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Effect of the clay mineral bentonite on ammonia inhibition of anaerobic thermophilic reactors degrading animal waste

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Abstract

Addition of bentonite or the waste product bentonite-bound oil counteracted to some extent the inhibitory effect of ammonia during thermophilic anaerobic digestion of cattle manure. In continuously-fed reactor experiments, addition of bentonite or bentonite-bound oil delayed the onset of the inhibition and aided process recovery after initial inhibition. The effect was observed only when the ammonia concentration was increased gradually, indicating that the major effect of bentonite and BBO was not through a direct antagonistic effect towards ammonia but through an increased process resistance to toxic compounds. In batch experiments bentonite had a similar stimulatory effect leading to a decreased lag phase and increased methane production rate in ammonia inhibited reactors.

Introduction

Anaerobic digestion is often subjected to failure due to disturbance of the balance between the different bacteria involved, caused by for instance toxic compounds contained in waste or a change in the loading of the reactor (De Baere et al. 1984). Elimination or control of toxic compounds is, therefore, of major importance. Ammonia ($NH_3^+NH_4^+$) is the most common toxin causing digester failure during anaerobic digestion of livestock wastes (Braun et al. 1981). The inhibitory level of ammonia has been the subject of numerous studies: the toxicity of ammonia depends on pH, temperature and inoculum. McCarty (1964) reported inhibition at ammonia concentrations in the range of 1.5 to 3.0 g-N/l, depending on the pH. Other studies showed that with inoculum adapted to ammonia methanogenesis could occur at ammonia, concentrations as high as 5 g-N/l (Van Velsen 1979). In experiments with thermophilic (55°C) continuously stirred reactors digesting cattle manure, we demonstrated that adaptation to an ammonia concentration of 4 g-N/l can be achieved by gradually increasing the ammonia concentration in the feed (Angelidaki & Ahring, in press). Higher concentrations were, however, found to be inhibitory for the process, stressing the importance of additives which can decrease ammonia toxicity.

Only a few studies have dealt with ways of decreasing ammonia inhibition. McCarty and McKinney (1961) found that addition of Mg^{2+} and Ca^{2+} had an antagonistic effect on ammonia inhibition. Sprott & Patel (1986) have likewise reported that certain cations (Ca^{2+} or Na^+) countered the toxic effects of ammonia on methane synthesis in pure cultures.

Clay minerals and other surface-active particles have been reported to influence microbial and enzymatic transformations of a variety of substances, including ammonium, sulfur, carbohydrates, proteinaceous materials, phenolics, vitamins, antibiotics and complex plant residues (Bremner 1954). Stotzky & Rem (1966) reported that the clay mineral montmorillonite stimulated respiration of a wide spectrum of bacterial species at all stages of their growth but especially by shortening the lag phase. Addition of vermiculite powder, which is structurally related to bentonite, and other biologically inert materials to cattle manure resulted in an increased biogas yield of 15 to 30% in batch experiments (Geeta et al. 1986). Furthermore, we showed that addition of bentonite reduces inhibition caused by long-chain fatty acids (Angelidaki et al. 1990).

In the present study, the effects of the clay mineral bentonite and the waste product bentonite-bound oil on thermophilic anaerobic digestion of cattle waste at different ammonia concentrations were examined. Experiments were made both in batch and in continuously-fed reactors.

Materials and methods

Reactor experiments

Prior to initiation of the experiments all reactors were operated at similar conditions for approx. two months. The experiments were performed in eight 4.5 l automated lab-scale reactors with a working volume of 3 l (Angelidaki & Ahring, in press). The reactors were automatically fed at intervals of 6 hr with a retention time (RT) of 15 days. The gas production was measured automatically with a gas meter (Angelidaki et al. 1992). The reactor temperature was kept at 55°C.

Experimental design

The eight reactors were divided into two groups. In the first group, referred to as the reference group, no extra ammonia was added. Two of the reactors were fed with cattle manure alone (control reactors), one was fed with cattle manure with the addition of 1.1% bentonite (bentonite reactor) and one was fed with cattle manure with 2% bentonite-bound oil (BBO reactor), corresponding to 1.1% bentonite.

