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Enhanced economic feasibility of excess sludge treatment: acid fermentation with biogas production

Mo-Kwon Lee^{1,2}, Yeo-Myeong Yun² and Dong-Hoon Kim^{1*}

Abstract

The excess sludge (ES) generated from wastewater treatment plants entails high expenditure for its treatment and disposal. In this study, firstly, the influence of mild-thermal (70 and 90 °C) and alkali-(pH 10 and 11) pretreatment methods on solubilization and acid generation from ES was investigated. The experimental results showed that the solubilization (SCOD/TCOD) increased as pretreatment intensity increased (70 °C < 90 °C < pH 10 < pH 11). However, organic acids generation was not consistent with the increased solubilization (pH 11 < 70 °C < 90 °C < pH 10). As a result of microbial analysis through next generation sequencing (NGS), it was observed that microbial community structure was greatly varied depending on the pretreatment methods. *Bacteroidetes* (70.8%), and *Firmicutes* (58.1%) were found to be dominant at thermal conditions of 90 °C and pH 10. Furthermore, the solid residue after acids generation was subjected to anaerobic digestion (AD) for CH₄ production. The economic assessment showed that the thermal pretreatment at 90 °C followed by acid recovery and AD process enhanced the net profit of the treatment process with a positive gain of 2.53 USD/ton of sludge. Meanwhile, the alkali-pretreatment at pH 11 showed a negative value of − 2.0 USD/ton of sludge.

Keywords: Anaerobic digestion, Excess sludge, Economic assessment, Organic acids, Solubilization

Introduction

The handling of excess sludge (ES) in municipal wastewater treatment plant is a huge economic burden, accounting for 30–40% of the total capital cost and 50% of plant operation cost [1, 2]. The reduction of sludge volume via anaerobic digestion (AD) is thus gaining a lot of attention, which converts organic wastes to CH₄-rich biogas, inactivates pathogens, and improves dewaterability of the sludge cake [3]. However, the digestion efficiency is often hindered by the low hydrolysis efficiency of ES. The main component of ES is microbial cells and their cell walls containing glycan strands, which cause resistance to biodegradation [4, 5]. To improve the digestion efficiency, various disintegration methods such as physical, chemical, thermal, alkali, microwave, and biological treatment or a combination of any two of these methods have been applied [6–12]. High-strength

of pretreatment warrants increased solubilization with enhanced digestion efficiency, but it could add an economic burden [13, 14].

On the other hand, valuable organic acids such as acetate, lactate, butyrate, and propionate can also be obtained during AD. They can be utilized as a substitute for methanol (0.45 USD/kg) that is commonly used as an extra carbon source in the denitrification process [15–17]. Organic acids are generated in the intermediate-stage of AD, which are finally converted to CH₄ unless methanogenesis is inhibited. Heat- and alkali-shock have often been used to inactivate methanogens in the seeding sources and ES. Yuan et al. [18] have reported that organic acids production from the ES was considerably improved under alkali condition (pH 10), and Xiao et al. [19] observed the 4.2 times higher organic acids production from heat-treated ES compared to raw sludge.

Without inoculum addition, ES can be self-fermented to organic acids under anaerobic condition. The indigenous bacteria such as *Clostridium*, *Anaerobranca*,

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Tissierella, and *Paludibacter* can degrade carbonaceous compounds in the sludge to various acids [20, 21]. This could be a simple process in terms of not requiring inoculum preparation and practical mean to conduct fermentation during the storage period. However, the organic acids yield is limited to less than 5%, and even the methanogenic activity that consuming produced acids has often been observed after a few days [19]. Therefore, pretreatment is essential, which can both increase organic acids generation and inhibit the methanogenic consumption. After producing acids, the remained solid residue obtained via liquid/solid separation can also be further digested to reduce sludge volume and gain CH_4 .

In the present study, three experimental scenarios were designed: 1). biogas production from raw ES by AD (S1), 2). biogas production from pretreated ES by AD (S2), and 3). organic acids generation from pretreated ES and biogas production from the solid residue (S3) (Fig. 1). Since a huge amount of energy consumption in the pretreatment would offset the beneficial effect, the pretreatment was applied in a mild range: the temperature at 70 °C and 90 °C, and pH at 10 and 11. In addition, the economic assessment was conducted using the experimental results. To our knowledge, this is the first report assessing the economic aspect of sludge pretreatments for AD with organic acids generation.

