Control of accumulated volatile fatty acids by recycling nitrified effluent

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Abstract

Background Volatile fatty acids (VFA) often accumulate in anaerobic digestion systems, decreasing pH levels and causing unstable operational performance and poor biogas production. The aim of this study is to improve anaerobic digestion efficiency by controlling/reducing the accumulation of VFAs in a continuous anaerobic digestion system.

Methods NO_3^- -N was added to the digester and its effects on VFAs were investigated. When the system reached an unstable condition with the accumulation of VFAs, the digester was fed at an organic loading rate of 6 kg COD (chemical oxygen demand)/ $\text{m}^3 \cdot \text{d}$ and 0.5Q of aeration tank effluent (1500 mg/L of NO_3^- -N) was recirculated.

Results With the addition of NO_3^- -N, VFAs were utilized during denitrification, after which methane production started. Furthermore, the accumulated VFAs could be used as a carbon substrate by denitrifying bacteria. After 56 d, a normal VFA concentration could be achieved. Methane production was 0.02–0.03 L CH_4/g VS higher with NO_3^- -N recirculation and feeding than that without feeding.

Conclusions The results show that the addition of NO_3^- -N is a potentially feasible method to control VFAs. Combined with recirculation and feeding, the method can be used to effectively prevent the inhibition of methanogenic microbial activities caused by accumulated VFAs and enhance denitrification and methane production in anaerobic digesters.

Keywords Simultaneous denitrification and methanogenesis (SDM) · Anaerobic digestion · VFA accumulation · NO_3^- -N recirculation

Background

Anaerobic digestion has proved to be one of the most suitable options for treating wastes rich in high-strength organic

carbon owing to its low sludge production and energy consumption compared to aerobic processes [1]. Anaerobic digestion involves a series of processes including hydrolysis, acidogenesis (acetogenesis), and methanogenesis. During hydrolysis, complex organic materials, such as polysaccharides, protein, and lipids, are degraded into soluble and simple organic materials, such as monosaccharides, amino acids, and long chain fatty acids [2, 3]. During acidogenesis, short-chain volatile fatty acids (VFAs) (C_2 – C_6) are formed, which are then oxidized into acetic acid, molecular hydrogen, and carbon dioxide during acetogenesis [4]. Carbon dioxide and methane are formed during methanogenesis.

The relationship between the performance of anaerobic digesters and VFA concentrations has attracted significant attention. At high concentrations, VFAs decrease the overall pH in anaerobic digesters, affecting the activities of methanogenic bacteria and ultimately causing instability in the performance of digesters [2, 4]. VFAs must be converted to acetic acid for methanogenesis and VFAs with more than four carbon chains cannot be used by methanogens [4]. Furthermore, at concentrations higher than 1400 mg/L, acetic acid inhibits the degradation of propionic acid; the concentration of propionic acid should be lower than 900 mg/L to keep the digesters running.

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Acetic acid and butyric acid concentrations of 2400 and 1800 mg/L, respectively, have no significant effects on the performance of digesters [5]. Nevertheless, for stable performance of the digesters, the suggested propionic acid to acetic acid ratio is >1.4 [6]. Gallert and Winter suggested that the concentration of propionic acid should be maintained below 6200 mg/L during the restart of the reactors in case of failure [7]. Even VFA concentrations of 6200–8500 mg/L had no significant inhibitory effect on methane production [8]. There have been various limited studies and results [9, 10].

Several factors including pH, temperature, carbon-to-nitrogen (C/N) ratio, and hydraulic retention time (HRT) are important for controlling the production of VFAs [11]. Several methods have been developed to avoid and control the accumulation of VFAs during anaerobic digestion. Bouallagui et al. suggested a tubular reactor or a two-phase reactor to separate acidogenesis and methanogenesis and improve stability [12]. The addition of co-substrates (i.e., codigestion with high nitrogen content) was considered effective in controlling VFAs and improving methane production [13]. Bouallagui et al. observed that codigestion of high nitrogen content improved methane production yield (8.1%) and achieved stable operation [12]. They recommended C/N ratios between 22 and 25 for anaerobic digestion of food wastes as well as co-substrates.

