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Microbial community structure in a thermophilic aerobic digester used as a sludge pretreatment process for the mesophilic anaerobic digestion and the enhancement of methane production

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HIGHLIGHTS

- A combined process was developed for sludge reduction and methane production.
- Biological TAD pretreatment highly increased soluble organic matters.
- Bacteria species using DGGE were examined in a combined process.
- Methanogen species using a real-time PCR were examined in the MAD.
- Methane enhancement by TAD pretreatment was observed in a combined process.

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ABSTRACT

An effective two-stage sewage sludge digestion process, consisting of thermophilic aerobic digestion (TAD) followed by mesophilic anaerobic digestion (MAD), was developed for efficient sludge reduction and methane production. Using TAD as a biological pretreatment, the total volatile suspended solid reduction (VSSR) and methane production rate (MPR) in the MAD reactor were significantly improved. According to denaturing gradient gel electrophoresis (DGGE) analysis, the results indicated that the dominant bacteria species such as *Ureibacillus thermophiles* and Bacterium thermus in TAD were major routes for enhancing soluble organic matter. TAD pretreatment using a relatively short SRT of 1 day showed highly increased soluble organic products and positively affected an increment of bacteria populations which performed interrelated microbial metabolisms with methanogenic species in the MAD; consequently, a quantitative real-time PCR indicated greatly increased *Methanosarcinales* (acetate-utilizing methanogens) in the MAD, resulting in enhanced methane production.

1. Introduction

The generation of waste activated sludge (WAS) continues to increase rapidly, and so more efficient methods are required for managing such organic materials. In Korea, the number of wastewater treatment plants (WWTP) has increased, and 8295 tons (dry weight) of WAS is generated daily (MOE, 2009). Recently, about 6241 tons (75% of total generation) of WAS per day were discharged or removed by ocean dumping, landfill, and incineration (MOE, 2009). However, these methods of WAS management are increasingly restricted by stringent regulation, rapidly shrinking landfill space, and public opposition. Thus, it is apparent that these methods will not be viable in the near future.

Anaerobic digestion (AD) has been widely used as an efficient means of treating such wastes, since it converts organic waste into biogas in the form of methane, which is a renewable energy source (Speece, 1983). Despite these advantages of AD, some limitations are inevitable; the rather slow digestion rate, long duration of solid retention time (SRT) (20–40 days), and the susceptibility of the process to variations in operating conditions (Chen et al., 2008). Thus, many researchers have focused on increasing digestion rate by promoting hydrolysis, which is generally regarded as the rate-limiting step of AD. Various physical, chemical, thermo-chemical, ultrasonic and freezing and thawing pretreatments have been applied to improve AD efficiency (Carrere et al., 2010). However,

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these conventional pretreatment methods are not economical, and especially chemical and freezing and thawing pretreatment methods could reduce methane yield owing to increased salinity and retaining of lipid or protein in the solid phase (Liu et al., 2008).

Autothermal thermophilic aerobic digestion (ATAD) may provide an efficient and environmentally friendly biological pretreatment that can be applied to municipal solid waste (MSW) (Kelly et al., 1993). The ATAD treatment process has advantages such as enteric pathogen inactivation, short hydraulic retention time (HRT), low operating costs and self-heating during microbial metabolism. In addition, it achieves relatively fast degradation of volatile suspended solids (VSS), effectively disrupts the microbial cells of sludge and significantly increases the soluble chemical oxygen demand (SCOD) concentration such as carbohydrate, protein, lipid and VFAs, since thermophilic microbes release proteases that are highly active in sludge degradation, which was the major known lytic enzyme during the hydrolysis of sludge (Yan et al., 2008). In this respect, the interest in ATAD process as sludge pretreatment and improved digestion processes has increased recently.

As another recent technological improvement for the sludge digestion processes, combined processes have been used to achieve high solid reduction. Some researchers reported that thermophilic aerobic digestion (TAD) process prior to mesophilic anaerobic digestion (MAD) showed greater reduction in volatile solids (VS) and pathogens compared to a single MAD process (Hasegawa et al., 2000; Pagilla et al., 1996). Also, two-stage anaerobic thermophilic and anaerobic mesophilic treatment methods are known to provide both efficient VS reduction and gas production (Han et al., 1997). Furthermore, it was suggested that the combined process addresses the notion that certain proportions of sewage sludge can be degraded only under anaerobic or aerobic conditions (Novak et al., 2003). In this respect, the combined anaerobic and aerobic digestion process can promote additional degradation of organic matter, leading to greater reduction in solids compared to a single AD process.

