



# Microbial community shifts in a farm-scale anaerobic digester treating swine waste: Correlations between bacteria communities associated with hydrogenotrophic methanogens and environmental conditions

Kyungjin Cho <sup>a</sup>, Seung Gu Shin <sup>b,\*</sup>, Woong Kim <sup>c</sup>, Joonyeob Lee <sup>b</sup>, Changsoo Lee <sup>d</sup>, Seokhwan Hwang <sup>b,\*</sup>

<sup>a</sup> Center for Water Resource Cycle Research, Korea Institute of Science and Technology, 39-1 Hawolgok-Dong, Seongbuk-Gu, Seoul 136-791, Republic of Korea

<sup>b</sup> School of Environmental Science and Engineering, Pohang University of Science and Technology, Pohang, Gyeongbuk 790-784, Republic of Korea

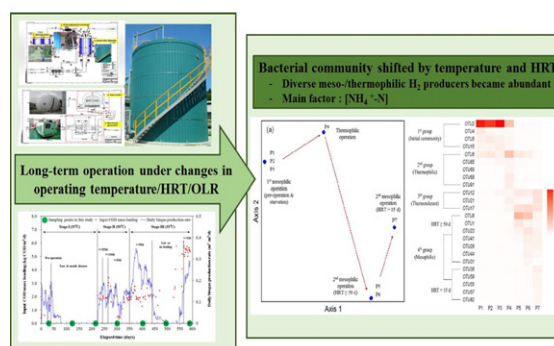
<sup>c</sup> Department of Environmental Engineering, Kyungpook National University, 80 Daehak-ro, Buk-gu, Daegu 41566, Republic of Korea

<sup>d</sup> School of Urban and Environmental Engineering, Ulsan National Institute of Science and Technology (UNIST), 50 UNIST-gil, Eonyang-eup, Ulju-gun, Ulsan 689-798, Republic of Korea

## HIGHLIGHTS

- Microbial community was elucidated in a farm-scale digester treating swine manure.
- Hydrogenotrophic methanogens were dominant except during the initial period.
- Bacterial community shifted by temperature and HRT but not by prolonged starvation
- Clostridiales and Bacteroidales were the major partner bacterial orders of HMs.
- Some mesophilic partner bacteria were positively correlated with  $[\text{NH}_4^+ - \text{N}]$ .

## GRAPHICAL ABSTRACT



## ARTICLE INFO

### Article history:

Received 31 March 2017

Received in revised form 19 May 2017

Accepted 20 May 2017

Available online xxx

Editor: Simon Pollard

### Keywords:

Anaerobic digestion

Swine waste

454 pyrosequencing

Hydrogen-producing bacteria

Syntrophic acetate oxidizing bacteria

Hydrogenotrophic methanogen

## ABSTRACT

Microbial community structure in a farm-scale anaerobic digester treating swine manure was investigated during three process events: 1) prolonged starvation, and changes of 2) operating temperature (between meso- and thermophilic) and 3) hydraulic retention time (HRT). Except during the initial period, the digester was dominated by hydrogenotrophic methanogens (HMs). The bacterial community structure significantly shifted with operating temperature and HRT but not with long-term starvation. Clostridiales (26.5–54.4%) and Bacteroidales (2.5–13.7%) became dominant orders in the digester during the period of HM dominance. Abundance of diverse meso- and thermophilic bacteria increased during the same period; many of these species may be  $\text{H}_2$  producers, and/or syntrophic acetate oxidizers. Some of these species showed positive correlations with  $[\text{NH}_4^+ - \text{N}]$  ( $p < 0.1$ ); this relationship suggests that ammonia was a significant parameter for bacterial selection. The bacterial niche information reported in this study can be useful to understand the ecophysiology of anaerobic digesters treating swine manure that contains high ammonia content.

© 2017 Elsevier B.V. All rights reserved.

## 1. Introduction

Global swine production has increased >3.5-fold during the past 40 years. Associated with the increasing swine production, swine

\* Corresponding authors.

