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Article

Effects of High-efficiency Digestion System and Microbial Diversity with Internal Recirculation

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Abstract

In this study, a combined sludge digestion process consisting of a mesophilic anaerobic digester (MAD) and a thermophilic aerobic digester (TAD) was developed. The effects of recirculation on the combined sludge digestion process were investigated. The volatile suspended solids (VSS) removal rate increased from 76% to 97% when internal recirculation was applied and remained stable until the end of the experiment. In contrast, the concentration of total suspended solids (TSS) increased slightly because of the accumulation of inert materials in the reactors. The methane production of the combined process showed the highest level of 260 mL/L/day in phase two and decreased to 170 mL/L/day as the strength of the pre-treatment decreased. At phase five, the influent and recirculated sludge were supplied without pre-treatment. During this phase, methane production rate decreased from 170 mL/L/day to 40 mL/L/day, and rapid accumulation of TSS and VSS occurred. However, the thermophilic aerobic digester was stable, and VSS accumulation was not observed. This indicates that the sludge removal ability of the combined process can be stably maintained without sludge pretreatment. Collectively, results of this experiment indicate that pre-treatment of influent sludge and internal recirculation of treated sludge from the TAD to the combined process are necessary for effective energy production and improved sludge removal in the combined process.

Keywords: *Combined sludge digestion process, Mesophilic anaerobic digester, Thermophilic aerobic digester, Pretreatment, Methane production, Internal sludge recirculation*

1. Introduction

Waste-activated sludge (WAS) treatment is challenging because it contains non-biodegradable cells, insoluble organic matter, and various hazardous components such as pathogens, toxic organic matter, and many types of heavy metals. Therefore, the treatment of WAS is very important for environmental protection. In Korea, the easiest way to get rid of WAS is to discharge it in the ocean. However, to protect the marine environment, dumping of untreated WAS to the ocean was completely banned by the London Convention in 2012[1] as it leads to ocean contamination and the red tide phenomena, leading to a heavy environmental burden. Therefore, WAS reduction technology has attracted the interest of researchers in Korea. Unlike other countries such as the USA and China, landfills of WAS are not common in Korea because of scarce land. In addition, the composting or incineration of WAS is difficult because WAS contains a large amount of moisture and releases hazardous materials when burned.

Anaerobic digestion is a widely known method for treating WAS because it produces renewable energy[2-6]. However, anaerobic digestion has several disadvantages, such as the requirement of a large area because of its slow reaction rate, long digestion time, sensitivity to operating conditions, and malodor production. As a substitute for anaerobic digestion, thermophilic aerobic digestion has been used to treat WAS because of its short solid retention time, stability of treated WAS, and the high activity of bacterial species under aerobic conditions. However, thermophilic aerobic digestion has disadvantages, including lack of bioenergy production, sludge separation problems, and high energy and cost requirements due to long-term aeration[7].

Recently, to resolve the problems of anaerobic and thermophilic aerobic digestion, combined anaerobic and aerobic sludge digestion has been suggested by several research groups[8,9]. A sludge digestion system consisting of anaerobic digestion followed by aerobic digestion was investigated, and it was found that the combined anaerobic and aerobic system showed 70% VS reduction[9]. The efficiency of a hyperthermophilic aerobic process coupled with a mesophilic digester was examined and it was found that intrinsic biodegradability increased by approximately 20~40%[8].

A high-efficiency digestion (HED) system was developed in a previous study by combining mesophilic anaerobic and thermophilic aerobic sludge digesters, which exhibited remarkable sludge digestion rates and methane production[10]. In this study, we attempted to improve