Results and discussion

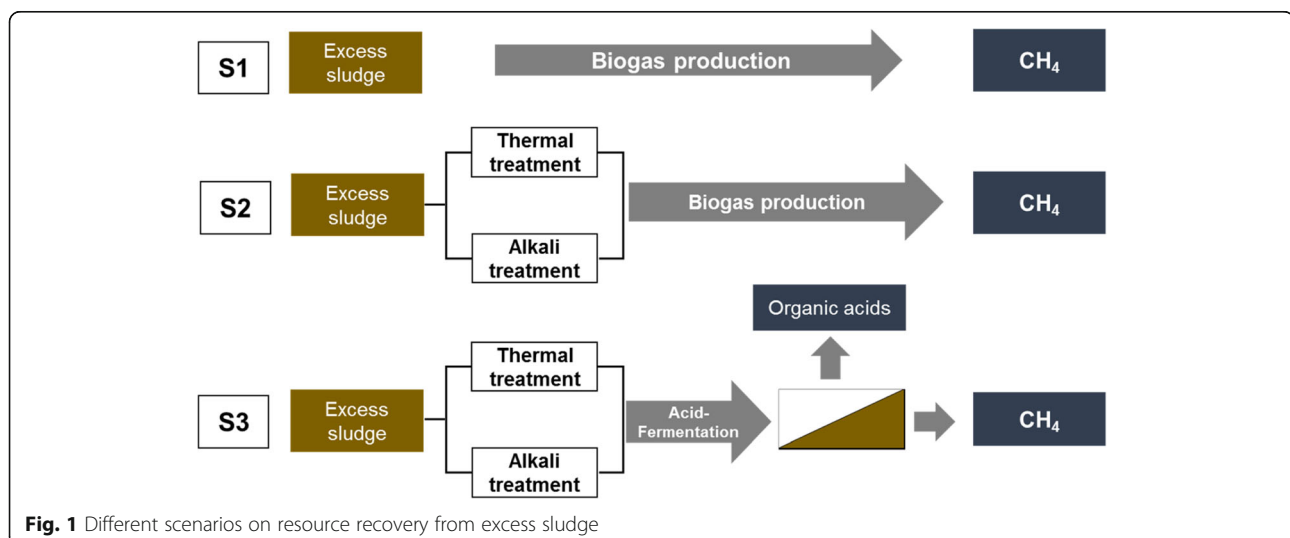
Effects of pretreatment

Soluble chemical oxygen demand (SCOD) is considered as the important factor to assess the bioavailability of the organic contents of the sludge [5]. As shown in Fig. 2, the soluble ratio of total chemical oxygen demand (TCOD) (SCOD/TCOD) increased after pretreatment. The highest solubilization ratio of

33.6% was observed from the alkali-pretreatment at pH 11. This value was similar to the report by Kavitha et al. [22], where 35% of sludge solubilization was observed under thermo-alkali-sonic pretreatment. In this study, the alkali pretreatment at pH 11 showed a maximum SCOD release of 8066 mg COD/L. On the other hand, alkali-pretreatment at pH 10 showed the moderate solubilization of 24.5% with a SCOD release of 5885 mg COD/L.

During the thermal pretreatment, which was operated for 30 min, an improvement in the ES solubilization ranging from 17.1 to 23.3% was observed. Similarly, Zhen et al. [23] reported that thermal pretreatment not only improved the sludge disintegration but also assisted in the removal of pathogen/odor. Such results are promising because of being obtained at a moderate temperature (70 and 90 °C). Contrary, most of the studies employed for the sludge disintegration via thermal pretreatment were studied in a higher temperature range of 121 to 210 °C, at which a limited impact of temperature on the sludge solubilization was harvested [24]. Moreover, these high temperatures led to the reduced sludge dewaterability with the generation of toxic substrates that hindered the sludge biodegradability [25]. Such drawbacks are overcome while applying moderate temperature-based pretreatment, which has additional cost advantages. Nevertheless, the latter requires more research in order to address the challenge of duration requested for effective solubilization.

The pretreated ES was subjected to acidogenic self-fermentation with an emphasis on organic acids generation and the results are shown in Fig. 3. It was found that organic acids' concentration decreased from 4.1 to 3.2 g COD/L by raising pretreatment pH from 10 to 11. Meanwhile, a contrary behavior was observed in the case of temperature increase from 70



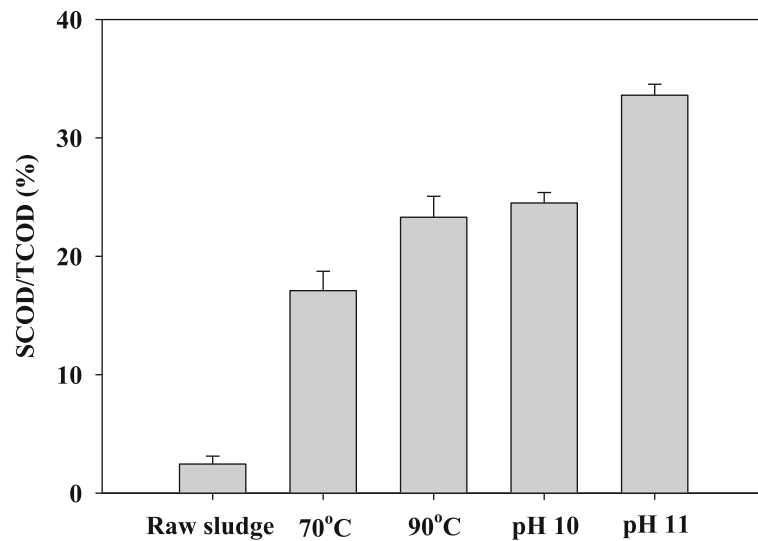


Fig. 2 The solubilization of excess sludge after pretreatment

to 90 °C, while acids' concentration was found to be augmented from 3.3 to 3.8 g COD/L. The maximum organic acids accumulation value was observed at pH 10. The main portions of produced organic acids were acetate (61–78%), propionate (8–11%), and butyrate (17–30%), which are known as the main byproducts of acidogenic bacteria [26–28]. The changes in the bacterial communities during acidogenic fermentation

affected the distribution of the organic acids accumulation and were summarized in the following section.

