Water Research 146 (2018) 275–287

Contents lists available at ScienceDirect

Water Research

journal homepage: www.elsevier.com/locate/watres

Inhibitory effects of ammonia on syntrophic propionate oxidation in anaerobic digester sludge

Chen Zhang [a, b], Quan Yuan [c], Yahai Lu [b, *]

[a] State Key Laboratory of Biochemical Engineering, Institute of Process Engineering, Chinese Academy of Sciences, Beijing, 100190, China
[b] College of Urban and Environmental Sciences, Peking University, Beijing, 100871, China
[c] State Key Laboratory of Environmental Geochemistry, Institute of Geochemistry, Chinese Academy of Sciences, Guiyang, 550081, China

ARTICLE INFO

Article history:
Received 15 June 2018
Received in revised form
30 August 2018
Accepted 21 September 2018
Available online 27 September 2018

Keywords:
Syntrophic propionate oxidation
Methanogenesis
Ammonia stress
Anaerobic digester
Stable isotope probe

ABSTRACT

Syntrophic propionate oxidation (SPO) coupled with methanogenesis is often inhibited under high ammonium concentrations in anaerobic digesters. However, the inhibitory mechanism remains poorly understood. We conducted two independent laboratory experiments with a swine manure digester sludge. In experiment I, RNA-based stable isotope probing (SIP) was applied to determine the active players of both bacteria and methanogens involved in SPO under different ammonium concentrations (0, 3 and 7 g NH_4^+–N L^{-1}). In experiment II, the dynamics of the bacterial community under ammonia stress was monitored using the 16S rRNA pyrosequencing and quantitative PCR under similar conditions as in experiment I but without the addition of external propionate. An additional higher ammonium treatment (10 g NH_4^+–N L^{-1}) was applied in experiment II to maximize the ammonia stress. We identified that the *Smithella* bacteria and the *Methanosaetaceae* and *Methanospirillaceae* archaea were the most active players involved in SPO and methanogenesis. We revealed that *Smithella*, *Methanosaetaceae* and *Methanospirillaceae* were moderately and severely inhibited at 3 and 7–10 g NH_4^+–N L^{-1}, respectively. However, the fermentative bacteria appeared to be more tolerant to ammonia stress. The microbial responses were corroborated with the accumulation of VFAs and the repression of methanogenesis under high ammonium conditions.

1. Introduction

Anaerobic digestion (AD) is widely used to degrade organic waste to biogas which helps reduce reliance on fossil fuels, mitigate emission of greenhouse gases, and lower environmental pollution (McKendry, 2002). In methanogenic AD systems, complex organic matter is ultimately converted to methane and carbon dioxide by an orchestration of fermentation, acetogenesis and methanogenesis (Schink, 1997). The acetogenesis step is catalyzed by bacteria that convert substrates such as fatty acids and alcohols into simple compounds such as H_2, CO_2, formate and acetate. This step is endergonic under standard conditions and can process only if the simple compounds produced are consumed by methanogens through a syntrophic association. Therefore, the acetogenesis is regarded to be critical in the AD processes (Junicke et al., 2016; Schink, 1997). Propionate, a key product during anaerobic decomposition, is degraded via the syntrophic propionate oxidation (SPO) which operates at near thermodynamic equilibrium (Stams and Plugge, 2009).

Several syntrophic propionate oxidation bacteria (SPOB) that grow in syntrophy with methanogens have been described, including the Gram-negative (*Syntrophobacter* and *Smithella*) and Gram-positive bacteria (*Pelotomaculum* and *Desulfotomaculum*) isolated from anaerobic digesters (McInerney et al., 2008). These SPOB have been found widespread in anoxic environments, such as peats, wetlands, rice paddy soils, sediments and subsurface petroleum reservoirs (Gan et al., 2012; Gray et al., 2011; Schmidt et al., 2016). Many environmental factors, such as temperature (Gan et al., 2012), hydraulic retention time (HRT) (Zamanzadeh et al., 2013) and levels of propionate (Ariesyady et al., 2007) and trace elements (molybdenum, tungsten and selenium) (Capson-Tojo et al., 2018; Worm et al., 2009) have been found to influence the SPOB communities. Stable isotope probing (SIP) allows the identification of microbial species that incorporate ^{13}C substrates into their DNA or RNA (Radajewski et al., 2000). This powerful technique has been used to investigate the active populations responsible for

