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Impact of a high ammonia-ammonium-pH system on methane-producing archaea and sulfate-reducing bacteria in mesophilic anaerobic digestion

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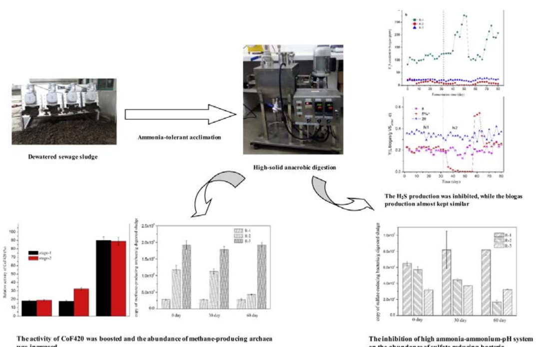
GRAPHICAL ABSTRACT



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ABSTRACT

A novel strategy for acclimation to ammonia stress was implemented by stimulating a high ammonia-ammonium-pH environment in a high-solid anaerobic digestion (AD) system in this study. Three semi-continuously stirred anaerobic reactors performed well over the whole study period under mesophilic conditions, especially in experimental group (R-2) when accommodated from acclimation period which the maximum total ammonia nitrogen (TAN) and free ammonia nitrogen (FAN) increased to 4921 and 2996 mg/L, respectively. Moreover, when it accommodated the high ammonia-ammonium-pH system, the daily biogas production and methane content were similar to those in R-1 (the blank control to R-2), but the hydrogen sulfide (H_2S) content lower than the blank control. Moreover, mechanistic studies showed that high ammonia stress enhanced the activity of coenzyme F_{420}. The results of real-time fluorescent quantitative polymerase chain reaction (PCR) showed that ammonia stress decreased the abundance of sulfate-reducing bacteria and increased the abundance of methane-producing archaea.

1. Introduction

Anaerobic digestion (AD) is widely used as a dependable method for the treatment of sewage sludge because it results in the effective degradation of contaminants and the production of green energy. Nevertheless, there are numerous limitations of the traditional AD process, in which a total solids (TS) level of 2–6% is maintained due to the high energy demands of maintaining the mesophilic (30–40 °C) or thermophilic (45–65 °C) conditions and the large-volume reactors (Yenigun and Demirel, 2013). High-solid AD, which involves a digestion system with a TS content exceeding 8% (Liao and Li, 2015), is a feasible method to eliminate these previous limitations (Liu et al.,

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2016). In such a system, the high ammonia concentration and pH are the important factors that stabilize the AD (Dai et al., 2016b), and ammonia inhibition is the main operational problem, particularly the handling and disposal of nitrogen-rich waste (Gao et al., 2015; Yenigun and Demirel, 2013).

During AD, hydrogen sulfide (H_2S) is produced as the metabolite of sulfates by sulfate-reducing bacteria. The sulfate-reducing bacteria compete with hydrolytic bacteria, acidogenic bacteria, acetogens and methanogens for organic substrates or hydrogen and can outcompete methane-producing archaea for organic substrates in a sulfate-rich reactor (Liu et al., 2015b). Furthermore, H_2S can easily diffuse through cell membranes into the cytoplasm and denature primary proteins (e.g., certain enzymes for maintaining well-balanced metabolism) (Chen et al., 2008; Elferink et al., 1994). Thus, the degradation of sulfates in sewage sludge is extremely undesirable due to the reduced methane yield and the malodor caused by H_2S (Liu et al., 2015c; Siles et al., 2010), which may lead to high toxicity and erosion and eventually induce the failure of whole AD system (Liu et al., 2015a).

Total ammonia nitrogen (TAN) is mainly composed of free ammonia nitrogen (FAN) and ionized ammonium nitrogen (NH_4^+) in an AD system (Yenigun and Demirel, 2013). Because of its high penetration ability of cell membranes, FAN is the dominant inhibitor of microorganisms (Muller et al., 2006). Previous studies have focused on determining the threshold concentrations of ammonia and sulfate that may inhibit AD. For instance, Siles et al. (2010) found that 620 mg free ammonia/L and 1400 mg SO_4^{2-}/L were the thresholds in terms of biogas yield. The FAN concentration is closely related with the pH and temperature of the reactor (Rajagopal et al., 2013), so explaining ammonia inhibition during AD is not comprehensive if only considering the ammonia concentration. High pH will give rise to a high ammonia level in a vested system, which can increase the intersystem pH because of ammonia ionization (Gao et al., 2015). Thus, the impact of the ammonia and sulfate concentrations on the AD system must be investigated by considering the ammonia-ammonium-pH system. However, few researchers have considered the impact of a high ammonia-ammonium-pH system on AD, especially for inhibiting H_2S production and affecting the competition between sulfate-reducing bacteria and methane-producing archaea.