pH has a major influence on the organic acids production and their optimal range between 5.0 to 6.5 was essential to improve the organic acids production. However, in this study, no initial pH adjustment was made during the self-acidogenic fermentation in order to reduce the chemical costs and assess the practical

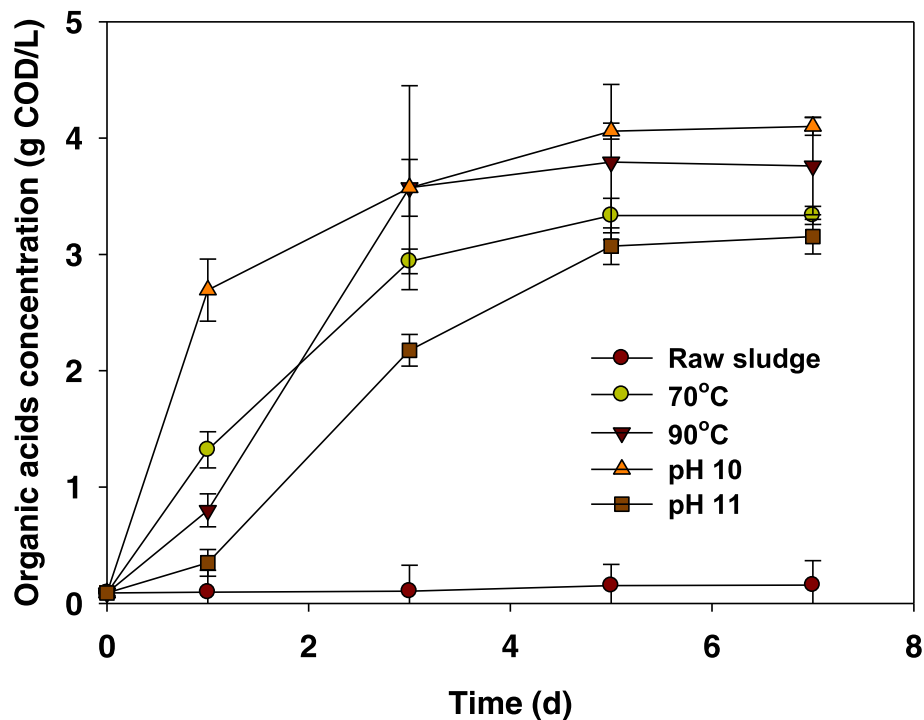


Fig. 3 Organic acids production profiles of pretreated sludges

applicability of the process. CH_4 production was detected after day 7 of the self-acidogenic fermentation, which inferred that long acclimatization time was required for the reactivation of methanogens after the heat and alkali-shock pretreatment [22]. Although pH 11 was effective in sludge solubilization, the acid accumulation was greatly affected than other conditions [27]. It seemed that high-alkali condition inhibits the acid-producing bacteria, resulting in the less production of organic acids [28]. After fermentation of 7 d, the pH value was observed in the range of 6.3 to 7.5 at the thermal and alkali conditions of pH 10. However, in case of pH 11, the pH value was slightly high over 8.9.

Microbial community

The microbial community analysis was performed via 454 pyrosequencing method to monitor the bacterial changes in the acidogenic fermentation of the pretreated ES. Figure 4 shows the microbial abundance at the phylum level distribution at the pretreated samples. The structure of the microbial community varied with pretreatment conditions. Most of the bacteria were classified as five major phyla (*Proteobacteria*, *Bacteroidetes*, *Firmicutes*, *Actinobacteria*, and *Fusobacteria*). *Proteobacteria* and *Bacteroidetes* were the major abundant in the control experiment (Raw-ES) and pH 11, which showed the lowest organic acids production. At 70 °C, 90 °C and pH 10 conditions, the dominance of *Firmicutes* was significantly increased to 13.9, 24.2, and 58.1%, respectively. The selective enrichment of the acid-producing

Firmicutes populations after the pretreatment step coincided with the observed increased proportion of the organic acids production. Also, as the thermal pretreatment intensity increased from 70 °C to 90 °C, *Bacteroidetes* was enriched to 30.8 and 70.8%, whereas it was decreased to 7–8% in the alkali pretreatment.

In order to further confirm the bacterial community, the genus level analysis was performed after removing the genus with low relative abundance (< 1%) (Table 1). *Parabacteroides* (19.5%) and *Clostridium* (6.3%), which are known as acetic acid producing bacteria and hydrogen producers, were slightly enriched at low temperature (70 °C) pretreatment and significantly increased at 90 °C to a value of 70.1 and 18.9%, respectively [29, 30]. This shows that variation within the thermal (70 and 90 °C) pretreatment affected the microbial groups and the sludge disintegration.

