Simultaneous Organic Solids Disintegration and Fermentative Hydrogen Production by Pretreated Sludge Generated from Wastewater Treatment Plant

Mijung Kim, Sechang Oh, Randeep Rakwal, Chunguang Liu, and Zhenya Zhang

***Abstract*—This study examines fermentative hydrogen production from untreated (raw) sludge and pretreated sludge by sterilization treatment (15, 30, 45, and 60 min) as substrate using mixed cultures in batch experiments under anaerobic thermophilic conditions. Longer treatment time was found to be highly effective for hydrolyzing organic matters in the sludge. Soluble chemical oxygen demand (SCOD) of pretreated sludge was 1.2 to 1.9-fold higher than that of untreated sludge. Sterilization treatment was found to accelerate and increase hydrogen production throughout the batch mode, but with no measurable methane production. Pretreated (30 min) sludge presented an optimal condition, resulting in maximum hydrogen yield (25.1 ml H_2/g-VS) and the highest hydrogen content (60.0%). Under the same conditions, enhanced hydrogen yield was 6.4-fold higher, which came with an additional benefit of efficient VS removal over the use of untreated sludge. This was attributed to destruction of solids in sludge during the solubilization process. Present findings have potential practical use in not only processes for efficient hydrogen production via anaerobic fermentation but also in waste treatment.**

***Index Terms*—Sewage sludge, sterilization treatment, hydrogen production, mixed cultures, anaerobic fermentation.**

I. Introduction

Worldwide, huge amounts of sewage sludge are discharged yearly. The sludge's are the organic waste generated from various stages of wastewater treatment plant. This also translates to a high cost requirement for disposal of sewage sludge and reuse in reclaimed land or for making concrete. Therefore, it is very important that we search for potential energy saving approaches by utilizing sewage sludge prior to waste disposal for a sustainable future environmentally friendly society [1]. Sewage sludge is composed of 95% water and 1-5% solids. The solid part contains a diverse population of microbes, organic matter, nutrients (nitrogen and phosphorus), and trace elements [2]. Based on these characteristics, the sludge can be used as inoculum and substrate for producing biogas (methane) through anaerobic digestion – a most commonly used process for sludge stabilization [1], [3].

Anaerobic digestion is widely used for sludge stabilization, as well as methane production using anaerobic microorganisms [4]. Despite the above benefits, a long sludge retention time is required for reducing the organic matter and producing methane by anaerobic digestion. Even after long periods (30-60 days) of digestion, the sludge degradation rate is low, due to presence of refractory materials that are hard to break during the anaerobic digestion period [5]. To counteract this disadvantage, several pretreatment methods, namely acid [6] alkali [7], thermal [8], ultrasonic [9], and microwave [10] have been proposed for dissolving organic solids in sewage sludge prior to anaerobic digestion. Eskicioglu *et al.* [11] reported an 31% increase in methane production from microwave pretreated sewage sludge. Li *et al.* [5] demonstrated alkaline pretreatment of sewage sludge prior to anaerobic digestion substantially increased the degradation rate of organic substances with the help of NaOH hydrolysis, and which resulted in a significant increase in the production of methane.

In the 21st century, biological hydrogen has attracted attention as a biofuel for the future. This is because hydrogen – a clean energy – can be directly used in fuel cells to generate electricity [1], [12]. Furthermore, hydrogen – an intermediary metabolite of anaerobic fermentation – is produced independently during the anaerobic digestion process [13]. Nevertheless, fermentative hydrogen production from sewage sludge has not been well investigated, primarily due to natural characteristics of low biodegradability that results in lower hydrogen yield [14]. Previous researches have reported improvement in fermentative hydrogen production by heat or sterilization methods. For example, Massanet-Nicolau et al. [3] found that pretreatment of sewage sludge by heat (70°C) and enzyme prior to fermentation significantly boosted the hydrogen yield. Xiao and Liu [15] demonstrated sewage sludge sterilization at 121°C consistently improved hydrogen production; hydrogen consumption still occurred in anaerobic self-fermentation of imperfectly sterilized sludge due to existence of other hydrogen-consuming bacteria.

With these backgrounds, a sterilization pretreatment step was added to the sewage sludge in this study in order to establish an effective method for sludge solubilization. The main aim of this study was to assess the feasibility of hydrogen production from pretreated sludge as a substrate by mixed cultures including hydrogen-producing bacteria, without pH control and additional nutrients under anaerobic thermophilic conditions, in order to i) determine the effects of various sterilization times on sewage sludge for organic solids disintegration and microbial activity, ii) compare the

Manuscript received June 9, 2013; revised August 18, 2013.

