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Short communication

Behavior of methanogens during start-up of farm-scale anaerobic digester treating swine wastewater

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ABSTRACT

The aim of this study was to monitor the changes in methanogenic community structures in an anaerobic digester (250 m^3 working volume) during start-up including prolonged starvation periods. Redundancy analysis was performed to investigate the correlations between environmental variables and microbial community structures. The anaerobic digester was operated for 591 days at alternating operating temperatures. In initial start-up period at stage I (35 °C), growth of various species of mesophilic aceticlastic methanogens (AMs) and hydrogenotrophic methanogens (HMs) was observed. *Methanobacteriales* species survived better than other methanogens under long-term starvation conditions. In stage II (50 °C), HMs became dominant over AMs as the operating temperature changed from mesophilic to thermophilic due to increase of ammonia inhibition. In stage III (35 °C), only the *Methanomicrobiales* population significantly increased during 50 days of HRT while *Methanobacteriales* dominated over 15 days of HRT. The influent pH negatively correlated with all methanogenic populations especially in stage II.
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1. Introduction

Swine manure causes environmental problems due to its high organic fraction and content of nutrients such as phosphorous and nitrogen. Anaerobic digestion (AD) is viable approach for the treatment of high strength organic wastes such as swine manure, since it produces renewable energy and valuable digested residues [1,2]. The start-up of an anaerobic digester, however, is a slow and critical step which can affect the subsequent process operation [3,4]. The process performance during the start-up period depends on a variety of factors, such as the temperature, the organic loading rate (OLR), hydraulic retention time (HRT), and digester design along with mixing [5–7].

Two distinct group of methanogens, aceticlastic or hydrogenotrophic, play a major role in producing methane [8]. Because hydrogenotrophic methanogens (HMs) are more resistant to toxic substances such as ammonia when compared with aceticlastic methanogens (AMs), conditions that favor the domination of HMs in AD systems treating swine wastewater would be desirable [1,9]. Operating temperature is one of the main factors to induce the predominance of the HMs in AD system [10]. Increasing the temperature results in the increase of free ammonia (FA) concentration, thus HMs predominate over AMs [11–15]. It is also possible that population of HMs can increase with decreasing the population of AMs at mesophilic condition if FA condition is maintained above 0.37 g/L [12,16,17]. HRT along with substrate also plays an important role in the predominance of a certain group in AD due to the different biokinetics among methanogens. In processes operated with long HRTs, essentially low substrate concentration, microbes with lower maximum specific growth rates and higher substrate affinity are likely to predominate over those with higher maximum specific growth rates but lower substrate affinities [18].

Engineers sometimes face with unexpected difficulties when anaerobic digesters are constructed in the field including swine farms. Foot-and-mouth disease (FMD), for example, is a highly contagious disease that affects a variety of domestic animals including pigs. A total 1795 outbreaks of FMD were reported from January 2006 to May 2011 in 42 countries [19]. If FMD occurs in pig farming area, the public access to the area is restricted for the disease control and the pigs are killed. Consequently, the substrate supply to anaerobic digester is usually stopped for several months [20]. The pipes in AD system may be frozen up at extremely low temperature, thus leading to the stop of substrate input [21–23]. If this starvation would last long enough that microbes undergo endogenous decay, removal of organic matter or gas production is limited to low values in anaerobic digester [24,25]. The aim of this study was to monitor the changes in methanogenic com-

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munity structures concomitant with the start-up of a farm-scale anaerobic digester treating swine wastewater, under unforeseen circumstances of starvation and changes of operating temperature and HRT.

2. Materials and methods

A farm-scale anaerobic digester with a working volume of 250 m^3 to treat swine wastewater was built at a pig farm in Pocheon, Korea. At the beginning of the operation, the anaerobic digester was inoculated with seeds collected from different anaerobic digesters treating alcohol processing wastewater and municipal sludge at thermophilic and mesophilic conditions, respectively. The pH was not controlled and the speed of agitation was maintained at 30 rpm.

The operation period of the AD process was divided into three stages according to the temperature change. Originally, we intended to let HMs be dominant in the AD reactor because HMs have greater resistance to toxic substances in swine wastewater than AMs [1,9]. During the first start-up attempt (i.e., stage I), the reactor was operated under mesophilic conditions (35 °C) expecting a predominance of HMs [1,2,26]. Due to an outbreak of FMD, however, substrate supply was completely stopped for 189 days (Fig. 1). Because AMs predominated during stage I, the AD operation resumed at thermophilic temperature (50 °C) to promote growth of HMs, and to exert more FA inhibitory effect to AMs (i.e., stage II). The HRT was subsequently decreased from 250 days through 100 days to 50 days. Finally, the operating temperature decreased to 35 °C while maintaining HRT at 50 days (i.e., stage III) after confirmation of predominance of HMs. The substrate supply was irregular for 124 days (Day 440 to Day 564) due to freezing problems. After then, the digester was restarted at 15 days HRT to compare the system behavior and its efficiency with the previous optimization study of gas production and COD removal using same swine wastewater [27,28].

The concentrations of total volatile fatty acids (TVFA) and biogas composition were analyzed by using the gas chromatography as previously described [2]. The pH and chemical oxygen demand (COD) were determined according to the procedures in Standard Methods [29]. Ammonium ion concentration was measured with ion chromatography (790 Personal IC, Metrohm, Switzerland). The total genomic DNA was extracted using an automated nucleic acid extractor (Magtration System 6GC, PSS, Chiba, Japan) [2]. The denaturing gradient gel electrophoresis (DGGE) analysis and DNA sequencing were performed as previously described [26]. Real time quantitative PCR (QPCR) analysis was performed using a LightCycler 480 (Roche Diagnostics, Mannheim, Germany) with four primer and probe sets for quantification of methanogens according to the protocols [30,31].

