Mesophilic–thermophilic–mesophilic anaerobic digestion of liquid dairy cattle manure

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Abstract The potential of a mesophilic–thermophilic–mesophilic anaerobic digestion system was investigated with respect to improvement of both digestion and sanitation efficiencies during treatment of liquid cattle manure. The pilot plant produced a high methane yield from liquid dairy cattle manure of 0.24 m^3 (kg VS_{fed})$^{-1}$. Considering the low system loading rate of 1.4–1.5 kg VS (m^3 d)$^{-1}$, digestion efficiency compared to conventional processes did not appear improved. The minimum guaranteed retention time in the tubular thermophilic reactor was increased compared to a continuously stirred tank reactor. Levels of intestinal enterococci in raw liquid manure as determined with cultivation methods were reduced by 2.5–3 log units to a level of around 10^2 cfu/mL. This sanitizing effect was achieved both during mesophilic–thermophilic–mesophilic and thermophilic–mesophilic treatment, provided the thermophilic digester was operated at 53–55°C. A change in feeding interval from 1 h to 4 h did not significantly alter methane yield and sanitation efficiency. It was proposed that a two-stage, thermophilic–mesophilic anaerobic digestion system would be able to achieve the same sanitizing effect and equal or better digestion efficiency at lower costs.

Keywords Cattle manure; mesophilic anaerobic digestion; pilot plant; sanitation; thermophilic anaerobic digestion

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Introduction

Anaerobic digestion (AD) of animal manure prior to spreading on agricultural land potentially reduces pathogen input from livestock farming into the environment. The sanitizing effect of AD is mainly dependent on process temperature, treatment time, and type of pathogenic organism. Thermophilic digestion of liquid animal wastes (at around 55 °C) effectively reduces the numbers of several pathogenic bacteria, viruses and parasite eggs, with temperature as the dominant inactivating factor (Olsen and Larsen, 1986; Larsen and Munch, 1990; Kearney *et al.*, 1993; Haas *et al.*, 1995). From studies of aerobic stabilization for sanitation of animal manure it was inferred that a combination of a first, mesophilic and a second, thermophilic treatment step could improve the inactivation of *Cryptosporidium* oocysts (Oechsner and Doll, 2000).

At the same time thermophilic AD operated at high loading rates may offer better energy efficiency than mesophilic digestion (Mackie and Bryant, 1995). A laboratory system for thermophilic–mesophilic anaerobic digestion of liquid dairy cattle wastes achieved a relatively high maximum methane recovery of 0.22 m^3 kg VS^{-1} fed, at an organic loading rate of 5.8 kg VS (m^3 d)$^{-1}$ and a retention time of 14 days (Sung and Santha, 2003). However, a corresponding full-scale system for the treatment of liquid dairy cattle manure (6.2 % VS) from about 2,500 animals could not be operated with success, primarily due to failure of the thermophilic digestion stage (Katers and Schultz, 2003).

A joint research project was initiated by a communal water supply company to investigate the potential of AD to control pathogen loads from livestock farming in sensitive

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areas (Effenberger *et al.*, 2003). Specific questions of our research that are addressed in this paper were: (i) can a mesophilic–thermophilic–mesophilic system improve both digestion and sanitation efficiencies of AD of liquid dairy cattle manure, and (ii) does the design of the thermophilic digester as a horizontal tubular reactor improve hydraulic efficiency during quasi-continuous feeding?

Health risks emerging from land application of biological wastes and the sanitation efficiency of different treatment processes for these wastes are typically evaluated by examining the occurrence and fate of indicator organisms such as (fecal) coliforms and fecal enterococci. While indicator organisms are quantified by culture-based techniques, a quantitative (reverse transcription) real-time PCR ((RT)qPCR) method for the quantification of various nucleic acid-containing organisms in liquid manure was developed within our joint project (Lebuhn *et al.*, 2003). Complementary use of these techniques is thought to improve both the efficiency and safety of hygienic monitoring (Lebuhn *et al.*, 2005).