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DOI: 10.7763/IJESD.2013.V4.425

effects of untreated sludge and pretreated sludge by sterilization treatment for fermentative hydrogen production, and iii) investigate 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.

II. Materials and Methods

A. Raw Materials

The substrate sewage sludge was obtained from a gravity sludge thickener line at the wastewater treatment plant in Ibaraki prefecture (Japan), and was stored at 4°C. 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. Anaerobic digester sludge was also taken 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.

B. Preparation of Hydrogen Producing Inocula

Hydrogen producing inocula were cultivated in a 250-ml of anaerobic bottle (SIBATA, Tokyo, Japan) using digested sludge as the original source of microorganisms and glucose medium was used as the substrate [16]. In order to inactivate hydrogen-utilizing bacteria and harvest hydrogen-producing bacteria, the digested sludge was heat treated at 100°C for 15 min [17]. The 200 ml of heat-treated sludge was mixed with glucose (8250 mg/l) containing medium, and cultured at 55°C for 24 hours [16]. 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 produced biogas, but without any detectable methane. This concoction was used as the mixed cultures for batch experiments.

C. Pretreatment Experiments

Sewage sludge was pretreated by sterilization treatment in an autoclave. The sewage sludge was sterilized for 15 min, 30 min, 45 min, or 60 min at a temperature of 121°C and pressure of 1.2 atm, followed by its use as a substrate for fermentative hydrogen production for determining 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 were estimated to determine solubilization rates of organic solids in the sludge. The optimal condition for four different kinds of sludge pretreatment time was assessed according to maximum hydrogen yield at each time point.

D. Fermentative Hydrogen Production from Untreated or Pretreated Sludge

Batch experiments were performed in 250-ml anaerobic bottles (SIBATA, Tokyo, Japan). The substrate to produce hydrogen was pretreated sludge by sterilization treatment. All reactors were filled with 135 ml of untreated or pretreated sludge and 15 ml of mixed cultures to obtain a working volume of 150 ml. Fermentation of only mixed cultures was used as the control. The initial pH was adjusted to 7.0 with 4N NaOH and 4N HCl during the hydrogen fermentation process. Rubber stoppers were used to seal the reactors, and the air was purged with N_2 to produce anaerobic conditions. The batch experiments were performed at 55°C for three days without any pH control. Performance indicators measured were – volatile fatty acids (VFAs) content, NH_4^+-N, SC, SCOD, and pH as well as the biogas concentration and composition. All reactor experiments were carried out in triplicate and the average values are presented.

E. Analytical Methods

Chemical oxygen demand (COD), SCOD, TS, and VS were measured in accordance with the standard methods [18]. Carbohydrate concentration was estimated by the phenol-sulfuric acid method with glucose as a standard solution [19]. The pH value was measured using a SevenGo proTM pH/Ion meter (SG8, METTLER TOLEDO). Concentration of NH_4^+-N was determined using an ion meter (TiN-9001, Toyo Chemical Laboratories Co., Ltd., Tokyo, Japan). The concentrations of metabolites such as acetate, propionate, and butyrate were 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°C 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 using a 0.45 μm membrane, and the filtrate was immediately analyzed at a flow rate of 1.0 ml/min. The ATP concentration 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°C) and a Porapak Q column (60°C) with nitrogen 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 feedstock added was evaluated based on gas contents of the gas produced. The hydrogen yield was calculated as follows:

$$Y_{H_2} = (V_1 - V_2)/M \tag{1}$$

where Y_{H_2} (ml/g-VS sludge added) is the hydrogen yield; V_1 (ml) is the cumulative hydrogen production from sludge samples during fermentation period; V_2 (ml) is the cumulative hydrogen production from control during fermentation period; M (g) is the VS (sludge) added. The VS removal efficiency was calculated by weight loss of VS values at the end of the hydrogen fermentation, relative to the starting content.