At the alkali-pretreatment condition of pH 10, the *Tissierella* and *Proteinclasticum* were the dominant bacterial group, accounting for 32.8 and 20.4% of the total number of microbial community. These genera seemed to play a major role in organic acids production. According to previous research, *Tissierella* was also involved in acetic acid and butyric acid production and *Proteinclasticum* was known to be involved in the hydrolysis of proteins and the production of acetic acid and butyric acid [30, 31]. At pH 11, the *Dechloromonas* was the major population occupying 41.3% while *Proteinclasticum* was found to be 2.0%. These results inferred that understanding microbial dynamics is essential for

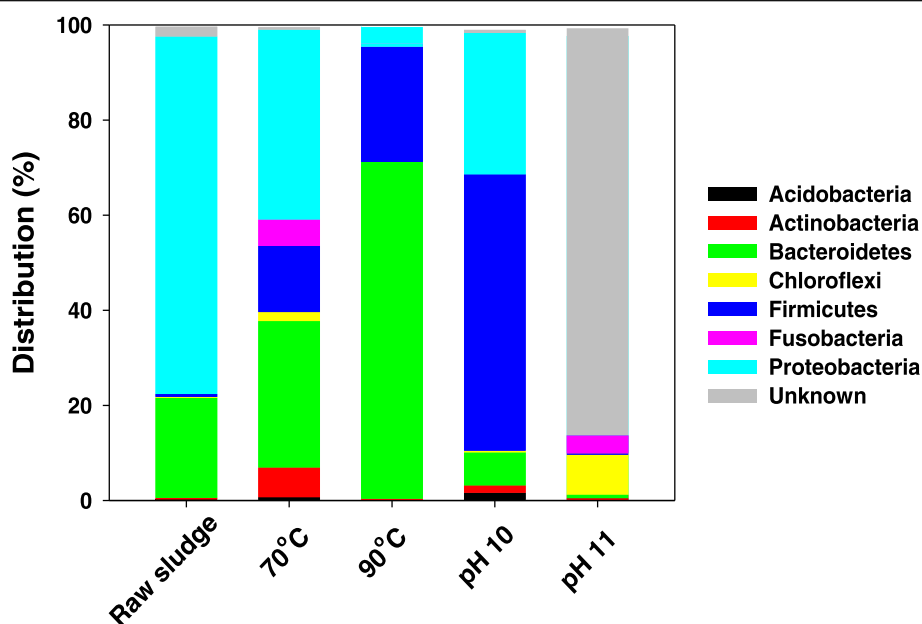


Fig. 4 Microbial community distribution at phylum level

Table 1 Phylogenetic classification of the dominant sequences (relative abundance > 1% at genus level) in the acidified broth of pretreated and raw ES

Phylum	Genus	Raw sludge	Thermal (70 °C)	Thermal (90 °C)	Alkali (pH 10)	Alkali (pH 11)
Proteobacteria	<i>Dechloromonas</i>	51.0	4.7	1.2	4.5	41.3
Bacteroidetes	<i>Flavisolibacter</i>	15.0	4.6	0.0	6.8	6.2
Proteobacteria	<i>Methylothermobacter</i>	7.0	0.8	0.1	0.0	4.1
Bacteroidetes	<i>Parabacteroides</i>	0.0	19.5	70.1	0.0	0.0
Proteobacteria	<i>Thermomonas</i>	1.4	8.1	0.2	9.9	1.1
Firmicutes	<i>Clostridium</i>	0.0	6.3	18.9	1.9	0.0
Actinobacteria	<i>Tetrasphaera</i>	0.2	5.9	0.3	0.8	0.5
Firmicutes	<i>Tissierella</i>	0.0	0.0	0.0	32.8	0.0
Firmicutes	<i>Proteinclasticum</i>	0.2	0.0	0.1	20.4	2.0
Proteobacteria	<i>Azospira</i>	1.7	9.0	0.3	1.5	18.8

optimizing the suitable pretreatment conditions for sludge disintegration and subsequent utilization for bioenergy production.

Biogas production and COD mass flow

Similar to the organics release, the biogas content and AD performances were varied among the tested conditions. The highest biogas yield of 39.8% was achieved at the pH of 10, followed by 90 °C (39.5%), pH 11 (37.8%), 70 °C (34.0%), and raw sludge (22%), respectively (Fig. 5). In case of S3 (where the biodegradable fraction of sludge was removed by centrifugation), the biogas yield ranged 18.0 to 25.0%. The solubilization at pH 11 was 1.4-fold higher than that of pH 10, but biogas production was

lower. These results showed that the solubilization ratio increased with the pretreatment strength increase, but biogas yield was inconsistent with solubilization [5, 32]. In this regard, Penaud et al. (1999) reported that when sludge was pretreated with excess alkali dosage, biodegradability can be decreased caused by the formation of refractory compounds due to the Maillard reaction [33]. Unlike the alkali pretreatment, no inhibitory effect such as Maillard reaction was found in thermal pretreatment at 90 °C, which corresponds to the low temperature range [8].

