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Sequential sludge digestion after diverse pre-treatment conditions: Sludge removal, methane production and microbial community changes

Sang Kyu Park [a], Hyun Min Jang [b], Jeong Hyub Ha [b], Jong Moon Park [c,*]

[a] Department of Chemical Engineering, Pohang University of Science and Technology, San 31, Hyoja-Dong, Pohang, Kyungbuk, Republic of Korea
[b] School of Environmental Science and Engineering, Pohang University of Science and Technology, San 31, Hyoja-Dong, Pohang, Kyungbuk, Republic of Korea
[c] Division of Advanced Nuclear Engineering, Pohang University of Science and Technology, San 31, Hyoja-Dong, Pohang, Kyungbuk, Republic of Korea

HIGHLIGHTS

- A sequential anaerobic–aerobic sludge digestion process was developed.
- Various pre-treatments were applied to examine the effects on methane production.
- Thermal–alkaline treatment significantly increased methane production.
- The most abundant archaeal species in the MAD was *Methanosarcinales* sp.
- The most abundant bacterial species in the TAD was *Ureibacillus* sp.

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ABSTRACT

A lab-scale sequential sludge digestion process which consists of a mesophilic anaerobic digester (MAD) and a thermophilic aerobic digester (TAD) was developed. Thermal, thermal–alkaline and long-term alkaline pre-treatments were applied to the feed sludge to examine their effects on sludge removal and methane production. Especially after thermal–alkaline pre-treatment, high COD removal was maintained; methane production rate was also drastically increased by improving the hydrolysis step of sludge degradation. Polymerase chain reaction–denaturing gel gradient electrophoresis indicated that bacterial communities were represented by three phyla (Firmicutes, Proteobacteria, Actinobacteria) and that *Clostridium straminisolvens* was the major bacterial species in MAD. Quantitative real-time PCR results indicated that *Methanosaeta concilli* was the major archaeal species in MAD, and that *Ureibacillus* sp. was the most abundant bacterial species in TAD.

1. Introduction

Waste activated sludge (WAS) is generated by the activated sludge process, which is a common biological method of treating municipal wastewater (Rittmann and McCarty, 2001). Recently, sludge generation has become a prominent environmental problem due to the marked increase in the number of wastewater treatment facilities. WAS treatment is troublesome problem because it generally contains pathogenic organisms, toxic organic matter, heavy metals and inorganic nutrients such as phosphate and ammonium that cause eutrophication (Campbell, 2000). Until recently in Korea, a large portion of sludge had usually been removed by ocean dumping (MOE, 2009). However, discharge of untreated WAS to the sea was banned by the London Convention 97 protocol in January of 2012 to protect the marine environment. Other WAS disposal methods such as landfill and incineration are rarely used in Korea due to increased land scarcity, low cost effectiveness and strict environmental regulations. Therefore, to protect environment, effective treatment of WAS is very important.

Anaerobic digestion is widely used to treat WAS because this process can degrade sludge, recover energy from the soluble organic matter in sludge, and kill pathogens (Speece, 1983). However, anaerobic digestion has several disadvantages including low organic removal (30–50%), long hydraulic digestion time of over 20 days, sensitivity to operating conditions, and large space requirement due to slow digestion rate (Chen et al., 2008). To increase the digestion rate, researchers have investigated various physical and chemical pre-treatment methods to accelerate the hydrolysis step by using thermal, alkaline, thermal–alkaline, ultrasonic and mechanical or ozone treatment (Tyagi and Lo, 2011). Applying these pre-treatment methods to WAS degradation improves sludge reduction and increases methane production. As a

* Corresponding author. Tel.: +82 54 279 2275; fax: +82 54 279 8299.
E-mail address: jmpark@postech.ac.kr (J.M. Park).

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substitute for anaerobic digestion, thermophilic aerobic digestion (TAD) has been used to treat WAS owing to TAD's advantages such as short solid retention time, self-heat generation, stability of treated WAS, and high activity of bacteria species (Kelly and Mavinic, 2003). However, one major drawback of this process is that a large proportion of WAS treated is just changed to carbon dioxide and aerobic cells rather than to methane, resulting in waste of useful organic resources without producing bioenergy.

Recently, a process that uses sequential anaerobic and aerobic digestion has been investigated as an improved digestion process for treatment of sludge based on the notion that a certain portion of sewage sludge degrades only under aerobic or anaerobic conditions (Novak et al., 2011). This process can make the additional reduction of solids compared to a single mesophilic anaerobic digestion (MAD). Meanwhile, TAD prior to MAD process increased the methane production since TAD is very effective for improving high concentrations of soluble organic matter (Pagilla et al., 2000; Jang et al., 2013). Therefore, the combined digestion process can make additional WAS degradation possible and provide better methane reduction than a single-stage digestion process.

In this study, a WAS treatment process that consists of sequential MAD and TAD was developed for efficient sludge reduction and methane production. Three pre-treatment methods for feed sludge were investigated to examine their effects on sludge reduction, methane production and microbial community changes. Like many biological processes, microbial community structures are closely linked to the removal efficiency and stability of sludge digestion. Microbial community analysis with respect to conventional anaerobic sludge digestion process has been reported previously (Shin et al., 2010), but no studies have reported microbial community analysis in a sequential MAD/TAD process with sludge pre-treatment. Therefore, this study included polymerase chain reaction–denaturing gel gradient electrophoresis (PCR–DGGE) and quantitative real-time PCR (qPCR) to examine the microbial community changes during sequential sludge digestion under diverse pre-treatment conditions of feed sludge.