Materials and methods

Pilot plant. The mesophilic–thermophilic–mesophilic treatment process was evaluated in a pilot biogas plant designed for the treatment of approximately 2,000 m^3 of liquid manure from dairy cattle per year. Animals were fed a total mixed ration of grass silage, hay, grain and mineral mix throughout the year. The biogas plant consisted of a sequence of two stirred tanks and a horizontal tubular reactor as described previously (Effenberger *et al.*, 2003). Two different modes of feeding were investigated, with the daily manure load supplied to the digesters in 21–22 batches (about hourly) or 5–6 batches (about every four hours). The plant was operated at the design feed rate of 5.5 m^3 liquid manure per day, with calculated hydraulic retention times (HRT) of digesters 1–3 of about 9, 8, and 27 days, respectively. The digested manure was stored in a covered tank (volume: 800 m^3) which was connected to the biogas collection system.

Monitoring and analytical methods. Monitoring of process parameters included: substrate quantities; digester temperatures; quantity and composition of biogas flows; production and own consumption of energy. Samples of raw and digested manure for various chemical analyses were taken from nine points along the treatment process (collection tank, digester 1, five points in digester 2, digester 3, and terminal storage tank). Sampling was done about once a week over most of the entire time of operating the plant and daily/every other day during limited periods of time. Analytical methods were based on German Standard Methods for the Examination of Water, Wastewater, and Sludge (Anon., 1981).

The total biogas production of the pilot plant was determined by taking daily readings of the biogas consumption of the engine. The data were normalized and corrected for the air flow that was introduced into the headspace of digester 1 to sustain biological desulphurization. The pilot biogas plant was considered to basically run at steady state when the 8-day moving mean of daily biogas consumption did not vary by more than 5% from day to day. Biogas composition in the gas pipe to the engine was analyzed with commercial gas analyzers. Methane and carbon dioxide were quantified by means of the infrared two-beam compensation method with pressure compensation (measuring error as specified: $\pm 2\%$). Hydrogen sulfide (after dilution) and oxygen were measured with electrochemical sensors (measuring error: $\pm 5\%$ in dilution and $\pm 0.2\%$, respectively).

Mean values of VS destruction, biogas and methane yields for the respective time periods were calculated from a mass balance based on the analyses of liquid samples of raw and digested manure, readings of biogas production, and measurements of biogas composition.

Tracer experiments in the horizontal tubular reactor. A known quantity of LiCl dissolved in liquid manure was introduced into the delivery pipe of digester 2 prior to feeding. The concentration of lithium in effluent samples was monitored over a time period corresponding to about three hydraulic retention times. Dried samples were digested with nitric acid in a microwave-accelerated digestion system (MARS®), and the lithium content was analyzed using atomic absorption spectrometry. The retention time distribution functions were characterized using the methods of moments approach as described by Haas *et al.* (1997). The minimum retention time was determined as the time of sampling after injection of the tracer when the tracer was first detectable in the effluent.

Microbiological monitoring. Various microbial parameters were monitored using selective cultivation and qPCR in parallel for five compartments: manure input from healthy cattle, digesters 1–3, and the terminal storage tank. Two different strategies for the evaluation of germ and specific genome reduction were applied: (a) random sampling of all compartments about once a month; and (b) charge tracing (follow-up of a specific charge) by consecutive sampling of the compartments corresponding to the respective calculated hydraulic retention times. The investigated bacterial parameters and the methods used have been reported by Lebuhn *et al.* (2003, 2004). Experiments with respect to monitoring the fate of oocysts of *Cryptosporidium parvum* during different anaerobic treatments of liquid manure were carried out in a model biogas plant and are described by Garcés *et al.* (2006).

Results

Anaerobic treatment process

Characteristics of liquid dairy cattle manure are summarized in Table 1. The data cover a time period of about five and a half months. The low starch content of between 0.11 and 0.44% (m/m) of TS in samples of raw manure indicated that the liquid manure did not contain significant amounts of undigested feed which would have raised the biogas yield. The high ash contents of more than 20% were attributed to fine grit from the concrete surface in the cattle stable.

Biogas production and composition. Operational parameters and values of biogas and methane production are shown in Table 2. No significant differences were observed for the two modes of operation. Values of the daily feed of liquid dairy cattle manure to the pilot plant and eight-day moving means of daily biogas production are shown in Figure 1. Data were evaluated for the time period from days 0–97 for hourly feeding and from days 142–189 for feeding every four hours, respectively.