The other group was fed identically to the reference group, apart from the ammonia concentration which was gradually increased by addition of extra ammonia in the form of NH_4Cl. When the experiment was initiated, the ammonia concentration was changed from 2.5 to 3 g-N/l. Further changes were made at day 42 (from 3 to 4 g-N/l) and at day 71 (from 4 to 5 g-N/l).

The methane yield of the reactors was estimated as the methane produced divided by the volatile solids added (l CH_4/gVS).

Substrate

The cattle manure used was obtained from the receiving tank of a Danish biogas plant.

The bentonite used had a cation exchange capacity (CEC) of approx. 0.85 meq/g. Bentonite consists mainly of montmorillonite (González-Pradas et al. 1991). A characteristic feature of this group of clay minerals is their layered structure. The layers are loosely bound one to another allowing water, numerous elements and organic matter to enter the spaces between layers.

The composition of the bentonite used was (w/w %) : SiO_2, 70; Al_2O_3, 12; FeO_3, 4; TiO_2, 2; MgO, 3; Na_2O, 0.4; K_2O, 0.8.

BBO is a waste product from the vegetable oil refining process, where bentonite is used to clean and decolorize vegetable oil, and contains high amounts of oil. In the decolorization process bentonite is used due to its ability to absorb dyes, chlorophylles, carrotenoides, etc from plant oils. The composition of the solids in BBO used was 33% oil

Table 1. Data on cattle manure and BBO used.

Substrate data		Cattle manure	BBO
Total solids	(%)	5.9	97
Volatile solids	(%)	4.2	43
Total nitrogen	(g-N/l)	3.7	
Ammonia nitrogen	(g-N/l)	2.5	
pH		7.8	

(rapeseed oil composition) and 55% bentonite (Angelidaki et al. 1990). The balance was organic material such as tocopherols, chlorophylls, steroids and phospholipids. Data of substrates used are shown in Table 1.

Batch culture experiments

The effect of bentonite on ammonia inhibition was also examined in batch culture experiments. BA-medium was used (Angelidaki et al. 1990) with 30 mM acetate as carbon and energy source. The ammonia content in BA-medium was 0.26 g-N/l. The medium contains 0.1 g/l yeast extract and was distributed anaerobically in 20 ml portions to 58 ml vials. Bentonite was added (0.3 g per vial) resulting in a content of 1.5 w/v %. The vials were inoculated with 5% digested manure from a lab-scale thermophilic reactor treating cattle manure with an ammonia content of 2.5 g-N/l. Triplicate vials were used. Ammonia was added as NH_4Cl from anaerobic stock solutions. In the control vials with no extra ammonia, anaerobic distilled water was added.

Analytical methods

Volatile solids, total solids and pH were determined using standard method (American Public Health Association 1975). CH_4 and CO_2 in the reactor experiments were determined by gas chromatography with TCD detection (Angelidaki et al. 1990). CH_4 produced during batch experiments was analysed by gas chromatography with a FID detector as previously described (Sørensen et al. 1991). Volatile fatty acids (VFA) were analysed by gas chromatograph equipped with FID detection (Sørensen et al. 1991). VFA are present as acetate equivalents. Total nitrogen and ammonia were determined by the Kjeldahl method.

Results

The methane yield of the two control reactors receiving only manure from the reference group was 0.20 l CH_4/gVS (STD=0.01) (Fig. 1a). Differences between the two reactors were usually lower than 10%. The methane yield in the reactor receiving 1.1% bentonite in addition to the manure was similar to the controls (Fig. 1a), indicating that bentonite alone did not have any effect on the biogas production. In the reactor receiving 2% BBO in addition to manure, the methane yield was slightly higher i.e approx. 0.23 l CH_4/gVS (std=0.01), as the extra organic material contained in the BBO is better degradable giving a higher yield in comparison to average organic material of the manure. Concentrations of the VFA were comparable and varied around 1 g/l as acetate in all 4 reactors (Fig. 1b).

