Optimization of biogas production from manure

Final project report EFP-04

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Publication date:
2007

Document Version
Publisher's PDF, also known as Version of record

Final Project Report

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September 2007

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Abstract

The main objective of the project was to improve biogas production from manures. This objective was addressed by investigating 1) the effect of different reactor configurations, 2) operational procedures, aiming to selectively retain/return degradable material in the reactor and 3) different posttreatments to improve the degradability of the undegraded material. Both lab-scale and pilot-scale experiments were carried out at the Institute of Environment & Resources, Technical University of Denmark. In the first experiment, the effect of serial digestion on process performance and methane production was compared to a conventional single CSTR process at 55°C. The total working volume (5 l) between the two methanogenic reactors of serial CSTR process was varied by distributing the volume between the first and second reactor at 90/10, 80/20, 70/30, 50/50, 30/70 or 13/87%. Results showed that serial CSTR process at 90/10, 80/20, 70/30, 50/50 or 30/70% volume distribution could produce 11-17.8% more biogas compared to single CSTR process under similar operating conditions. The increased biogas production was mainly from the second reactor of the serial process, which accounted for 16-18% of the total biogas production. At 13/87 ratio, no significant increase in biogas production was noticed. Both single and serial CSTR processes were stable when operated 90/10, 80/20, 70/30 or 50/50% volume distributions and also during an organic pulse load (19.6 to 65.3 g/l reactor volume). Results from pilot-scale studies showed that serial digestion with 77/23% volume distribution produced 1.9-6.1% more biogas compared to that obtained during one-step CSTR operation. However, temperature was found to have a strong influence on the methane production and process performance of the second reactor of a serial CSTR process.

In the second experiment, the effect of temperature (10 & 55°C) and microbial activity on passive separation of digested cow manure was investigated in vertical columns (100 cm) with an aim to improve solids retention time within the reactor and improve biogas production. Results showed that greatest degree and rates of passive separation took place when the temperature during the settling was maintained at 55°C and after 24 hrs of incubation. Higher temperature and lower viscosity, and probable higher biological activity aided the separation process at 55°C than at 10°C. The effect of continuous mixing (control), mixing for 10 min per day (minimal mixing) and withholding mixing for 2 hrs prior to feeding (intermittent mixing) on biogas production was evaluated in three lab-scale CSTRs. Results showed that minimal and intermittent mixing improved biogas productions by 12.5% and 1.3% respectively over continuous mixing. Intermittent mixing also resulted in stratification of solids with higher solids content in the top and bottom layers compared to middle layer. Similar result was also noticed in pilot-scale plant when intermittent mixing was sequenced with continuous mixing. Biogas yields improved from 2.5 to 14.6% when the reactor was operated under intermittent mixing compared to continuous mixing. The effect of mixing intensities (minimal, gentle or vigorous) in batch assays at 55°C showed that when the process was overloaded by high substrate to inoculum ratio (40/60), gentle (35 times per minute) or minimal mixing (10 minutes mixing before feeding) was advantageous compared to vigorous mixing (110 times per minute). On the other hand, under low substrate to inoculum ratio (10/90), gentle mixing was the best. The study thus indicated that mixing schemes and intensities have some effect on anaerobic digestion of manures.

In the third experiment, the effect of eight different post-treatments on improving the biogas production of fibres separated from thermophilically digested cow manure was studied in batches at 55ºC. Results showed that only partial aerobic treatment (air flow rate at 0.28 l/min g total solids) and grinding (mortar and pestle) improved methane yields while chemical treatments (NaOH or CaO at 40g/kgVS) resulted in more or less similar methane yields to that of untreated fibres. Treatments such as microwave irradiation (300-700 W), conventional boiling and wet oxidation (195ºC, 12 bar of O_2 for 10 min.) improved the soluble chemical oxygen demand content while ultrasound irradiation did not affect the fibres' SCOD content. Thus, the present results showed that biogas production from manure can be improved from 7 to 18% by either adopting serial digestion of manure, mixing the reactor intermittently with a 2 hour mixer blocking prior to feeding or by partial aerobic treatment or grinding of the fibers separated from the digested manure. However, the optimal volume distributed between the two methanogenic reactors in serial digestion could be either 70/30 or 50/50% and/or effluent should be removed from the middle layer after 2 hrs of settling. The improved methane recovery with the tested posttreatment needs further investigation with respect to the costs, efforts and energy inputs.

