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Pathways in bacterial and archaeal communities dictated by ammonium stress in a high solid anaerobic digester with dewatered sludge

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HIGHLIGHTS

- Metagenomic comparisons of microbial profiles under ammonium stress were conducted.
- The microbes showed higher abundance in ammonium-related genes under ammonium stress.
- The reduction of some amino-acid-related genes was observed under ammonium stress.
- No enhancement of hydrogenotrophic pathways was observed under ammonium stress.

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ABSTRACT

Metagenomic comparisons of microbial profiles were conducted to investigate differences between the samples from steady (Day 42), ammonium-adjusting (Day 63), and ammonium-stressed (Day 102) periods during the 110-day operation of a high solid anaerobic digester of sewage sludge. Comparing to the steady period, biogas production was slightly inhibited after ammonium adjustment, during which the microbes showed higher abundance in 6 of the total 22 ammonium-related genes. In addition, among the 19 amino-acid-related genes, 9 genes involved in amino acid generation and utilization were reduced, which partially revealed the reason of deterioration of volatile solids (VSs) degradation following ammonium stress. Furthermore, although the acetoclastic pathway was to some extent inhibited with the decrease of biogas amount and content, no enhancement of genes involved in hydrogenotrophic methanogenesis was observed, elucidating the distinct role of ammonium stress in directing bacterial community structure toward the enhanced syntrophic acetate oxidation reaction.

1. Introduction

A common approach employed in modern sludge treatment facilities mainly comprises a biological treatment step of anaerobic digestion which can simultaneously stabilize organic wastes and offer a sustainable renewable energy resource (Appels et al., 2008). Recently, high solid anaerobic digestion processes (with total solid inputs over 10%) have been widely implemented due to the advantages of a higher organic loading rate, lower energy requirements for heating, and employing a smaller digester volume than traditional low solid anaerobic digestion processes (Guendouz et al., 2008). However, in high solid systems, high-strength ammonium is usually formed through the degradation of protein existed in the input sludge, which imposes limitations on digester performance when ammonium levels exceeds the tolerance levels of microbes involved in the anaerobic digestion processes (Chen et al., 2008; Vanwonterghem et al., 2014). So far, ammonium has been proved to be a decisive factor that can drive microbial community structure to be significantly imbalanced in both laboratory-scale (Dai et al., 2015; Li et al., 2017) and full-scale (Li et al., 2015) cases.

To date, researchers have investigated the effects of excess ammonium on the shift of bacterial and archaeal communities by molecular biological methods such as denaturing gradient gel electrophoresis (DGGE) (Verhamme et al., 2011), fluorescent in situ hybridization (FISH) (Calli et al., 2005), and 16S rRNA gene sequencing (De Vrieze et al., 2015). Alsouleman et al. (2016) reported that acetotrophic methanogens are more susceptible to ammonium stress than hydrogenotrophic ones, and methanogenesis is assumed to be more readily suppressed by ammonium than hydrolysis and acidogenesis. However, although the reduction of biogas has been preferentially ascribed to the inhibition of methanogenesis (Sung and Liu, 2003), a comprehensive understanding of ammonium inhibition on specific microbes in

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complicated anaerobic digestion systems is still lacking (Wang et al., 2015). For instance, Fotidis et al. (2013) reported that syntrophic acetate oxidation (SAO), along with the hydrogenotrophic pathway, could become dominant in most experiments when ammonium concentration exceeds the threshold values. On the contrary, Calli et al. (2005) stated that the acetoclastic pathway was still predominant under ammonia stress, and that there was no shift of the microbial communities in both low and high total ammonium nitrogen (TAN) conditions (Hao et al., 2015). Dai et al. (2016) found that *Methanosarcina* was dominant with enhanced SAO during the initial 'ammonium inhibition', which partially explains the abovementioned contradictions because either pathway could be triggered in *Methanosarcina* (Phelps et al., 1985). With the recent development of metagenomic analysis based on high-throughput sequencing (Solli et al., 2014; Guo et al., 2015; Pore et al., 2015), attaining complete understanding of microbial composition and the functional roles of specific microorganisms on the genetic level has become possible, and microbial responses to ammonium stress can thus be clarified.

The objective of the current study was to investigate the effects of elevated ammonium stress on both digester performance and the functional adaptation of microbial communities in a long-term high solid anaerobic digester with dewatered sludge, attempting to provide an accurate and comprehensive understanding of the microbiome in an anaerobic digester facing ammonium stress. A high solid digester with dewatered sludge was operated for 110 days to compare the performances with normal (3000 mg/L) and overloaded (5000–6000 mg/L) ammonium concentrations. The inherent mechanism of ammonium inhibition and the shift of metabolic pathways under ammonium stress were thus clearly understood by characterizing the metagenomic community composition and functional traits, which could inform the manipulation of microbes to avoid the deterioration of digester performance under ammonium stress.