Mean values (standard deviations) of methane, oxygen, and hydrogen sulfide concentrations in the mixed biogas supplied to the engine were 55.9 (1.7) % (v/v), 0.8 (0.4) % (v/v), and 137 (206) ppm. The methane content in the biogas from the thermophilic digester was significantly lower than in the biogas from the mesophilic digester 1; no

Table 1 Composition of samples of liquid dairy cattle manure taken from the delivery pipe to digester 1 (mean values ± 1 standard deviation; n = 24)

Total solids	% (m/m)	7.8 ± 0.8
Volatile solids	% of TS (m/m)	77.6 ± 3.0
pH	–	7.4 ± 0.1
VFA	mg Hac/L	6,844 ± 530
Alkalinity	mg $CaCO_3$/L	12.1 ± 0.5
NH_4-N	mg/L	1,963 ± 216

Table 2 Operational parameters and observed biogas yields of the pilot plant

Feeding interval		1 hour	4 hours
Loading rate of digester 1	kg VS $(m^3\,d)^{-1}$	6.6	6.8
Loading rate of digester 2	kg VS $(m^3\,d)^{-1}$	5.9	6.2
Loading rate of digester 3	kg VS $(m^3\,d)^{-1}$	1.7	1.7
Loading rate of whole system	kg VS $(m^3\,d)^{-1}$	1.4	1.5
Biogas yield	m^3 (kg VS_{fed})$^{-1}$	0.41	0.41
Methane yield	m^3 (kg VS_{fed})$^{-1}$	0.24	0.24
Methane productivity	m^3 $(m^3 d)^{-1}$	0.33	0.34

significant differences were observed between the biogas composition in the four separate headspaces of the thermophilic digester 2 (Table 3; $\alpha = 5\%$). The system of maintaining a biological desulphurization process by introducing air into the digester headspaces was not very reliable as indicated by the high standard deviation of the hydrogen sulfide content in the biogas.

Solids degradation. Mean gravimetric volatile solids destruction up to digester 3 was 35%. Half of this VS destruction occurred in digester 1 whereas digester 2 accounted for only 20% or less. The additional VS destruction that occurred in the terminal storage tank could not be evaluated with sufficient accuracy for the reported time periods because only a few samples of digest were available and water was added to dilute the digest prior to land spreading.

VFA levels in samples from digesters and storage tank. Total VFA levels in samples from digester 1 were mostly around 1,000 mg/L (expressed in mg of acetate per litre; mean value ± standard deviation: 1,084 ± 327 mg/L). The digester was prone to foaming if heated without frequent agitation (operated every 10 min for 10 min). Values of pH in digester samples ranged between 7.5 and 8.5 throughout the entire monitoring period, and digester failure was not observed. VFA concentrations in digester 2 were about twice as high as in digester 1 (2,113 ± 984 mg/L). VFA levels in all samples from digester 3 were below 700 mg/L (437 ± 112 mg/L) but higher again (up to 1,000 mg/L) in samples of digest taken from the terminal storage tank. Mean values of acetate and propionate concentrations were 11.7 and 2.5 mM in samples from digester 1, and 21.4 and 5.1 mM

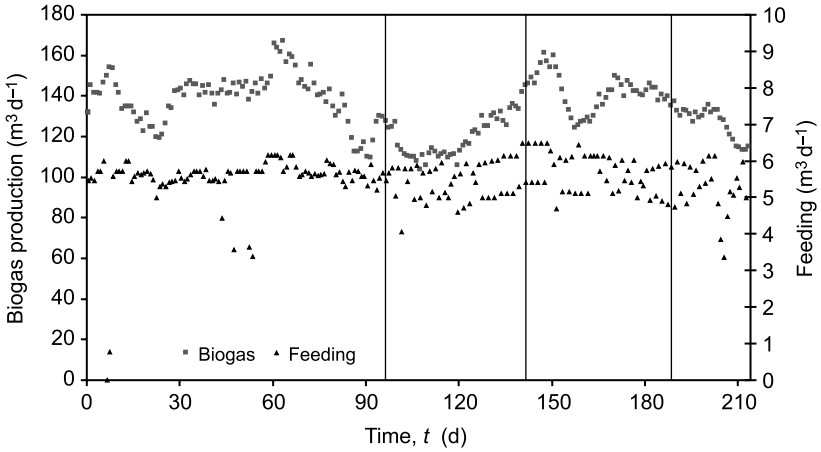

Figure 1 Daily feed of liquid manure to the pilot plant and eight-day moving mean of daily biogas production over the evaluated time period; change of feeding mode on day 98