E-mail addresses: [candlit@postech.ac.kr](mailto:candlit@postech.ac.kr) (S.G. Shin), [shwang@postech.ac.kr](mailto:shwang@postech.ac.kr) (S. Hwang).

manure has been attracting attention as a bio-resource due to its high nutrition contents. Anaerobic digestion (AD) is a good method to treat swine waste, because the process can produce energy and fertilizer (Zhang and Jahng, 2010). AD is performed by harmonious activity of different microbial groups including hydrolyzing/fermenting bacteria and methanogenic archaea. The optimal growth conditions and responses to inhibitory compounds vary greatly among anaerobes, so their populations may vary significantly and asynchronously over time, even within a single digester (Yenigün and Demirel, 2013). Although process functionality may be tolerant to such variations in some cases, microbial population changes can cause process imbalance or even failure of AD (Fernandez et al., 1999). For this reason, great efforts have been devoted to understanding microbial community structures in AD processes under transient conditions (Cho et al., 2013).

Methanogens are broadly classified into two distinct groups: hydrogenotrophic (HMs) and acetoclastic methanogens (AMs). HMs are more resistant than AMs to toxic materials such as ammonia, so HMs often dominate over AMs under harsh operating conditions (Yenigün and Demirel, 2013). Due to the high ammonia content of swine waste, CH<sub>4</sub> production via the hydrogenotrophic pathway has been regarded as the key methanogenic pathway in AD processes that treat this waste (Fotidis et al., 2013; Lee et al., 2015). The growth of HMs is closely related to the formation of a partnership (usually as syntrophism) with a bacterial community that produces H<sub>2</sub>, which is the substrate for HMs (Sasaki et al., 2012). Recently, Narihiro et al. (2015) suggested that a preferential partnership between H<sub>2</sub>-producing bacteria and HMs may exist. Moreover, use of ammonia-tolerant microbial consortia (i.e., partner bacterial community associated with HMs) as an inoculum could increase the efficiency of an AD process operated under high ammonia conditions (Fotidis et al., 2013). Therefore, identification of the H<sub>2</sub>-producing bacteria coupled with HMs in the AD processes treating swine manure should be a valuable addition to our knowledge of microbial management (Carballa et al., 2015).

Anaerobic H<sub>2</sub> production can be classified into two categories according to the substrate type. First, H<sub>2</sub> can be produced by fermentation of the macro-organic molecules. For example, family Syntrophomonadaceae produces H<sub>2</sub> syntrophically by degrading long-chain fatty acids under methanogenic conditions (Hatamoto et al., 2007). *Coprothermobacter* spp. are protein degraders as well as H<sub>2</sub> producers, and can significantly interact with HMs (Gagliano et al., 2015). Second, H<sub>2</sub> can be produced from acetate by syntrophic acetate oxidizing bacteria (SAOB). Because acetate can also be directly utilized by AMs to produce methane, the activity of SAOB associated with HMs is regarded as an alternative, as well as competitive, methanogenic pathway via acetate. Syntrophic acetate oxidation (SAO) has been suggested as the major acetate catabolism in anaerobic digesters that treat nitrogenous substrates such as animal manure (Fotidis et al., 2013). To date, only six SAOB species have been isolated: three thermophilic (*Thermotoga lettingae*, *Thermacetogenium phaeum*, and strain AOR) and three mesophilic (*Clostridium ultunense*, *Syntrophaceticus schinkii*, and *Tepidanaerobacter acetatoxydans*) (Fotidis et al., 2013). Strain AOR (an acetate-oxidizing, rod-shaped bacterium) was initially co-isolated with *Methanobacterium* sp. strain THF from a thermophilic anaerobic digester treating lignocellulosic urban solid waste (Zinder and Koch, 1984). Recently, potential SAOBs have been suggested at higher taxonomic levels; for example, Lee et al. (2015) has suggested that phylum Spirochaetes may be SAOB. In addition, order Clostridiales may perform SAO in anaerobic digesters (Lu et al., 2014). However, information on the H<sub>2</sub>-producing partner bacteria of HMs in AD process treating swine manure remains limited.

