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Thermophilic anaerobic digestion of livestock waste: the effect of ammonia

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Abstract. Ammonia concentrations of 4 g N/l or more inhibited thermophilic digestion of cattle manure. A stable digestion of cattle manure could be maintained with ammonia concentrations up to 6 g N/l after 6 months of operation. However, the methane yield was reduced and the concentration of volatile fatty acids increased from 1 to 3 g/l as acetate, compared to controls with an ammonia concentration of 2.5 g N/l. The temporary strong inhibition following an one-step increase in ammonia concentration was reduced by applying a gradual increase. The specific methanogenic activity of ammonia-inhibited reactors (6 g N/l) with acetate or hydrogen as substrate was reduced by 73 and 52%, respectively. Tests of ammonia toxicity on the acetate- and hydrogen-utilizing populations showed a higher sensitivity of the aceticlastic compared to the hydrogenotrophic methanogens; the specific growth rate for the aceticlastic methanogens was halved at ammonia concentrations of 3.5 g N/l, compared to 7 g N/l for the hydrogenotrophic methanogens.

Introduction

Inhibition during anaerobic digestion of livestock waste is often caused by high ammonia concentration. In addition to ammonia ($NH_3 + NH_4^+$) livestock waste contains compounds that readily release ammonia when degraded, e.g. urea and proteins. Especially for swine and poultry manure, the total ammonia concentration is often higher than 4 g N/l (Angelidaki and Ahring 1991).

Many investigations have dealt with the ammonia inhibition level, but the results are conflicting and have been obtained under different conditions, such as the pH, temperature and inoculum used. McCarty (1964) reported ammonia inhibition to occur at concentrations from 1.5 to 3.0 g N/l at pH levels above 7.4, whereas inhibition occurred for all concentrations higher than 3 g N/l at all pH levels tested. Likewise Koster and Lettinga (1984) reported ammonia inhibition to occur at 1.7 g N/l at pH 7.5.

Much higher inhibitory levels have however been reported by other authors. Van Velsen (1979) showed in batch experiments, with inoculum adapted to high concentrations of ammonia, that methanogenesis occured after a lag phase at ammonia concentrations as high as 5 g N/l.

Only a few investigations have dealt with ammonia inhibition at thermophilic temperatures. Zeeman et al. (1985) reported an initial inhibition at 1.7 g N/l at 50° C. Hashimoto (1986) found ammonia inhibition at about 2.5 g N/l for both mesophilic and thermophilic reactors when the reactors were not previously acclimatized to ammonia. However, the corresponding values was 4 g N/l for thermophilic reactors previously acclimatized to ammonia concentrations between 1.4 and 3.3 g N/l. In their experiments the effluent pH was approx. 7.2.

Free ammonia (NH_3) has been suggested as the active component causing ammonia inhibition. A level of 80 mg N/l of free ammonia has been proposed as the minimum inhibitory level (Koster and Lettinga 1984; De Baere et al. 1984). McCarty and McKinney (1961) and Braun et al. (1981) found 150 mg N/l to be the inhibitory free ammonia concentration. As the free ammonia fraction increases with temperature and pH, the ammonia level tolerated at high pH and thermophilic temperatures would be expected to be low. Biogas reactors operating with livestock waste often have a high pH (about 8) and, especially at thermophilic temperatures, the free ammonia concentration will be up to ten times higher than the free ammonia concentrations reported as inhibitory.

In the present study we examined the effects of addition of different ammonia concentrations and the possibility of adaptation to ammonia during anaerobic thermophilic digestion of cattle manure in continuously fed lab-scale reactors. The specific methanogenic activity (SMA) of an uninhibited and an ammonia-inhibited reactor are reported. Finally, we examined the effect of various ammonia concentrations on thermophilic aceti-

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clastic and hydrogenotrophic methanogens in batch experiments.

Materials and methods

Continuously fed reactor experiments

Substrate. Cattle manure, obtained from a Danish biogas plant was used as substrate and was provided in one batch for each of two experiments. The natural ammonia concentration of the first batch was 1.5 g N/l. The batch used for the second experiment had a higher ammonia content and was therefore diluted with tap water resulting in a final ammonia concentration of 2.5 g N/l. Data of the two batches are given in Table 1.

The experiments were performed in six 4.5-l, automated lab-scale reactors with a working volume of 3 l as shown in Fig. 1. The reactors had a slowly moving stirring blade (60 rpm), which was activated for 1 min everys 3 min. Gas production was measured automatically with a gas meter as previously described (Angelidaki et al. 1992). The reactor temperature was kept at 55° C, and the retention time (RT) was 15 days.