Table 3 Measured composition of biogas in the headspaces of individual digesters and as delivered to the engine (mean values ± 1 standard deviation)

	CH_4 % (v/v)	CO_2 % (v/v)	O_2% (v/v)	H_2S ppm
Digester 1 (n = 11)	53.8 ± 1.1	32.9 ± 3.5	2.4 ± 0.4[§]	n.d.
Digester 2/1 (n = 6)	50.3 ± 2.1	46.4 ± 2.3	0.9 ± 0.3	n.d.
Digester 2/2 (n = 6)	48.8 ± 2.8	49.7 ± 2.0	0.8 ± 0.2	n.d.
Digester 2/3 (n = 6)	50.5 ± 2.7	47.8 ± 2.4	1.0 ± 0.2	n.d.
Digester 2/4 (n = 6)	50.7 ± 1.7	47.2 ± 1.4	0.9 ± 0.2	n.d.
Digester 3* (n = 12)	55.0 ± 1.0	37.0 ± 0.6	1.4 ± 0.4	n.d.
Gas pipe to engine (n = 240)	55.9 ± 1.7	36.2 ± 2.1	0.8 ± 0.4	137 ± 206

n.d., not determined; *mixed biogas from digesters 1–3; [§] injection of air into the headspace of digester 1 for desulphurization

in samples from digester 2, respectively. Iso-butyrate, butyrate, and iso-valerate were detectable in a few samples from digesters 1 and 2 at maximum concentrations of 1.3, 1.0 and 1.1 mM, and 1.5, 1.2 and 1.7 mM, respectively.

Retention time distribution of tubular digester. Results of the two tracer tests that were performed in the thermophilic horizontal reactor (digester 2) of the pilot plant are summarized in Table 4. The maximum tracer concentration was measured in effluent samples taken about 48 h after injection of the tracer.

Hygienic investigations

Table 5 shows results for intestinal enterococci which gain more and more attraction as indicator germs for the evaluation of hygienization during anaerobic digestion. Mean values of colony forming units are reported for time periods when the reactor chain ran: (i) at suboptimal conditions (temperatures of 48–55 °C in the thermophilic digester) and hourly feeding, (ii) at optimal conditions (55 °C in digester 2) and a 4 h-feeding interval, and (iii) as thermophilic–mesophilic system with hourly feeding and digester 1 operated at about 20 °C.

According to Table 5, we consistently obtained a reduction in levels of intestinal enterococci of 2.5–3 log units when digester 2 ran at 55 °C (4 h feeding interval), whereas the reduction was lower (1.5–2 log units) when digester 2 ran suboptimally and was fed at 1 h intervals. The latter data were obtained during an earlier time period than that referred to above. A slight rise in numbers of intestinal enterococci was observed in samples from the terminal storage tank.

Since intestinal enterococci are more thermotolerant, the log-reduction for these organisms was lower than for fecal coliforms. Bacterial spore formers such as *Bacillus cereus* and *Clostridium perfringens* were (almost) not compromised (Lebuhn *et al.*, 2004; 2005). An improvement of sanitation efficiency of the mesophilic–thermophilic–mesophilic process compared to a single thermophilic treatment with respect to *Cryptosporidium parvum* oocysts was not found (Garcés *et al.*, 2006).