When the ammonia concentration was increased to 4 g-N/l, at day 42, in the feed to the second group of reactors, the methane yield decreased in both the control and the bentonite reactors (Fig. 2a). The process seemed to adapt to this concentration of ammonia and the methane production gradually increased after 30 and 18 days in the control and bentonite reactors, respectively. The BBO reactor did not show any decrease in the methane production at this ammonia concentration. When 5 g-N/l was introduced at day 71, methane production dropped in all the reactors, especially in the control reactors (Fig. 2a). The methane yield of the control reactors decreased to less than 0.10 l CH_4/gVS, and in the bentonite and BBO reactors to 0.12 and 0.14 l CH_4/gVS, respectively. Following this initial drop, methane production in the bentonite and the BBO reactor gradually increased, reaching the same level as before inhibition. The control reactors did, however, not recover to the same extent and the methane yield was 0.13 l CH_4/gVS at the end of the experiment. In the bentonite and the in BBO reactors the methane yields at the end of the experiment were 0.20 and 0.22 l CH_4/gVS, respectively.

The sudden drop in methane production in the bentonite reactor at day 119 was due to accidental loss of almost half of the reactor content. After this accident the feed was adjusted to correspond to 15 days retention time, which should in principle result in the same methane yield. For 25 days afterwards, however, a somewhat lower yield was observed.

VFA concentration (Fig. 2b) was stable at approx. 1 g/l as acetate until the ammonia concentra-

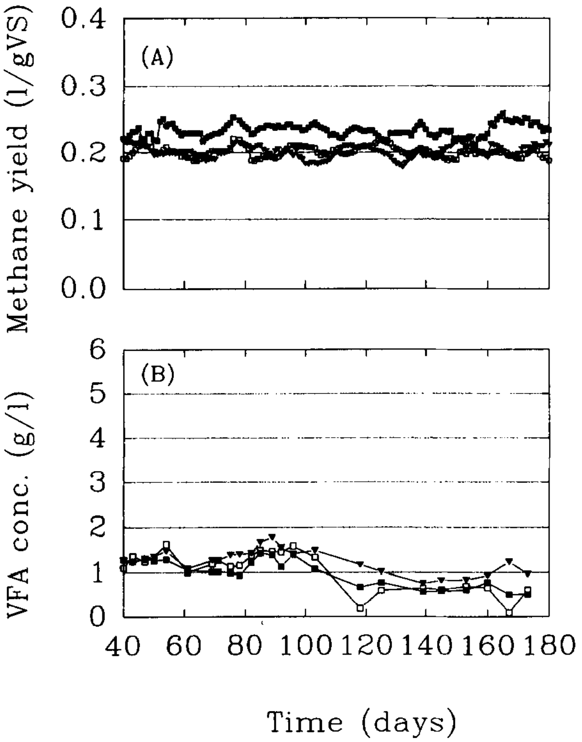

Fig. 1. Continuously-fed reactor experiment with no extra ammonia addition. (a) methane yield (l methane produced/gVS in a 5 days average), (b) VFA concentration (calculated as acetate). Symbols: ▼, average from the two control reactors; □, with bentonite addition, ■, with BBO addition.

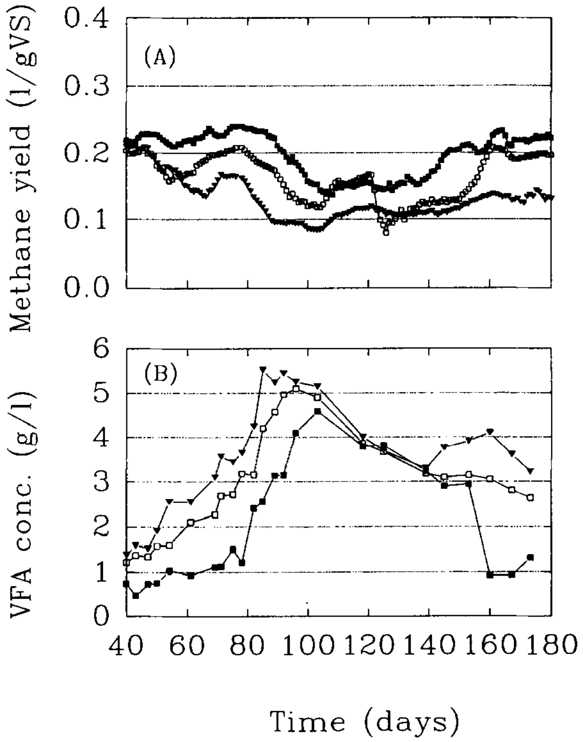

Fig. 2. Continuously-fed reactor experiment with increasing ammonia concentration. (a) methane yield (l methane produced/gVS in a 5 days average), (b) VFA concentration (calculated as acetate). At day 42 ammonia concentration was changed from 3 to 4 g-N/l, and at day 71 from 4 to 5 g-N/l. Symbols as in Fig. 1.