Experimental design. Two series of reactor experiments were performed. In the first experiment the effect of adding ammonia to a level of 4 and 6 g N/l was compared to the performance of control reactors with an ammonia concentration of 1.5 g N/l. In the second experiment the effect of 6 g N/l was compared to the effect of a gradually increasing concentration of ammonia. The N concentration was increased at intervals of 30 days (corresponding to 2 RT) and the levels were 2.5, 3, 4, and 5 g N/l. The results obtained in this experiment were compared with those from contol reactors with 2.5 g N/l ammonia. In both experiments the extra ammonia was added to the feed as NH_4Cl. Duplicate reactors were operated for each concentration tested. As the variation between duplicate reactors was in general small (< 10%) mean values are reported.

Table 1. Data on cattle manure used

Substrate data	Experiment I	Experiment II[a]
Total solids (%)	6.4	5.9
Volatile solids (%)	4.5	4.2
Total nitrogen (g N/l)	n.d.[b]	3.7
Ammonia nitrogen (g N/l)	1.5	2.5
pH	7.6	7.8
Volatile fatty acids	7.4	7.6

[a] After dilution with tap water
[b] Not determined

SMA test

The SMA of the control reactors and the reactors receiving 6 g N/l ammonia were compared. The tests were performed in 58 ml serum vials containing 21 ml BA medium (Angelidaki et al. 1990) adjusted to pH 7.9 (corresponding to the pH of the reactors) with NaOH and a gas phase of N_2/CO_2 (approx. 90:10) in order to keep the pH at 7.9–8.0 during the experimental period. Acetate (30 mM) or 200 kPa of H_2/CO_2 (80:20) were applied as substrates and the methane produced was compared to vials without substrate added. The vials were inoculated anaerobically with 25% (v/v) reactor content and were incubated in a shaking water bath at 55° C. The SMA was estimated as the initial methane production rate per gram biomass (volatile solids, VS). The mean activity found in the control vials (without substrate addition) was subtracted from activities found in the experimental vials.

Effects of ammonia on methanogenic populations

The effect of different concentrations of ammonia on aceticlastic and hydrogenotrophic methanogenic populations was tested in batch experiments, using 5% (v/v) digested manure as inoculum in BA medium (content of ammonia in BA medium was 0.26 g N/l). The pH in these experiments was adjusted to 7.2–7.3, considered to be the optimum pH for these bacterial groups. As substrate, 30 mM acetate or 200 kPa H_2/CO_2 (80:20) was applied. For the acetate series, the range of concentrations tested was 0.26–13 g N/l, and 0.26–20 g N/l for the H_2/CO_2 series. The specific growth rates of the aceticlastic or hydrogenotrophic populations were estimated by a semi-logarithmic plot of methane production versus time. Each experiment was run in triplicate and all experiments were repeated.

Analytical methods

VS, total solids and pH were determined using standard methods (American Public Health Association 1985). CH_4, CO_2 and volatile fatty acids (VFA) were measured by gas chromatography as

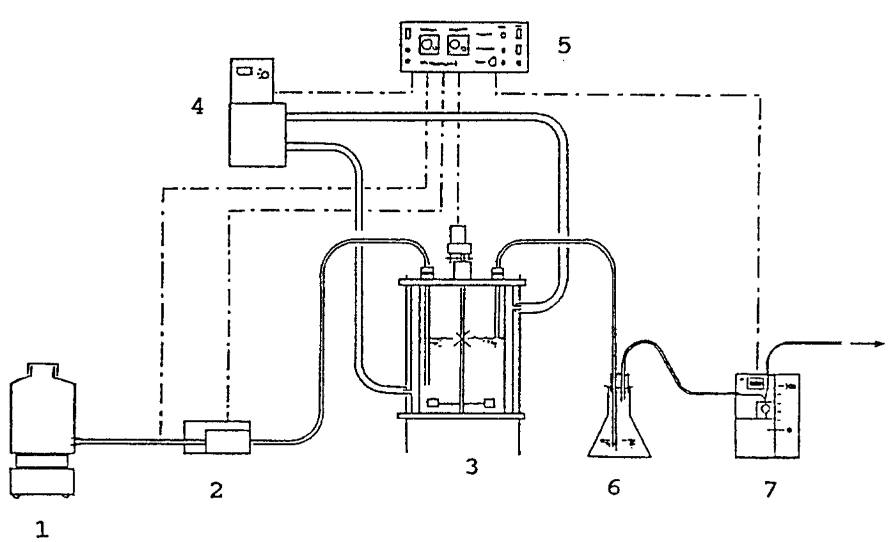

Fig. 1. The reactor set-up: *1,* substrate flask on a magnetic stirrer; *2,* pump; *3,* reactor; *4,* water bath; *5,* controller; *6,* gas-separating bottle; *7,* gas meter

