



Correlations between bacterial populations and process parameters in four full-scale anaerobic digesters treating sewage sludge



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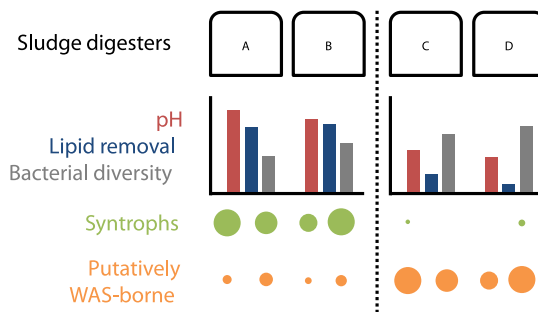
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HIGHLIGHTS

- Protein and lipid removals were positively intercorrelated while carbohydrate wasn't.
- Bacterial diversity and evenness were lower in digesters with better performance.
- "Richness" of major bacterial genera was higher in digesters with better performance.
- Poor-performance digesters almost lacked fatty acid and protein-utilizing syntrophs.
- Putatively WAS-borne bacteria were more abundant in digesters with poor performance.

GRAPHICAL ABSTRACT



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ABSTRACT

Process parameters and bacterial populations were investigated in four full-scale anaerobic digesters treating sewage sludge. Although the four digesters were operated under similar conditions, digesters A and B had higher pH (7.2–7.4) and lipid removal efficiencies (>50%) than C and D (pH 6.1–6.4; average lipid removal <16%). Bacterial richness, diversity, and evenness were higher in digesters C and D. Among the top-populated genera, ten (group I) were more abundant in digesters A and/or B; they were putative syntrophic fatty acid or protein/amino acid-utilizers. In contrast, fifteen others (group II) were less abundant in A and/or B and included potentially dormant/dead cells originated from activated sludge. Despite the overall richness trend, the presence of the 25 genera in groups I/II was greater in digesters A and B (24) than in C and D (17); this observation suggests that group I bacteria might be essential in AD of sewage sludge.

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1. Introduction

Management of excess sludge contributes up to half of the total operating cost of municipal wastewater treatment plants (WWTPs)

(Zhou et al., 2010). Anaerobic digestion (AD) has relatively low operational cost and is energy-efficient, so it has been widely used to achieve the organic reduction of excess sewage sludge. AD of organic materials involves a series of biochemical reactions: hydrolysis, acidogenesis, acetogenesis, and methanogenesis. The final methanogenesis step is performed by a specialized group, the methanogens, of which the phylogeny and physiology are

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relatively well characterized (Benedict et al., 2012). In contrast, the former three reactions are mediated by complex bacterial consortia that cooperatively decompose macromolecules and produce substrate that the methanogens can use.

Maintenance of active bacterial populations is particularly essential in AD of particulate organic materials such as sewage sludge, because hydrolysis is often the rate-limiting step in such digesters (Zhou et al., 2010). Bacteria are also responsible for the syntrophic metabolism of thermodynamically-unfavorable reactions, such as degradation of propionate, coupled to electron-accepting partners (Carballa et al., 2015). Accordingly, to improve the design and operation strategies of AD processes, the complex bacterial communities must be understood. It should be also noted that the AD of sewage sludge involves high concentrations of microorganisms in the influent sludge, because WAS is basically composed of cellular biomass (Zhou et al., 2010). Therefore, caution is required while interpreting data generated with DNA-based molecular approaches, such as fingerprinting and sequencing methods, for analyzing bacterial communities in anaerobic sludge digestion, due to the potential ambiguity of distinguishing active and dormant bacteria with such methods (Rivière et al., 2009; Sundberg et al., 2013).

The microbial structures and the performance of AD are influenced by many factors such as substrate characteristics and operational conditions. Substrate characteristics determine which compounds enter the anaerobic food chain and in turn influence which microorganisms thrive in the system. In primary sludge and waste activated sludge (WAS), complex organic matters such as carbohydrates, proteins, and lipids, account for most of the organic compounds, along with volatile fatty acids (VFAs) (Mahmoud et al., 2004; Miron et al., 2000). Drastic microbial responses to different substrates (i.e., carbohydrates, proteins, or lipids) have been recently reported in laboratory-scale continuous bioreactors (De Francisci et al., 2015). Successful operation of AD processes also requires adequate levels of nutrients and salts. The effects of operational conditions, such as temperature, hydraulic retention time (HRT), organic loading rate, and mixing regime, on the performance and microbial community structure in AD have been demonstrated (Li et al., 2014). Although microbial populations in general depend on such external factors and substrate characteristics, the populations also affect environmental parameters inside a digester. For example, the pH of an AD process is not only dependent on the acidity and alkalinity of the influent, but also on the activities of acid-mediated anaerobes in the system (De Francisci et al., 2015).