The main objective of this study was to investigate the impact of a high ammonia-ammonium-pH system on the competition between sulfate-reducing bacteria and methane-producing archaea by evaluating the biogas yield coefficient, performance, key enzyme activity, sulfate reduction and H_2S production. This study was carried out in three laboratory-scale, semi-continuously stirred, anaerobic reactors under mesophilic conditions (35 ± 1 °C). Sulfate-reducing bacteria and methane-producing archaea during different periods in the AD system were absolutely quantified through real-time fluorescent quantitative PCR.

2. Materials and methods

2.1. Substrate and inoculum

The dewatered sewage sludge used as the substrate for AD was obtained from the Anting wastewater treatment plant in Shanghai, China, which treated domestic sewage using an inverted A/A/O process. The sludge was obtained by collecting primary and excess sludge and then dewatered using a dehydrator (a high-molecular-weight polyacrylamide flocculant). Dewatered sludge was collected every 30–40 days and kept at 4 °C after sufficient mixing. The main characteristics (average values plus the standard deviations of duplicate tests) of the feedstock were as follows (Table 1): pH 7.31 ± 0.15, 20.03 ± 0.93 g/L TS, 599.20 ± 10.03 g/kg-TS VS (volatile solids), 5850.3 ± 103.4 mg/L TA (total alkalinity), and 759.9 ± 35.2 mg/L TAN. All the substrates were preheated to approximately 35 °C before feeding into the reactors.

Table 1
Characteristics of the inoculum and dewatered sewage sludge.

Parameter	Measuring unit	Inoculum	Dewatered sewage sludge
pH	/	8.07 ± 0.13	7.31 ± 0.15
TS (total solids)	g/L	14.01 ± 0.74	20.03 ± 0.93
VS (volatile solids)	g/kg-TS	463.40 ± 9.64	599.20 ± 10.03
TA (total alkalinity)	mg/L	13848.4 ± 135.8	5850.3 ± 103.4
Total ammonia nitrogen (TAN)	mg/L	4120.7 ± 112.1	759.9 ± 35.2

Data are the averages and standard deviations of duplicate tests.

The mesophilic seed sludge was obtained from an anaerobic reactor that had been steadily operated for a couple of months, which treated the dewatered sewage sludge by an AD process. The main characteristics (average values plus the standard deviations of duplicate tests) of the inoculum were as follows (Table 1): pH 8.07 ± 0.13, 14.01 ± 0.74 g/L TS, 463.40 ± 9.64 g/kg-TS VS, 13848.4 ± 135.8 mg/L TA, and 4120.7 ± 112.1 mg/L TAN.

Three semi-continuously stirred tank reactors (R-1, R-2, and R-3) with a liquid working volume of 6.0 L were developed in this study. The stirring cycle consisted of 10 min of stirring and a 10 min break and was continuous. Biogas volumes were measured by wet gas meters every day. The daily feeding operation was carried out by pushing substrate through the feeding piston. The designed solid retention time in each of the three reactors was 20 days. R-1, R-2, and R-3 were fed with the previously described substrate; however, the feedstock of R-1 and R-2 was diluted with water to approximately 5% TS (about 190 mg/L TAN), and the feedstock of R-3 was the dewatered sewage sludge without dilution (759.9 ± 35.2 mg/L TAN). R-1 served as a blank control for R-2, and the TAN concentration of R-2 was increased to a higher level (approximately 3000–5000 mg/L TAN) than that in R-1 by adding NH_4HCO_3 while feeding the reactor (see Fig. 1). The temperature of all reactors was controlled at 35 ± 1 °C. Prior to this study, the three reactors had been operated with good performance for approximately six months while dynamically maintaining the physicochemical properties listed above, except without feeding NH_4HCO_3 in R-2.

2.2. Ammonia-tolerant acclimation strategy

During acclimation, the TAN concentration (including ionized ammonium nitrogen and FAN) in R-2 (the initial TAN concentration was the same as the R-1, about 1200 mg/L) was increased stepwise from approximately 3000–5000 mg/L. To reinforce the tolerance of the

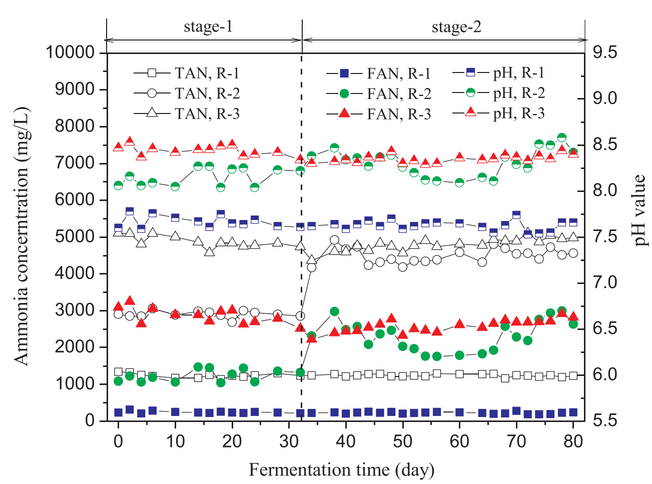

Fig. 1. Changes in TAN, FAN and pH in the three reactors during domestication.