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Acclimation to extremely high ammonia levels in continuous biomethanation process and the associated microbial community dynamics

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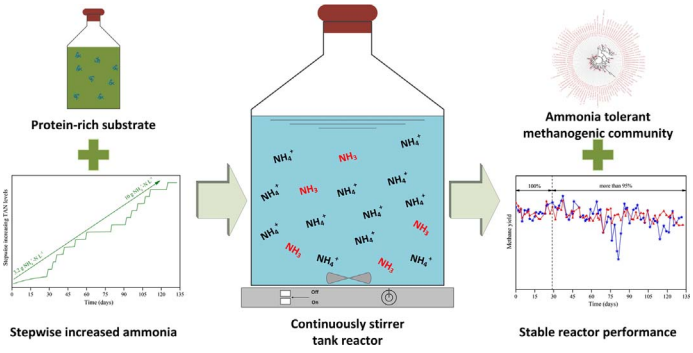
GRAPHICAL ABSTRACT



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ABSTRACT

Acclimatized anaerobic communities to high ammonia levels can offer a solution to the ammonia toxicity problem in biogas reactors. In the current study, a stepwise acclimation strategy up to 10 g NH_4^+-N L^{-1}, was performed in mesophilic (37 ± 1 °C) continuously stirred tank reactors. The reactors were co-digesting (20/80 based on volatile solid) cattle slurry and microalgae, a protein-rich, 3rd generation biomass. Throughout the acclimation period, methane production was stable with more than 95% of the uninhibited yield. Next generation 16S rRNA gene sequencing revealed a dramatic microbiome change throughout the ammonia acclimation process. *Clostridium ultunense*, a syntrophic acetate oxidizing bacteria, increased significantly alongside with hydrogenotrophic methanogen *Methanoculleus* spp., indicating strong hydrogenotrophic methanogenic activity at extreme ammonia levels (> 7 g NH_4^+-N L^{-1}). Overall, this study demonstrated for the first time that acclimation of methanogenic communities to extreme ammonia levels in continuous AD process is possible, by developing a specialised acclimation AD microbiome.

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1. Introduction

Anaerobic digestion (AD) is a sustainable technology that can produce biogas and nutrient-rich bio-fertilizer from a broad variety of residual biomass (e.g. agricultural waste, food waste, and sewage sludge) (Karim et al., 2005). AD is a complex biological process, which comprises four main steps: hydrolysis, acidogenesis, acetogenesis and methanogenesis, with a variety of microorganisms mediating each step. Acetate is the main precursor of methane production which follows two major methanogenic pathways: a) aceticlastic pathway and b) hydrogenotrophic pathway (syntrophic acetate oxidation (SAO) coupled with hydrogenotrophic methanogenesis). Aceticlastic pathway is mediated by *Methanosarcinaceae* spp. and *Methanosaetaceae* spp., while *Methanomicrobiales* spp., *Methanobacteriales* spp., *Methanococcales* spp., *Methanopyrales* spp. and *Methanocellales* spp. mediate the hydrogenotrophic partway (Lyu and Lu, 2015). Furthermore, there is also evidence showing that some members of *Methanosarcinaceae* spp. can perform both aceticlastic and hydrogenotrophic pathways (Liu and Whitman, 2008).

Many compounds (e.g. ammonia, sulphide, light metal ions, heavy metals, organics etc.) can affect the AD microbial community, cause reactor instability with low methane yield. Ammonia is the major toxicant of the commercial AD reactors and usually derives from the degradation of urea and protein-rich substrates, such as slaughterhouse wastewater and food waste etc. Latterly, microalgae, a 3rd generation biomass, has been considered as biomass for biogas production, because it does not compete with food supply and has a high methane potential. However, the use of protein-rich microalgae as AD substrate has been proven to be very difficult due to its high nitrogen content (Maity et al., 2014). Total ammonia (TAN) is presented as ammonium ion (NH_4^+) and free ammonia (NH_3, FAN) depending on the pH and temperature of the aqueous phase. FAN is believed to be the most toxic form of TAN due to its high permeability into cell membrane (Massé et al., 2014). Different inhibition thresholds were reviewed in literature (Yenigün & Demirel, 2013). However, it is generally accepted nowadays that TAN and FAN loads above 3 g NH_4^+-N L^{-1} and 0.15 g NH_3-N L^{-1} can inhibit AD process and lower the potential methane yield of AD reactors (Nielsen and Angelidaki, 2008; Yenigün and Demirel, 2013).

