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Biohydrogen Production from Sterilized Sewage Sludge as a Substrate Using Mixed Cultures

Mijung Kim [1, +, †], Sechang Oh [2], Randeep Rakwal [1,3], Chunguang Liu [1], Zhenya Zhang [1]

[1] Graduate School of Life and Environmental Sciences, University of Tsukuba, Tsukuba, Ibaraki 305-8577, Japan

[2] Graduate School of Comprehensive Human Science, University of Tsukuba, Tsukuba, Ibaraki 305-8574, Japan

[3] Organization for Educational Initiatives, University of Tsukuba, Tsukuba, Ibaraki 305-8572, Japan

Abstract. This study investigates biohydrogen production from sewage sludge as substrate by mixed cultures using batch experiments under thermophilic anaerobic conditions and four different sterilization times (15, 30, 45, and 60 min). Increasing the sterilization time caused a decrease in the total solids (TS) and volatile solids (VS), however, the soluble chemical oxygen demand (SCOD) of sterilized sludge increased. The SCOD of sterilized sludge was 1.2 to 1.9 times higher than that of raw sewage sludge. Sterilization treatment was found to accelerate and increase biohydrogen production throughout the batch experiment, but with no measurable methane production. The maximal biohydrogen yield from sterilized sludge at 15, 30, 45, and 60 min was 16.8, 25.1, 25.2 and 25.5 ml H_2/g-VS, respectively, which was 4.3 to 6.5 times higher than that obtained with raw sewage sludge (3.9 ml H_2/g-VS). Our results show that 30 min is the optimal sterilization time for sewage sludge. Under these optimal conditions, overall VS removal (solubilization and anaerobic process) in sterilized sludge was 41.4%, and which was 1.5 times higher than that seen with raw sludge. The findings of our study have potential practical use in not only processes for efficient biohydrogen production but also in waste treatment.

Keywords: Sewage sludge; Sterilization treatment; Biohydrogen production; Mixed cultures; Anaerobic digestion.

1. Introduction

Massive amounts of sewage sludge, such as the organic waste during various stages of wastewater treatment, are generated every year worldwide. This means that there is a high cost requirement for waste treatment and reuse in reclaimed land or for making concrete. Therefore, it is important to search for potential energy saving approaches by utilizing sewage sludge prior to waste treatment for a sustainable future environmentally friendly society. The sewage sludge is composed of 95% water and 1-5% solids. The solid part in sewage sludge contains a diverse population of microbes, organic matter, nutrients (nitrogen and phosphorus), and trace elements [1]. Based on these characteristics, sludge can be used as the inoculum and substrate for producing biogas (methane) through anaerobic digestion – a most commonly used process for sludge stabilization [2]. Therefore, anaerobic digestion is widely used for sludge stabilization, as well as methane production using anaerobic microorganisms [3].

Despite the above benefits, a long sludge retention time is required for reducing organic matter and producing methane by anaerobic digestion. Even after long periods (30-60 days) of digestion, sludge degradation rate is low, due to the presence of refractory materials that are hard to break during the anaerobic digestion period [4]. In order to overcome this disadvantage, several pretreatment methods, such as acid [5],

[+] Corresponding author. Tel.: + (8129-861-2900); fax: +(8129-861-2900).
E-mail address: (mijungkim1003@gmail.com; mijung-kimu@aist.go.jp).

alkali [6], thermal [7], ultrasonic [8], and microwave [9] have been proposed for dissolving organic solids in the sewage sludge prior to anaerobic digestion. Eskicioglu et al. [10] reported an increase of 31% in methane production from the microwave pretreated sewage sludge. Li et al. [4] demonstrated that alkaline pretreatment of sewage sludge prior to anaerobic digestion substantially increased the degradation rate of organic substances with the help of NaOH hydrolysis, which resulted in a significant increase of methane production.