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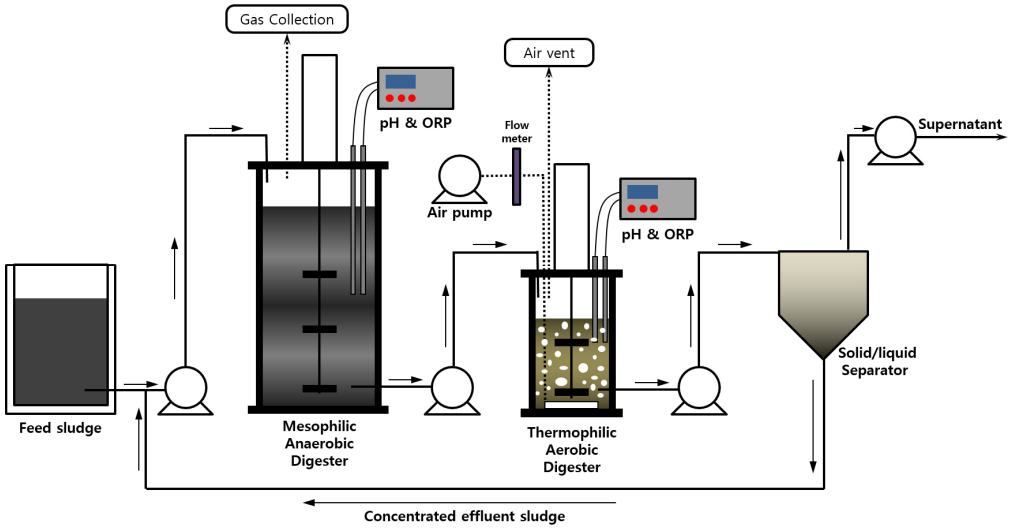

Figure 1. Schematic diagram of lab-scale combined sludge digestion process with internal recirculation.

the sludge removal rate of the HED process and achieve the zero sludge discharge with internal recirculation from TAD using a solid-liquid separation unit, leading to a significant improvement in sludge removal from WWTP. We also investigated the effects of various pre-treatment methods on the feasibility of enhancing methane production and sludge reduction. In addition, changes in the microbial communities in the mesophilic anaerobic and thermophilic aerobic sludge digesters were examined using quantitative PCR (qPCR).

2. Materials and methods

2.1. Reactor configuration

A schematic of the lab-scale combined digestion system is shown in Figure 1. The combined digestion system consisted of a mesophilic anaerobic digester (MAD), thermophilic aerobic digester (TAD), solid/liquid separator, and thermo-alkaline treatment reactor. Two double-jacketed stirred reactors with working volumes of 6 L and 1.5 L due to the difference of reaction rate between MAD and TAD were used, respectively. Temperatures of MAD and TAD were maintained to 35 °C and 55 °C using a water circulator, respectively. To prevent decay of influent sludge, temperature of feed tank was maintained at 4 °C using a water circulator. A compressor and bubble diffuser were used to supply air to the TAD and the airflow rate was maintained at 2 L/min. pH and oxygen reduction potential (ORP) probes were installed on MAD and TAD, respectively. Sludge was supplied and withdrawn from each reactor using peristaltic pumps. Owing to the pumping speed limitation, the pumps were operated four times per day for 10 min each time using a timer and relay. Stable and continuous pumping operations were achieved by adding a control relay to the pump. The solid retention times (SRT) of MAD and TAD in phase 1 were 40 and 10 d, respectively. From phases 2 to 5, the SRT of each reactor decreased to 30 days and 7.5 days based on the experimental data as the recirculation of effluent sludge was introduced.

2.2. Reactor operation

The inocula for MAD and TAD were obtained from a combined sludge digestion system described in our previous study[10]. Influent sludge was prepared by mixing concentrated activated sludge and primary raw sludge at a ratio of 6:4. The influent feedstock was frozen, stored in the freezer to prevent decay, and thawed before feeding. The characteristics of the feed sludge used in the laboratory-scale HED system are presented in Table 1.

The laboratory-scale HED system was operated in five phases. In the first phase, a solid/liquid separation unit was not used and the influent sludge was thermal-alkaline pre-treated before being fed into the combined digestion system. Subsequently, the effluent from the HED system

Table 1. Influent Characteristics Used in This Experiment

Parameters	Phase I	Phase II	Phase III	Phase IV	Phase V
pH	7.0 ± 0.00	7.0 ± 0.00	7.6 ± 0.11	7.0 ± 0.00	7.4 ± 0.17
TCOD (g/L)	51.94 ± 3.3	54.33 ± 1.3	55.15 ± 2.6	57.26 ± 2.2	55.14 ± 1.7
SCOD (g/L)	18.32 ± 2.05	17.21 ± 1.89	14.61 ± 1.27	13.16 ± 2.11	4.42 ± 0.35
TSS (g/L)	33.25 ± 2.22	38.69 ± 2.55	44.26 ± 2.35	46.31 ± 1.38	52.24 ± 3.59
VSS (g/L)	29.16 ± 1.63	32.04 ± 2.14	35.36 ± 3.48	35.28 ± 3.24	43.22 ± 3.26
Soluble Protein (g/L)	4.31 ± 0.38	3.84 ± 0.15	2.91 ± 0.14	3.15 ± 0.33	0.61 ± 0.03
Soluble Carbohydrate (g/L)	3.52 ± 0.24	3.23 ± 0.37	2.87 ± 0.22	2.75 ± 0.18	0.47 ± 0.08