III. Results and Discussion

A. Characteristics of the Untreated or Pretreated Sludge

Physicochemical characteristics of the untreated (raw) sludge and pretreated sludge's by sterilization treatment were analyzed before being used in hydrogen fermentation experiments. Results for the untreated sludge and pretreated sludge's are shown in Table I. The untreated sludge contained 2.01% TS, 1.59% VS, 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 pretreated sludge's, TS and VS of the sludge decreased with increasing sterilization times, relative to initial content of TS and VS in the untreated sludge. Pretreated sludge for 60 min showed the highest disintegration rate of 21.9% (TS) and 23.9% (VS), followed by the pretreated sludge for 45 min of 19.9% (TS) and 22.0% (VS). TS and VS of the pretreated sludge for 30 min were disintegrated by 19.4% and 21.4%, respectively. For the pretreated sludge for 15 min, the disintegration rate of TS and VS was only 11.9% and 13.2%, respectively. In contrast, SCOD, SC, NH_4^+-N, and total volatile fatty acids (TVFAs) of all the pretreated sludge's increased with sterilization times. The SCOD concentration for pretreated sludge's is quite similar among 30 min, 45 min and 60 min sterilization times (Table I). However, SCOD concentration of pretreated sludge (15 min) was lower than that obtained with the three pretreated sludge's (30, 45, and 60 min). The highest concentrations of SCOD (3353 mg/l), SC (422 mg/l), NH_4^+-N (1001 mg/l), and TVFAs (1249 mg/l) were obtained for the 60 min pretreated sludge.

TABLE I: THE CHARACTERISTICS OF SEWAGE SLUDGE BEFORE AND AFTER PRETREATMENT

Factors	Untreated sludge	Pretreated sludge with sterilization treatment Pretreatment time (min)			
		15	30	45	60
TS (%)	2.01	1.77	1.62	1.61	1.57
VS (%)	1.59	1.38	1.25	1.24	1.21
TCOD (mg/l)	12715	ND	ND	ND	ND
SCOD (mg/l)	1725	1828	3038	3248	3353
TC (mg/l)	3341	3076	2928	2829	2811
SC (mg/l)	59	257	341	388	422
NH_4^+-N (mg/l)	780	804	901	992	1001
TVFAs (mg/l)	865	1004	1802	2245	1980
pH	6.4	6.4	6.4	6.4	6.4

TS, total solids; VS, volatile solids; TCOD, total chemical oxygen demand; SCOD, soluble chemical oxygen demand; TC, total carbohydrate; SC, Soluble carbohydrate; NH_4^+-N, ammonia nitrogen; TVFAs, total volatile fatty acids. ND, not determined. The percentages were calculated on the basis of dry weight.

Table I shows that the main components of SCOD in pretreated sludge are carbohydrates, NH_4^+-N, and VFAs. This data implies that organic matters in sewage sludge were released from microbial cells, and subsequently these insoluble solid organics are converted into soluble substances [15]. Compared to an initial SCOD concentration of 1725 mg/l in untreated sewage sludge, SCOD concentration in pretreated sludge's increased 1.2 to 1.9-fold with different sterilization times. This is in agreement with the result of Feng [20], who showed a 2.7-fold increase in SCOD concentration from pretreated sludge by ultrasonic treatment. Moreover, there was only a small increase in ammonia amounts from pretreated (autoclave treatment) sludge compared to ammonia concentration in untreated sludge. Feng et al. [21] also reported a small amount of NH_4^+-N increase from the ultrasonic pretreated sludge. It is likely that the nitric organics, such as proteins and nucleic acid, were hardly degraded by heat-shock treatment into smaller soluble molecular components [22].

B. Microorganism Activity

The microbial quantity and activity can be gauged by the ATP concentration value, a good indicator of metabolically active cells and an index of microbial density in anaerobic fermentation [17], [23]. Hence, the ATP concentration was measured in untreated and pretreated sludge's by sterilization treatment. Theoretically, large numbers (concentration) of microorganisms would contribute to a higher ATP concentration. In reality, ATP concentration of the untreated sludge (0.579 μmol/l ATP value), was much higher than that of the pretreated sludge's (Fig. 1). Post-sterilization treatment, the ATP concentration sharply decreased. Moreover, in the 60 min pretreated sludge, the ATP value was zero. It can be inferred that not only hydrogen-consuming bacteria (mostly methanogens), but also endospores from microorganisms such as hydrogen-producing bacteria and acid-forming bacteria are effectively suppressed under longer sterilization times [24]. Sterilization treatment of pretreated sludge could also improve anaerobic digestibility due to an increase in the soluble carbon and nitrogen after the pretreatment process [9, 25, 26]. Based on the above result, further experiments from the pretreated sludge as substrate by mixed cultures for hydrogen production were conducted through anaerobic fermentation.