Recently, biohydrogen has been attracting increasing attention as a biofuel for the future, because hydrogen as a clean energy can be directly used in fuel cells to generate electricity [2]-[11]. Additionally, hydrogen as an intermediary metabolite of anaerobic digestion can be produced independently during the anaerobic digestion process. However, biohydrogen production from sewage sludge is not well studied, primarily due to its natural characteristics of low carbohydrate content (<10%) and biodegradability, which results in lower hydrogen yield [11]. It is well known that in biohydrogen production, carbohydrates are the more favored substance than lipid or protein [12]. Previous researches have reported an improvement in biohydrogen production by heat or sterilization methods. For example, Massanet-Nicolau et al. [2] found that pretreatment of sewage sludge by heat (70 ℃) and enzyme prior to fermentation has a significant effect on the hydrogen yield. Xiao and Liu [13] reported sewage sludge sterilization at 121 ℃ consistently improved biohydrogen production; hydrogen consumption still occurred in the anaerobic self-fermentation of imperfectly sterilized sludge due to existence of other hydrogen-consuming bacteria.

With this background, in the present study we used a sterilization pretreatment step to the sewage sludge in order to establish an effective method for sludge solubilization. The main purpose of this study was to assess the feasibility of biohydrogen production from sterilized sewage sludge as a substrate by mixed cultures, without additional nutrients under anaerobic thermophilic conditions. The effects of various sterilization times on sewage sludge for biohydrogen production were determined. In addition, we also examined the changes in pH, soluble chemical oxygen demand (SCOD) including metabolites, carbohydrates, and ammonia nitrogen (NH_4^+-N) as well as the volatile solids (VS) removal during the hydrogen fermentation process.

2. Materials and Methods

2.1. Raw materials

Sewage sludge as the substrate was taken from a gravity sludge thickener line at the wastewater treatment plant in Ibaraki prefecture (Japan), and was stored at 4 ℃. The pH, alkalinity as $CaCO_3$, and volatile suspended solids (VSS) of the sewage sludge were 6.4, 4.8 g/l, and 7.4 g/l, respectively. The sewage sludge was sterilized in an autoclave, and was sterilized for 15, 30, 45, or 60 min at a temperature of 121 ℃ and pressure of 1.2 atm, and then used as a substrate for hydrogen fermentation to determine the optimal sterilization time. The effects of five factors, i.e., soluble carbohydrate (SC), NH_4^+-N, SCOD, disintegration rate of organic matter, and ATP value from the sterilization sludge was estimated to determine the solubilization rates of organic solids in the sludge. The optimal condition for the four different kinds of sludge pretreatment time was assessed according to maximal hydrogen yield at each time point. Anaerobic digester sludge was also obtained from the wastewater treatment plant, and was used as seed sludge. The total solids (TS), VS, and pH of the digested sludge were 0.8%, 0.5%, and 7.2, respectively. In order to inactivate the hydrogen-utilizing bacteria and harvest hydrogen-producing bacteria, the digested sludge was heat treated at 100 ℃ for 15 min [14]. The 200 ml of heat-treated sludge was mixed with glucose (8250 mg/l) containing medium, and cultured at 55 ℃ for 24 hours [15]. The medium contained 11 inorganic supplements (mg/l): NH_4Cl 1300, KH_2PO_4 250, $MgCl_2 \cdot 6H_2O$ 125, $FeSO_4 \cdot 7H_2O$ 5, $ZnCl_2$ 0.5, $NiCl_2 \cdot 6H_2O$ 0.5, H_3BO_4 0.5, $NaMoO_4 \cdot 2H_2O$ 0.5, $MnCl_2 \cdot 6H_2O$ 2.5, KI 2.5, and $CoCl_2 \cdot 6H_2O$ 2.5. The mixed culture showed high glucose degradation efficiency of 92.4% and over 50.0% of hydrogen content in the biogas produced, but with no detectable methane. This concoction was used as the mixed cultures for batch experiments.

