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Effects of lipids on thermophilic anaerobic digestion and reduction of lipid inhibition upon addition of bentonite

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Summary. The effect of bentonite-bound oil on thermophilic anaerobic digestion of cattle manure was investigated. In digestor experiments, addition of oil was found to be inhibitory during start-up and the inhibitory effect was less pronounced when the oil was added in the form of bentonite-bound oil compared to when the oil was added alone. After adaption of the digestors, very rapid degradation of oil was observed and more than 80% of the oil was degraded within a few hours after daily feeding. In batch experiments, glyceride trioleate was found to be inhibitory to thermophilic anaerobic digestion when the concentrations were higher than 2.0 g/l. However, addition of bentonite (a clay mineral) at concentrations of 0.15% and 0.45% was found to partly overcome this inhibition. Addition of calcium chloride in concentration of 3 m*M* (0.033% w/v) showed a similar positive effect on the utilization of oil, but the effect was lower than with bentonite.

Introduction

In the eight large full-scale biogas plants in Denmark, receiving manure from several farmers and daily treating from 50–300 tons of manure each, addition of waste from slaughterhouses and food industries has increased dramatically in recent years. Some of these additives have a high content of lipids and thus a large potential for biogas production. Improved performance of thermophilic digestion, with respect to gas yield and stability, has been observed in the large-scale biogas plant in Vegger, North Jutland, after addition of a waste product from the plant oil refinery industry called Bentonite-Bount-Oil (BBO). Bentonite, consisting mainly of the clay mineral montmorillonite, is used in plant oil refineries for cleaning and decolourizing vegetable oils. This refining process results in a waste product with a high oil content.

In a biogas digestor, neutral lipids (fat and oil) are hydrolysed by extracellular hydrolytic enzymes produced by fermentative bacteria to long-chain fatty acids (LCFA) and glycerol. The main part of the energy content of the oils is conserved in the LCFA, which are then further fermented by hydrogen-producing acetogenic bacteria via beta-oxidation (Weng and Jeris 1976). The products of this degradation (acetate and hydrogen) are finally converted into biogas (methane and carbon dioxide) by methanogenic bacteria (Bryant 1979).

The methane yield from oil is higher than from most other organic materials. The theoretical gas yield of glyceride trioleate (GTO) is, for example, 1.4 Nm^3 per kilogram of oil (Nm^3 = volume at 0° C) and 1 bar) with a methane content of 70% (Buswell and Neave 1930; Weng and Jeris 1976). In comparison, manure typically results in a gas yield of approx. 0.4 Nm^3 per kilogram of added organic matter, with a lower methane content. Therefore, waste with a high content of oil constitutes an attractive substrate for biogas production.

LCFAs have, however, been reported to cause inhibition of bacterial growth and biogas production at relatively low concentrations (Nieman 1954; Demeyer and Henderickx 1967; Henderson 1973; Hanaki et al. 1981; Sheu and Freese 1972; Rinzema et al. 1989). In the present paper we further study the effect of lipids, added in the form of oil, during the thermophilic anaerobic digestion of cattle manure, as well as the potential effect of simultaneous addition of bentonite.

Materials and methods

Digestor experiments

Substrate. Cattle waste consisting of a mixture of manure and urine from Vegger biogas plant, with a concentration of 8.9% total solids and 5.9% volatile solids was used. This waste was blended and kept frozen at −20° C until required. The total nitrogen concentration of the cattle waste used as primary substrate was approx. 3.7 g/l, and the ammonium concentration was 2.4 g NH_4^+-N/l.

The BBO (bentonite-bound oil) used in combination with the

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cattle waste consisted of 97% total solids and 43% volatile solids. The composition of the solids was 33% oil (low-erucic-acid rapeseed oil) and 57% bentonite. The balance was organic material such as tocopherols, chlorophylls, steroids, and phospholipids (Niels Lang Mathiesen, personal communication).