tion in the feed was increased to 4 g-N/l. This resulted in an increase in the VFA concentration of the control and the bentonite reactors. VFA concentrations in the reactor with BBO addition increased only after the ammonia concentration was elevated to 5 g-N/l. After 160 days the VFA concentration of the BBO reactor stabilized and returned to the same level as before extra ammonia was introduced.

Batch culture experiments

Methane production from the control vials with no extra ammonia was alike in all vials, independent of addition of bentonite (Fig. 3a). When extra ammonia was added to the medium the methane production rate decreased and the lag phase increased (Fig. 3b,c,d). In the vials with bentonite the increase in lag phase was slightly lower than in vials without bentonite. This effect was more pronounced as the ammonia concentration increased, i.e the lag phase was shortened by 3, 7 and more than 25 days by addition of bentonite for ammonia concentrations of 2, 5 and 7 g-N/l, respectively, compared to controls with 0.26 g-N/l. In addition to the shortening of the lag phase, bentonite had a positive effect on the methane production rate (Fig. 3b, 3c, 3d). Bentonite did not influence the methane yield from acetate. The ultimate methane yield from acetate was the same in all vials except for the vials with 7 g-N/l ammonia and no bentonite, where no methane was found when the experiment was terminated.

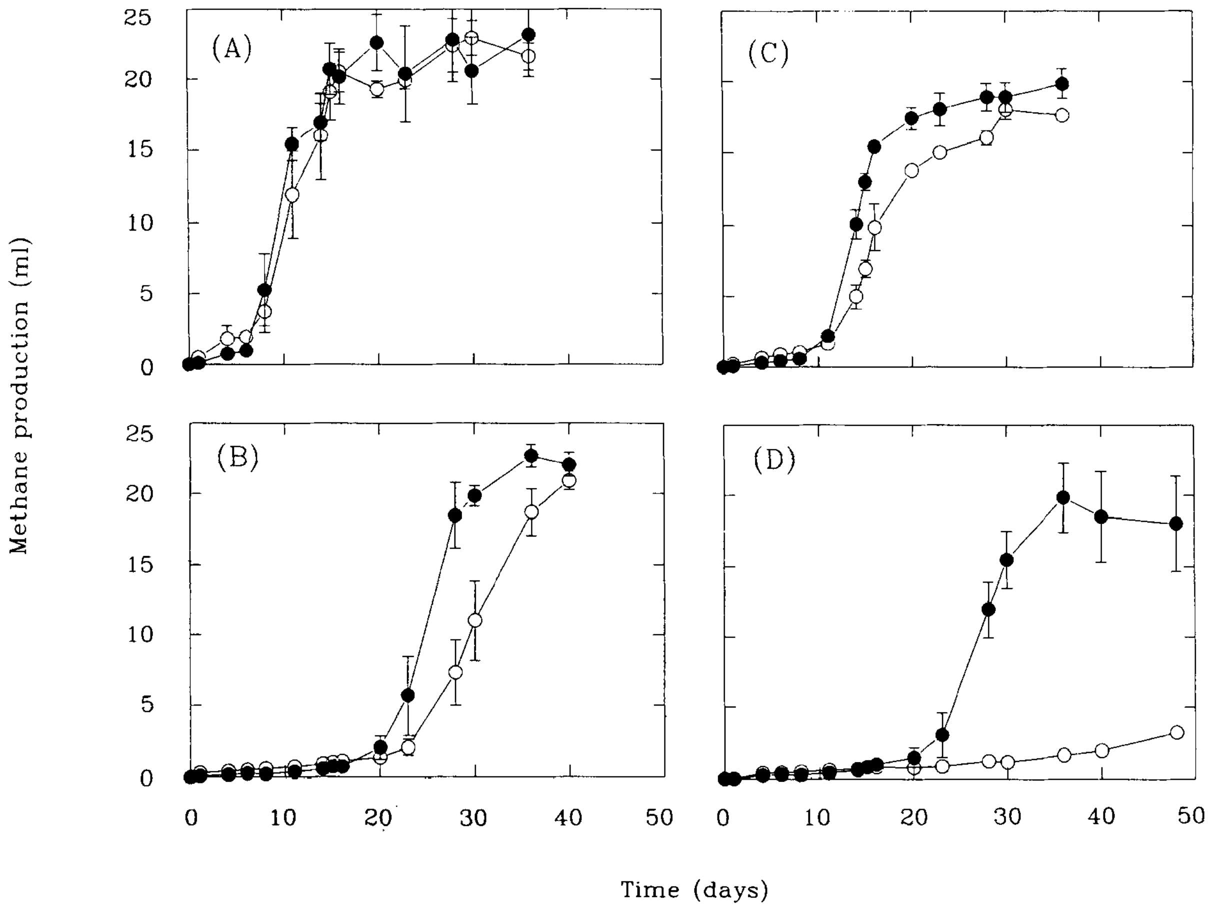

Fig. 3. Batch experiment at various ammonia concentration. (a) no extra ammonia addition, (b) 2 g-N/l ammonia, (c) 5 g-N/l ammonia and (d) 7 g-N/l ammonia. Symbols : ○, without bentonite addition; ●, with bentonite addition. Bars are the standard deviation of the mean.

Discussion

The methane yield from digestion of cattle manure with an ammonia concentration of 2.5 g-N/l was approx. 0.20 l CH_4/gVS. This yield is similar to results from previous experiments where the ammonia content was 1.5 g-N/l (Angelidaki and Ahring 1992, in press) indicating that the process was not inhibited by this increase in ammonia concentration.

The clay mineral bentonite did not exhibit any effect on the methane production or the level of the VFA found in uninhibited biogas reactors (the reference group) (Fig. 1). The same result was obtained from the batch experiments (Fig. 3a). This is in contrast to earlier reports where surface active materials, such as vermiculite and bentonite, have been shown to increase methane production (Geeta et al. 1986; Schoberth 1979).

