentation of the sterilized sludge decreased from 6.81 to 6.56. VFA were the main liquid by-products of the anaerobic fermentation. Acetate was the major composition of VFA, and the second was butyrate. The SCOD increased because some insoluble materials were hydrolyzed and converted into soluble materials. The degradation of carbohydrates played an important role in the hydrogen production of the sterilized sludge.

Acknowledgement

This work is financially supported by National Nature Science Foundation of China (No. 50538090).

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