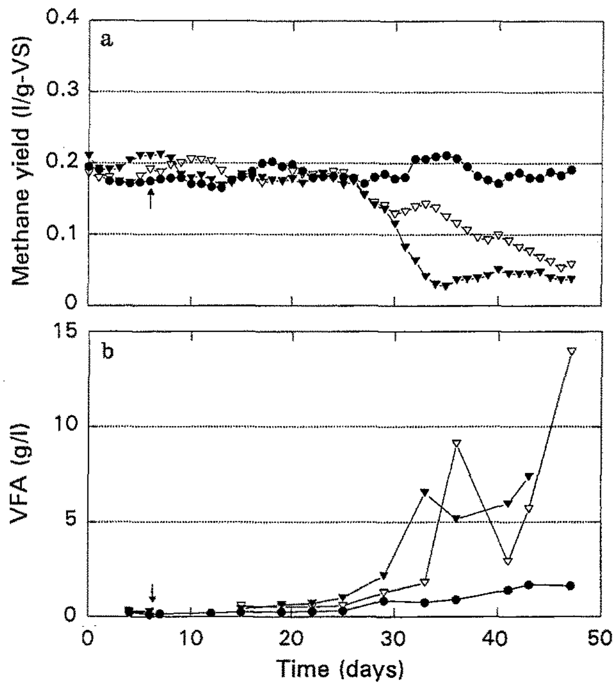

Fig. 2a, b. Continuous reactor experiment I. **a** Methane yield (5 days average). **b** Volatile fatty acids (VFA) concentration (calculated as acetate). The *arrows* indicate when the addition of ammonia was started: ●, control (1.5 g N/l); ▽, 4 g N/l, ▼, 6 g N/l. VS, volatile solids

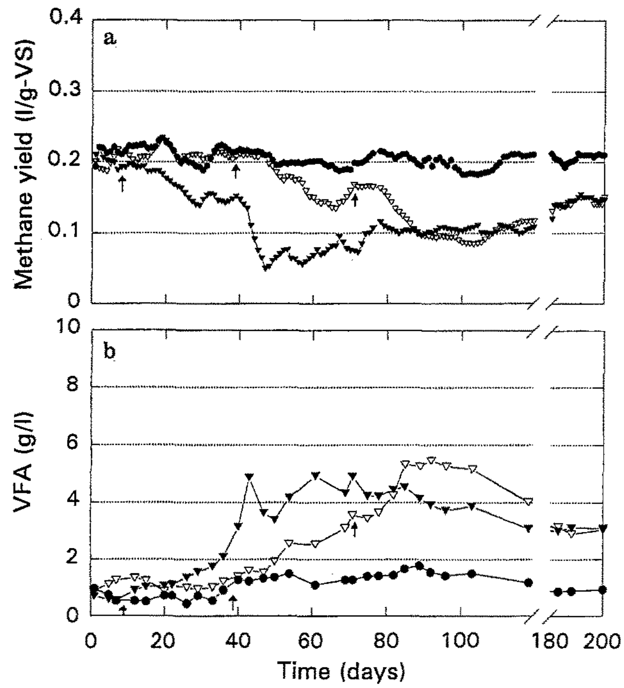

Fig. 3a, b. Continuous reactor experiment II. **a** Methane yield (5 days average). **b** VFA concentration (calculated as acetate). Ammonia was introduced at day 7 at a concentration of 6 g N/l or stepwise from 3 to 5 g N/l. The *arrows* indicate the stepwise increase in ammonia concentration to 3, 4 and finally 5 g N/l in the reactors receiving increasing concentrations of ammonia: ●, control (2.5 g N/l); ▽, increasing ammonia concentrations; ▼, 6 g N/l

previously described (Angelidaki et al. 1990). Total ammonia content was determined by the Kjeldahl method.

The free ammonia concentration was calculated from the equilibrium relationship:

$$[NH_3] = \frac{[T-NH_3]}{\left(1+\frac{H^+}{k_a}\right)}$$

where $[NH_3]$ and $[T-NH_3]$ are the free and the total ammonia concentrations, respectively, and k_a the dissociation constant, with the value of $38.3 \cdot 10^{-10}$ at 55° C; during calculations, the appropriate pH values were used.

