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Long-term high-solids anaerobic digestion of food waste: Effects of ammonia on process performance and microbial community

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ABSTRACT

A long-term high solids anaerobic digestion of food waste was conducted to identify microbial mechanisms of ammonia inhibition during digestion and to clarify correlations between ammonia accumulation, microbial community dynamics (diversity, composition, and interactions), and process stability. Results show that the effects of ammonia on process performance and microbial community were indirectly caused by volatile fatty acid accumulation. Excess free ammonia blocked acetate metabolism, leading to process instability. Accumulated acetate caused feedback inhibition at the acetogenesis stage, which resulted in considerable accumulation of propionate, valerate, and other long-chain fatty acids. This high concentration of volatile fatty acids reduced the abundance of syntrophic acetogenic bacteria and allowed hydrolytic fermentative bacteria to dominate. The normally interactive and orderly metabolic network was broken, which further exacerbated the process instability. These results improve the understanding of microbial mechanisms which contribute to process instability and provide guidance for the microbial management of anaerobic digesters.

1. Introduction

Food waste (FW) is one of the largest waste streams in the world and is being generated at an ever-increasing rate. The high theoretical yield of methane from FW, which ranges from 0.4 to 0.5 L g VS^{-1} (Li et al., 2018), has heightened interest in using anaerobic digestion (AD) to recover energy from FW (Amha et al., 2017). High-solids AD processes, in which total solid (TS) inputs are greater than 10%, have been developed and are attracting widespread attention due to the advantages of higher organics loading rates (OLR), lower energy requirements for heating and pumping, lower degree of raw material processing, and smaller digester volumes than traditional low-solids AD processes (Gao et al., 2015; Li et al., 2017).

FW typically has a TS content of 18.1–30.9% (Braguglia et al., 2018) and is suitable for high-solids AD processes. However, FW also has a high protein content, which increases ammonia release during hydrolysis (Gao et al., 2015; Tao et al., 2017). Though low levels of ammonia can increase the buffering capacity of AD, high concentrations usually lead to declines in gas production and potential digester operation failures (Astals et al., 2013; Mahdy et al., 2017; Sun et al., 2016). Ammonia is a major environmental factor influencing biomethanation in full-scale anaerobic digesters. Ammonia inhibition will be more prominent in high-solids AD processes with low water content (Poirier et al., 2017; Tao et al., 2017). Several methods to counteract ammonia inhibition (including dilution of feeding streams, air stripping, bioaugmentation, co-digestion, ammonia-binding ions, and struvite precipitation) have been proposed in the literature (Mahdy et al., 2017; Sun et al., 2016). Though some methods have proven successful in laboratory research, the high cost and technical challenges associated with these methods prevent full-scale implementation (Mahdy et al., 2017). Therefore, operators usually leave digesters to operate under "inhibited steady state" conditions. Under such conditions, overall profitability may be reduced by as much as 30% (Li et al., 2018).

Microbial resource management is an important developing field in recent years. The aim of microbial resource management is to optimize process efficiency and stability by initiating changes in the microbial community (Carballa et al., 2015; Li et al., 2018; Poirier et al., 2016b). Therefore, many studies have explored the effects of ammonia on microbial communities in anaerobic digesters. But most studies have focused on acute toxicity experiments in batch reactors to investigate inhibition thresholds, changing microbial composition, or metabolic pathways under different ammonia gradients (Lü et al., 2013; Poirier et al., 2016b). Some researchers investigated the effects of ammonia stress on AD processes in semi-continuous experiments, but these studies also focused on biogas conversion computation and shifting microbial communities and metabolic pathways (Li et al., 2017; Niu et al., 2013; Shimada et al., 2014). A few studies considered the tolerance response to *in situ* ammonia stress in anaerobic digesters. For example,

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Gao et al. (2015) carried out ammonia acclimation in a continuous AD of solid residual kitchen waste by increasing ammonia stress in a stepwise fashion in a continuously-stirred tank reactor (CSTR) with a 50 L active volume. Results showed that the anaerobic reactor functioned well during acclimation at an average ammonia concentration of 4275 mg L^{-1}; ammonia stress enhanced the activity of coenzyme F_{420} and the microbial community shifted from acetotrophic methanogens to hydrogenotrophic methanogens. Sun et al. (2016) investigated the feasibility of co-digestion of chicken manure and maize silage in 5 L digesters. They found that the threshold and total inhibition levels of biogas production occurred at 7 and 9 g N L^{-1}, respectively. During high N input, the predominant methanogenic pathway was hydrogenotrophic, but it reverted to acetotrophic during lower N input. Overall, the available literature primarily covers biogas production, inhibition thresholds, metabolic pathways, and microbial community dynamics of AD processes under a wide range of ammonia conditions. Few researchers have fully explored the inhibitory mechanism of ammonia, especially the origin of community disturbances during the course of instability, the interactions among microbial communities, and the correlations between environmental variables, microbial community succession, and process stability.

The current study focused on the effects of *in situ* ammonia stress on process performance in a long-term, high-solids AD of FW in a CSTR with a 30 L working volume. Related changes in microbial composition and behavior were investigated as well. The objectives of this study were to investigate the linkages between ammonia accumulation, microbial dynamics (diversity, composition, and interaction), and process stability, then identify microbial mechanisms of ammonia inhibition and provide guidance for microbial resource management of anaerobic digesters.

