The various microbial activity at different ammonia nitrogen concentrations for thermophilic and mesophilic biogas processes

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Abstract
Anaerobic digestion of substrates with high content of nitrogen may results in an acetate conversation performed by the slow-growing syntrophic acetate oxidizing bacteria (SAOB). In the present experiment, a mixture of slaughterhouse waste, household waste and glycerol was digested at short hydraulic retention time (17 days) and at high organic loading rate (6.5 g VS/L d). Digestion was performed in the presence of a process additive containing trace elements in an experimental matrix including different temperatures (38/55°C) and diverse ammonium concentrations. The daily methane production was equal in all systems. However, an unexpectedly large difference in biogas production rate was observed. Mesophilic temperature and low ammonia concentration resulted in 87-99% higher biogas production rate compared to both mesophilic and termophilic digestion at high ammonia levels. The microbial analysis confirmed that SAOB were abundant in all reactors, suggesting that acetate degradation works well even though neither aceticlastic methanogens nor syntrophic acetate oxidizing bacteria theoretically should thrive in an environment with high ammonia concentration or short hydraulic retention times.

Keywords
anaerobic digestion, ammonium inhibition, microbial activity, thermophilic, mesophilic

INTRODUCTION

In the last years there has been an increased interest for renewable energy sources. Additional knowledge regarding the anaerobic degradation process is essential in order to fulfil the demand for biogas, to increase the production and achieve stable processes.

The effect of ammonium (NH_4^+) and ammonia (NH_3) on the biogas production process has been investigated by many different research groups (Ek *et al.* 2011; Prochazka *et al.* 2012; Schnürer and Nordberg 2008). Inhibition of the aceticlastic methanogenesis and thereby a decreased specific methane production may occur if the ammonium concentration raises above 3 g NH_4–N/L and the ammonia concentration is in the range of 0.13-0.33 g NH_3-N/L (Schnürer and Nordberg 2008). The acetate-utilizing methanogens are normally dominating the microbial activity. High ammonium concentrations have however been shown to cause changes in the microbial community and an increased importance of the syntrophic acetate oxidizing bacteria, SAOB (Schnürer and Nordberg 2008). Anaerobic digestion with SAOB as the dominating acetate-utilizing microorganisms has lower degradation rates of acetate compared to the aceticlastic pathway (Schnürer and Nordberg 2008; Schnürer *et al.* 1999). Long hydraulic retention time (HRT) has been shown to be one way to accomplish a stable degradation process at ammonium concentrations of 5-6 g NH_4-N/L (Ek *et al.* 2011), since the doubling time of SAOB is up to 28 days compared to 2-8 days for the aceticlastic methanogens (Schnürer and Nordberg, 2008).

The aim of this study was to evaluate the anaerobic digestion process at subnormal conditions including short HRT and high organic loading rate. This study includes three different processes in laboratory scale: one mesophilic process with high ammonia concentration, one mesophilic process with low ammonia concentration and one thermophilic process with high ammonia concentration.

MATERIALS AND METHODS

Experimental setups and operational conditions

Three semi-continuous laboratory-scale reactors (Moestedt *et al.* 2012; Nordell *et al.* 2011), with an active volume of 9 L, were used in this study. The reactors were denoted reactor T (thermophilic, 55°C, low ammonium), reactor N (mesophilic, 38°C, high ammonium) and reactor C (mesophilic, 38°C, low ammonium). The reactors were fed semi-continuous with 24 hours interval. The experiment lasted for 90 days and a start-up period was applied prior the experiment to stabilize the inoculum. For day 1 to 26 the OLR was held constant at 4.7 g VS/L d (HRT = 18.3 days), and was thereafter increased to 6.5 g VS/L d (HRT = 17.6 days) for the rest of the experiment. The substrate for all three reactors was food waste (59-82% of OLR), slaughterhouse waste (13-18% of OLR) and glycerol (0-28% of OLR). An in-house developed process additive similar to (Ejlertsson 2006) was continuously added to the reactors. Reactor N was fed with additional urea to gain an ammonia concentration comparable to the expected concentration in the thermophlic reactor T.

Experimental monitoring and analyses

A MGC-10 milligas counter (Ritter, Germany) was used to measure the volumetric gas production and a methane gas sensor (Bluesense, Germany) was used to detect the methane gas. The concentration of volatile fatty acids (VFAs) was analysed using a Clarus 550 gas chromatography (Perkin Elmer, USA) with a packed Elite-FFAP column for acidic compounds (Jonsson and Borén 2002). The concentration of ammonium nitrogen (as a sum of soluble NH_4-N and NH_3-N) was analyzed according to FOSS Tecators application sub note 3502 with a Kjeltec 8200 (FOSS in Scandinavia, Sweden).The concentration of ammonia nitrogen was calculated as a fraction from the concentration of ammonium nitrogen, the pH and the temperature (Hansen *et al.* 1998). The biogas production rate and the kinetics were calculated according to Nordell *et al.* (in preparation, a). *Clostridium ultunense (Cu), Syntrophaceticus schinkii (Ss), Tepidanaerobacter acetatoxydans (Ta), Methanosaetaceae (Msa), Methanomicrobiales (Mm)* and *Methanosarcinaceae (Msc)* were analysed according to Westerholm *et al.* (2011), in all three reactors (day 78).