Table 2. Primer Sets for qPCR Used in This Experiment

Target group	Primer	Sequence (5`-3`)	Amplicon size (bp)
Bacteria	BAC338F	ACTCCTACGGGAGGCAG	468
	BAC805R	GACTACCAGGGTATCTAATCC	
Archaea	ARC787F	ATTAGATACCCSBGTAGTCC	273
	ARC1059R	GCCATGCACCWCCTCT	
Ureibacillus	Urei1137F	GCCGTACAAATACGGAGGAA	280
	Urei1416R	ACCGACTTCGGGTGTTACAG	
Methanosarcinales	MSL812F	GTAAACGATRYTCGCTAGGT	354
	MSL1159R	GGTCCCCACAGWGTACC	
Methanomicrobiales	MMB282F	ATCGRTACGGGTTGTGGG	506
	MMB832R	CACCTAACGCRCATHGTTTAC	
Methanobacteriales	MBT852F	CGWAGGGAAGCTGTTAAGT	343
	MBT1196R	TACCGTCGTCCACTCCTT	
Methanococcales	MCC495F	TAAGGGCTGGGCAAGT	337
	MCC832R	CACCTAGTYCGCARAGTTTA	

is discharged directly without recirculation. For thermal-alkaline pre-treatment, a 4N NaOH solution was added to increase the pH of the influent sludge to 12 and stirred for 1 h. Then, the sludge was heated to 121 °C with autoclave for 1 h and neutralized with 4N HCl solution after cooling to room temperature. After 75-d of operation, the second phase began with the introduction of a liquid/solid separator and a sludge recirculation instrument. The effluent sludge was concentrated using a liquid/solid separator, clear supernatant was discharged, and concentrated effluent sludge was recirculated and mixed with the influent sludge before pre-treatment. The pre-treatment conditions of the mixed influent sludge were the same as those in Phase 1.

At phase 3, the HED system was operated for 160 days after reaction began and the pre-treatment condition was changed to only heat pre-treatment with autoclave at 121 °C and for 1 h. The purpose of this change was to reduce the cost of pre-treatment. For the same purpose, the pre-treatment conditions of the fourth phase were changed to alkaline treatment. As a result of the previous batch treatment, the optimal conditions for alkaline pre-treatment alone were determined to take place at 3.3 h, pH 11, and room temperature. In phase 5, the raw influent sludge was supplied to the combined process, which was operated continuously without any pre-treatment.

2.3. Analytical methods

Volatile suspended solids (VSS), total suspended solids (TSS), chemical oxygen demand (COD), and ammonium concentration were analyzed according to standard methods[11]. To measure soluble components such as SCOD, ammonium ions, carbohydrates, and proteins, samples were centrifuged (5000 rpm, 30 min) and the supernatant was filtered through a 0.45-μm syringe filter (Whatman, USA). Protein and carbohydrate contents were measured using the Lowry-Folin method and phenol−sulfuric acid methods, respectively. The pH and ORP of MAD and TAD were measured using a pH/ORP meter (Mettler Tolledo, Switzerland). Methane gas from the MAD was collected using a Tedlar bag and analyzed using gas chromatography (Model 6890N, Agilent Inc., USA) with a pulsed discharge detector (PDD).

2.4. Microbial community analysis

2.4.1. Total DNA extraction

50 μL of mixed sludge from each reactor was sampled and washed with 1 ml of PBS buffer. After centrifugation with 14000 g for 5 min, supernatant was decanted and sludge pellet was suspended 100 μL of Tris-HCl (pH 8.0) buffer. The total genomic DNA of the prepared samples was extracted immediately using a Nucleospin Soil Kit (Macherey-Nagel, Germany) according to the manufacturer's instructions. The extracted genomic DNA was eluted in 50 μL of deionized water and stored at -20 °C until use.