Table 4 Results of tracer experiments in the horizontal tubular digester 2

Feeding interval	1 hour	4 hours
Hydraulic retention time, HRT, days	8.5	8.7
Mean calculated retention time, θ, days	6.4	7.1
Minimum retention time, hours	9	8

Table 5 Intestinal enterococci in compartments of the digester chain for: (i) suboptimal conditions and 1 h feeding interval, (ii) optimal conditions and 4 h feeding interval in (a) random sampling and (b) charge tracing experiments, respectively, and (iii) operation as thermophilic–mesophilic system with digester 1 used only for pre-heating of the raw manure

Intestinal enterococci (cfu/mL)	Manure collection tank	Digester 1 (mesophilic)	Digester 2 (thermophilic)	Digester 3 (mesophilic)	Terminal storage tank
(i,a) 48–55 °C	1.9×10^4	2.3×10^3	2.0×10^2	8.3×10^1	1.9×10^2
(i,b) 48–55 °C	1.4×10^5	3.5×10^3	2.4×10^3	3.6×10^3	4.0×10^3
(ii,a) 55 °C	1.9×10^4	2.3×10^3	7.5×10^1	5.0×10^1	8.7×10^1
(ii,b) 55 °C	2.5×10^5	2.3×10^4	4.9×10^2	2.9×10^2	2.9×10^2
(iii)	n.d.	3.5×10^4	1.6×10^2	1.3×10^2	6.5×10^1

cfu, colony forming units; n.d., not determined

Discussion

Generally, a comparison of methane yields from liquid cattle manure will always be limited by the tremendous amount of diverseness and variability in the composition of different liquid manures. Table 6 compares the methane yield from liquid dairy cattle manure observed in the pilot plant with different values reported in literature. The methane yield of the mesophilic–thermophilic–mesophilic AD system is the highest in this compilation. However, the experimental data fit quite well into a trend of a linear decrease of methane yield with increasing loading rate except for the highest value of methane yield reported for the thermophilic–mesophilic system. Therefore it appears that our system did not show improved digestion efficiency compared to conventional processes under the specified loading conditions. This also becomes clear when considering the low methane productivity (Table 2).

The rationale for having a thermophilic digester upstream of a mesophilic one is the increased hydrolysis rate at higher temperatures. Thus the second digester receives high concentrations of dissolved organic compounds which are degraded more efficiently at mesophilic temperatures (Christ, 1999). It was stated that in order to take full advantage of the higher digestion efficiency of thermophilic digestion, reactors have to be operated at short HRT and high organic loading rate (Mackie and Bryant, 1995). In our pilot plant the system loading rate was limited by the first, mesophilic stage.

The above-stated amount of VS degradation in our pilot plant up to digester 3 does not account for the high methane yield observed. An additional 7% degradation with respect to VS in the raw manure occurred in the terminal storage tank as estimated from older data. The increased VFA levels that were observed in the terminal storage tank indicate ongoing digestion processes. Angelidaki *et al.* (2005) reported similar observations in post-digestion systems of centralized co-digestion plants in Denmark.

Table 6 Compilation of methane yields from liquid cattle manure

System, scale (reference)	Organic loading rate kg VS*$(m^3{*}d)^{-1}$	Methane yield $m^{3}{*}(kg\ VS_{fed})^{-1}$
Guideline value, agricultural plants (KTBL, 2005)	3.5	0.15
37 °C, semi-technical (Lampel, 1984)	2.9	0.20
Mesophilic, agricultural plants (Gosch, 1984)	4.1	0.17
50 °C, laboratory (Elmashad *et al.*, 2001)	2.1	0.20
55 °C, laboratory (Angelidaki and Ahring, 1993)	2.8	0.19
55 °C/38 °C, laboratory (Sung and Santha, 2003)	4.5	0.15
	5.8	0.22
38 °C/50–55 °C/35–42 °C (this work)	1.4–1.5	0.24

The resulting total VS destruction in the pilot plant of approximately 42% is in good accordance with the maximum value of 41.5% reported by Sung and Santha (2003) for their temperature-phased AD system. In this system the first, thermophilic stage accounted for 69–76% of the total observed VS degradation. This means that digesters 1 and 2 of our pilot plant together achieved only about 2/3 of the performance of the first stage of the thermophilic–mesophilic system, however at a more than four times longer HRT. On the other hand, due to the high proportion of roughage in the cattle feed, the liquid manure used in our studies very likely contained considerably higher amounts of cell-wall constituents (around 45% of dry matter) that are slowly digested.