* Corresponding author.
E-mail address: luyh@pku.edu.cn (Y. Lu).

https://doi.org/10.1016/j.watres.2018.09.046
0043-1354/

SPO in rice field soils (Gan et al., 2012; Lueders et al., 2004b). However, a direct identification of the microbial populations responsible for SPO in anaerobic digester sludge still remains unavailable.

This limitation of energy conservation predicts that any subtle disturbances to the AD system could affect the functioning of the SPO community and propionate degrading. Accumulation of ammonium produced from the degradation of protein-rich materials is known to inhibits SPO and methanogenesis in the anaerobic digesters that treat livestock manure (Chen et al., 2008; Montag and Schink, 2016), wherein free ammonia is considered to be the main source of ammonium stress (Rajagopal et al., 2013). Under methanogenic conditions, SPO involves at least three different groups of microbes, namely SPOB, hydrogenotrophic methanogens and aceticlastic methanogens. Presumably, inhibition of any individual group would result in an inhibition of the whole SPO process (Capson-Tojo et al., 2017; Stams and Plugge, 2009; Van Velsen, 1979). A consensus seems to have suggested that syntrophic bacteria and methanogenic archaea have different levels of sensitivity to ammonia, but the details appear contradictory. Calli et al. (2005) indicated that SPOB were more sensitive than methanogenic archaea to free ammonia in anaerobic reactors, while other studies suggested just the opposite (Müller et al., 2006; Weaterholm et al., 2011). Wiegant and Zeeman (1986) proposed that it was the inhibition of the hydrogenotrophic methanogens that resulted in an accumulation of propionate, which further inhibited the aceticlastic methanogens. Likewise, the exact responses of either aceticlastic or hydrogenotrophic methanogens to ammonia varied across a few studies (Jarrell et al., 1987; Kato et al., 2014; Westerholm et al., 2012; Zhang et al., 2014).

To gain more insight into the effects of ammonium on microbes involved in SPO and methanogenesis, we conducted two independent laboratory experiments. In the experiment I, RNA-SIP technique was applied to identify the active organisms involved in SPO in a sludge sample collected from a full-scale swine manure digester (Zhang et al., 2014), and in the experiment II a batch experiment with a wide range of ammonium concentrations was conducted to determine the responses of the entire microbial community in fermentation and methanogenesis to ammonia stress.

2. Materials and methods

2.1. Anaerobic incubation

Activated sludge was collected from a full-scale anaerobic bioreactor (continuous stirred-tank reactor) treating swine manure in a livestock farm located in Bei Langzhong Village, Shunyi District, Beijing. The physicochemical characteristics of swine manure and activated sludge are shown in Supplemental Table S1. The sludge, swine manure and the experimental procedures have been described previously (Zhang et al., 2014). Briefly, 10 g sludge and 1 g swine manure (wet weight) were mixed with 30 mL of 50 mM Hepes buffer (N-2-hydroxyethylpiperazine-N′-2-ethanesulfonic acid) in 100-mL glass bottles. This yielded a substrate/inoculum (S/X) ratio of 3.3 in volatile solid (VS) basis. Prior to mixing, the Hepes buffer was supplemented with NH_4Cl to create a final concentration of 0, 3, 7, 10 NH_4^+–N L^{-1}, respectively (referred to as 0-, 3-7- and 10-N treatments) and autoclaved. The pH of the sludge slurries was adjusted to 7.0 with either 1 M HCl or NaOH. The bottles of sludge slurries were then closed with butyl rubber stoppers, flushed with N_2 and incubated under dark at 35 °C.