Digestor operation. Six 1-l serum bottles, each with a 0.6-l working volume, were used in the digestor experiments. The digestor bottles were placed in a water-bath maintained at 55° C and were continuously stirred by magnetic stirring. Gas production was measured by a liquid displacement system. The six digestors were initially started by mixing (1:1) raw cattle manure and digested cattle manure, obtained from the thermophilic biogas plant in Vegger, resulting in a 4.5% volatile solid content. After 55 days of batch digestion gas production decreased and the volatile fatty acid (VFA) concentrations were below 1.0 g/l in all digestors. The contents of the six digestors were then cross-inoculated in order to obtain equal starting conditions in all six digestors.

After the batch period, the digestors were operated as semi-continuous fed stirred tanks for a period of 76 days. Two digestors were fed with raw cattle manure alone and served as control digestors. The third digestor was fed with cattle manure with 1.65% bentonite added, corresponding to the amount of bentonite in 3% BBO. The fourth digestor was fed with cattle manure with 1% oil added, extracted with diethyl ether from BBO and corresponding to the amount of oil in 3% BBO. The two last digestors were fed with cattle manure with the addition of 3% and 6% BBO respectively.

For the three digestors receiving manure supplemented with BBO or oil, the relative oil utilization was calculated as the methane production measured minus the methane production of the control digestors relative to the theoretical methane yield of the amount of oil added.

Batch experiments

The effect of various concentrations of GTO (0-5 g/l), was tested in 117 ml vials, containing 40 ml BA medium (see below) and supplemented with 0.1 g/l yeast extract; 2.5 ml of thermophilic digested manure from one of the control digestors was used as inoculum. GTO was used, because oleate was the dominating fatty acid in the BBO used, accounting for 55% of the total fatty acids.

The effect of bentonite and calcium chloride on oil utilization was tested in similar vials, containing either of these compounds together with oil. The vials were incubated at 50° C and all experiments were performed in triplicate. Methane production and the concentration of volatile fatty acids were followed. The relative oil utilization was calculated as described for the digestor experiments. The pH of all vials was constant throughout the experiment at approx. pH 7.0.

Medium. BA medium was prepared from the following stock solutions (chemicals in g/l of double distilled water): (A) NH_4Cl, 100; NaCl, 10; $MgCl_2 \cdot 6H_2O$, 10; $CaCl_2 \cdot 2H_2O$, 5; (B) $K_2HPO_4 \cdot 3H_2O$, 200; (C) resazurin, 0.5; (D) trace metal and selenite solution: $FeCl_2 \cdot 4H_2O$, 2; H_3BO_3, 0.05; $ZnCl_2$, 0.05; $CuCl_2 \cdot 2H_2O$, 0.038; $MnCl_2 \cdot 4H_2O$, 0.05; $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$, 0.05; $AlCl_3$, 0.05; $CoCl_2 \cdot 6H_2O$, 0.05; $NiCl_2 \cdot 6H_2O$, 0.092; ethylenediaminetetraacetate, 0.5; concentrated HCl, 1 ml; $Na_2SeO_3 \cdot 5H_2O$, 0.1; (E) vitamin mixture according to Wolin et al. (1963). To 974 ml of redistilled water, the following stock solutions were added: A, 10 ml; B, 2 ml; C, 1 ml; D, 1 ml; E, 1 ml. After boiling with extra water to the original volume, the mixture was cooled under gassing with 80% N_2–20% CO_2. Cysteine hydrochloride (0.5 g) and $NaHCO_3$ (2.6 g) were added and the medium was dispensed and autoclaved. Before inoculation the vials were reduced with $Na_2S \cdot 9H_2O$ to a final concentration of 0.025%.

Analytical methods

Volatile solids, total solids, and pH were determined as described in American Public Health Association (1975).

Samples for VFA analysis were acidified to pH 2 with HCOOH and centrifuged. The supernatant was filtered through a 0.45 μm membrane filter, and analysed in a gas chromatograph equipped with a flame ionization detector. The column was 60/80 carbopack C/0.3% carbowax 20M/0.1% H_3PO_4.