Redundancy analysis (RDA) was performed to investigate the correlations between environmental variables and microbial community structures (CANOCO 4.5, Plant Research International, the Netherlands) [2]. In this study, the results of methanogenic population from the QPCR assay, COD removal and daily biogas production rate (DBP) were used as dependent variables and five different physico-chemical parameters, HRT, OLR, pH, COD and inflow (Q), were used as independent variables.

Table 1
Identification of amplified 16 S rRNA gene sequences excised from the DGGE gels.

Species	Order	Band number	Similarity (%)
Methanobrevibacter gottschalkii	MBT	8	99
Methanothermobacter crinale	MBT	16,17,18,19,20	99
Methanoculleus bourgensis	MMB	1,2,3,4,6,7,9,11	99
Methanoculleus receptaculi	MMB	13	99
Methanospirillum hungatei	MMB	5	98
Methanosaeta concilii	MSL	15	97
Methanosarcina mazei	MSL	10,12,14	99

3. Results and discussion

3.1. Stage I (Day 0 to Day 224): mesophilic operation

In the initial start-up period (Day 0 to Day 35), average OLR and DBP were 1.7 kg/m^3/day and 0.10 m^3/m^3/day, respectively. After the outbreak of FMD, DBP decreased to 0.03 m^3/m^3/day (Fig. 1).

During the initial start-up period, the band intensity of three species of HMs, *Methanobrevibacter gottschalkii*, *Methanospirillum hungatei* and *Methanoculleus bourgensis* and two species of AMs, *Methanosaeta concilii* and *Methanosarcina mazei*, showed increasing patterns (Fig. 2 and Table 1). Among these species, band intensity of *M. bourgensis* and *M. mazei* increased noticeably, while the band intensity of *Methanothermobacter crinale* decreased (Fig. 2, and Table 1). These variations would be caused by the operating temperature because the mesophilic conditions could encourage the growth of mesophilic methanogens [32–34]. Moreover, a relatively low ammonia concentration due to a dilution effect is likely a crucial factor that encouraged growth of the methanogens in the initial start-up period. In general, the dilution effect of ammonia can be followed by adding the seed and the feedstock together into a digester during the start-up period. Because the pH and total ammonia (TA) were maintained at 7.7 ± 0.1 and 2.2 ± 0.2 g/L, respectively, FA concentration was only 0.14 ± 0.01 g/L [16]. This

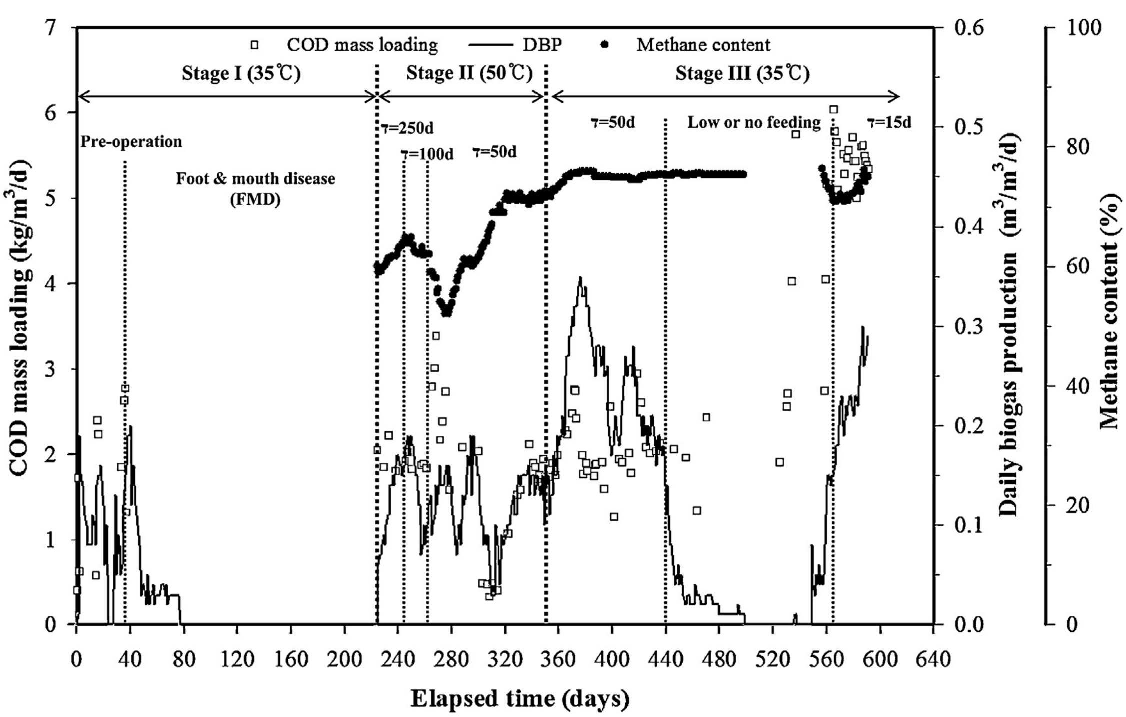

Fig. 1. Profiles of COD mass loading, DBP and methane content in the farm-scale anaerobic digester during the whole elapsed time.

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