2. Materials and methods

2.1. Characteristics of inoculum and feedstock

The inoculum, with total solids (TS) of 13.6% and volatile solids/TS (VS/TS) of 46.6%, was collected from a lab-scale high solid anaerobic digester of dewatered sludge under mesophilic condition. The input dewatered sludge was obtained from a typical domestic wastewater treatment plant (WWTP) in Shanghai, China, with TS and VS/TS of 20.1% and 53.9%. Before being fed into the digester, the dewatered sludge was heated to 35 °C from a storage temperature of 4 °C.

2.2. Digester operation

The digester (HLZ-AR (DV)-9, Shanghai, China), with a nominal volume of 9 L, was equipped with a helix-type stirrer and operated under mesophilic conditions (35 ± 1 °C) at a solid retention time (SRT) of 20 days. The digester was intermittently stirred at a speed of 60 rotations per minute (rpm) at a 10 min on and off interval. As previously reported by Dai et al. (2016), inoculum sludge (4.5 kg) and dewatered sludge (4.5 kg) were both initially adjusted to a TS of 15% and added to the digester. The biogas generation was recorded daily with a wet gas meter (LMF-1, Qingdao, China). The TS of the daily input sludge was also diluted to 15% with tap water. Feeding and discharge occurred once a day in the 110-day operation, which included four periods: (I) start-up from day 1 to 40; (II) steady state period from day 41 to 55, where TAN concentrations were spontaneously maintained at 3000–3500 mg N/L; (III) ammonium adjusting period from day 56 to 70, where the TAN concentration was progressively increased to 5000 mg N/L by mixing ammonium chloride into the feeding sludge; (IV) ammonium stressed period from day 71 to 110, during which TAN concentration was maintained within 5000–6000 mg N/L.

2.3. Analytical methods

Samples were taken to analyze biogas content, pH, TS, VS/TS, volatile fatty acids (VFAs), alkalinity and TAN. Methane and carbon dioxide content were measured by a gas chromatograph (GC112A, INESA, China) with a thermal conductivity detector equipped with GDX-102 packed column (2 m × 4 mm). pH was measured with a pH meter (S210, METTLER, Switzerland). TS, VS, alkalinity, and TAN were determined every two or three days by following the protocols in Standard Methods for the Examination of Water and Wastewater (APHA, 2005). To determine VFAs, the supernatant was collected after the centrifugation of the sample at 13,000 rpm for 20 min. Prior to VFA analysis by a gas chromatograph (GC) (2010 plus, Shimadzu, Japan) with a flame ionization detector (FID) and a capillary column (30 m × 0.25 mm ID × 0.25 μm, Rtx-WAX, Restek, USA), the filtrate was acidified to adjust pH to approximately 2.0 by the addition of formic acid.

2.4. Library construction and sequencing for metagenomics analyses

The DNA of digestate samples from day 42, 63, and 102, which represent the stable (Period II), ammonium adjustment (Period III), and sub-optimal periods (Period IV), respectively. The paired-end library was constructed using M220 Focused-ultrasonicator™ (Covaris, Woburn, Massachusetts, USA) to excise and extract ≈300-bp fragments. Dual-index adapters containing the full complement of sequencing primer hybridization sites were ligated to the Blunt-end fragments using TruSeq™ DNA Sample Prep Kit (Illumina, San Diego, CA, USA). Following amplification (10 cycles) and denaturation with sodium hydroxide, libraries were pooled and loaded onto an Illumina cBot (Clark et al., 2011). The 3 samples were individually sequenced in one flow cell lane with 2 × 150 bp paired-end format on an Illumina Genome Analyzer (Hiseq2500, Illumina Inc., San Diego, CA, USA) at Majorbio Bio-Pharm Technology Co., Ltd. (Shanghai, China) using a TruSeq SBS kit v3-HS (Illumina, San Diego, CA, USA). All the raw metagenomic datasets have been deposited into NCBI's Sequence Read Achieve with the accession number of SAMN06972165.

2.5. Assembly of metagenomic datasets and ORF annotation

The raw reads were trimmed with a minimum quality score of 20 and a minimum read length of 20 bp by SeqPrep (https://github.com/jstjohn/SeqPrep), and no ambiguous bases were allowed (Albertsen et al., 2012). Assembly of clean reads was achieved using SOAPdenovo software (http://soap.genomics.org.cn, Version 1.3) at a Kmer of 31 (Luo et al., 2012). Scaffolds were extracted with a length of over 500 bp which were further broken into contigs without gaps.

MetaGeneAnnotator (http://metagene.cb.k.u-tokyo.ac.jp/) was used for gene prediction, taxonomy assignment, and functional annotation of open reading frames (ORFs) of contigs and singletons from metagenomic samples (Noguchi et al., 2008). The translation of the predicted ORFs with a length of over 100 bp to amino acid sequences was conducted using the NCBI translation table (http://www.ncbi.nlm.nih.gov/Taxonomy/taxonomyhome.html/index.cgi?chapter=tgencodes#SG1). The translated amino acids were subsequently annotated using BLASTP search (Version 2.2.25) against the NCBI nr database with the parameters for BLAST set as the E-value cutoff = 1e-5, num_alignments = 250, and num_descriptions = 500. The taxonomic assignment of the small-subunit (SSU) rRNA genes was then carried out using BLAST against