2.2. Biohydrogen production from sterilized sewage sludge

Batch experiments were performed in 250-ml anaerobic bottles (SIBATA, Tokyo, Japan). The sterilized sludge was used as the substrate to produce hydrogen. All reactors were filled with 135 ml of raw or sterilized sewage sludge and 15 ml of mixed cultures to obtain a working volume of 150 ml. Fermentation of only mixed cultures was used as a control. The initial pH was adjusted to 7.0 with 4N NaOH and 4N HCl during the hydrogen fermentation process. The reactors were sealed with rubber stoppers, and the air was purged with N_2 to produce anaerobic conditions. The batch experiments were performed at 55℃ for three days. The volatile fatty acids (VFAs) content, NH_4^+-N, SC, SCOD, and pH as well as the biogas concentration and composition were measured as performance indicators. All reactor experiments were carried out in triplicate and the average values are presented.

2.3. Analytical methods

The chemical oxygen demand (COD), SCOD, TS, and VS were measured in accordance with the standard methods [16]. Carbohydrate concentration was estimated by the phenol-sulfuric acid method with glucose as a standard solution [17]. A SevenGo proTM pH/Ion meter (SG8, METTLER TOLEDO) was used to determine the pH value. The concentration of NH_4^+-N was determined using an ion meter (TiN-9001, Toyo Chemical Laboratories Co., Ltd., Tokyo, Japan). The concentration of metabolites such as acetate, propionate, and butyrate was analyzed by HPLC (JASCO Co., Japan) equipped with a UV/VIS and a COSMOGEL 5C18-AR-II Packed Column (4.6 × 250 mm) at 40℃ using 20 mM phosphate buffer (pH 2.5) as the mobile phase. The sample was centrifuged at 10,000 rpm for 10 min, after which the supernatant was filtered (0.45 μm membrane) and the filtrate was immediately analyzed at a flow rate of 1.0 ml/min. The ATP value was measured using a Bac Titer-Glo™ Microbial Cell Viability Assay (Promega, USA). The composition of the biogas, including hydrogen, methane, and carbon dioxide, was determined by gas chromatography (GC-8A, SHIMADZU, Japan) using a thermal conductivity detector (TCD, 80℃) and a Porapak Q column (60℃) with N_2 as the carrier gas. Biogas was collected in 50-ml plastic syringes, and volumes were directly read by the scale on the syringes. The effect of amount of each substrate added was evaluated based on the gas contents of the gas produced. The VS removal efficiency was calculated by weight loss of VS values at the end of the hydrogen fermentation, relative to the starting content.

3. Results and Discussion

3.1. Characteristics of the sterilized sludge

Physicochemical characteristics of the raw sewage sludge and sterilized sewage sludges were analyzed prior to their use in hydrogen fermentation experiments. The results obtained for the raw and sterilization sludges are shown in Table 1. The raw sludge contained 2.01% TS, 1.59% VS, 0.579 μmol/l ATP, 3341 mg/l total carbohydrate (TC), 59 mg/l SC, 12715 mg/l total chemical oxygen demand (TCOD), and 1725 mg/l SCOD. In all sterilized sludges, the TS and VS decreased with increasing sterilization times, relative to initial content of TS and VS in the raw sludge. The 60 min sterilized sludge showed the highest disintegration rate of 21.9% (TS) and 23.9% (VS), followed by the sterilized sludge for 45 min of 19.9% (TS) and 22.0% (VS). TS and VS of the sterilized sludge for 30 min showed a disintegration rate of 19.4% and 21.4%, respectively. For the 15 min sterilized sludge, the disintegration rate of TS and VS was only 11.9% and 13.2%, respectively.