Samples for LCFA analysis were taken after vigorous shaking of the digestor contents. Samples were acidified to pH 2 with HCOOH, centrifuged, and the supernatant was extracted with C_6H_{10} and methylated with BF_3. The LCFA were then quantified by gas chromatography using a flame ionization detector and an SP 2330 capillary column.

Ammonium and total nitrogen content were determined by the Kjeldahl method.

Both CH_4 and CO_2 were determined with a gas chromatograph using a thermal conductivity detector. The 4.5-ft-long glass column was packed with Poropack Q (80/100).

Results

Digestor experiments

The two control digestors exhibited parallel behaviour during the experimental period. Variation in gas production between the two digestors was less than 10%, indicating a fermentation process with good reproducibility (data not shown). The performance of the digestor fed with manure and bentonite was similar to the two control digestors, with a variation of less than 10%, indicating no apparent effect of addition of bentonite (data not shown).

During the start of continuous feeding (Fig. 1) the digestor receiving cattle waste with oil addition showed signs of inhibition. The calculated oil utilization was negative in the beginning, indicating that the oil inhibited methane production from substrates other than oil. The digestor with 6% BBO added showed reduced oil utilization compared to the digestor with 3% BBO

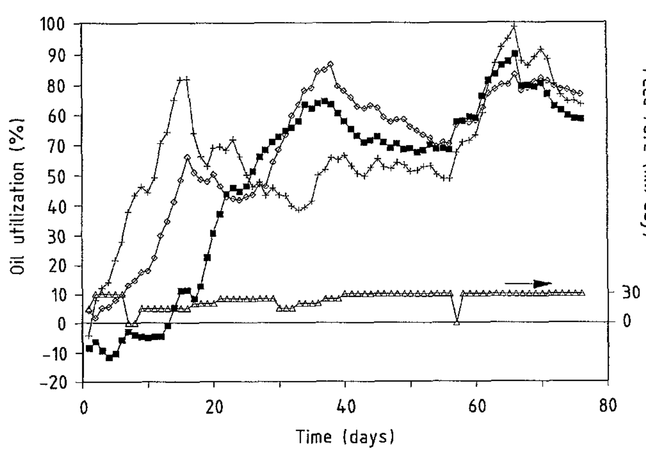

Fig. 1. The relative oil utilization of continuously fed digestors calculated as 10 days average, operated with cattle manure as the main substrate and various additions of oil: ■, with addition of 1% oil extracted from bentonite-bound oil (BBO); +, with addition of 3% BBO (approx. 1% oil); ◇, with 6% BBO; Δ, feed rate (30 ml/day corresponds to 20 days hydraulic retention time)

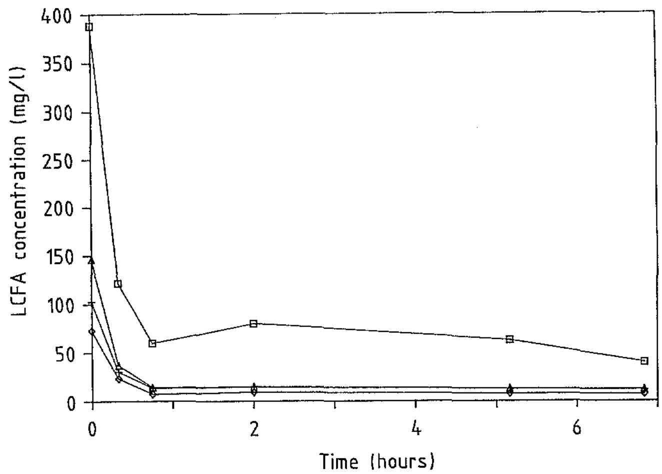

Fig. 2. Degradation of oil after daily feeding of the digestor receiving cattle manure as the main substrate and supplemented with 6% BBO for a period of 55 days: □, total long-chain fatty acid (LCFA) concentration; +, concentration of palmitic acid; ◇, concentration of stearic acid; △, concentration of oleic acid