In experiment I, the sludge slurries were first preincubated at 35 °C for 30 days. Then, the slurries were incubated under the following conditions: a) without propionate, b) with unlabeled propionate and c) with [U–^{13}C] propionate (99 atom%, Sigma-Aldrich, American) at 35 °C for 201 h. Three levels of N were applied, i.e. the 0-, 3- and 7-N treatments. The total amount of propionate for 0-N and 3-N treatments was 8 mM, which was segmented into two doses with equal quantity applied at 0 and 82 h, respectively, for strengthening the ^{13}C labeling. For the 7-N treatment, the addition of 4 mM propionate occurred only once at 0 h. In experiment II, the slurries were incubated for 86 days without preincubation and without addition of propionate. Swine manure added at the beginning was the only substrate for acetogenic and methanogenic activity. An entire range of N concentrations in medium, i.e. 0, 3, 7, 10 g NH_4^+–N L^{-1}, were applied. All incubations were carried out in triplicate.

2.2. Chemical analysis

The gaseous samples were taken form headspace for analyzing the concentration and $^{13}C/^{12}C$ ratios of CH_4 and CO_2 according to the methods previously (Zhang et al., 2014). Liquid samples were collected and centrifuged, then the supernatants were filters and stored at −20 °C as described previously (Zhang et al., 2014). Volatile fatty acids were measured using high performance liquid chromatography (Sykam, Gilching, Germany) with refractive index and UV detectors, with a detection limit of ca. 5 μM ^{13}C abundance in volatile fatty acids from the liquid samples were measured using a HPLC system (Spectra System P1000, Thermo Finnigan, USA) equipped with an ion exclusion column (Aminex HPX-87-H, Bio-Rad, Munich, Germany) and coupled to a Finnigan LC IsoLink (Thermo Electron Corporation, Germany). Isotope ratio values were detected using IRMS (Finigan MAT delta plus advantage) (Penning and Conrad, 2007). The pH of incubation medium and concentration of NH_4^+–N was measured as described previously (Zhang et al., 2014). The free ammonia (NH_3–N) was calculated based on NH_4^+/NH_3 equilibrium, taking into account the concentration of NH_4^+–N, temperature, pH (Rajagopal et al., 2013) and the ionic strength of medium according to Pitzer's ion-interaction approach (Hafner and Bisogni, 2009).

2.3. Nucleic acid extraction and gradient centrifugation

Simultaneous extraction of DNA and RNA in sludge samples was conducted as previously described with modification (Noll et al., 2005). RiboGreen (Invitrogen, American) was used to quantify RNA content in the extracts. The RNA was density resolved by equilibrium density gradient centrifugation in CsTFA (GE Healthcare, American) under the conditions reported previously (Lueders et al., 2004a). After centrifugation, the cesium trifluoroacetate buoyant density of each fraction was determined, and RNA was precipitated and re-suspended in nuclease-free water for subsequent quantitative and qualitative community analyses. Control gradients were conducted with RNA from unlabeled sludge samples. Reverse transcription PCR (RT-PCR) was performed using cDNA Synthesis Kit (TaKaRa), following the protocol described previously (Yuan et al., 2011).

2.4. Quantitative analysis of rRNA

Bacterial and archaeal 16S rRNA from gradient fractions was quantified using quantitative (real-time) PCR in a 7500 real-time PCR system (Applied Biosystems) with the primer pair Ba519f/907r (Lueders et al., 2004a) and Ar364f/934r (Kemnitz et al., 2005) respectively. The standards, that had a dilution series (factor 10) corresponding to 1.2×10^8 to 1.2×10^2 copies μl^{-1}, were prepared from an *in vitro* transcript of bacteria and archaea clones from our sludge samples using the Riboprobe *in vitro* Transcription System