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$_3^-$ 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)/m$^3$·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.
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 (N2) 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 NO3−-N and NO2−-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 NO3−-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 | 156,000 |
| TCODcr (mg/L)    | 169,000–182,000 | 175,000 |
| SCODcr (mg/L)    | 101,000–134,000 | 118,000 |
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 COD$_{cr}$/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.

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          |

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 (H$_2$SO$_4$) 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 COD/m$^3$·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 COD/m$^3$·d. At R2, the accumulation of VFAs was induced by increasing the OLR to 10 kg COD/m$^3$·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 COD/m$^3$·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 × 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
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].

\[
M = M_o(1-e^{-kt})
\]  

(1)

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

\[
\ln \frac{S}{S_0} = -kt
\]

(2)

where \(S_o\) 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 (kg COD/m³·d) | 6  | 10 | No feeding | 6 |
|       | Recirculating NO₃⁻-N | No | No | No | No |
| Set 2 | OLR (kg COD/m³·d) | 6  | 10 | No feeding | 6 |
|       | Recirculating NO₃⁻-N | No | No | 0.5Q (1500 mg/LNO₃⁻-N) | No |
| Set 3 | OLR (kg COD/m³·d) | 6  | 10 | 6  | 6  |
|       | Recirculating NO₃⁻-N | No | No | 0.5Q (1500 mg/LNO₃⁻-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 NO₃⁻-N was added (Fig. 3b) and only 31% was degraded without the addition of NO₃⁻-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 NO₃⁻-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
COD/m$^3$ 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/m$^3$ d at R2, VFAs increased to 16,000 mg/L COD after 100 d and methane production dropped dramatically (0.12 L CH$_4$/g VS at R2 compared to 0.32 L CH$_4$/g VS at R1). Once OLR was stopped and 0.5Q (1500 mg/L of NO$_3^-$-N) of aeration tank effluent was recirculated, normal VFA concentration was attained after 46 d. Methane production was 0.08 L CH$_4$/g VS lower at R3 compared to that in Set 1 likely because of the recirculated NO$_3^-$-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/m$^3$ 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 CH$_4$/g VS higher at R3 than that in Set 2.

**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.
fate or other inorganic electron acceptors, such as Fe(III) competition with methanogens [26].

Therefore, the time as the time taken to reach 60% methane in biogas was set as the time taken to reach a VFA concentration of 3000 mg/L. Poggi-Varaldo et al. used the stabilization 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 NO3\textsuperscript{−}-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.

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\textsubscript{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\textsuperscript{−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\textsuperscript{−1}). Set 3 was expected to take the longest time to stabilize due to denitrification of recirculated NO\textsubscript{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 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 NO3\textsuperscript{−}-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.

Set 1 operation without the recirculation of NO3\textsuperscript{−}-N or feeding OLR (Set 3), the longest stabilization time was expected due to the denitrification of recirculated NO3\textsuperscript{−}-N and utilization of introduced VFAs; however the system stabilized earlier (stabilization time: 56 d, specific reaction rate: 0.02774 d\textsuperscript{−1}) than the Set 1 operation without the recirculation of NO3\textsuperscript{−}-N or feeding (stabilization time: 72 d, specific reaction rate: 0.03161 d\textsuperscript{−1}). This may be attributed to the faster denitrification that the introduction of VFA. Recirculation of NO3\textsuperscript{−}-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 NO3\textsuperscript{−}-N provides an economically viable option for such plants.

Table 4 Specific reaction rate, k and stabilization time

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

Table 5 Denitrification rate constant k\textsubscript{den} and methanogenesis rate constant k\textsubscript{me} for each set

|                      | Set 1   | Set 2   | Set 3   |
|----------------------|---------|---------|---------|
| Denitrification rate constant, k\textsubscript{den} (d\textsuperscript{−1}) | 0.1009  | 0.1173  | 0.1159  |
| Methanogenesis rate constant, k\textsubscript{me} (d\textsuperscript{−1}) | 0.1062  | 0.1496  | 0.1590  |

Conclusions

A continuous anaerobic digestion system was operated to control accumulated VFAs. Accumulation of VFAs occurred with an OLR of 10 kg COD/m\textsuperscript{3}·d. Recirculation of NO3\textsuperscript{−}-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 NO3\textsuperscript{−}-N and no feeding) were 46 d and 0.04396 d\textsuperscript{−1}, respectively. By recirculating NO3\textsuperscript{−}-N and feeding OLR (Set 3), the longest stabilization time was expected due to the denitrification of recirculated NO3\textsuperscript{−}-N and utilization of introduced VFAs; however the system stabilized earlier (stabilization time: 56 d, specific reaction rate: 0.02774 d\textsuperscript{−1}) than the Set 1 operation without the recirculation of NO3\textsuperscript{−}-N or feeding (stabilization time: 72 d, specific reaction rate: 0.03161 d\textsuperscript{−1}). This may be attributed to the faster denitrification that the introduction of VFA. Recirculation of NO3\textsuperscript{−}-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 NO3\textsuperscript{−}-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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Simultaneous denitrification and methanogenesis; TS, VS, Volatile fatty acids; References Not applicable.