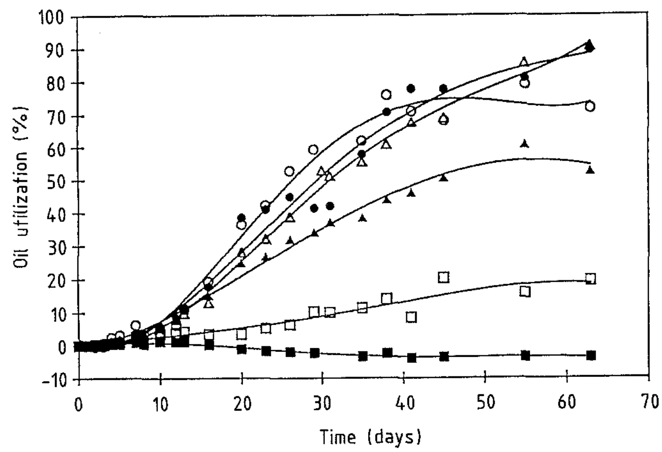

Fig. 3. Relative oil utilization from batch experiments with various amounts of glyceride trioleate (GTO) as carbon source: ○, 0.5 g/l; ●, 1.0 g/l; △, 1.5 g/l; ▲, 2.0 g/l; □, 2.5 g/l; ■, 5.0 g/l

addition (Fig. 1). However, in both digestors some oil was utilized from the start of continuous feeding. The results obtained indicate that oil can act as a potential inhibitor, and that bentonite reduces this effect. After an adaption period of approx. 55 days, all three digestors exhibited nearly the same degree of oil utilization, ranging between 70%–90%.

The concentration of LCFA in the liquid phase of the adapted digestor receiving manure supplemented with 6% BBO was followed during a daily feeding cycle. The results (Fig. 2) shows a very fast degradation of the LCFA.

Batch experiments

The methane production in batch experiments containing various concentrations of GTO, inoculated with digested cattle manure unadapted to oil, showed clear signs of inhibition at higher concentrations of GTO (Fig. 3). When initial GTO concentrations were higher than 2.0 g/l the calculated oil utilization decreased and inhibition of methane production from substrates other than oil was found at 5.0 g/l. However, no accumulation of VFA was observed in these vials and the concentrations were similar to control vials without GTO addition (data not shown).

Addition of bentonite together with 1.5 g/l GTO resulted in a positive, stimulatory effect on oil utilization (Fig. 4). The stimulatory effect of bentonite was the same for both bentonite concentrations tested (1.5 and 4.5 g/l). Control vials, with no oil added, produced small amounts of methane from the organic material in the inoculum. No differences were observed in control vials with or without bentonite added (data not shown).

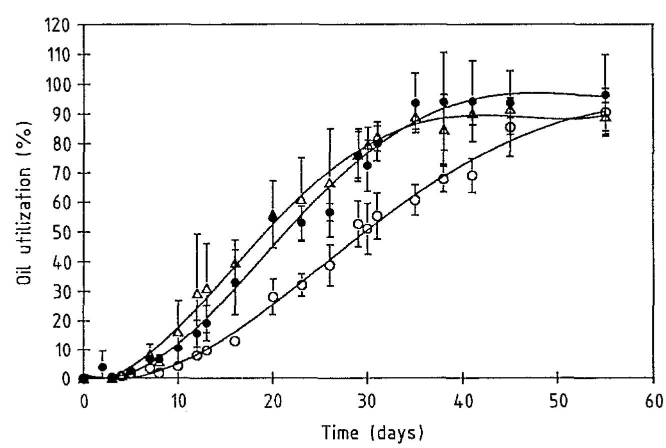

Fig. 4. Relative oil utilization from a batch experiment with addition of 1.5 g/l GTO as carbon source and different amounts of bentonite: ○, without bentonite addition; ●, with 1.5 g/l bentonite; △, with 4.5 g/l bentonite