In contrast, the SCOD, SC, NH_4^+-N, and total volatile fatty acids (TVFAs) of all the sterilized sludges increased with the sterilization times. The SCOD concentration for sterilized sludges was found to be similar among the 30, 45 and 60 min sterilization times, but lower in the 15 min sterliization (Table 1). The highest concentration of SCOD (3353 mg/l), SC (422 mg/l), NH_4^+-N (1001 mg/l), and TVFAs (1249 mg/l) was obtained with the 60 min sterilized sludge. From Table 1, it can be seen that the main components of SCOD in sterilized sludge are carbohydrates, NH_4^+-N, and VFAs. This data suggests that organic matters in the sewage sludge are released from microbial cells, and these insoluble solid organics will be converted into soluble substances [13]. Compared to an initial SCOD concentration of 1725 mg/l in the raw sludge, the SCOD concentration in sterilized sludges increased 1.2 to 1.9 times with different sterilization times. This is in agreement with the result of Feng [18], who reported a 2.7 times increase in SCOD concentration from pretreated sludge by ultrasonic treatment. Moreover, there was only a small increase in ammonia amounts

from sterilized (autoclave treatment) sewage sludge compared to ammonia in the raw sludge. It is possible that the nitric organics, such as proteins and nucleic acid, were hardly degraded by heat-shock stress into smaller soluble molecular components [19]. Feng et al. [20] also reported a small amount of NH_4^+-N increase from the ultrasonic pretreated sludge.

Table 1: Composition of raw sewage sludge and sterilized sewage sludges.

Parameters	Raw sewage sludge	Sterilized sewage sludges			
		15 min	30 min	45 min	60 min
Total solids (TS, %)	2.01	1.77	1.62	1.61	1.57
Volatile solids (VS, %)	1.59	1.38	1.25	1.24	1.21
Total chemical oxygen demand (TCOD, mg/l)	12715	-	-	-	-
Total carbohydrate (TC, mg/l)	3341	-	-	-	-
Soluble chemical oxygen demand (SCOD, mg/l)	1725	2035	3038	3248	3353
Soluble carbohydrate (SC, mg/l)	59	257	341	388	422
Total volatile fatty acids (TVFAs, mg/l)	865	962	1021	1182	1249
Ammonia nitrogen (NH_4^+-N, mg/l)	780	804	901	992	1001
ATP value (μmol/l)	0.579	0.009	0.004	0.002	0.000
pH	6.4	6.4	6.4	6.4	6.4

The percentages were calculated on the basis of dry weight.

The microbial quantity and activity can be gauged by the ATP value (concentration), which is a good indicator of metabolically active cells and an index of microbial density in anaerobic digestion [14]. Therefore, we measured the ATP concentration in raw and sterilized sludges. Theoretically, large numbers of microorganisms would contribute to a higher ATP concentration. In reality, the ATP concentration of the raw sludge was much higher than that of the sterilized sludges. After sterilization, the ATP concentration sharply decreased. Moreover, in the 60 min sterilized sludge experiment, the ATP value was zero (Table 1). It can be inferred that not only the hydrogen-consuming bacteria (methanogens), but also the endospores form microorganisms such as acid-forming bacteria and hydrogen-producing bacteria were effectively suppressed under longer sterilization times [21]. Sterilized sewage sludge could also improve the anaerobic digestibility because of an increase in the soluble carbon and nitrogen after the heat-shocking pretreatment process [8]-[23]. Based on the above result, further experiments from the sterilized sludge as substrate by mixed cultures for biohydrogen production were conducted through anaerobic digestion.