2.4.2. Quantitative PCR

Quantitative PCR (qPCR) was performed to analyze the microbial community dynamics of the combined MAD and TAD processes. To analyze the microbial population, seven known primer sets were used for total bacteria, total archaea, *Ureibacillus*, and four different methanogenic orders: *methanobacteriales*, *methanosarcinales*, *methanomicrobiales*, and *methanococcales*. All the primer sets used in this study are listed in Table 2. The QPCR was performed using a 7300 Real-Time PCR system (Applied Biosystems). 20 μL of qPCR mixture containing 1 μL of each primer and 1 μL of template DNA was prepared with Power SYBR Green PCR mastermix (Applied Biosystems). A cycle of qPCR consisted of initial denaturation for 10 min at 94 °C; 40 cycle of 10 sec at 94 °C and combined annealing, and an extension for 30 sec at 60 °C.

3. Result and discussion

3.1. Total suspended solid (TSS) changes

Figure 2 shows the total suspended solid (TSS) component profiles

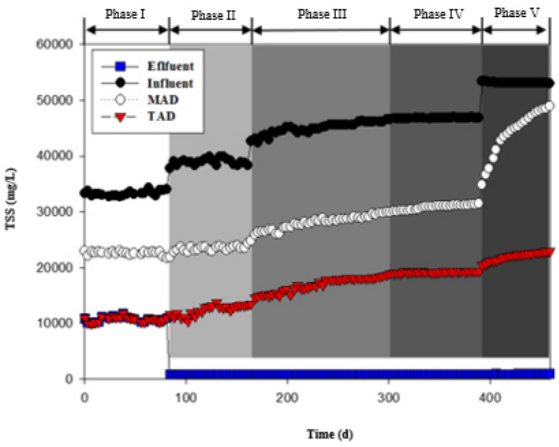

Figure 2. Profiles of TSS concentrations.

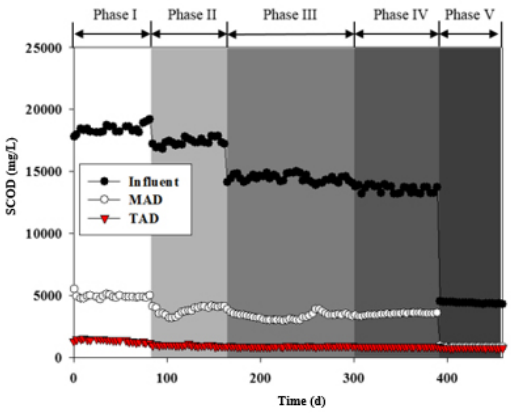

Figure 3. Profiles of soluble COD at different phases.

of the combined sludge digestion process. The TSS concentration in the influent sludge increased from 33.25 g/L in Phase 1 to 46.31 g/L in Phase 4. In phase 2, the main reason for the TSS increase might be the supply of concentrated TAD sludge because recycled TAD sludge is highly concentrated, and the percentage of inert solids is also higher than that of the initial feed sludge. As the pre-treatment conditions weakened in phase 3, the amount of soluble organic matter decreased, and the concentration of TSS in phase 3 increased to 44.26 g/L. The TSS concentrations of the influent sludge in phases 3 and 4 were almost the same because the strengths of the thermal and alkaline pre-treatment were almost similar. In the combined MAD and TAD, TSS accumulation was observed after recirculation of the concentrated effluent sludge from the TAD. Accumulated solids are considered inert solid materials, such as dirt, inorganic compounds, and sand. As fixed solids accumulated, the difference between the TSS and VSS concentrations increased continuously. The VSS concentration of the influent feed sludge showed almost the same pattern as that of the TSS. However, in the combined process, VSS accumulation did not occur in comparison with TSS until the end of phase 4. This result indicates that sludge removal performance was stably maintained until Phase 4. However, during phase 5, the sludge removal efficiency of the MAD rapidly decreased. Both the TSS and VSS accumulated rapidly. However, sludge removal by TAD was only slightly affected when there was no pre-treatment of the feed sludge. The reason for this unexpected breakdown of MAD was that raw sludge was supplied without any pre-treatment in phase 5. Without pre-treatment, the TAD microorganisms that flowed to the MAD could survive for a long period and inhibit microbial activity. There are some reports on the presence of facultative microorganisms in ATAD process[12].