Considering the biogas yield achieved, and the volume of the solid/liquid fraction after centrifugation, the COD mass flow at different scenarios is depicted in Fig. 6. The

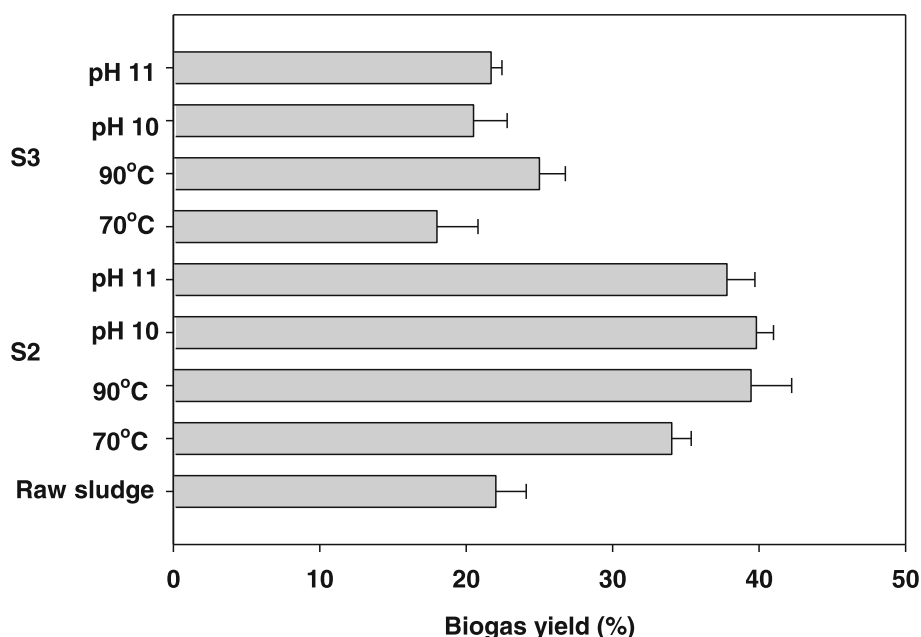
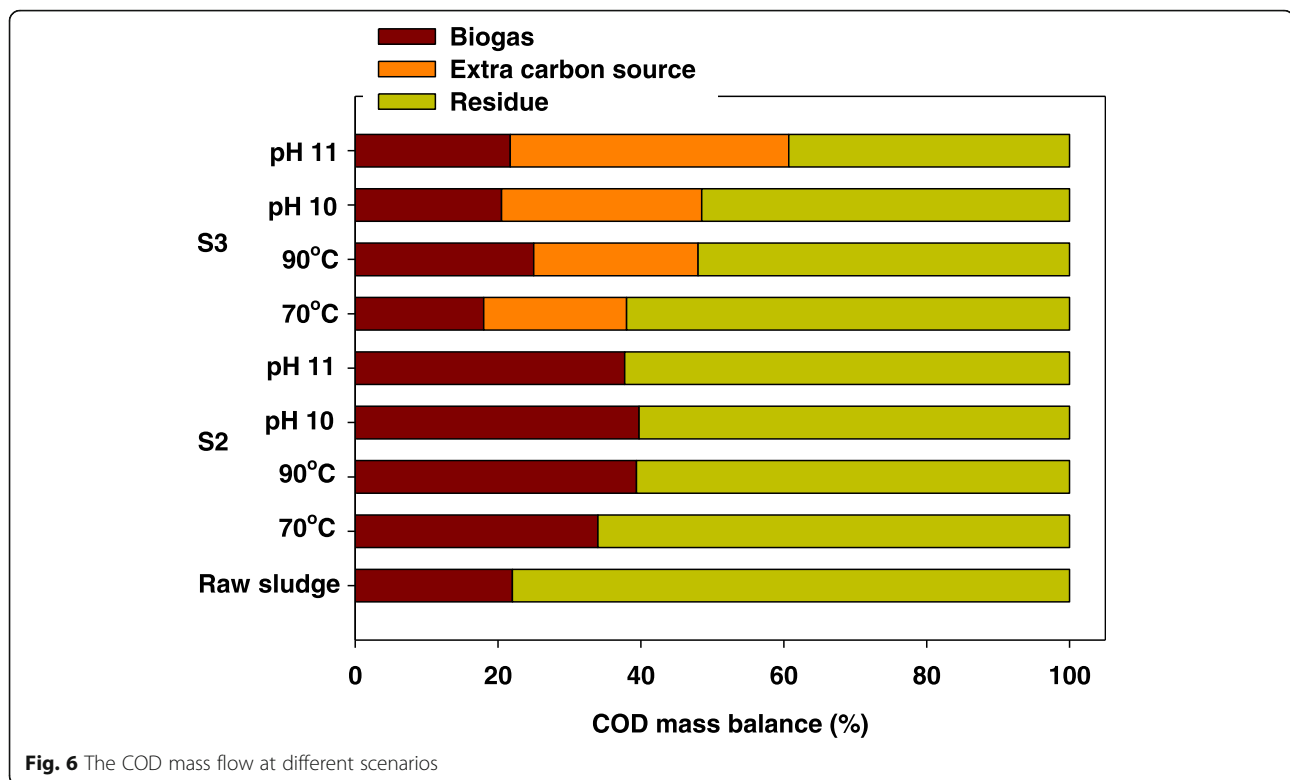