Food waste and livestock wastewater in South Korea have high nitrogen content that causes eutrophication when discharged into water bodies. Insufficient removal of ionized ammonia and nitrate during anaerobic digestion often leads to odor problems and inhibitory effects on the overall digestion process [14]. Denitrification is a biological process of nitrate reduction (performed by a large group of heterotrophic facultative or strict anaerobic bacteria) to produce molecular nitrogen (N_2) through a series of intermediate gaseous nitrogen oxide products. In anaerobic respiration, denitrifying microorganisms use nitrate and nitrite instead of oxygen as electron acceptors, oxidizing organic matter that serves as an electron donor [15]. Biological denitrification provides a practical alternative to physical/chemical techniques for nitrate removal [1, 16]. In a simultaneous denitrification and methanogenesis (SDM) process, methane and nitrogen gas are produced. Wastewater can serve as an organic carbon source for denitrification and the remaining wastes are converted to methane. SDM in a single reactor offers economic advantages, such as low substrate cost as well as low material, energy, and space consumption. As such, substantial effort has been dedicated to achieving SDM in anaerobic digestion processes [1, 15].

Many anaerobic digestion facilities in South Korea have low digestion efficiency because of excessive organic loading, resulting in inefficient methanogenesis and VFA accumulation. Therefore, new techniques are needed to improve the efficiency of digesters. Tilche et al. reported the application of the SDM process in a single anaerobic digester with 96%

COD (chemical oxygen demand) and 92% nitrogen removal, accounting for 80% of the overall denitrification capacity [17]. Park et al. observed that denitrification occurred when nitrate entered the anaerobic digester and organic substrate was utilized during denitrification, after which methanogenesis occurred [18]. Hendriksen and Ahring achieved almost 100% nitrogen and carbon removal in treating wastewater that contained a mixture of VFA and nitrate by SDM under the conditions of carbon surplus [19]. Lin and Chen reported that after denitrification and complete exploitation of NO_2^- -N and NO_3^- -N, surplus methanol was converted to methane in a co-mixed culture [16]. Sage et al. used a dairy effluent as the carbon source for denitrification and Elefsiniotis et al. used VFAs produced from industrial and municipal wastewater as the carbon source for denitrification [20, 21].

The purpose of this study is to improve the efficiency of a laboratory-scale anaerobic digester using accumulated VFAs and an aerobic treatment effluent containing NO_3^- -N. We attempted to reduce the accumulation of VFAs in the anaerobic digester and simultaneously achieve methanogenesis by recirculating the aerobic treatment effluent.

Methods

Food waste characteristics

Food waste was collected from a local municipal food waste treatment plant in Cheongju, South Korea. The food waste was ground and sieved (2 mm), and its characteristics were analyzed without any pretreatment (Table 1).

Data availability All data generated or analyzed during this study are included in this published article and its [supplementary information files](#).

Experimental methods

Batch test

The inoculum was prepared using 300 mL of sterilized nutrient media with 30 mL of food waste in a 635-mL

Table 1 Characteristics of food waste

Parameters	Range	Average
pH	3.8–4.2	4.0
TS (mg/L)	201,000–221,000	211,000
VS (mg/L)	156,000–168,000	136,000
TCOD _{cr} (mg/L)	169,000–182,000	175,000
SCOD _{cr} (mg/L)	101,000–134,000	118,000

serum bottle [21]. Batch tests were conducted following the biochemical methane potential (BMP) test described in Owen and Chynoweth to simultaneously determine VFA reduction and methane production [22]. Duplicate bottles of VFAs were prepared with and without nitrate to compare the effect of NO_3^- -N. In the duplicate bottles, 50% acetic acid, 50% propionic acid, and 500 mg/L of KNO_3 were used and the concentration of VFAs was set at 10,000 mg $\text{COD}_{\text{cr}}/\text{L}$. A blank sample without the addition of VFAs was also prepared. The duplicate bottles were assayed for BMP using a sample concentration of 2 g volatile solids (VS)/L. The pH was adjusted to 7 using 1 N NaOH and 1 N HCl, and 1.2 g/L NaHCO_3 was added to prevent the pH from decreasing due to VFA production. After being purged with nitrogen gas, the serum bottle was incubated at 35 °C. Initially, samples were collected every 6 h for analysis. After being incubated for 24 h, samples were collected every 24 h for 164 h. The configuration and the operational conditions of the batch test reactor are described in Fig. 1 and Table 2, respectively.