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

References

1. Andalib M, Nakhla G, McIntee E, Zhu J. Simultaneous denitrification and methanogenesis (SDM): review of two decades of research. Desalination. 2011;279:1–14.
2. Appels L, Baeyens J, Degreve J, Dewil R. Principles and potential of the anaerobic digestion of waste-activated sludge. Prog Energy Combust Sci. 2008;34:755–81.
3. Lee WS, Chua ASM, Yeoh HK, Ngoh GC. A review of the production and applications of waste-derived volatile fatty acids, Chem Eng J. 2014;235:83–99.
4. Wang Q, Kuninobu M, Ogawa HI, Kato Y. Degradation of volatile fatty acids in highly efficient anaerobic digestion. Biomass Bioenergy. 1999;16:407–16.
5. Wang Y, Zhang Y, Meng L, Wang J, Zhang W. Hydrogen-methane production from gasline manure: effect of pretreatment and VFAs accumulation on gas yield. Biomass Bioenergy. 2009;33:1131–8.
6. Buyukkamaci N, Filibeli A. Volatile fatty acid formation in an anaerobic hybrid reactor. Process Biochem. 2004;39:1491–4.
7. Gallert C, Winter J. Propionic acid accumulation and degradation during restart of a full-scale anaerobic biowaste digester. Bioprocess Technol. 2008;99:170–8.
8. Wang Y, Zhang Y, Wang J, Meng L. Effects of volatile fatty acids concentrations on methane yield and methanogenic bacteria. Biomass Bioenergy. 2009;33:848–53.
9. Nielsen HB, Uellendah H, Ahring BK. Regulation and optimization of the biogas process: propionate as a key parameter. Biomass Bioenergy. 2007;31:820–30.
10. Siegert I, Banks C. The effect of volatile fatty acid additions on the anaerobic digestion of cellulose and glucose in batch reactors. Process Biochem. 2005;40:3412–8.
11. Wang K, Yin J, Shen D, Li N. Anaerobic digestion of food waste for volatile fatty acids (VFAs) production with different types of inoculum: effect of pH. Bioprocess Technol. 2014;161:395–401.
12. Bouallagui H, Ben Cheikh R, Marouani L, Hamdi M. Mesophilic biogas production from fruit and vegetable waste in tubular digestor. Bioprocess Technol. 2003;86:85–90.
13. Garcia-Peña EI, Parameswaran P, Kang DW, Canul-Chan M, Krajmalnik-Brown R. Anaerobic digestion and co-digestion processes of vegetable and fruit residues: process and microbial ecology. Bioprocess Technol. 2011;102:9447–55.
14. Rajagopal R, Massé DI, Singh G. A critical review on inhibition of anaerobic digestion process by excess ammonia. Bioprocess Technol. 2013;143:632–41.
15. Akunna J, Bizeau C, Moletta R, Bernet N, Heduit A. Combined organic carbon and complete nitrogen removal using anaerobic and aerobic upflow filters. Water Sci Technol. 1994;30:297–306.
16. Lin YF, Chen KC. Denitrification and methanogenesis in a co-methylized mixed culture system. Water Res. 1995;29:35–43.
17. Tilche A, Borzone G, Forner M, Indulli L, Stanze L, Tesini O. Combination of anaerobic digestion and denitrification in a hybrid upflow anaerobic filter integrated in a nutrient removal treatment plant. Water Sci Technol. 1994;30:405–14.
18. Park SM, Park NB, Seo TK, Jun HB. Effects of denitrification on acid production in a two-phase anaerobic digestion process. J Korean Soc Environ Eng. 2008;30:628–36.
19. Hendriksen HV, Ahring BK. Combined removal of nitrogen and carbon in granular sludge: substrate competition and activities. Antonie Van Leeuwenhoek. 1996;69:33–9.
20. Sage M, Daufin G, Gésan-Guiziou G. Denitrification potential and rates of complex carbon source from dairy effluents in activated sludge system. Water Res. 2006;40:2747–55.
21. Elefsiniotis P, Wareham DG, Smith MO. Use of volatile fatty acids from an acid-phase digester for denitrification. J Biotechnol. 2004;114:289–97.
22. Owen JM, Chynoweth DP. Biochemical methane potential of municipal solid waste (MSW) components. Water Sci Technol. 1993;27:1–14.
23. American Public Health Association, American Water Works Association, Water Pollution Control Federation, and Water Environment Federation. Standard methods for the examination of water and wastewater. 22nd ed. Washington DC: American Public Health Association, American Water Works Association, Water Environment Federation; 1998.
24. Owen WF, Stuckey DC, Healy JBJ, Young LY, McCarty PL. Bioassay for monitoring biochemical methane potential and anaerobic toxicity. Water Res. 1979;13:485–92.
25. Scholten JC, Stams AJM. The effect of sulfate and nitrate on methane formation in a freshwater sediment. Antonie Van Leeuwenhoek. 1995;68:309–15.
26. Klüber HD, Conrad R. Effects of nitrate, nitrite, NO, and N2O on methanogenesis and other redox processes in anoxic rice field soil. FEMS Microbiol Ecol. 1998;25:301–18.
27. Roy R, Conrad R. Effect of methanogenic precursors (acetate, hydrogen, propionate) on the suppression of methane production by nitrate in anoxic rice field soil. FEMS Microbiol Ecol. 1999;28:49–61.
28. Tugtas AE, Tezel W, Pavlostathis SG. An extension of the anaerobic digestion process mode no. 1 to include the effect of nitrate reduction processes. Water Sci Technol. 2006;54:41–9.
29. Tugtas AE, Pavlostathis SG. Inhibitory effects of nitrogen oxides on a mixed methanogenic culture. Biotechnol Bioeng. 2007;96:444–55.