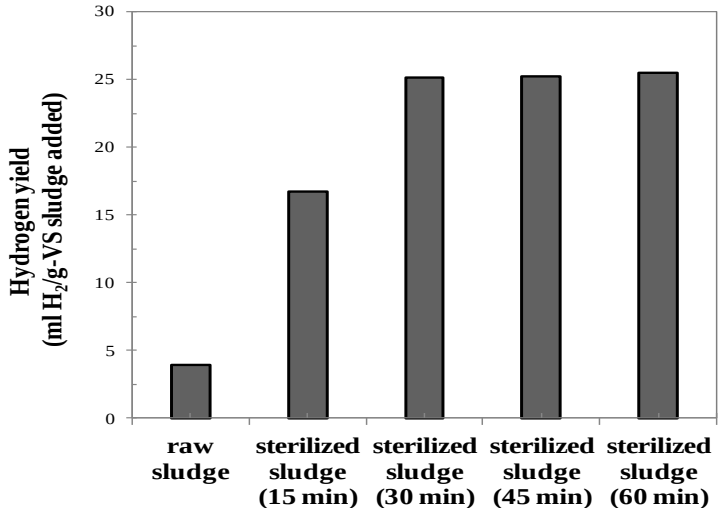

Fig. 1: Hydrogen yields from the raw sludge and sterilized sludges at 121 ℃ by mixed cultures.

3.2. Effect of sterile sludges on biohydrogen production

The hydrogen yield from raw sludge and four different kinds of sterilized sludges is shown in Fig. 1. Biohydrogen production from all sterilized sludges increased with sterilization time. The sewage sludge sterilized at 121 ℃ for 60 min gave the highest hydrogen yield (25.5 ml H_2/g-VS sludge added) during three days. The maximal hydrogen yield on day three from sterilized sludge for 45 min, 30 min, and 15 min was 25.2 ml H_2/g-VS, 25.1 ml H_2/g-VS, and 16.8 ml H_2/g-VS, respectively. For comparison, the raw sludge as the substrate was investigated for its ability to produce hydrogen. Under this scenario, the daily hydrogen production was negligible, and the maximal hydrogen yield obtained was only 3.9 ml H_2/g-VS sludge added on day three. When raw sludge was used as the substrate, hydrogen content decreased on a daily basis, while the methane content increased during the fermentation period (data not shown). This result clearly demonstrated the negative effects of raw sewage sludge on hydrogen fermentation performance.

Accordingly, the maximum hydrogen yields from the sterilized sludges for 15, 30, 45, and 60 min was about 4.3 to 6.5 times higher than that of the raw sludge (Fig. 1). Kang et al. [24] reported that during the fermentation of pretreated (heat or alkaline) sludge, there was a significant increase in biohydrogen production. Xiao and Liu [13] showed that maximal hydrogen yield of the sterilized and raw sludges by anaerobic self-fermentation without any extra-seeds was 16.26 ml H_2/g-VS and 0.35 ml H_2/g-VS, respectively. Contrastingly, those yields [13] were markedly lower than the results obtained in this study from the sterilized sludges (16.8-25.5 ml H_2/g-VS) and raw sludge (3.9 ml H_2/g-VS) using mixed cultures. This phenomenon may be attributed to the higher biomass used in cultivation of the mixed cultures in this study [25], [26]. As shown in Fig. 1 and Table 1, maximal hydrogen yield and solubilization rate for sludge treated for the longest duration (60 min) was almost equal to those of treated sludge for 30 and 45 min. These results indicate toward an optimal condition of 30 min heat-shock pretreatment of sewage sludge for enhanced hydrogen production. Under optimum conditions, the VS of solubilization and anaerobic fermentation process at the end of the batch experiment was reduced by 21.4% (Table 1) and 20.0% (Table 2), respectively, relative to the starting content. This means that the total VS removal efficiency was 41.4%, which was 1.5 times higher than the value obtained with the raw sludge in anaerobic fermentation process, and which could be attributed to the breakdown of organic solids in sludge during the solubilization.

Table 2: Concentration of the soluble substances and VS removal at the end of batch experiments.