3.2. Soluble COD, pH, and ORP changes

The profiles of the soluble components during the entire experiment are shown in Figure 3. Concentration of soluble COD in feed sludge in phase 1 was greatly increased compared to the raw sludge by the thermal-alkaline pre-treatment and the amount of SCOD produced increased from 4.42 g/L in the raw sludge to 18.32 g/L in phase 1. The soluble COD comprises organic acids, carbohydrates, proteins, and lipids. Therefore, the profiles of SCOD, carbohydrates, and proteins exhibited similar patterns. With internal sludge recirculation, the SCOD concentration of the influent decreased from 18.32 g/L in phase 1 to 17.21 g/L in phase 2, mainly because of the introduction of TAD sludge, which contains a low SCOD with solid cell walls, leading to a decrease in sludge solubilization efficiency. In contrast, the concentration of soluble components in the influent decreased more with the change in pre-treatment conditions in phases 3 and 4, as sole thermal and alkaline pre-treatment were less efficient than the combined thermal-alkaline pre-treatment method. In phase 5, the concentration of soluble organic compounds drastically dropped to 4.4 g/L due to the lack of pre-treatment of the feed sludge. The methane production ability of MAD was significantly inhibited by the depletion of easily usable substrates. Moreover, microorganisms in the MAD started to compete with facultative microorganisms from the TAD, which had high substrate utilization activity in phase 5. In contrast, TAD showed a stable performance owing to high microbial activity during the entire experimental period.

The pH and ORP profiles are shown in Figure 4. The pH of the influent in phases 1, 2, and 4 was maintained at 7.0 because neutralization after alkaline pre-treatment was performed. As shown in the results, the pH value did not deviate from the normal operation range, although some fluctuations were observed in the influent and MAD. The ORP value in MAD remained about -520 mV, which corresponds to strict anaerobic condition while the ORP value in TAD was approximately 100 mV. The ORP value was maintained even in phase 5 in the combined MAD and TAD processes.

3.3. Methane production

Throughout the experiment, methane production rate was continuously measured. Figure 5 shows methane production in the MAD. In phases 1 and 2, with thermal-alkaline pre-treatment, the methane production rate of the combined TAD-MAD process was over 250 mL/L/day showing the effectiveness of the combined thermal-alkaline pre-treatment in enhancing methane production. With sole pre-treatment of thermal and alkaline conditions compared to thermal-alkaline

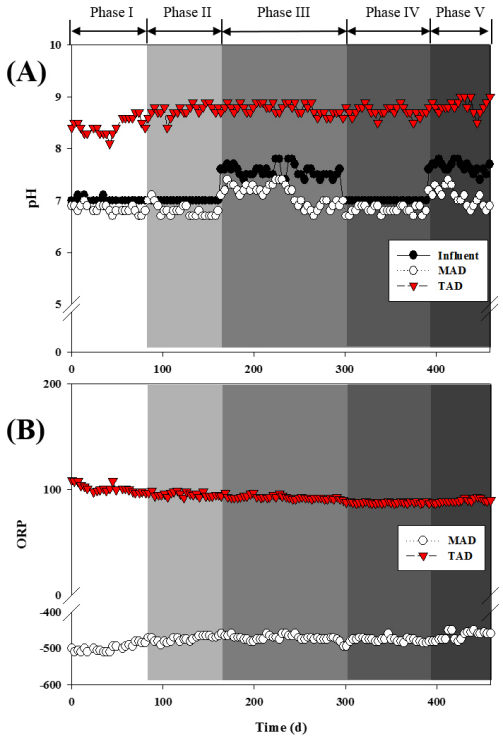

Figure 4. Profile of pH and ORP.

treatment at phases 3 and 4, methane production decreased to approximately 170 mL/L/day due to the decrease in soluble organic matter. This result indicated that the strength of the pre-treatment directly affected the methane production rate. Indeed, methane production without pre-treatment during Phase 5 significantly decreased to below 40 mL/L/day. This result is lower than the start-up period in our previous study, in which no pre-treatment was performed[10]. These findings indicate that the inflow of TAD microorganisms into the MAD reactor, resulting in a shortage of soluble products, seriously damaged the stable methane production of the MAD.