Results

Addition of ammonia to a total concentration of 4 or 6 g N/l to the feed of reactors fed with cattle manure containing 1.5 g N/l (at day 7) resulted in a decrease in the methane yield after 3 weeks (Fig. 2a). The methane yield decreased from approx. 0.2 to 0.05 l CH_4/g VS for both reactors, corresponding to 25% of that of the control reactors receiving no extra ammonia. The decrease was faster for the reactors with 6 g N/l ammonia (Fig. 2a). The VFA concentration for both reactors receiving additional ammonia increased as the methane yield decreased (Fig. 2b).

In the second experiment the basic level of ammonia was 2.5 g N/l. The methane yield and the VFA concentration were the same as for the control reactors in the previous experiment, showing that 2.5 g N/l ammonia had no apparent effect on the biogas process compared to manure with 1.5 g N/l ammonia (Fig. 3a, 3b).

As found in the first experiment, addition of 6 g N/l resulted in a decreased methane yield, to approx. 0.05 l CH_4/g VS. The VFA concentration increased from about 1 g/l to above 4 g/l as acetate. However, after approx. 2 RT (30 days) the methane yield increased again to approx. 0.1 l CH_4/g VS and the VFA level decreased to 3 g/l as acetate (Fig. 3a, b). In the reactors receiving substrate with a gradually increasing concentration of ammonia, 3 g N/l did not result in any changes in methane yield and VFA concentration compared to the controls. Addition of 4 g N/l, however, resulted in an increase in the VFA concentration, followed by a decrease in methane yield. After 2 RT the methane yield partially recovered although the VFA level still increased.

When 5 g N/l was introduced, serious process failure occurred and the methane yield dropped to 0.1 l CH_4/g VS, i.e. the same level as the reactors receiving 6 g N/l from day 7. At the end of the experiment (200 days), reactors with both strategies of ammonia increase (instant and gradual) stabilized at a methane yield of 0.15 l CH_4/g VS and a VFA concentration of 3 g/l (Fig. 3a, b).

In both experiments the basic pH level in the reactors was approx. 7.9. Accumulation of VFA in the inhibited reactors resulted in a lowering of pH to approx. 7.5.

SMA test

The activity of the aceticlastic and the hydrogenotrophic methanogenic populations was significantly lower in the reactors receiving 6 g N/l ammonia. The decrease in the activity was higher (73%) for the aceticlastic population than for the hydrogenotrophic methanogens (52%) (Table 2). Microscopic examination of diluted samples showed numerous clusters of *Methanosarcina* indicating that this was the predominant genus of the aceticlastic methanogens.

Ammonia toxicity experiment

The maximum growth rates were 0.62 day^{-1} and 0.11 h^{-1} for the aceticlastic and hydrogenotrophic methanogens, respectively. The inhibitory effect of ammonia was in general stronger for the aceticlastic than for the hydrogenotrophic methanogens with initial inhibition occurring at an ammonia concentration of approx. 2 g N/l for the aceticlastic and 3.5 g N/l for the hydrogenotrophic methanogens (Fig. 4). Growth rates were reduced to 50% of the uninhibited value at 3.5 and 7 g N/l (250 and 500 mg N/l free NH_3) for the acetate-utilizing and hydrogenotrophic methanogenic bacteria, respectively.

Table 2. Specific methanogenic activity (SAM) of reactors under different states of ammonia inhibition

Reactors[a]	Substrate	SMA (μmol/g VS/h)	Reduction (%)[b]
Control	Acetate	23.3±3.1	
Ammonia	Acetate	6.0±0.6	73
Control	H_2/CO_2	24.0±1.7	
Ammonia	H_2/CO_2	11.5±2.0	52

VS, volatile solids

[a] The control reactor received 2.5 g N/l ammonia introduced with the cattle waste while the ammonia reactor received ammonia to a total level of 6.0 g N/l

[b] Reduction of the SMA of the ammonia reactor compared to the uninhibited reactor

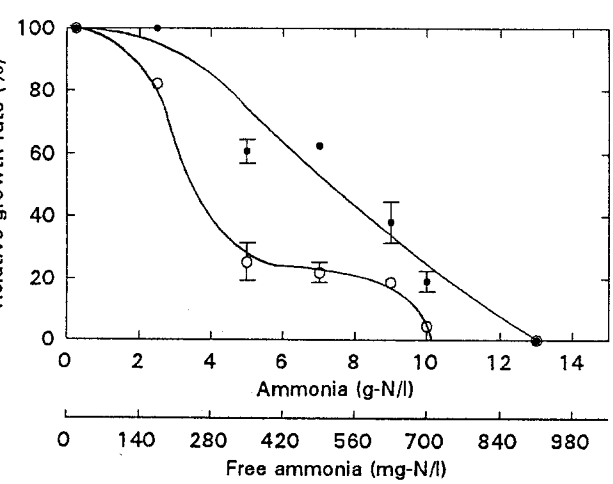

Fig. 4. Reduction in the specific growth rate as a function of ammonia: ○, acetate; ●, hydrogen as substrate

