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Short Communication

Changes in bacterial and archaeal communities in anaerobic digesters treating different organic wastes

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HIGHLIGHTS

- BMP tests were conducted using representative organic wastes.
- Biomethanation of food wastes produced a high yield of methane.
- Bacterial communities distinctly shifted in the presence of different organic wastes.
- The dominance of the main methanogens slightly shifted in between the different ADs.

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ABSTRACT

The goal of this study was to characterize microbial communities in anaerobic batch digesters treating different representative organic sources (sewage sludge, food waste, septage). Among the digesters, the anaerobic digester of food waste had the highest methanogen density, producing a peak value methane yield of 813.2 mL CH_4/g VS. In all the digesters, acetoclastic *Methanosarcinales* and hydrogenotrophic *Methanomicrobiales* were the most dominant methanogen groups, but their proportion among the methanogens varied depending on the organic sources. The bacteria community in the anaerobic digestion (AD) of food waste and septage was distinctly different from that found in the AD of sewage sludge (primary sludge and waste activated sludge). Shifts in both bacterial and archaeal community structures could be related to differences in chemical properties, production, and accumulation of intermediates digested from organic wastes having different characteristics. These findings could prove useful in optimizing the microbial community to enhance AD process treating organic wastes.

1. Introduction

Anaerobic digestion (AD) has long been used for the stabilization of particulate organic wastes such as waste activated sludge, food waste and animal manure. Later, it was successfully adapted for the treatment of industrial and domestic wastewaters. AD consists of a series of microbiological processes that convert organic compound to methane and carbon dioxide as well as reduce volatile solids (Bitton, 2005). In AD of wastes, consortia of microorganisms, comprising bacterial and archaeal groups transform complex, high molecular weight organic compounds to methane. The bacterial group hydrolyzes and ferments organic compounds to form hydrogen and organic acids, while methanogens in archaeal groups produce methane using intermediates such as formate, hydrogen and acetate (Lee et al., 2009; Lim et al., 2013). For the complete bioconversion of organic wastes to methane, synergistic, sequential interactions between the various groups of microorganisms are implicated in anaerobic digesters. Therefore, a basic understanding of the microbial communities in anaerobic digesters is required to fundamentally improve AD technology.

In particular, previous studies on bacterial and methanogenic archaeal communities in anaerobic digesters have shown that microbial communities in anaerobic digesters can shift with changes in operating conditions such as temperature, organic loading and feedstock (Lee et al., 2009; Kim et al., 2011, 2013). However, little information currently exists on the quantitative as well as qualitative differences in bacterial and methanogenic communities in anaerobic digesters treating different organic

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wastes. Identification of microbial communities and their functions is necessary to adequately control process conditions, leading to possible improvement both in efficiency and stability as well as in designing reactors for anaerobic digesters. In this study, therefore, the primary objective was to characterize microbial communities in anaerobic batch digesters treating different representative organic sources (i.e. wastewater sludge, food waste, septage) by applying a combination of different molecular techniques, denaturing gradient gel electrophoresis (DGGE) for qualitatively assay, and quantitative polymerase chain reaction (qPCR) for quantitative assay. The results of this study provide insight into microbial communities in anaerobic digesters treating representative organic wastes.

2. Materials and methods

2.1. Biochemical methane potential test

Four different municipal organic waste sources were selected for this study. Primary sludge (PS) and waste activated sludge (WAS) were collected from a municipal wastewater treatment plant (WWTP) in Anyang City, Korea. Food waste (FW) and septage were sampled from a FW and septage treatment facility in Anyang, Korea (Table 1). Biochemical methane potential (BMP) tests were performed for 50 days in 160 mL serum bottles with a working volume of 100 mL (Cabbai et al., 2013). The bottles were initially inoculated with anaerobic sludge from an anaerobic digester treating PS in the WWTP. The inoculum and substrate were mixed at a ratio of 1:1 based on the concentration of volatile solids (VS).

2.2. Chemical analysis

Biogas composition was analyzed by gas chromatography (HP 5890, PA, USA) equipped with a thermal conductivity detector (TCD). Total solids (TS), VS, chemical oxygen demand (COD), total nitrogen (TN) and total phosphorus (TP) were analyzed according to standard methods (APHA, 1998). NH_4^+, PO_4^{3-} and Cl^- were measured with an ion chromatograph (ICS-1000, Dionex Co., USA).