A field-scale anaerobic digester is usually operated under defined conditions to maximize process stability. However, undesired events can occur, and operating parameters may be changed intentionally (Cho et al., 2013). In such cases, shifts of the anaerobic community structure must be monitored, because they likely affect the function of

the system. For example, operating temperature has a crucial influence on microbial activity and community composition in bioprocess systems (Gao et al., 2011). Temperature can be manipulated as a start-up strategy while inoculating mesophilic sludge to a thermophilic AD process (Kim and Speece, 2002), but may also fluctuate due to failure of heating, or if temperature is not controlled (e.g., ambient digesters) (Gao et al., 2011). Hydraulic retention time (HRT) also strongly influences digester efficiency, and the dominance or exclusion of certain microbial groups in an AD process. Feeding of the digester may be limited or even stop during process imbalance, mechanical failure or maintenance, or substrate unavailability (Cho et al., 2013). Farm-scale AD plants have been widely implemented to treat agricultural wastes such as swine manure; over 4000 on-farm AD plants are currently operated in Germany (Wilkinson, 2011). Because farm-scale AD processes are normally installed at large pig farms (Holm-Nielsen et al., 2009), protein- and ammonia-rich swine manure can be regarded as a main substrate. Therefore, the process performance and microbial community in farm-scale AD processes can be significantly different compared with conventional AD plants treating activated sludge because the major CH<sub>4</sub> production pathway could be different (Riviere et al., 2009; Westerholm et al., 2016). However, there are limited reports available on the microbial characterization in farm-scale AD processes, especially during long-term operation.

The objective of this study was to investigate the partner bacterial community of HMs in a farm-scale anaerobic digester treating swine manure under changes of operating temperature and HRT. The bacterial community structure was examined using 454 pyrosequencing analysis over a 1.5-year period along with three process events (i.e. 1) prolonged starvation, and changes of 2) operating temperature (between meso- and thermophilic) and 3) HRT). Statistical techniques were used to help understand the bacterial community shifts with respect to variations in process parameters.

## 2. Materials and methods

### 2.1. Farm-scale anaerobic digester and reactor operation

A farm-scale anaerobic digester with a working volume of 250 m<sup>3</sup> was built in a pig farm (Pocheon, Korea). The anaerobic digester was inoculated with two types of anaerobic seed sludge: one type collected from a thermophilic digester (treating brewery wastewater) and one type collected from a mesophilic digester (treating sewage sludge). After inoculation, the AD process was operated for 591 days. The operation periods were classified into three stages according to the operational temperature (Table 1; Fig. S1); stage I: 1st mesophilic operation period (Day 0 to Day 224, P1–P3), stage II: thermophilic operation period (Day 224 to Day 348, P4), and stage III: 2nd mesophilic operation period (Day 348 to Day 591, P5–P7). During stage I, the operation of the AD process was suspended for 189 days (Day 35 to Day 224) (P2–P3) due to an outbreak of foot-and-mouth disease (FMD). After the digester was re-started, the HRT was gradually decreased from 250 days to 50 days during stage II (P4). During stage III, the AD process was operated

**Table 1**

Sample information used for physico-chemical and molecular analyses with process events.

Sample	Elapsed time (days)	Process events
P1	30	1st mesophilic operation (35 °C); pre-operation
P2	121	1st mesophilic operation (35 °C); starvation due to FMD
P3	212	1st mesophilic operation (35 °C); starvation due to FMD
P4	303	Thermophilic operation (50 °C); HRT = 50 days
P5	405	2nd mesophilic operation (35 °C); HRT = 50 days
P6	487	2nd mesophilic operation (35 °C); HRT > 50 days
P7	588	2nd mesophilic operation (35 °C); HRT = 15 days

Acronym: FMD, foot-and-mouth disease.

Download English Version:

<https://daneshyari.com/en/article/5750374>

Download Persian Version:

<https://daneshyari.com/article/5750374>

[Daneshyari.com](https://daneshyari.com)