Fig. 5 The biogas yield (actual CH₄ production/theoretical maximum) of various samples (S1 = anaerobic digestion of raw excess sludge, S2 = anaerobic digestion of pretreated excess sludge, and S3 = organic acids generation + anaerobic digestion of solid residue after acids generation)



separation efficiency on a volume basis (liquid:solid) were 81:19, 82:18, 81:19, and 84:16 at 70 °C, 90 °C, pH 10, and pH 11, respectively. The percentage of COD on liquid/solid fraction were 20/80, 23/77, 28/72, and 39/61, at 70 °C, 90 °C, pH 10, and pH 11, respectively. The total COD included biogas production, extra carbon source, and residues. In the case of S3, the COD from the biogas was lower than S2, but the COD from the external carbon source increased with the pretreatment strength, and consequently, the amount of available COD was higher in S3. At pH 11, available COD of S3 was 61% but at S2 it was about 38%. However, it is difficult to evaluate without comparing cost and benefits. Therefore, the economic assessment was conducted focused on cost and benefit in the next section.

Economic assessment

The cost-benefit analysis of the sludge treatment was considered by calculating the energy returns and input required for handling 1 ton of sludge, and the results are shown in Fig. 7. The energy analysis was performed by comparing the energy obtained in the form of CH₄ with the energy used for sludge pretreatment and digestion process. The amount of CH₄ that can be obtained from 1 ton of raw sludge and pretreated sludge at 70 °C, 90 °C, pH 10, and pH 11 were 2.85, 3.31, 3.34, and 3.17 m³, respectively, corresponding to an electrical energy of 18.4, 28.4, 32.9, 33.2, and 31.5 kWh, respectively (1.0 m³

of CH₄ is equal to 35.85 MJ based on low calorific value). The input energy used during digestion was assumed to be 14.7 kWh [22]. For the thermal-pretreatment at 70 °C and 90 °C, 22.5 and 26.0 kWh of additional energy is required, respectively.

Based on the achieved biogas yield and TCOD concentration of the raw sludge (S1), 3.96 kg of volatile solids (VS) reduction and 1.85 m³ of CH₄ production per ton of sludge can be expected, corresponding to the economic benefit of 1.29 USD/ton of sludge [24]. The VS reduction was calculated considering that 1 kg VS is equivalent to 1.33 kg COD [34].

In case of S2, the improvement of biodegradability resulted in the increase of CH₄ production almost by double, when compared to S1. The thermal-pretreatment resulted in the net profit of 2.0 and 2.3 USD/ton of sludge at 70 and 90 °C respectively, whereas the chemical cost needed for alkali pretreatment was higher and resulted in a negative profit of -0.1 and -2.0 USD/ton of sludge at pH 10 and 11, respectively. The cost for the chemical used in the pretreatment was calculated on the basis of KOH usage (1.5 and 2.4 kg KOH at pH 10 and 11, respectively) and its price of 2.0 USD/kg KOH [35]. Owing to the high pretreatment cost, the alkali-pretreatment resulted in a negative gain of the process [36].

S3 seems to be an appropriate strategy for improving the economic feasibility from the process, due to their