Although the combined process provides a useful alternative method for sludge reduction and methane production, research on the combined process is in its infancy. Thus, the objective of the present study was to investigate the feasibility of a combined TAD–MAD process for the treatment of sludge; to assess the potential advantages (i.e., greater VSS reduction, VSSR and increased methane production rate, MPR); and to ascertain the optimum SRT of TAD within a combined system. In addition, there are no previous reports on the microbial community and population dynamics in continuous combined TAD–MAD process under different operating conditions. Thus, this study also aimed to elucidate the qualitative and quantitative microbial community structures and population changes in the combined TAD–MAD process using denaturing gradient gel electrophoresis (DGGE) and real-time PCR analysis.

2. Methods

2.1. Feed-sludge preparation

The feed sludge for this study was a mixture of primary and secondary sludge samples, collected from the municipal WWTP in Daegu, Korea. The plant's activated sludge process treats 520,000 m^3/d of domestic wastewater. Prior to mixing, all material was filtered by 1.0 mm sieve to remove inert matter, and then the primary and secondary sludge samples were mixed thoroughly (proportions 3:7 v/v). The mixed sludge was transferred to 3-L bottles and stored at −25 °C until use. The physical/chemical characteristics of the feed sludge used in this study are presented in Table 1.

2.2. Reactor setup

The design of the combined process is shown in Fig. 1. It consists of a TAD process (R_1) followed by MAD process (R_2); and a single MAD process (R_3) acting as a control. R_1 serves as a biological pretreatment process, and R_2 as sludge digestion and methane production. R_1 was seeded with sludge from a successfully operated ATAD pilot plant in Daejeon, Korea, while R_2 and R_3 were seeded with mesophilic anaerobic sludge from a treatment plant in Daegu, Korea, respectively. Prior to continuous operation, all reactors were operated in batch mode for two weeks. The reactors were fed four times a day, using a peristaltic pump (Cole-Parmer®) controlled by a timer and relay. During the experimental period, feeding and discharge were conducted simultaneously. R_2 and R_3 were operated at SRT of 40 days in phases I, II and III, while R_1 was operated under different SRTs (4, 2 and 1 day) in order to investigate the effects of TAD pretreatment on anaerobic digestion performance. Meanwhile, in phase IV, R_2 was operated at SRT of 39 days, and R_1 was operated at SRT of 1 day in order to make the total control volume the same as R_3. All reactors were operated as a CSTR with complete mixing, so that HRT and SRT in the system were equal. More detailed operating conditions are described in Table 2.

2.3. Physical and chemical analytical methods

Standard Methods (APHA-AWWA-WEF, 1998) were used to determine total suspended solids (TSS), VSS, total COD (TCOD), total nitrogen (TN), alkalinity and total phosphorus (TP). After centrifugation for 30 min at 5000 rpm, supernatant was filtered by 0.45-μm syringe filter (Whatman, USA) to measure SCOD, ammonia (NH_4^+–N), soluble TN (STN), and soluble TP (STP). The carbohydrate and protein concentrations were measured using the phenol–sulfuric acid method (DuBois et al., 1956) and Lowry–Folin method (Lowry et al., 1951), respectively. The concentrations of nitrite (NO_2^-), nitrate (NO_3^-), and orthophosphate (PO_4^{3-}–P) were determined by ion chromatography (ICS-100, DIONEX Co., USA). The pH and oxidation/reduction potential (ORP) in each reactor were con-

Table 1
Characteristics of feed sludge.

Parameter	Value (average ± S.D.)
pH	6.61 ± 0.06
Alkalinity (g $CaCO_3$/L)	1.72 ± 0.06
TSS (g/L)	51.51 ± 5.22
VSS (g/L)	35.56 ± 2.47
TCOD (g/L)	62.56 ± 7.96
SCOD (g/L)	7.96 ± 0.8
Nitrogen	
TN (g/L)	4.51 ± 0.77
STN (g/L)	0.96 ± 0.12
NH_4^+–N (g/L)	0.90 ± 0.09
NO_2^- (g/L)	–
NO_3^- (g/L)	–
Phosphorus	
TP (g/L)	1.81 ± 0.12
STP (g/L)	0.43 ± 0.02
PO_4^{3-} (g/L)	0.42 ± 0.01
Total VFAs (g COD/L)	1.54 ± 0.16
Acetic acid (g COD/L)	1.54 ± 0.16
Butyric acid (g COD/L)	–
Propionic acid (g COD/L)	–
Isobutyric acid (g COD/L)	–
Isovaleric acid (g COD/L)	–
Protein (g COD/L)	1.81 ± 0.29
Carbohydrate (g COD/L)	0.63 ± 0.13