Discussion

Inhibition of the biogas process was observed when the ammonia concentration was increased to 4 g N/l or more in the continuously fed biogas reactors. The methane yield decreased to 25%, with both 4 and 6 g N/l added, compared to the controls with 1.5 g N/l ammonia. When ammonia was introduced gradually, the process was unaffected up to 3 g N/l and only slightly affected at 4 g N/l, with signs of recovery after 1 RT. At a concentration of 5 g N/l, process performance was seriously affected and reached the same reduced level as the reactors fed with 6 g N/l from the start of the experiment. After prolonged exposure to ammonia the reactors with ammonia concentration of 5 or 6 g N/l stabilized at a level of 0.15 l CH_4/g VS and 3 g/l VFA (Fig. 3a, b).

The experiments clearly demonstrate that it will be possible to obtain a stable digestion of manure with ammonia concentrations exceeding 4 g N/l after an initial adaption period. However, the methane yield will be lower (approx. 25% lower than for uninhibited reactors) and the VFA level will be higher than in reactors with a lower ammonia load.

Growth of methanogenic bacteria was inhibited by ammonia levels above 2 g N/l (Fig. 4), while a concentration of 4 g N/l was needed to affect the performance of the continuously fed biogas reactors. In a continuously fed reactor inhibition is only detected when the reduction of the growth rates of the active biomass approaches the dilution rate used. In contrast a reduced growth rate will directly affect the outcome of a batch experiment.

The concentrations of free ammonia calculated for the reactor experiments are high. At 2.5 g N/l, corresponding to the controls of the second experiment, the calculated free ammonia concentration (pH = 7.9) was 550 mg N/l. At 4 g N/l ammonia, where the first signs of inhibition occurred, the calculated free ammonia concentration was approx. 900 mg N/l (pH = 7.9). However, the actual pH of the reactors dropped to approx. 7.7, due to the accumulation of VFA, resulting in a free ammonia concentration of approx. 650 mg N/l. This concentration of free ammonia resulted in a reduction in the growth rates of the aceticlastic methanogens to 20%, in the experiments with ammonia toxicity (Fig. 4). Growth rates at this level are still sufficient to retain the active biomass within the reactor at the RT used in the reactor experiments. However, ammonia concentrations higher than 4 g N/l resulted in growth rates of the aceticlastic methanogens close to the RT, resulting in a decreased methane yield and an increased VFA concentration in the reactors.

Process instability due to ammonia resulted in VFA accumulation, which again led to a lowering of the pH and thereby decreased the concentration of free ammonia in the reactor. This decrease in free ammonia could explain the observed ability of the process to stabilize even with high ammonia concentration and with a lower but stable methane yield.

The SMA of the acetate-utilizing methanogens of the ammonia-inhibited reactor decreased more than the hydrogenotrophic populations. Both the SMA test and the ammonia toxicity experiment showed that it is the aceticlastic methanogens that are primarily affected by ammonia. This result is in accordance with other reports for mesophilic methanogens (Robbins et al. 1989; Bhattacharya and Parkin 1989; Sprott and Patel 1986). However, Zeeman et al. (1985) and Wiegant and Zeeman (1986) suggested that the hydrogenotrophic methanogens are more sensitive towards ammonia than the aceticlastic methanogens under thermophilic conditions, which is not supported by our experiments.

Inhibition of the aceticlastic populations showed a sigmoidal pattern. The same pattern of inhibition of the aceticlastic populations was observed by Poggi-Varaldo et al. (1991), who found that the bacterial growth rate and the specific acetate-uptake rate were affected by the free ammonia concentration in a three-stage pattern: initial inhibition, plateau and final inhibition. This inhibition pattern could indicate that two inhibition mechanisms are involved, acting at different concentration levels. The hydrogenotrophic populations exhibited, however, a more linear pattern of inhibition.

In large biogas plants a lowering of the gas yield has serious economic consequences. Therefore, the ammonia concentration should be checked and if possible kept at 4 g N/l or lower for maximum biogas production. If high ammonia concentrations cannot be avoided, the results indicate that a longer RT would be beneficial.

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