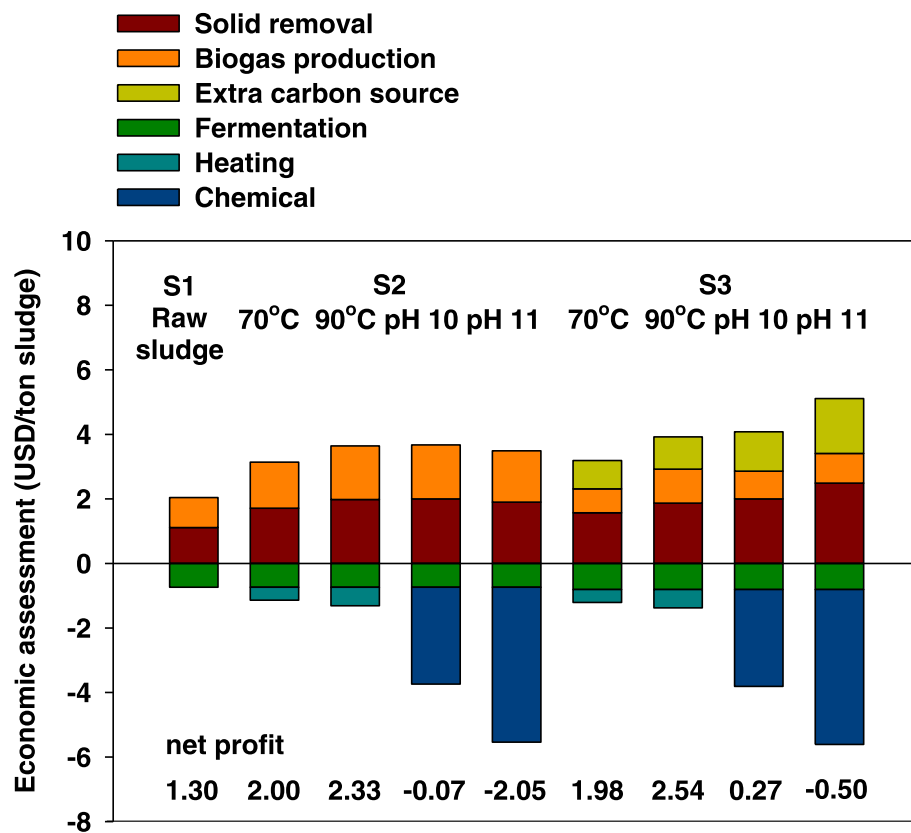


Fig. 7 Economic assessment of various scenarios (S1 = anaerobic digestion of raw excess sludge, S2 = anaerobic digestion of pretreated excess sludge, and S3 = organic acids generation + anaerobic digestion of solid residue after acids generation)

additional carbon energy source in the form of organic acids provided by the self-acidogenic fermentation. The additional carbon source obtained from pretreatment sludge of 1 ton at 70 °C, 90 °C, pH 10, and pH 11 were 4.9, 5.5, 6.8, and 9.4 kg COD, respectively. 60% of the obtained SCOD was available as a carbon source [37] and was calculated as a price corresponding to methanol equivalents (1.5 kg COD/kg CH₃OH, 0.45 USD/kg CH₃OH). For example, the benefit of S3 pretreatment at 90 °C was approximately 1.0 USD, calculated as follows: 5.5 kg COD/1.5 kg COD/kg CH₃OH × 0.6 × 0.45 USD/kg CH₃OH.

During the S3 conditions, the alkali-conditions of pH 10 and 11 slightly improved the economic benefit than the S2 conditions and showed a value of 0.3 and −0.5 at a pH of 10 and 11, respectively. The additional economic benefit by the self-acidogenic fermentation was offset by the high cost of alkali-pretreatment [38, 39]. Compared with alkali-pretreatment, the thermal pretreatment seems to be a feasible pretreatment option to enhance the economic benefits of the process. Based on the results obtained at the S3 condition, thermal pretreatment at 90 °C improved a net economic benefit of 2.53 USD/ton of sludge.

Conclusions

This study evaluated the various scenarios of sludge disintegration based on AD technologies on economic cost and biodegradability. The outcomes showed that alkali pretreatment significantly improved the sludge solubilization and organic acids accumulation, whereas thermal treatment showed moderate sludge solubilization, but better energy recovery. The organic acids production from ES was 3.7 and 4.1 g COD/L, which accounted for 15 and 17% of total COD at 90 °C and pH 10, respectively. Thermal pretreatment at 90 °C showed an increased abundance of *Bacteroidetes* (70.8%) and *Firmicutes* (24.2%) with a biogas yield of 39.5%. In terms of economic benefits, the thermal pretreatment at 90 °C, followed by acidogenic fermentation and AD process (S3) provided a net economic benefit of 2.53 USD/ton of sludge, attributed by the additional supply of external carbon source generated during the acidogenic fermentation.

Methods

Inoculum and feedstock

The inoculum for biogas production was taken from an anaerobic digester in a local wastewater treatment plant

in Korea. The pH, alkalinity, and volatile suspended solids (VSS) concentrations of the digester sludge were 7.5, 2.4 g CaCO₃/L, and 5.6 g/L, respectively. Prior to AD, it was shredded by a blender smaller than 1.0 mm. The ES used was taken from the recycling pumping line of activated sludge process located at the same local wastewater treatment plant. The characteristics of ES were as follows: pH 7.0, TCOD 24,000 mg/L, SCOD 590 mg/L, total solids (TS) 22,800 mg/L, VS 18,000 mg/L, total nitrogen (TN) 2200 mg N/L, ammonia 360 mg NH₄-N/L, and carbohydrate concentration 410 mg Carbo. COD/L.

Experiment

In the alkali-pretreatment, 6 N KOH solution was added to 0.5 L of ES sample to reach pH values of 10 and 11. The samples were mixed for 0.5 h using a magnetic stirrer at 150 rpm at 20 °C. In the thermal pretreatment, 0.5 L of ES was heated by using a water bath at 70 °C and 90 °C for 0.5 h. To minimize evaporation during heating, the beaker was covered with aluminum foil.

The biogas production was conducted using the serum bottles having an effective volume of 100 mL (total volume of 250 mL). The ES, inoculum, diluting water, and 1 mL of trace element solution made according to Kim et al. 2006 [40] were added to set a substrate concentration and inoculum to substrate ratio at 3.0 g COD/L, and 2.0 (g VSS/g COD). After adjusting an initial pH to 7.5 ± 0.1 using 2 N KOH and 2 N HCl solutions, the serum bottles were purged with N₂ gas for 5 min to establish an anaerobic condition. In case of acids generation for S3, pretreated ES was added to the serum bottle without pH adjustment for 7 d under anaerobic condition. No extra-inoculum and diluting water were added to obtain self-generated organic acids from ES. After acid fermentation, the broth was centrifuged at 6000 rpm for 10 min, and the solid residue was only further used for biogas production. All bottles were placed in a shaking incubator controlled at 35 °C and 150 rpm. The tests were carried out in duplicate, and the results were averaged.