Continuous tests

Continuous tests were conducted using the laboratory-scale system (Fig. 2). The anaerobic methane reactor was a continuously stirred tank reactor (CSTR) with 25 L working capacity. The operational temperature was fixed at 35 °C as the chemostat was installed in a temperature controlled room at 35–37 °C. The volume of biogas produced was measured by connecting the reactor to a biogas collector placed in an acidic

Table 2 Operational conditions of the batch test reactor

Parameters	Blank	Without nitrate	With nitrate
Initial pH	7.0		
VS (mg/L)	2000		
VFAs (mg/L as COD)	—	1000	1000
KNO_3 (mg/L)	—	—	500

(H_2SO_4) and saturated NaCl solution of pH 1.0. The anaerobic methane reactor was maintained at HRTs of 20–25 d for 240 d.

Three sets of experiments were conducted with operational conditions described in Table 3. For Set 1, the initial organic loading rate (OLR) was 6 kg $\text{COD}/\text{m}^3\cdot\text{d}$ for 60 d at Reactor 1 (R1). This initial OLR value was selected because the average OLR in anaerobic digestion of food waste ranges between 4 and 6 kg $\text{COD}/\text{m}^3\cdot\text{d}$. At R2, the accumulation of VFAs was induced by increasing the OLR to 10 kg $\text{COD}/\text{m}^3\cdot\text{d}$. At R3, feeding was stopped and after 70 d of operation without feeding, the concentration of VFAs became normal. At R4, the reactor was fed with the initial OLR.

For Set 2, the reactor was operated under the same conditions as Set 1, except for recirculating NO_3^- -N with 0.5Q of aeration in the tank effluent without feeding at R3. For Set 3, the reactor was operated under the same conditions as Set 2, except for feeding at the OLR of 6 kg $\text{COD}/\text{m}^3\cdot\text{d}$ along with the recirculation of 0.5Q of NO_3^- -N at R3.

Analytical methods

NO_3^- -N was measured using ion chromatography (SDV50A, Youngling Co., Korea) with absorbance detector (UV725S, Younglin Co., Korea). SCOD_{cr} (soluble chemical oxygen demand) was measured after filtration through a 1.25- μm membrane and TCOD_{cr} (total chemical oxygen demand) was measured using the closed-reflux, colorimetric-chrome method [23]. VFAs were analyzed by high-performance liquid chromatography (HPLC 9600, YoungLin, Korea) with an absorbance detector (UV725S, Younglin, Korea). Biogas contents were analyzed using a gas chromatograph (Series 580 GC, Gow Mac Instrument Co., USA) equipped with a thermal conductivity detector and a 1.8 m $\times$ 2 mm stainless steel column pack with Porapak Q (80/100 mesh, Sigma Aldrich, USA). Helium was used as the carrier gas at a flow rate of 15 mL/min. The operating temperatures of the column, injector, and detector were 50, 80, and 90 °C, respectively. Alkalinity, pH, VS, and total solids (TS) were measured using standard methods [23].

The degradation of each sample was assumed to follow a first-order rate of decay. Methane production rate

Fig. 1 The configuration of batch test reactor

Fig. 2 Flow chart and actual picture of the process for recirculating $\text{NO}_3\text{-N}$

constant (k) and ultimate methane yields (M_o) were determined using the method of Owen et al. [24]. The rate constants of methane production, denitrification, and methanogenesis were calculated based on Owen and Chynoweth [22].

$$M = M_o(1 - e^{-kt}) \quad (1)$$

The specific degradation rate for VFAs, k , was calculated using Eq. 2,

$$\ln \frac{S}{S_0} = -kt \quad (2)$$

where S_0 is the initial concentration of VFAs and S is the reduced concentration of VFAs at time t .