2. Methods

2.1. Reactor operation

The feed sludge was obtained by mixing primary and secondary sludge (2:3 v:v) collected from municipal wastewater treatment plants (WWTPs) in Daejeon. Primary and secondary sludges were first filtered through 1.0-mm sieves to remove large particles of inert matter, then stored at −25 °C until use.

A lab-scale MAD/TAD sequential sludge digestion system was used (Fig. 1). MAD was inoculated with samples from anaerobic digesters of municipal WWTPs in Daegu. The subsequent TAD was seeded from another lab-scale TAD process which was operated stably. Throughout the experiments (250 d), heating plates were used to maintain the temperature of MAD at 35 °C and the temperature of TAD at 55 °C. A water circulator was used to maintain the temperature of the feed sludge tank at 4 °C to prevent the feed sludge from decaying. MAD and TAD were operated at SRTs of 40 and 10-d with working volumes of 6 and 1.5 L, respectively. Air was supplied (2 L/min) to TAD using an air pump equipped with sparger. The feed sludge was fed into MAD every 6-h using a peristaltic pump controlled by a timer and a relay. Digesters were fed and discharged simultaneously.

2.2. Feed sludge pre-treatment

The combined sludge digestion process used in this study was operated in four different phases with different sludge pre-treatment strategies. In phase I, feed sludge was supplied to the system without any pre-treatment. In phase II, the feed sludge was thermally pre-treated (121 °C, 1 h) before it was supplied to the combined process. In phase III, feed sludge was pre-treated using a thermal–alkaline method: the pH of feed sludge was adjusted to 12 by adding 5-N NaOH solution and stirring for 1 h at room temperature, then thermally treated (121 °C, 1 h), cooled to room temperature and neutralized by adding 4-N HCl solution. The cooling time was about 2 h. In phase IV, the pH of feed sludge was adjusted to 12 by adding 5-N NaOH solution and stirred continuously for 7-d. The pH of feed sludge was checked and adjusted daily during this long-term alkaline pre-treatment. After 7-d of alkaline pre-treatment, the feed sludge was neutralized using 4-N HCl solution and supplied to the feed tank. These treatments affected the characteristics of the feed sludge for each phase (Table 1).

2.3. Analytical methods

Standard methods (APHA, 1998) were used to determine total COD, TSS, VSS, TN, TP and alkalinity. To measure soluble COD, soluble TN, soluble TP, ammonia, carbohydrate and protein, samples were centrifuged (5000 rpm, 30 min) and the supernatant was filtered through a 0.20-μm syringe filter (Whatman, USA). Protein and carbohydrate contents were measured using the Lowry–Folin method and the phenol–sulfuric acid method, respectively. The pH and oxidation reduction potential (ORP) of MAD and TAD were measured using a pH/ORP meter (Mettler Tolledo, Switzerland). Methane concentration was analyzed using a gas chromatograph (Agilent, USA) equipped with a pulsed discharge detector. Total volume of biogas was measured using water displacement methods, and methane production rate of MAD was calculated from methane concentration and biogas volume.

2.4. Microbial community analysis

2.4.1. Total DNA extraction

Mixed sludge (50 μL) from each reactor was sampled and washed with 1 mL of PBS buffer; the mixture was centrifuged at 14,000*g* for 5 min, then the supernatant was decanted and the sludge pellet was suspended in 100 μL of Tris–HCl (pH 8.0) buffer. Total genomic DNA of prepared samples was extracted immediately using a nucleospin Soil kit (Macherey-Nagel, Germany) according to manufacturer's instruction. Extracted genomic DNA was eluted in 50 μL of deionized water and stored at −20 °C until usage.

2.4.2. PCR–DGGE analysis

PCR for DGGE using designed primer sets (Table 2) (Muhling et al., 2008; Yu et al., 2005) was performed to amplify 16s rRNA genes of bacteria and archaea in the reactors. The PCR cycle consisted of initial denaturation at 94 °C for 10 min; 35 cycles of denaturation at 94 °C for 30 s, annealing at 55 °C for 30 s, extension at 72 °C for 30 s; and final extension at 72 °C for 10 min.

DGGE was conducted using a DCODE system (BioRAD). PCR product (20 μL) was loaded onto 8% polyacrylamide gel with a 40–60% denaturing gradient. DGGE condition was 600 min running time at 70 V and 60 °C in 0.5× TAE buffer. After electrophoresis, polyacrylamide gel was stained with ethidium bromide and analyzed under UV using Gel DOC XR (BioRAD, USA). Visible bands in the gel were excised and DNA in the excised gel was extracted with 40 μL of deionized water.

Extracted DNA was amplified by PCR using the same primer set without the GC clamp. PCR product was sequenced (Solgent Co.), and then BLAST (http://blast.ncbi.nlm.nih.gov/Blast.cgi) was used to compare sequences with the GenBank database. Sequence align-