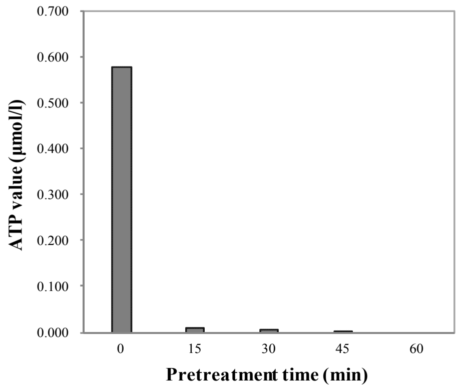

Fig. 1. ATP Values in the untreated sludge and pretreated sludge's by sterilization treatment.

C. Biogas Production from Untreated or Pretreated Sludge by Mixed Cultures

Fig. 2 shows the change in accumulated biogas and corresponding biogas contents (hydrogen and methane) in the reactors with untreated sludge or four different kinds of pretreated sludge's by sterilization treatment. Cumulative biogas production during three days from untreated sludge and pretreated sludge's for 15 min, 30 min, 45 min, and 60 min was 104 ml, 61 ml, 78 ml, 85 ml, and 86 ml, respectively (Fig. 2a). The control reactor containing mixed cultures without additives did not generate any biogas. Biogas production in the pretreated sludge's significantly increased till 24 h. After 24 hrs, biogas production only slightly increased till the end of the experiments (Fig. 2a). This demonstrated that cultivating mixed cultures, which include hydrogen-producing bacteria, could readily use soluble organics (carbon and nitrogen) in the sterilization sludge, resulting in rapid hydrogen generation [27, 28]. In addition, hydrogen content in the pretreated sludge's was observed between the range of 28.2% and 60.0% (Fig. 2b) with no methane detected (Fig. 2c) during the fermentation period. In contrast, biogas production from untreated sludge using mixed cultures increased gradually (Fig. 2a), and the methane content increased slightly from 15.9% to 27.0% (Fig. 2c) during the fermentation period. However, hydrogen content decreased significantly from a peak at 8 h (16.1% of the biogas) to almost negligible levels after 48 h (Fig. 2b). A possible reason may lie in the presence of a variety of microorganisms, including hydrogen-consuming bacteria such as methanogens in the untreated sludge [29].

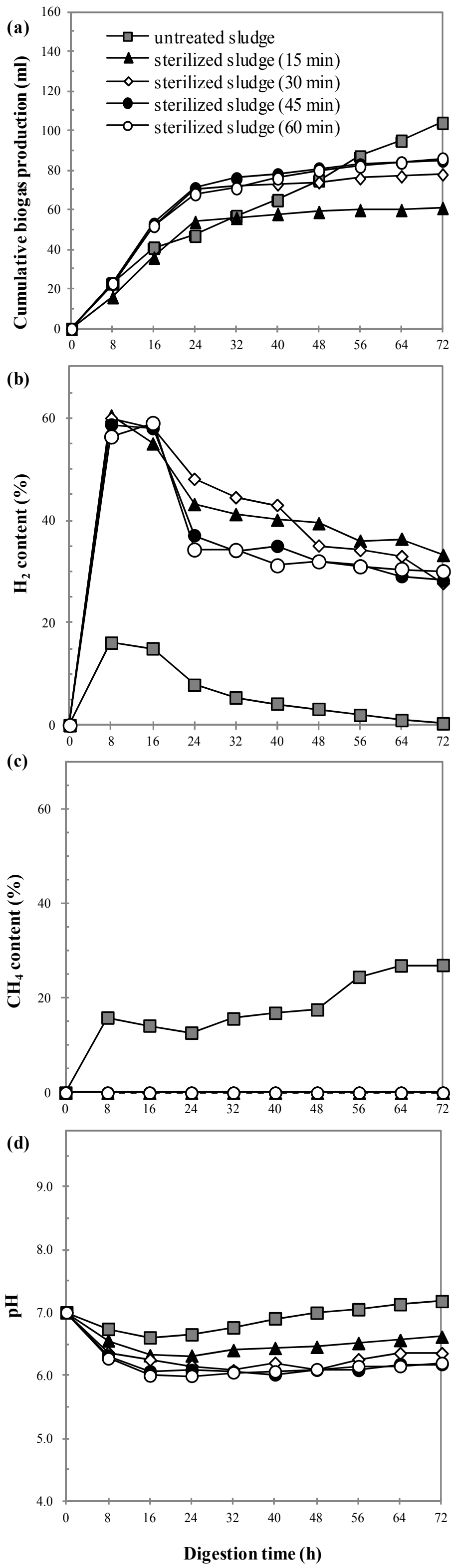

Fig. 2. Cumulative biogas production (a), contents of the H_2 (b) and CH_4 (c) and daily variations in pH (d) for the five bioreactors during the fermentation period.