RESULTS AND DISCUSSION

General process performance

All three reactors were performing well during the whole experiment and the methane production was equal in all three reactors (Table 1). The two reactors with high ammonia concentration (reactor T/N) suffered from moderate VFA concentration (1.3 g/L in average), while the VFA concentration in reactor C was undetectable (Table 1). However, even though the mesophilic reactor C was fed with the same substrate mixture as the thermophilic reactor T, the pH was 8.0 in reactor T compared to 7.6 in reactor C (Table 1). Moreover, the mineralization ratio of nitrogen was 25% higher in the thermophilic reactor T. This may have been caused by multiple factors such as; higher degradation rate of nitrogen rich compounds, lower microbial abundance or decreased assimilation of nitrogen.

Table 1. Mean values of methane production, VFA, ammonium, ammonia, Kjeldahl-N and pH.

	Reactor T	Reactor N	Reactor C
Specific CH_4 production (NmL/g VS)	469 ± 26	469 ± 41	473 ± 30
VFA (g/L)	1.3 ± 1.2	1.3 ± 0.7	<0.1
Ammonium (g NH_4-N/kg)	2.5 ± 0.1	5.6 ± 0.2	2.0 ± 0.2
Ammonia – calculated (g NH_3-N/kg)	0.86 ± 0.05	0.75 ± 0.09	0.1 ± 0.01
Kjeldahl-N (g Kjel-N/kg)	4.3 ± 0.2	7.9 ± 0.3	4.3 ± 0.1
pH	8.1 ± 0.1	8.1 ± 0.1	7.6 ± 0.1
Aceticlastic methanogens (log gene c.)	Msc (6), Msa (7)	-	Msa(6)
SAOB (log gene c.)	Ta (7), Ss (6)	Ss (6), Cu (5)	Ss (6), Cu (5)
Hydrogenotrophic methanogens (log gene c.)	Mm (9)	Mm (6)	Mm (7)

Microbial activity

The high concentration of ammonia in reactor N (0.75 g NH_3-N/kg) and reactor T (0.86 g NH_3-N/kg) should theoretically result in selection for SAOB since aceticlastic methanogens are sensitive to ammonia and are inhibited in the range of 0.13-0.33 g NH_3-N/kg (Schnürer *et al.* 1994; Schnürer and Nordberg 2008). Microbial analysis with qPCR showed that the slow-growing SAOB, *C. ultunence*, determined by Schnürer and Nordberg (2008), was present in both of the mesophilic reactors C and N, but undetected in reactor T. Furthermore, the reactors were running at short HRT of 17-18 days, which according to previously published literature is shorter than the doubling time for *C. ultunence* (Schnürer and Nordberg (2008). The reason why SAOB were present in this study could be due to the fact that the substrate mixture contained a process additive consisting of iron, hydrochloric acid and trace elements (Ejlertsson 2006; Nordell *et al.* In preperation, b). Moreover, Nordell *et al.* (In preperation, b) have showed that addition of the similar process additive decreased the concentration of VFA with 90% and increased the biogas production rate in a mesophilic system where SAOB was the dominating acetate degradation pathway, at moderate ammonium concentration (3.2 g/L) and HRT of 30 days.

The time to reach $Y_{½,biogas}(t)$ in reactor C was 4.7 hours in average at the OLR of 6.5 g VS/L d (day 41 to 90). The biogas production rate was significantly different between the three reactors at $Y_{½,biogas}(t)$. The biogas production rate in reactor C was approximately 747 NmL/g VS h, which can be compared with the thermophilic reactor T and the mesophilic reactor N, which only produced 398 NmL/g VS h and 375 NmL/g VS h, respectively. Thus, the biogas production rate was 87-99% higher in reactor C compared to reactor T and reactor N. This result indicates that the microbial activity was higher in reactor C compared to reactor N and reactor T. A high microbial activity in reactor C correlates well with the low VFA concentration, see Table 1. The similarity of the biogas production for reactor T and reactor N as well as the VFA concentration, was unexpected since these two reactors were operated at different temperatures (55°C/38°C) and at different ammonium levels (2.5 g/L compared to 5.6 g/L).