Economic assessment

The economic assessment was carried out for three different scenarios, considering the input cost and obtained values. Pretreatment (heat and chemical dose), and electricity cost for stirring, and heating during the fermentation were included in the input cost. The obtained values were calculated based on the amount of produced CH₄, organic acids, and the reduction of sludge. The economic benefit from ES reduction (0.28 USD/kg VS) was calculated using according to Kavitha et al. [22]. The energy needed for heating was calculated using the following Eq. (1) [41].

$$\text{Energy (heat)} Q = \rho \times V \times C_p \times (T_f - T_i) \quad (1)$$

, where Q is the heat energy needed to heat the sludge (kJ), ρ is the density of sludge (kg/m³), V is the volume of sludge (m³), C_p is specific heat of sludge (kJ/kg.°C) (4.2 kJ/kg.°C), and T_i and T_f are initial and final temperatures (°C) of the sludge, respectively.

For the same economic criteria, all energy costs were calculated based on the commercial natural gas price of 0.014 USD/MJ [42]. The obtained value from the organic acids generation was calculated by considering the carbon source cost based on methanol.

Analytical methods

The concentrations of TS, VS, COD, TN, VSS, and ammonia were measured by standard methods [43]. The carbohydrate concentration was determined by the colorimetric method [44]. The produced biogas was adjusted to the standard conditions of temperature (0 °C) and pressure (760 mmHg) (STP). The CH₄ and CO₂ content in the biogas were measured by gas chromatography (GC, Gow Mac series 580) equipped with a 1.8 m × 3.2 mm Porapak Q (80/100 mesh) column using N₂ as a carrier gas. The temperatures of injector, detector, and column were kept at 80, 90, and 80 °C, respectively. Organic acids were analyzed by a high performance liquid chromatography (HPLC) (LC-10A model, SHIMADZU Co.) with an ultraviolet (215 nm) detector (UV1000, SHIMADZU) and a 100 mm × 7.8 mm fast Acid Analysis column (HPX-87H, Bio-Rad Lab.) using 0.005 M H₂SO₄ as a mobile phase. The liquid samples were pretreated with a 0.20 µm membrane filter before injection to HPLC. Biogas yield was calculated by comparing the actual production of CH₄ produced to the theoretical maximum (350 mL CH₄/g COD) [5].

Microbial community analysis

To analyze the change in bacterial communities, the next generation sequencing (NGS) method was used. Samples were obtained for Deoxyribonucleic acids (DNAs) extraction after pretreatment. DNA in the samples was extracted and purified using an Ultraclean Soil DNA kit (Cat #12800–50; Mo Bio Laboratories, Inc., USA) and an Ultra Clean Microbial DNA Isolation Kit (Mo Bio Laboratories, CA, USA). Then the preparation of libraries and the next procedure for emPCR was performed as previously described method [45]. The 16S universal primers 27F (5'GAGTTTGATCMTGGC TCAG3') and 800R (5'TACCAGGGTATCTAATCC3') were used for amplifying the 16 s rRNA genes [46]. After the PCR reaction, products were purified using AMPure beads (Beckman coulter). Sequencing was then performed using a 454pyrosequencing Genome Sequencer FLX Titanium (Life Sciences, CT, USA), following the

manufacturer's instructions, by a commercial sequencing facility (Macrogen, Seoul, Korea).

The sequencing data from the pyrosequencing were analyzed with the software MOTHUR for pre-processing (quality-adjustment, barcode split), identification of operational taxonomic units (OTUs), taxonomic assignment, community comparison, and statistical analysis. The methods for sequence filtration and trimming were done as previously described [46]. The sequences spanning the same region were then realigned with the NCBI BLAST database (www.ncbi.nlm.nih.gov).

Abbreviations

AD: Anaerobic digestion; COD: Chemical oxygen demand; DNA: Deoxyribonucleic acid; ES: Excess sludge; GC: Gas chromatography; HPLC: High performance liquid chromatography; NGS: Next generation sequencing; OTU: Operational taxonomic unit; SCOD: Soluble chemical oxygen demand; STP: Standard conditions of temperature and pressure; TCOD: Total chemical oxygen demand; TN: Total nitrogen; TS: Total solids; VS: Volatile solids; VSS: Volatile suspended solids

Acknowledgements

Not applicable.

Funding

This work was supported by INHA UNIVERSITY Research Grant (INHA-55800-01). The funding form comprised of financial support and providing facilities required for samples' analysis.

Availability of data and materials

Please contact the corresponding author for data requests.

Authors' contributions

M-KL performed experiments of fermentation and wrote this part in the manuscript, while Y-MY contributed with the microbial analysis and its discussion writing. D-HK was responsible for making the experimental design and revising the whole manuscript. All authors have read and approved the final manuscript.

Competing interests

The authors declare that they have no competing interests.

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Received: 22 November 2018 Accepted: 8 April 2019

Published online: 16 May 2019

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