These microorganisms would aid in the consumption of hydrogen along with a generation of methane and carbon dioxide. Nonetheless, pretreatment of sludge via sterilization is a feasible substrate for hydrogen production using mixed anaerobic cultures.

D. Effect of Untreated or Pretreated Sludge on Fermentative Hydrogen Production

Hydrogen yield (calculated from equation 1) from the untreated sludge and four different kinds of pretreated sludge's by sterilization treatment is shown in Table II. Hydrogen production from all pretreated sludge's increased with sterilization time. The sewage sludge pretreated at 121°C for 60 min gave highest hydrogen yield (25.5 ml H_2/g-VS sludge added) during three days. The maximum hydrogen yield on day three from pretreated 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, untreated sludge as the substrate was investigated in its ability to produce hydrogen. Under this scenario, daily hydrogen production was negligible, and the maximum hydrogen yield obtained was only 3.9 ml H_2/g-VS sludge added on day three. When untreated sludge was used as substrate, hydrogen content decreased on a daily basis (Fig. 2b), while the methane content increased (Fig. 2c) during the fermentation period. This result clearly demonstrated the negative effects of untreated sludge on hydrogen fermentation performance. Consequently, maximum hydrogen yields from the pretreated sludge's for 15 min, 30 min, 45 min, and 60 min was about 4.3 to 6.5-fold higher than that of the untreated sludge. Kang et al. [30] reported a significant increase in fermentative hydrogen production during the fermentation of pretreated (heat or alkaline) sludge.

TABLE II: HYDROGEN YIELDS FROM THE UNTREATED SLUDGE AND STERILIZED SLUDGE'S AT 121°C BY MIXED CULTURES

Pretreatment time (min)	0	15	30	45	60
Maximum H_2 yield (ml H_2/g-VS sludge added)	3.9	16.9	25.1	25.3	25.6

Xiao and Liu [15] reported the maximum hydrogen yields of sterilized sludge and raw sludge by anaerobic self-fermentation as 16.26 ml H_2/g-VS and 0.35 ml H_2/g-VS, respectively. Contrastingly, those yields [15] were markedly lower than the results obtained in this study from the pretreated sludge's (16.8-25.5 ml H_2/g-VS) and untreated sludge (3.9 ml H_2/g-VS) using mixed cultures. This phenomenon may be attributed to higher biomass used in cultivation of mixed cultures in this study [31], [32]. As shown in Table II and Table I, the maximum hydrogen yield and solubilization rate for sludge treated for the longest duration (60 min) was almost equal to those of treated sludge for 30 min and 45 min. These results indicated an optimal condition of 30 min sterilization 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 were reduced by 21.4% and 20.0%, respectively, relative to the starting content (Fig. 3). This means that total VS removal efficiency was 41.4%, which was 1.5-fold higher than the value obtained with untreated sludge in anaerobic fermentation process, and which can be attributed to the breakdown of organic solids in sludge during the solubilization process.

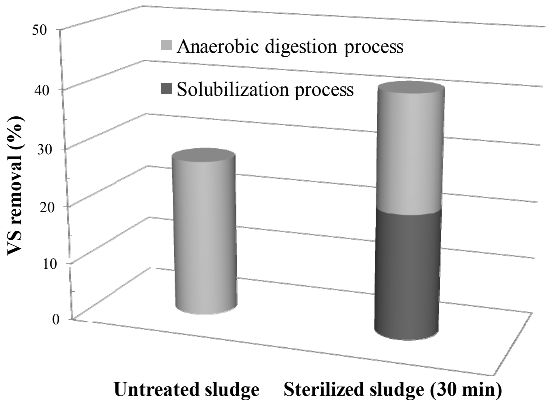

Fig. 3. VS removal efficiency in the untreated sludge and sterilized sludge.