Keywords: anaerobic digestion, biogas, manure, mixing strategy, post-digestion, serial CSTR, temperature, serial operation.

EFP 04 project

EFP-04 research project was carried out at the Institute of Environment & Resources, Technical University of Denmark during Oct 2004-07. This project was funded by a grant from the Danish Energy Agency (EFP-04 J.Nr. 33030-0017). This final project report summarizes on various research objectives set forth, scientific achievements including the progress, various research activities performed, results achieved and papers published.

1. Background

Cattle manure is the one of the main substrate for many biogas plants in Danish Centralised biogas plants. A large fraction *ca*. 40-50% of the total solids (TS) of cattle manure is present in the form of fibres. In a biogas process with a typical hydraulic retention time (HRT) of 15-30 d, only a part of the fibres are degraded producing an average methane yield of 0.20-0.25 m^3 kg^{-1} volatile solids $(VS)_{added}$ compared to the theoretical yield of 0.40-0.45 m^3 kg^{-1} VS_{added} for cow manure (Hartmann et al., 2000). The need to explore the unused methane potential of manures, which is about 25% of the theoretical methane, led us to explore different strategies to improve biogas production manure based biogas plants.

1.1. Research objectives and approach

1. To investigate different reactor configurations for biogas plants for achieving more effective biogas production and more stable operation. Under this objective the performance of serial digestion of manure with distribution volumes of 90/10, 80/20, 70/30, 50/50, 30/70 or 13/87 between the two methanogenic reactors were compared to that of a one-step CSTR reactor operated under similar process conditions (I).
2. To investigate operational procedures, aiming to selectively retain/return degradable material in the reactor. At first, passive separation of digested manure within the reactor was studied to identify the optimum time interval for effluent removal prior to feeding. Subsequently, the effect of continuous, minimal and intermittent mixing on biogas production was investigated (II).
3. To investigate methods for degradation of undegraded material, by returning without/with post-treatment to the main reactor. The effect of different post-treatments such as partial aerobic treatment, grinding, chemical treatments (NaOH or CaO), wet oxidation, microwave irradiation, conventional boiling and ultrasound irradiation on improving

the biodegradability and methane yields of fibres separated from thermophilically digested cow manure was studied (III).

1.2. Experimental Approaches

Keeping in line with the above project objective, several lab-scale and pilot-scale experiments were carried out at the Institute of Environment & Resources, Technical University of Denmark during 2004-07. Data from a full-scale plant with post-digestion process was also included in this report.

1. Optimisation of biogas production from manure through serial digestion: lab-scale and pilot-scale studies (I)
2. The effect of temperature on the post-digestion in a serial operation process on the process performance and methane production (I).
3. The effect of temperature and microbial activity on passive separation of digested cow manure (II).
4. The effects of mixing intensity on methane production during thermophilic anaerobic digestion of manure: lab-scale & pilot-scale studies (II).
5. Effect of post-treatments on biodegradability and methane recovery from fibres separated from thermophilically digested cow manure (III).

Project overview

The time schedule and an overview of the project are presented in Table 1 and 2. The pilot-scale plant was built and operated successfully within the designed budget. All major lab-scale and pilot-scale experiments were successfully executed and completed within the schedule time.

TIME SCHEDULE

The project was started in autumn 2004 as per the revised time schedule shown below and was completed successfully within the time schedule and is shown below.

Table 1. Time schedule for the project.

Research phase	2004		2005		2006		2007	
Start of the project, Identification and Procuring reactor material, Literature surveys		x	x					
Serial digestion expt. in laboratory			x	x				
Lintrup registration/evaluation			x	x				
Pumping out/Stirring expt in lab and Pilot-scale plant.				x	x	x		
Experimental Modelling				x	x	x	x	
Full-scale application of modelling results at Pilot-scale plant						x	x	
Economic evaluation of methodology							x	x
Final reporting								x

Table 2. Overview of progress of the EFP 04 Research project 2004-07.