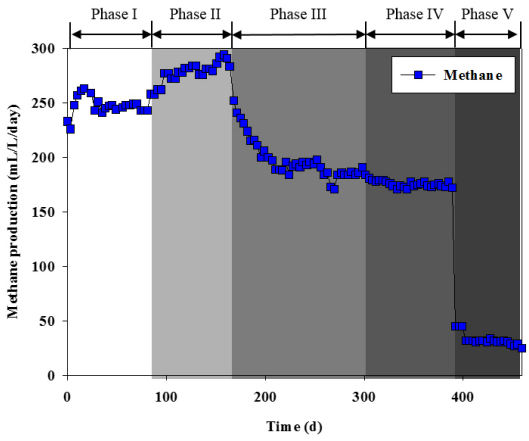

Figure 5. Methane production rate in the combined process at different phases.

3.4. Quantitative PCR results

The population of microorganisms in a bioreactor is closely related to its performance and stable operation[13-16]. Therefore, microbial community analysis is important for understanding biological systems. qPCR was performed to investigate the microbial population in the combined sludge digestion process, and the results are shown in Figure 6. The total bacterial population in the MAD did not change significantly during the entire experiment. However, in the case of archaeal species, the population rapidly decreased in phase 5. The total population of archaea in phase 1 was 8.6×10^8 copies/mL, but decreased to 3.2×10^8 copies/mL at phase 5, leading to a decrease in methane production. Most of the archaea that disappeared were *methanosarcinales*, a major methanogenic order in the sludge digestion system. The population of *methanosarcinales* decreased from 7.9×10^8 copies/mL at phase 1 to 1.7×10^8 copies/mL at phase 5, whereas the population of other methanogenic orders was maintained normally. This result indicates that the acetogenesis step conducted by specific bacterial species did not occur well in phase 5; therefore, the *methanosarcinales* group showed a low population owing to a shortage of

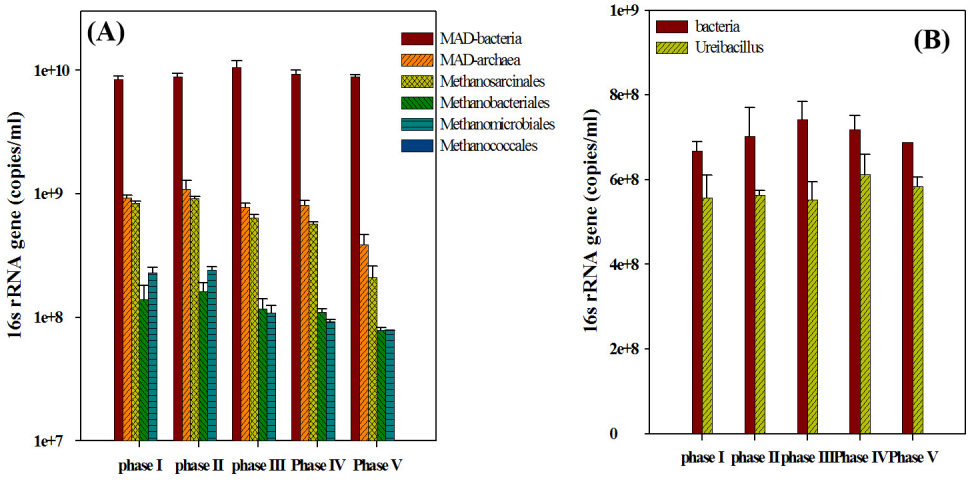

Figure 6. qPCR results of the combined sludge digestion process

acetic acid for the substrate.

The microbial population of the TAD was stably maintained during the entire experimental period. The population of *Ureibacillus*, which shows high cell degradation activity, was maintained at over 70% in the TAD.

4. Conclusions

We successfully modified a combined sludge digestion process with internal recirculation to improve sludge removal and methane production. With the application of internal recirculation of the effluent sludge, sludge removal increased from 65% to 95% of TSS. However, the accumulation of inorganic material was observed, and the flow of concentrated TAD sludge to the MAD without pre-treatment caused serious operational problems in the combined process, especially for methane production. Meanwhile, the methane production of the combined process showed the highest level of 260 mL/L/day in phase 2 with the thermal-alkaline pre-treatment. The population of *methanosarcinales* which is a major methanogenic order decreased from 7.9 $\times 10^8$ copies/mL at phase 1 to 1.7 $\times 10^8$ copies/mL at phase 5, leading to a decrease in methane production. Taken together, these results indicate that the pre-treatment of influent sludge is essential to maintain effective energy production in the anaerobic digester, and internal sludge recirculation from the TAD reactor to the combined process leads to improved sludge removal.

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