The causes of the low VS degradation in the thermophilic digester of our pilot plant are not clear. A prolonged inhibitory effect on methane production and VS removal during AD of dairy cattle manure at 35 °C was found for ammonia nitrogen concentrations of and above 1,500 mg/L, corresponding to free ammonia concentrations of and above 600 mg/L (Sterling *et al.*, 2001). With a mean value of about 550 mg/L, calculated free ammonia concentration in the thermophilic digester were close to this level. However, considering a propionate concentration of about 10 mM as a possible sign of process inhibition (Hartmann *et al.*, 2004), the observed VFA concentrations in the thermophilic digester 2 were not critical. The same applies to digester 1. The fact that this reactor was prone to foaming in conjunction with failures of the agitator may be attributed to the consistency of the liquid manure. The digester contents still exhibited viscous properties similar to those of raw liquid manure and contained considerable amounts of solids which might have hampered the release of the produced biogas from the liquid phase.

Based on the retention time distribution as determined from the tracer experiments the horizontal tubular reactor can be characterized as a real stirred tank. The same was inferred from data on biogas composition (Table 3) and chemical analyses (data not shown) which exhibited no significant differences between the four sections of the reactor. The discrepancy between hydraulic and calculated mean retention times indicate a considerable proportion of stagnant space due to insufficient radial mixing of the digester contents by the paddle mixer. Due to the delayed first appearance of the tracer the minimum retention time in this digester was still significantly longer than in a completely mixed tank. The mean retention times calculated from experimental moments did not differ significantly for the two different feeding intervals (Table 4).

A reduction in numbers of thermotolerant intestinal enterococci of about 1 log unit was observed in digester 1 for both feeding intervals. This is remarkable given the mesophilic temperature level, and is thought to be caused mainly by chemical factors. According to additional data (not shown) there were no differences in sanitation efficiency of digester 2 due to the change in the feeding interval. Since the retention time distribution determined from the tracer tests was essentially the same for both feeding intervals, the lower sanitation efficiency during hourly feeding is attributed to temperatures considerably below 55 °C (Table 5). Digester 3 did essentially not alter remaining numbers of intestinal enterococci.

Based on hygienic monitoring of centralized biogas plants in Denmark, Larsen and Munch (1990) inferred that a reduction in numbers of intestinal enterococci by 3 to 4 log units to a level of 10^2 cfu/mL would indicate sufficient sanitation with respect to vegetative bacteria and intestinal parasites. The latter level was surpassed in our experiments only in one case when the thermophilic digester ran at 48 °C (Table 5). When digester 2 ran optimally, enterococci levels in effluent samples of the digester chain were reduced by 2.5–3 log units, regardless of the feeding interval. The same degree of sanitation was observed when the system was run as a thermophilic–mesophilic process with digester

1 used only for preheating the substrate to about 20 °C. Meanwhile the suitability of the relatively heat resistant intestinal enterococci as indicator organisms and realistic standards is still being discussed in the European Union. Of course, inactivation of other indicator and pathogenic organisms has to be taken into account in order to guarantee that the digested residue is hygienically safe.

Conclusions

The investigated mesophilic–thermophilic–mesophilic AD system at pilot scale produced a high methane yield from dairy liquid cattle manure when compared to values from agricultural biogas plants. However, considering the low overall loading rate the system did not show improved digestion efficiency compared to conventional processes and was thus comparably expensive.

The first, mesophilic digester in series limited the system loading rate. The reasons for the observed poor VS destruction in the thermophilic digester could not be clarified.

The retention time distribution of the horizontal tubular digester derived from tracer tests could be characterized as that of a real stirred tank with delayed breakthrough and incomplete mixing. The minimum guaranteed retention time in the reactor was increased in comparison with a continuously stirred tank reactor. No significant differences in RTD could be observed for feeding intervals of 1 hour and 4 hours.

Both during mesophilic–thermophilic–mesophilic and thermophilic–mesophilic treatment, levels of intestinal enterococci in raw liquid manure were reduced by 2.5–3 log units to a level of around 10^2 cfu/mL, provided the thermophilic digester was operated at 53–55 °C. A marginal re-growth of these organisms seemed to occur in the terminal storage tank.

Based on the data stated above, a two-stage, thermophilic–mesophilic AD system may be able to achieve the same sanitizing effect and equal or better digestion efficiency at lower costs than the mesophilic–thermophilic–mesophilic system.

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