Table 3 Operational conditions of continuous reactors

	R1	R2	R3	R4
Set 1				
OLR ($\text{kg COD/m}^3\cdot\text{d}$)	6	10	No feeding	6
Recirculating $\text{NO}_3^- \text{-N}$	No	No	No	No
Set 2				
OLR ($\text{kg COD/m}^3\cdot\text{d}$)	6	10	No feeding	6
Recirculating $\text{NO}_3^- \text{-N}$	No	No	0.5Q (1500 $\text{mg/LNO}_3^- \text{-N}$)	No
Set 3				
OLR ($\text{kg COD/m}^3\cdot\text{d}$)	6	10	6	6
Recirculating $\text{NO}_3^- \text{-N}$	No	No	0.5Q (1500 $\text{mg/LNO}_3^- \text{-N}$)	No

Results

Batch tests

The blank sample is denoted as VFAs (Blank) and samples with extra VFAs are denoted as VFAs in Fig. 3a. The results of the batch test Set 1 are shown in Fig. 3a. When there was no accumulation of VFAs, such as in the blank sample, methane production was higher in the blank (0.8 L) compared to that in the samples (0.5 L) with accumulated VFAs.

Figure 3b shows the results of the batch tests with VFAs and with the addition of nitrate. In the first 6 h, 65% of the initial concentration of VFAs was degraded when $\text{NO}_3^- \text{-N}$ was added (Fig. 3b) and only 31% was degraded without the addition of $\text{NO}_3^- \text{-N}$ (Fig. 3a).

SDM was observed during the batch tests and accumulated VFAs were clearly used up by denitrifying bacteria as their carbon source. The sharp increase in cumulative methane production and decrease in the concentration of VFAs at 6 h also suggest that the anaerobic digestion system reached a stable condition sooner and the methanogens started to produce methane faster than they would in the system without $\text{NO}_3^- \text{-N}$.

Continuous tests

Biogas and methane production during the experiment is described in Supplementary materials figure S1 (Additional File 1). In Set 1 (Fig. 4), OLR at 10 kg

Fig. 3 VFA concentration and cumulative methane production from batch tests: **a** without nitrate; **b** with nitrate

COD/m³·d was introduced in R2 to induce the accumulation of VFA (16,000 mg/L COD) and after 100 d, methane production dropped dramatically as expected. Once OLR was stopped at R3 to return to normal conditions, the normal VFA concentration (1700 mg/L COD) was reached in approximately 72 d. Similar to Set 1, in Set 2 (Fig. 4), after introducing 10 kg COD/m³·d at R2, VFAs increased to 16,000 mg/L COD after 100 d and methane production dropped dramatically (0.12 L CH₄/g VS at R2 compared to 0.32 L CH₄/g VS at R1). Once OLR was stopped and 0.5Q (1500 mg/L of NO₃⁻-N) of aeration tank effluent was recirculated, normal VFA concentration was attained after 46 d. Methane production was 0.08 L CH₄/g VS lower at R3 compared to that in Set 1 likely because of the recirculated NO₃⁻-N inducing denitrification, in which VFAs were utilized as the carbon source for methane conversion. In Set 3 (Fig. 4), after accumulation of VFAs, OLR at 6 kg COD/m³·d was applied and 0.5Q of aeration tank effluent was recirculated. After 56 d, the normal VFA concentration was reached (3000 mg/L as COD). Methane production was 0.02–0.03 L CH₄/g VS higher at R3 than that in Set 2.

Fig. 4 VFA concentration and cumulative methane production for three sets of operation (**a** Set 1; **b** Set 2; **c** Set 3)

Discussion

Batch tests

When VFA accumulation occurs, the buffering capacity is depleted and the pH level is decreased, inhibiting microbial activity for methanogenesis [5]. In Fig. 3a, the sample with accumulated VFAs suppressed the methanogenic activity, resulting in lower methane production than the blank. This observation confirms that the accumulation of VFAs affects methanogenesis.

Table 4 Specific reaction rate, k and stabilization time

Parameter	Set 1	Set 2	Set 3
Specific reaction rate, k (d^{-1})	0.02774	0.04396	0.03161
Time taken for stabilization (d)	72	46	56
R square (R^2)	0.987	0.944	0.972

Figure 3b shows that the addition of NO_3^- -N enables the denitrification process and accumulated VFAs are utilized as carbon sources. Subsequently, methanogenesis occurs, confirmed by a dramatic increase in cumulative methane production.