Factors	Raw sewage sludge	Sterilized sewage sludge (30 min)
Acetate (%)	13.9	33.5
Propionate (%)	86.1	20.3
Butyrate (%)	0.0	46.2
TVFAs (mg/l)	548	1659
Carbohydrates (mg/l)	32	284
NH_4^+-N (mg/l)	1521	1878
SCOD (mg/l)	2224	3923
VS removal efficiency (%)	27.1	20.0

3.3. Biological context evaluation

Table 2 summarizes the obtained soluble substances at the end of the batch tests from the raw sludge or sterilized sludge (30 min) using mixed cultures. The SCOD of raw sludge and sterilized sludge was 2224 mg/l and 3923 mg/l, respectively. The SCOD of both the sludge samples increased, and this is due to the hydrolysis of sludge in the anaerobic fermentation process. In the sterilized sludge case, major SCOD was metabolites (1659 mg/l), NH_4^+-N (1878 mg/l), and carbohydrates (284 mg/l). In the case of raw sludge, the SCOD consisted mainly of NH_4^+-N (1521 mg/l), metabolites (548 mg/l), and carbohydrates (32 mg/l). It was suggested that the hardly degradable microbial cell walls in sludge were broken up by sterilization treatment into organic substances (Table 1). The organic substances were then more easily hydrolyzed and/or

converted into additional SCOD compared to the raw sludge in anaerobic digestion process, thereby increasing the SCOD concentration [27].

In general, biohydrogen production is accompanied with production of metabolites, such as VFAs [28]. Hence, the variation of VFAs in the fermentation process is a useful indicator, especially the butyrate/acetate (B/A) ratio, for monitoring biohydrogen production in the thermophilic fermentation process [29]. The results shown in Table 2 indicate that approximately 3 times more VFAs were produced with the sterilized sludge (30 min) in comparison with the raw sludge. High concentration of acetate (33.5%) and butyrate (46.2%) were observed when the pH was in the range of 6.0-6.3 in the sterilized sludge (30 min) during the fermentation period. The results indicate that VFAs accumulate during the fermentation process, and are not converted into methane. This might be one of the reasons for a decline in the overall pH of the sterilized sludge. In this case, the B/A ratio was approximately 1.4. This correlates to a higher yield for biohydrogen production with higher butyrate content [30]. This result indicates that the sterilized sludge by heat-shock treatment using mixed culture might increase biohydrogen production via the butyrate and acetate fermentation pathway [29]. Zhu and Béland [31] also reported that the butyrate concentration was much higher than acetate concentration from sucrose medium using pretreated digested wastewater sludge by heat-shock, acid, base, and aeration for producing hydrogen. On the other hand, a higher concentration of propionate (86.1%) along with relatively lower concentration of acetate (13.9%), and lack of butyrate was found in the pH range of 7.0-7.2 in the raw sludge throughout the experiment. This could be due to the utilization of VFAs for biogas production (mostly carbon dioxide and methane) resulting in a lower VFAs value along with higher NH_4^+-N value and a simultaneous increase in pH (data not shown). As a result, the change in pH and SCOD including VFAs and NH_4^+-N during the fermentation period is a significant factor for efficient biohydrogen production under the thermophilic anaerobic conditions.

4. Conclusion

In this study, we demonstrate the enhanced solubilization of insoluble matters into soluble organics in the sewage sludge by sterilization in an autoclave (121 ℃ and 1.2 atm) with different time periods (15 to 60 min), along with a subsequent improvement in hydrogen productivity. Results showed that longer treatment time is efficient for improving the hydrolyzability of organic mattes in the sludge. All sterilized sludges did not produce methane in the biogas throughout the experiment, while the raw sludge produced methane throughout the experiment. Compared with the raw sludge, the sterilized sludge significantly enhanced the biohydrogen production by mixed cultures upon anaerobic digestion. However, this was not the case with sterilized sludges for 30, 45, and 60 min. The 30 min sterilized sludge presented the optimum condition, resulting in the maximum hydrogen yield (25.1 ml H_2/g-VS sludge added) and highest hydrogen content (60.0%). Under the same conditions, overall VS reduction (solubilization and anaerobic process) was 41.4%, which was 1.5 times higher than the value obtained with the raw sludge. This was attributed to the destruction of solids in sludge during the solubilization.

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