Due to the complexity of process-microbial interactions and the limited understanding thereof, most AD processes are operated based on empirical knowledge without microbe-based management (Carballa et al., 2015). The limited access to information about microbial populations and interactions, especially in commercial digesters, has been one of the major reasons for such a convention. Recent advances in high-throughput sequencing have greatly reduced the time and price required to characterize microbial populations. Accordingly, correlations between key bacterial taxa and some process indicators have been reported (Carballa et al., 2015). However, the bacterial species that are responsible for given process parameters are not yet fully understood in full-scale AD of sewage sludge. The functions of the bacterial species could be inferred by comparing multiple AD plants in parallel and collecting process and microbial data simultaneously (Rivière et al., 2009).

This study aimed to find correlations among bacterial populations and between various process indicators in four full-scale anaerobic digesters treating sewage sludge, which were each sampled every three months for a year. The four AD systems were operated at similar conditions (i.e., reactor configuration, temperature, and HRT), but their organic removal efficiencies were dissimilar.

Bacterial populations were analyzed using 454 pyrosequencing, which generates longer sequence lengths than other conventional high-throughput sequencing methods. The structures and dynamics of the bacterial taxa were presented using multivariate analysis and discussed in relation to variance in process parameters.

2. Material and methods

2.1. Site descriptions, sample collection, and physicochemical analyses

The four anaerobic digesters investigated in this study were independently built and operated, and were located at four different full-scale municipal WWTPs in South Korea: A (Seoul), B (Daegu), C (Incheon), and D (Asan city). The digesters were fed with a mixture of primary sludge and WAS (Table 1). All the four digesters were continuously-stirred tank reactors (CSTRs) with mesophilic (35 °C) operation, and had similar HRTs (average HRTs between 30 and 36 d).

Influent and digester samples were collected from the bioreactors every three months for one year ($n = 4$ for each site; 16 in total): between October 2010 and September 2011 at sites A and C and between April 2011 and March 2012 at sites B and D. The influent samples were taken from pipes conveying mixed substrate into the digester, and the digester samples were taken from digestate circulating through heat exchangers. Approximately 200 mL of sample was collected in sterile plastic containers, stored in a mobile refrigerator at 4 °C, and transported to the laboratory within 24 h.

All physicochemical analyses were conducted in duplicate. The pH of the samples was measured using an electronic pH meter (Cole Parmer, Vernon Hills, IL). Chemical oxygen demand (COD) and solids estimation, including volatile solids (VS), were conducted according to the procedures in Standard Methods as described previously (Shin et al., 2015). Volatile fatty acid (VFA; C₂–C₆) concentrations were analyzed using a gas chromatograph (6890 plus, Agilent, Palo Alto, CA) equipped with an Innovax capillary column and a flame ionization detector. Carbohydrate concentration was measured as previously described using the phenol-sulfuric acid method (Shin et al., 2015). Total Kjeldahl nitrogen (TKN) and NH₄⁺-N concentrations were measured using the Kjeldahl method as previously reported (Shin et al., 2015). Organic nitrogen was estimated as the difference between TKN and NH₄⁺-N; for 1 g of organic nitrogen, 6.25 g of protein was assumed. Lipid concentration was analyzed as previously described using gravimetry after extraction of lipids by solvent (chloroform:methanol, 1:2 v/v) (Shin et al., 2015). The concentrations of cations and anions were measured using two identical ion chromatographs (790 Personal IC, Metrohm, Switzerland).

Table 1
Summary of the operational parameters of the four full-scale anaerobic digesters.

Parameter	Digester			
	A	B	C	D
Location	Seoul city	Daegu city	Incheon city	Asan city
Reactor type	CSTR ^a	CSTR	CSTR	CSTR
Temperature (°C)	35	35	35	35
Volume (m ³)	9903	6568	1400	1600
Feed	Sewage sludge ^b	Sewage sludge	Sewage sludge	Sewage sludge
HRT ^c (d)	35.5	28.7–31.5	30	32–40
Biogas production ^d (m ³ /m ³ substrate)	18–23	No data	5.7–11	3.7–4.5

^a Continuous stirred tank reactor.

^b Primary and secondary sludge from the municipal WWTP where each digester is located at.

^c Hydraulic retention time.

^d Approximate value over the period of monitoring.

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