The results of SDM show that denitrifiers and methanogens exist in the same habitat/test conditions. In the blank, the addition of nitrate induced a competition for carbon uptake among denitrifiers and methanogens; therefore, methanogenesis was inhibited by nitrate. Various researchers reported that N-oxides have inhibitory effects on methane production [25–29], as observed in rice fields [26, 27] and sediments [25]. The inhibitory effects of nitrates on methane production are caused by several mechanisms, including toxic effects on methanogenic microbial communities, competition between denitrifiers and methanogens for H_2 , and temporary accumulation of sulfate or other inorganic electron acceptors, such as Fe(III) that activate sulfate or iron-reducing bacteria and induce competition with methanogens [26].

Continuous tests

Set 1 stabilized after approximately 72 d and the VFA degradation rate constant was $0.02774 d^{-1}$ (Table 4). In Set 2, the system returned to normal after 46 d and the VFA degradation rate constant was at its highest value ($0.04396 d^{-1}$). Set 3 was expected to take the longest time to stabilize due to denitrification of recirculated NO_3^- -N and utilization of introduced VFAs; however, stabilization was achieved earlier (56 d) than in Set 1. This may be related to the higher rate of denitrification than the rate of introduction of VFAs. In this study, the stabilization time was set as the time taken to reach a VFA concentration of 3000 mg/L. Poggi-Varaldo et al. used the stabilization time as the time taken to reach 60% methane in biogas in the batch mode after inoculation [29]. Therefore, the

Table 5 Denitrification rate constant k_{den} and methanogenesis rate constant k_{me} for each set

	Set 1	Set 2	Set 3
Denitrification rate constant, k_{den} (d^{-1})	0.1009	0.1173	0.1159
Methanogenesis rate constant, k_{me} (d^{-1})	0.1062	0.1496	0.1590

stabilization times in this study could not be compared with the results of previous studies. In addition, individual systems have their own stabilization times that may not be applicable for comparative analyses. Recirculation of NO_3^- -N into the anaerobic digester enabled denitrifiers to utilize and reduce VFAs for denitrification. Based on the results of this study, feeding of VFAs is recommended for SDM.

Denitrification and methanogenesis rate constants for each set were calculated based on Eq. 1. Denitrification occurred during the 125th–170th or 180th d depending on the set and methanogenesis occurred during the 170th or 180th–230th d. In each set of experiments, higher rate constants were achieved for methanogenesis than for denitrification (Table 5).

Conclusions

A continuous anaerobic digestion system was operated to control accumulated VFAs. Accumulation of VFAs occurred with an OLR of 10 kg COD/ $m^3 \cdot d$. Recirculation of NO_3^- -N into the anaerobic digester reduced VFA concentrations by inducing denitrification and using VFAs as the carbon source. Stabilization time and specific reaction rate of Set 2 (recirculation of NO_3^- -N and no feeding) were 46 d and $0.04396 d^{-1}$, respectively. By recirculating NO_3^- -N and feeding OLR (Set 3), the longest stabilization time was expected due to the denitrification of recirculated NO_3^- -N and utilization of introduced VFAs; however the system stabilized earlier (stabilization time: 56 d, specific reaction rate: $0.02774 d^{-1}$) than the Set 1 operation without the recirculation of NO_3^- -N or feeding (stabilization time: 72 d, specific reaction rate: $0.03161 d^{-1}$). This may be attributed to the faster denitrification that the introduction of VFA. Recirculation of NO_3^- -N into the anaerobic digester enabled denitrifiers to utilize and reduce VFAs for denitrification. The results of this study should be applicable to the plants that face the problem of VFA accumulation. Instead of stopping OLR and waiting for the system to stabilize, continuous feeding with recirculation of NO_3^- -N provides an economically viable option for such plants.

Authors' contributions JG Park and B Lee were major contributors to the writing of the manuscript. SY Jo and JS Lee analyzed and interpreted the data. HB Jun analyzed the data and provided advice in writing the manuscript. All authors have read and approved the final manuscript.

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Compliance with ethical standards

Ethics approval and consent to participate Not applicable.

Consent for publication Not applicable.

Competing interests The authors declare that they have no competing interests.

Abbreviations *BMP*, Biochemical methane potential; *CSTR*, Continuously stirred tank reactor; *OLR*, Organic loading rate; *SDM*, Simultaneous denitrification and methanogenesis; *TS*, Total solid; *VFAs*, Volatile fatty acids; *VS*, Volatile solid; *SCOD*, Soluble chemical oxygen demand; *TCOD*, Total chemical oxygen demand; *HRT*, Hydraulic retention time

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