When the process was inhibited by ammonia, however, a positive effect of both bentonite and BBO was observed, resulting in less drastic changes in the biogas production and more rapid recovery of the process. This clearly demonstrates that an increased resistance to ammonia inhibition is introduced by addition of bentonite. An even more pronounced effect was observed in reactors where BBO was added. Besides the effect of bentonite in the BBO, the extra substrate added seems to counteract the inhibition by ammonia.

When ammonia was increased directly from 2.5 to 6.0 g-N/l, neither bentonite nor BBO seemed to have the same effect in reversing the ammonia inhibition (data not shown). Consequently, the effect of bentonite and BBO was probably a stabilization of the process, and not a directly "curing" effect.

The exact mechanism of bentonite on ammonia inhibition has not yet been revealed. Stotzky & Rem (1966) observed that although montmorillonites buffering capacity was one of the mechanisms by which montmorillonite stimulated bacteria, additional mechanisms were involved. In our experiments the buffering capacity of the bentonite was of no significance, as manure is already very strongly buffered by ammonia and bicarbonate. There was no significant difference in pH in the reactors where bentonite or BBO were added in comparison to the control reactors.

The cation exchange capacity of bentonite (0.85 meq/g) is further too low to entirely explain the reversal of ammonia inhibition by a direct chemical binding of ammonia by bentonite. In the amount used in our experiment bentonite could at a maximum only exchange 130 mg-N/l ammonia.

The presence of cations such as Ca^{2+} and Na^{+} in bentonite could partly explain the observed effect since these ions have been shown to counteract the inhibitory effect of ammonia (McCarty & McKinney 1961; Sprott & Patel 1986).

During recent years many large scale joint biogas plants have been established in Denmark. These plants receive raw materials from several farmers along with industrial waste, for instance food industries. The mixing of several wastes leaves the possibility of appropriate raw material management in order to achieve a more stable digestion and to maximize biogas production. Thus, addition of certain types of wastes with properties similar to bentonite could, beside the actual treatment of these wastes, be a cheap way to counteract inhibitory effects of different toxins.

Acknowledgement

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