2. Materials and methods

2.1. Substrates and inoculums

FW was collected each week from a school dining facility and was ground into a homogenized slurry after the removal of bones, eggshells, napkins, plastic, and other indigestible materials. The FW slurry was stored at −18 °C in self-sealing bags and thawed for one day at 4 °C prior to use. The pH of FW was 6.4 ± 0.2; TS and volatile solids (VS) contents were 28.41 ± 0.62% and 26.46 ± 0.67%, respectively; C/N was 14.73 ± 0.34%; and the contents of protein, crude fat, and carbohydrate were 19.96% ± 0.34%, 19.87% ± 0.21%, and 53.36% ± 2.10%, respectively. The microbial inoculum was collected from a lab-scale, high-solids anaerobic digester treating FW under mesophilic conditions. Feeding was stopped for about 2 months before inoculation. Inoculum characteristics were as follows: pH 7.54 ± 0.3, TS 26.15 ± 0.60%, VS 15.95 ± 0.56%, and C/N 10.02 ± 0.15%.

2.2. Reactor and operation

A CSTR with total volume of 50 L and working volume of 30 L was used in this study. A mechanical stirrer (45 rpm) intermittently mixed the AD for 5 min every hour throughout the experiment. A thermostat water jacket was applied to hold the temperature at 36 ± 1 °C. The digester was operated in a daily fill-and-draw mode; namely, substrates were fed into the digester and effluents were withdrawn once per day. For an efficient injection of solid substrate, the substrate (volume = Q) was fed after two times dilution with inside sludge (volume = 2Q). A consistent OLR of 3 g VS L^{-1} d^{-1} was used, based on previous research suggesting that this OLR is a "safety OLR," which carries no risk of overloading the digester (Li et al., 2014; Shi et al., 2016). This protocol assures that ammonia accumulation will be the only factor leading to process perturbation after long-term operation. In addition, no external source of ammonia (such as ammonia-nitrogen) was added to the digester. Therefore, the gradual accumulation of ammonia was produced entirely by FW degradation under conditions of a consistent OLR. The consistent OLR was given a hydraulic retention time of approximately 90 days. After 232 days of operation, the methane yield decreased by more than 50%, and a large amount of foam was observed. The foam expanded to the top of the reactor, and the solid particles (microorganisms or suspended solids) among the foam clogged the gas outlet, leading to the termination of the experiment.

2.3. Performance parameter analysis

Biogas production and pH were measured every day; biogas composition, TS, VS, volatile fatty acids (VFAs), and alkalinity (TA) were determined every 3 days; and total ammonia-nitrogen (TAN) was determined every 3, 2, and 1 days, when free ammonia-nitrogen (FAN) was less than 100 mg L^{-1}, between 100 and 150 mg L^{-1}, and greater than 150 mg L^{-1}, respectively. The monitoring methods for the above parameters and the equation for calculating VS removal rate (VS_r) can be found in previous reports (Li et al., 2014). The total volatile fatty acid (TVFA) concentration is equal to the sum of VFAs. FAN was calculated according to the following equation (Tao et al., 2017):

$$\mathrm{FAN} = \mathrm{TAN} \times \left(1 + \frac{10^{-\mathrm{p}H}}{10^{-\left(0.09018 + \frac{2729.92}{T(K)}\right)}}\right)^{-1}$$

In addition, fat content of FW was measured by national standard GB-T 5009.6-2003. Protein content was estimated by multiplying total nitrogen content by 6.25. Elemental contents (C, H, N, S) were quantified using an elemental analyzer (Elementar Vario ELIII, Germany). Carbohydrate content was calculated by subtracting the content of fat and proteins from VS content. Oxygen content was calculated by subtracting the content of C, H, N, and S from VS content.

2.4. Microbial community structure analysis

2.4.1. DNA extraction, PCR amplification, and Illumina sequencing

Based on changes in TAN concentration and reactor performance, 10 digestate samples were collected on days 36, 99, 127, 139, 152, 172, 189, 212, 223, and 232. The reasoning behind the timing of sample collection is given in Section 3.3.1. Genomic DNA was extracted from each sample with the E.Z.N.A Soil DNA kit (OMEGA, USA) following the manufacturer's instructions. The DNA quality was verified using agarose gel (1.0%) electrophoresis, and DNA concentrations were determined using a Nanodrop 2000 (Nanodrop Technologies, Wilmington, DE). Each sample was extracted in triplicate, then the combined DNA solution was stored for the following analysis.

Amplification of partial 16S rRNA gene was performed using GeneAmp® 9700 (ABI) with the following primer pairs: 338F (5′-ACT CCTACGGGAGGCAGCAG-3′)/806R (5′-GGACTACCAGGGTATCT AAT-3′) for bacteria and Arch344F (5′-ACGGG GYGCAGCAGGCG-CGA-3′)/Arch915R (5′-GTGCTCCCCCGCCAATTCCT-3′) for archaea. The PCR amplification conditions for bacteria were as follows: initial denaturation at 95 °C for 3 min, 25 cycles of denaturing (95 °C; 30 s), annealing (55 °C; 30 s), and extension (72 °C; 1 min); and a final elongation (72 °C, 10 min). The amplification program for archaea was similar to that for bacteria except that the number of cycles was 35 rather than 25. The PCR products were purified using AxyPrep DNA gel Recovery Kit (AXYGEN, USA) and quantified using QuantiFluor™-ST (Promega, USA) according to the manufacturer's protocol. Purified amplicons were pooled in equimolar portions and paired-end sequencing was performed (2 × 300 bp) on an Illumina MiSeq platform (Majorbio Bio-Pharm Technology Co., Ltd., Shanghai, China) according to standard protocols. Raw sequencing reads were deposited in the NCBI Sequence Read Archive database with accession No. SRP076068.

2.4.2. Sequence data processing and analysis

Raw FastQ files were demultiplexed and quality-filtered using