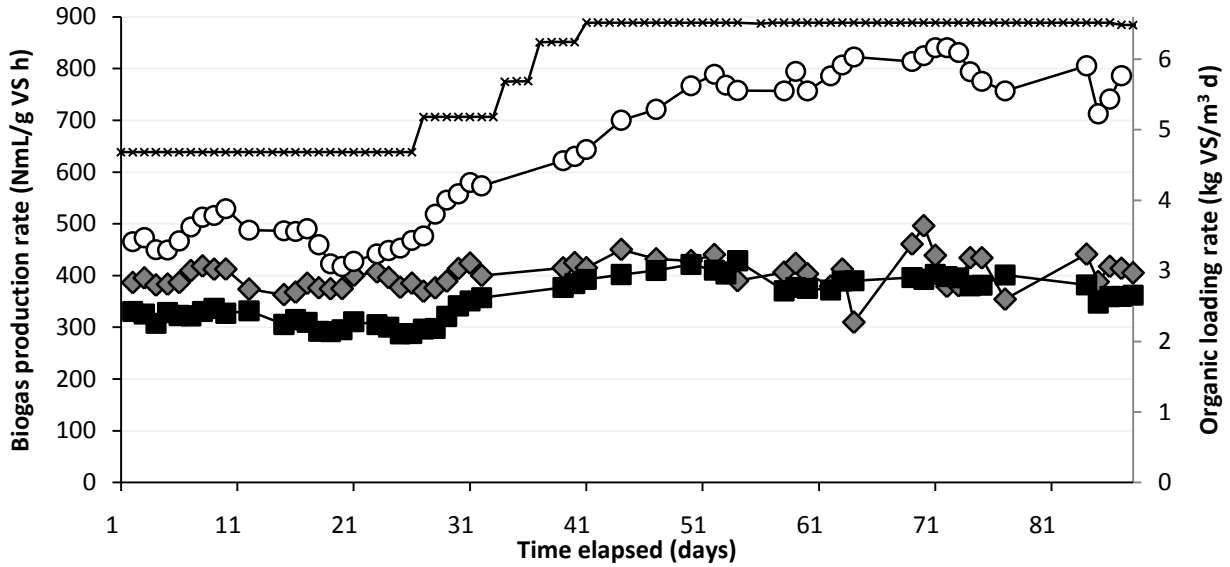

Figure 1. The OLR (-x-) and the biogas production rate (r_{biogas}) of the daily biogas production for reactors: reactor C (○), reactor T (♦) and reactor N (■).

qPCR analysis showed a diversity in the microbial communities responsible for acetate degradation among the compared reactors. Aceticlastic *M. saetaceae*, which converts acetate into methane, was present in both reactor C and reactor T – despite high ammonia concentration in reactor T (Table 1). However, both reactor C and T also had a high microbial density of SAOB (Table 1). In the absence of *M. saetaceae,* the most nitrogen tolerant SAOB *C. ultunence* dominated in reactor N indicating

that SAOB was the dominant acetate degradation pathway, in agreement with Karakashev *et al.* (2006), see Table 1. However, due to the short HRT (17 days) in the reactors, our results contradict Schnürer and Nordberg (2008) where *C. ultunence* was shown to have a doubling time of 28 days.

The results also indicate that high ammonium concentration, and not ammonia, may be the cause of the concluded dominance of SAOB in reactor N since reactor N and reactor T has similar ammonia concentrations but different ammonium (Table 1). Similar microbial composition of reactor T and C indicates that a different microbiological activity, rather than composition, caused the observed difference of the biogas production rate (Figure 1). High ammonia concentrations were found to be negatively correlated with the biogas production rate (Figure 1) since reactor T and reactor N had similar biogas production rate while reactor C, with low ammonia concentration, had a more rapid biogas production rate.

CONCLUSIONS

In this study, it is clearly shown that it is possible to achieve both a stable mesophilic and thermophilic anaerobic degradation process at low HRT and at high ammonia concentrations. No difference in daily methane production was found. However, an unexpectedly large difference in biogas production rate was observed. Mesophilic temperature and low ammonia concentration resulted in 87-99% higher biogas production rate compared to both mesophilic and termophilic digestion at high ammonia levels. Finally, the microbial analysis confirmed that SAOB were present in all reactors, suggesting that acetate degradation works well even though neither aceticlastic methanogenesis nor syntrophic acetate oxidizing bacteria theoretically should thrives an environment with high ammonia concentration or short retention times.

ACKNOWLEGDEMENTS

The authors would like to express their gratitude to Yasna Calderon for the technical assistance in the laboratory and to Anna Schnürer at Swedish University of Agricultural Sciences for facilitating access to equipment and material required for qPCR analyses.

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