E. Biological Context Evaluation

The change in pH is illustrated during the anaerobic fermentation process of untreated or pretreated sludge in Fig. 2d. In case of untreated sludge, the pH value first decreased slightly followed by an increase gradually from 7.0 to 7.2. This pH condition is not suitable for fermentative hydrogen production, but is rather a representative of the best condition for methanogens survival [27]. Indeed, a small amount of hydrogen was produced daily from untreated sludge by mixed culture throughout the experiment (Fig. 2). By contrast, the final pH value in all bioreactors with pretreated sludge as the substrate was lower than the initial pH of 7.0. In case of pretreated sludge's by sterilization treatment, the final pH ranged from 6.2 to 6.6. In addition, the pH in pretreated sludge's (30 min, 45 min, and 60 min) dropped sharply from the start (7.0) to 16 h (6.0-6.2), and then remained between 6.0 and 6.4 until end of the batch experiment. One possible reason for this pH variation tendency may be that the degradation of organic matters like carbohydrates in sludge coincided with the production of VFAs [33]. After 16 hrs, it is possible that the proteins in sludge were degraded resulting in the release of NH_4^+-N with time. Hence, a slight increase in pH was seen. The NH_4^+-N can neutralize organic acids produced in the anaerobic process [34].

Table III summarizes obtained soluble substances at end of the batch experiments from the untreated or pretreated sludge (sterilization treatment for 30 min) using mixed cultures. The SCOD of untreated sludge and pretreated sludge was 2224 mg/l and 3923 mg/l. The SCOD of both the sludge samples increased, and which is due to the hydrolysis of sludge in the anaerobic fermentation process. In the case of pretreated sludge, major SCOD was metabolites (1659 mg/l), NH_4^+-N (1878 mg/l), and carbohydrates (284 mg/l). In the case of untreated sludge, the SCOD consisted mainly of NH_4^+-N (1521 mg/l), metabolites (548 mg/l), and carbohydrates (32 mg/l). It was proposed that the hardly degradable microbial cell walls in sludge were broken up by sterilization treatment into organic substances. The organic substances were then easily hydrolyzed and/or converted into more SCOD compared to the untreated sludge in anaerobic digestion process, thereby increasing the SCOD concentration [35].

In general, the fermentative hydrogen production is accompanied with production of metabolites, such as VFAs [33, 36, 37]. Therefore, variation of VFAs in the fermentation process is a useful indicator, especially the butyrate/acetate (B/A) ratio, for monitoring hydrogen production in the thermophilic fermentation process [38]. As shown in Table III, approximately 3-fold more VFAs were produced with the sterilized sludge (30 min) in comparison with the untreated sludge. High concentrations 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 (Fig. 2d).

TABLE III: CONCENTRATION OF THE SOLUBLE SUBSTANCES AT THE END OF BATCH EXPERIMENTS

Factors	Untreated sludge	Sterilized sludge (30 min)
Acetate (%)	13.9	33.5
Propionate (%)	86.1	20.3
Butyrate (%)	0.0	46.2
TVFAs (mg/l)	548	1659
SC (mg/l)	32	284
NH_4^+-N (mg/l)	1521	1878
SCOD (mg/l)	2224	3923

Results indicate that VFAs accumulate during the fermentation process, and are not converted into methane in all the pretreated sludge's cases (Fig. 2c). This might be a reason for the observed decline in the overall pH of the sterilized sludge (Fig. 2d). In the case of pretreated sludge (30 min), the B/A ratio was approximately 1.4. This correlates to a higher yield for hydrogen production with higher butyrate content [39]. This result suggests that the pretreated sludge by sterilization treatment using mixed culture might increase hydrogen production via the butyrate and acetate fermentation pathway [38]. Zhu and Béland [40] also reported that 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 were observed in pH range of 7.0-7.2 in the untreated sludge throughout the experiment (Table III). This could be due to the utilization of VFAs for biogas production (mostly carbon dioxide and methane) resulting in the lower VFAs value along with higher NH_4^+-N concentration and a simultaneous increase in pH (Fig. 2d), thereby causing the overall hydrogen production to decline. This resulted in an increase in the pH, and finally to digester failure. As a result, the change in pH and SCOD including VFAs and NH_4^+-N during the fermentation period are very important factors for efficient hydrogen production under anaerobic thermophilic conditions.

ACKNOWLEDGMENT

Authors greatly appreciate Dr. Dong-Yeol Lee (Environmental Technology Team of the GS Engineering & Construction Co. Ltd.) for a critical reading of the paper and constructive comments therein.

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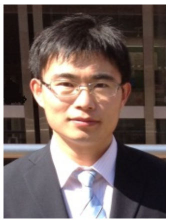

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