2.3. Molecular microbial analysis

Total DNA from the sludge was extracted and purified using a Nucleo Spin® Soil kit (MACHEREY–NAGEL, Germany) according to the manufacturer's protocol. Bacterial and archaeal communities were analyzed via PCR–DGGE using the primer set BAC338F/805R and ARC787F/1059R, respectively, with a GC-clamp as previously described (Yu et al., 2005; Kim et al., 2013). Upon confirmation of the excisions as single bands via a secondary DGGE run, the bands were re-amplified, purified with QIAEX II (Qiagen, CA), and sequenced (ABI3730XL DNA analyzer, Applied Biosystems, CA). Sequences were aligned using MEGA 4.0 and analyzed using BLAST. Applying previously documented protocols (Yu et al., 2005; Shin et al., 2010), qPCR assays were conducted in triplicate on an Applied Biosystems 7300 qPCR system (Applied Biosystems, Forster City, USA) using six primer and probe sets targeting 16S rRNA genes of different microbial groups, the domains bacteria and archaea, and the methanogenic orders *Methanobacteriales*, *Methanococcales*, *Methanomicrobiales*, and *Methanosarcinales*.

3. Results and discussion

3.1. BMP tests for the selected organic wastes

A series of BMP tests using the selected organic wastes was performed for 45 days; the results represented in Table 1 were obtained on the final day of tests. Between the four selected organic wastes, the cumulative methane yield from FW in the anaerobic digesters revealed the highest value, while the lowest value was obtained from septage digestion, attaining 813.2 and 241.4 mL CH_4/g VS, respectively. Despite an expected high theoretical methane potential (646.8 mL CH_4/g VS) of septage based on its chemical formula ($C_{12.3}H_{46.6}O_{5.9}N$), the experimental methane production of septage actually achieved less than 27.9% of the theoretical methane potential. This reflects that the existence of a low carbon fraction in septage is challenging for the feed of an anaerobic digester (Lee et al., 2013). The AD of PS and WAS produced similar methane yields, corresponding to 323.5 and 277.8 mL CH_4/g VS. Sewage and manure are materials lacking the most potential for AD since the biodegradable material has already had much of the energy content taken out by the animals that produced it (Lemmer and Oeschsner, 2001). Therefore, many digesters necessarily operate with co-digestion of two or more types of feedstock. Provided with a better balance of nutrients in feed stock, digestion of co-substrates can improve biogas production (Kim and Kang, 2015). Considering the almost unlimited potential of FW having a large quantity of readily biodegradable carbon, co-digestion of FW with other organic wastes might overcome the problems of separate digestion of each substrate alone.

3.2. Microbial community

Archaeal and bacterial communities in anaerobic digesters treating the four selected organic wastes were analyzed according to their DGGE results. Most of the archaeal sequences were phylogenetically affiliated within the methanogen species of two orders, hydrogenotrophic *Methanomicrobiales* (A1, 2, 4, 5, 9 and 11) and acetoclastic *Methanosarcinales* (A8 and 12) (Table 2). Among the six *Methanomicrobiales*-related bands, two bands were closely related to *Methanolinea* species, *M. tarda* (A2 and 5), identified as the major hydrogenotrophic methanogen group in anaerobic digester systems (Kim et al., 2013, 2014). The bacterial community was composed of the phyla *Bacteroidetes*, *Firmicutes*, *Actinobacteria* and *Proteobacteria* (Table 2). Among the various bands, B2 and 9 were detected in all samples and were affiliated with the order *Clostridiales* whose members are frequently found in AD processes

Table 1
Characteristics of four selected organic wastes and values obtained from BMP tests.

Plant	Characteristics of organic wastes										Results of BMP tests					
	TS (%)	VS (%)	TCOD (mg/L)	SCOD (mg/L)	T-N (mg/L)	NH_4^+-N (mg/L)	T-P (mg/L)	PO_4^{3-}-P (mg/L)	Cl^- (mg/L)	n-hexane (mg/L)	Biogas production (mL biogas/g VS)	CH_4 (%)	CO_2 (%)	N_2 (%)	H_2S (ppm)	VS reduction (%)
PS	2.0	1.31	22200	1283	480	249	69	16	134	988	323.5	70.2	25.5	1.6	465	61.7
WAS	2.59	1.72	27400	700	2170	198	158	25.3	96	2575	277.8	69.2	26.5	1.5	460	54.6
FW	8.09	6.72	129000	73667	1580	271	784	120.7	2090	2232	813.2	64.3	33.9	1.8	250	66.6
Septage	0.69	0.57	10200	1027	380	219	43	11.7	143	620	241.4	68.3	26.9	1.6	500	67.7

PS: primary sludge; WAS: waste activated sludge; FW: food waste.