To solve the ammonia problem, many solutions have been proposed in recent years. For example, dilution of the reactor content with water (Nielsen and Angelidaki, 2008); air stripping (Zhang et al., 2012); addition of absorbing material (Hansen et al., 1999); lowering the operating temperature (Angelidaki and Ahring, 1994); co-digestion with high carbon content substrate (Tsapekos et al., 2017); microbial electrochemical cell (Zhang & Angelidaki, 2015) and bioaugmentation with syntrophic acetate oxidizing bacteria (SAOB) or methanogens (Fotidis et al., 2014b; Westerholm et al., 2012). However, many of these methods are technically complex connected with high operational costs leading to limited applicability. Acclimation of microbial consortia to high ammonia levels could provide a practical and cost-effective method to digest protein-rich substrates (Yenigün and Demirel, 2013). A large number of investigations has demonstrated that methanogenic inocula have a high ammonia adaptation potential (Koster and Lettinga, 1988; Parkin et al., 1983). Additionally, a recent research showed that fast and efficient acclimatization of anaerobic consortia to high ammonia levels is possible in batch and fed-batch reactors (Tian et al., 2017). However, limited information (if any) is available in literature about successful acclimation using continuous reactors (e.g. continuously stirred tank reactor (CSTR)) at extremely high ammonia levels (> 7 g NH_4^+-N L^{-1}). Lack of successfully acclimatizing the process to extremely high ammonia levels could probably be attributed to the washout effect, by which inhibited microorganisms not growing fast enough at a specific retention time were washed out from the reactor (Tian et al., 2017). Washout is limiting operation at high rates continuous reactors without any microbial support matrix (e.g. granules).

Previous microbial community assessment studies during different acclimation processes to high ammonia levels, have reported controversial results. On one hand, there are studies indicating that the acclimation process to high ammonia levels caused a shift from aceticlastic to hydrogenotrophic methanogenesis (Schnürer and Nordberg, 2008; Werner et al., 2014). On the other hand, *Methanosarcinaceae* spp. associated aceticlastic pathway has been found to be predominant at high ammonia levels (Calli et al., 2005; Karakashev et al., 2005). A more insightful and detailed understanding about the microbiome would be of great importance in further optimization of stable AD process, thus it is important to elucidate the microbial community changes during acclimation of continuous AD process to extremely high ammonia levels.

Therefore, the main aim of the present study was to use, for the first time, CSTR reactors fed with protein-rich microalgae (3rd generation biomass) as the main substrate, to successfully acclimatize methanogenic consortia to extremely high ammonia levels (> 7 g NH_4^+-N L^{-1}) overcoming the microbial washout effect. An additional aim was to reveal the effect of the ammonia acclimation process in the continuous reactors on the AD microbiome dynamics using next generation 16S rRNA gene sequencing.

2. Material and methods

2.1. Inoculum and feedstock

The inoculum derived from a full-scale mesophilic (37 ± 1 °C) biogas plant (Hashøj, Denmark), fed with 70–90% animal manure and 10–30% food industrial organic waste. Two substrates were used in this study: cattle slurry and microalgae *Chlorella vulgaris*. Cattle slurry was obtained from Hashøj Municipality, Denmark. It was sieved and stored at −21 °C until use. Microalgae *C. vulgaris* (> 50% protein in dry matter), as a protein-rich substrate, was grown in mineral salt medium (MBBM-2N) in a raceway pond with continuous illumination at 25 °C and pretreated according to a previous methodology (Mahdy et al., 2015) after cultivation and harvest. Specifically, a biological catalyst (Protease, Alcalase 2.5, Novozymes, Denmark) was used to hydrolyse microalgae at pH 8. Subsequently, the inactivation of the enzyme was done by heating the hydrolytic broth to 75 °C for 30 min. Then the pretreated microalgal biomass was stored into freezer until use. The basic characteristics of the inoculum and substrates were shown in Table 1. Ammonium chloride (NH_4Cl, Sigma-Aldrich) was used as ammonia source.

2.2. Experimental setup

Two lab-scale mesophilic (37 ± 1 °C) CSTR reactors were used in this study (R1 and R2) as duplicate. Each reactor had a 2.3 and 1.8 L total and working volume, respectively, and was equipped with an influent and an effluent bottle, a feeding peristaltic pump, an electrical

Table 1
Characteristics of the inoculum and substrates.

Parameter	Inoculum value ± SD[a]	Cattle slurry value ± SD[a]	Microalgae value ± SD[a]
Total solids-TS (g L^{-1})	33.20 ± 0.19	32.90 ± 0.02	160.00 ± 0.24
Volatile solids-VS (g L^{-1})	19.80 ± 0.18	23.00 ± 0.04	138.66 ± 0.18
Total Ammonium nitrogen-TAN (g NH_4^+-N L^{-1})	4.58 ± 0.02	1.10 ± 0.12	3.44 ± 0.36
Total Kjeldahl nitrogen-TKN (g N L^{-1})	5.01 ± 0.13	1.49 ± 0.01	14.29 ± 0.18
Volatile fatty acids-VFA (mg L^{-1})	76.08 ± 5.75	8936.97 ± 50.51	2668.94 ± 68.91

[a] Standard deviation.