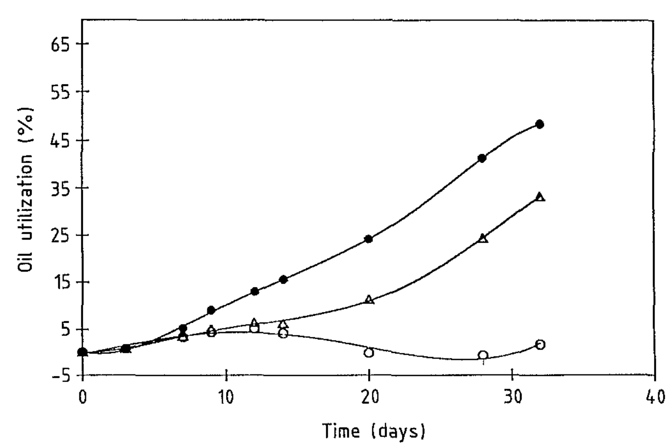

Fig. 5. Relative oil utilization from a batch experiment with 2.5 g/l GTO as carbon source: ○, with no addition; ●, with addition of bentonite (4.0 g/l); △, with addition of $CaCl_2$ (3 m*M*)

A second series of vials was prepared to further investigate the effect of bentonite at other concentrations of GTO (1.0 and 2.5 g/l) and to investigate if the positive effect of bentonite could be a result of its calcium cation content. As found in the first vial experiment, neither bentonite nor calcium had any effect on methane production in the controls without oil (data not shown).

At 1.0 g/l GTO, bentonite showed a slight, but not significant positive effect and calcium had no apparent effect on gas yield (data not shown). However, at a concentration of 2.5 g/l GTO added, 4.0 g/l bentonite as well as 0.33 g/l (3 m*M*) calcium chloride stimulated oil utilization (Fig. 5) but the effect was slightly more pronounced with bentonite than with calcium.

Discussion

The results obtained showed a significant increase in gas yield upon addition of oil to thermophilic biogas digestors fed with cattle waste. Oil added to adapted digestors was shown to be almost totally converted to biogas. The degradation course of the LCFAs after feeding (Fig. 2) revealed a fast degradation of oil added to adapted digestors.

Increased inhibition, at batch experiments with inoculum unadapted to oil, was found upon addition of more than 2.0 glyceride trioleate at thermophilic conditions. Based on the fact that no accumulation of VFAs was found in the vials containing GTO, inhibition seems to be effective at the first stages of the degradation, i.e. affecting the hydrolytic and/or the acetogenic bacteria. When the concentration of GTO was 5 g/l, methane production was less than from the control vials, indicating inhibition of methane producing bacteria (Fig. 3).

Recent experiments in our laboratory show that oleate is inhibitory at much lower concentrations than GTO. This indicates that the toxicity of oil depends on the relative rate of LCFA formation (hydrolysis) compared to degradation (beta-oxidation).

The data obtained from the present experiments showed that bentonite had a stimulating effect on oil utilization at oil concentrations that would otherwise result in inhibition. A possible explanation for this effect could be the flocculating capacity of bentonite. Bentonite, as well as other clay minerals, attract molecules to form flocs that, after reaching a certain size, will precipitate. By this mechanism bentonite might be able to bind oil on its surface and thereby lower the effective oil concentration in the liquid phase. Another possibility is a direct effect of the cations found in bentonite. Calcium has been shown to limit the concentration of dissolved LCFAs (Roy et al. 1985). A positive effect on oil utilization upon addition of calcium was also found in the present study.

The finding that bentonite and calcium ions have a stimulating effect on oil degradation can be of value in cases where oil is added to digestors normally fed with manure, which are not likely to contain a well-adapted oil-degrading population. In these cases, addition of bentonite might allow a faster adaption period which would be important for biogas plants receiving substrate from many sources (e.g. from food-processing industries), where significant variations in amount and composition are likely to occur. The digestor experiment does not indicate any benefit from adding bentonite to a digestor with a well-adapted bacterial population.

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