S.No.	Activity	Status
1	Identification and Procuring reactor material,	**Completed**
2	Fabrication, building and assembling of pilot-scale plant Reactor 1	**Completed**
3	Reactor 2	**Completed**
4	Literature surveys	**Completed**
4	Serial CSTR experiments Laboratory-scale studies (90/10, 80/20, 70/30, 50/50, 30/70 & 13/87 volume distribution ratios between the two serial reactors). Pilot-scale studies (77/23 volume distribution ratio between the two serial reactors).	**Completed**
5	Experimental Modelling Lab-scale studies on serial CSTR process	**Completed**
6	Operational strategies experiment (Pumping out/Stirring) Lab-scale studies 1. Minimal, intermittent and continuous mixing strategies 2. Initial substrate to inoculum ratio and mixing intensity Pilot-scale plant. Intermittent (2 hr blocking prior to feeding) and continuous stirring (5 min on/off)	**Completed**
7	Post-treatment or recycling of separated solids Lab-scale studies 1.Effect of chemical, wet-oxidation, mechanical grinding on methane potential of fibers 2. Effect of partial aerobic treatment, wet-oxidation, micro-wave, sonification and thermal treatments on methane potential of fibers	**Completed**
8	Lintrup registration/evaluation Residual methane potential of Lintrup biogas plant process	**Completed**
9	Final reporting	**Completed**

PUBLICATION

Total four research papers have been produced. Two papers were accepted for publication and are in press. One paper has been accepted for AD11 Conference (Sep 2007, Birsbane) and one paper has been submitted on 18 Sep. 2007 (see appendix).

Kaparaju, P. and Angelidaki, I. 2007. Effect of temperature and microbial activity on passive separation of digested cow manure. Biores. Technol. (in press, DOI: 10.1016/j.biortech2007.02.003).

Kaparaju, P., Ellegaard, L. and Angelidaki, I. 2007. Optimisation of biogas production from manure through serial digestion: lab-scale and pilot-scale studies (Submitted to Biotechnology and Bioengineering).

Kaparaju, P., Buendía, I., Ellegaard, L and Angelidaki, I. 2007. Effects of mixing on methane production during thermophilic anaerobic digestion of manure: lab-scale and pilot-scale studies. Bioresour Technol. In press. DOI: 10.1016/j.biortech.2007.09.015.

Kaparaju, P. and I. Angelidaki, I. (2007). Effect of post-treatments on biodegradability and methane recovery of fibres separated from thermophilically digested cow manure. Accepted for oral presentation at Anaerobic Digestion Conference, AD11. To be held in Brisbane, Australia. Sep. 2007.

Abbreviations

CLSM Confocal Laser Scanning Microscope

CSTR Continuous Stirred Tank Reactor

FISH Fluorescent *in situ* Hybridization

GC Gas chromatography

HRT Hydraulic Retention Time

LCFA Long chain fatty acids

SRT Solids retention time

TS Total Solids

VS Volatile Solids

VFA Volatile Fatty Acids

Experiment 1

1.1. The effect of reactor configuration on the process performance and biogas production in a serial process (I)

Lab-scale experiments

In this project, the possibility to optimize biogas production from manure through different reactor configurations was investigated. The process performance of a serial digestion process consisting of two CSTR connected in series with a volume distribution of 90/10, 80/20, 70/30, 50/50, 30/70 or 13/87% between the two methanogenic reactors operated with a total HRT of 15 d was compared with a single CSTR operated at 15 d HRT and 55°C. Without phasing, this study used the first reactor as a main mixed-culture reactor, not hydrolysis/acidogenesis stage, and the second reactor as a recovery stage or effluent polishing.

Background

Anaerobic digestion of livestock manure for biogas production is commonly carried out in conventional biogas plants operated as single step continuous stirred tank reactor (CSTR) either under constant mesophilic (30-37°C) or thermophilic (50-55°C) conditions. The reason for loss of degradable matter is due to "short-circuit" of a portion of the feed which staying in the reactor for a much shorter time than the nominal retention time.

Methodology

Substrate characteristic

Fresh cow manure was obtained from Snertinge biogas plant (Denmark). Substrate was stored at –20°C during the experimental period. A part of the frozen manure was thawed at room temperature and the prepared feed was stored at 4°C for 2-3 days. The composition of the prepared feed is shown in Table 1.

Reactor setup and operations

Single CSTR process was carried out in CSTR (R1) with a HRT of 15 days. Serial CSTR process (R2 and R3) was constructed using two CSTR reactors connected in series with a total HRT of 15 days and volume distribution of 90/10, 80/20, 70/30 or 50/50%. The reactor capacities and working volumes in the experiments

are shown in Table 2. Reactors were built from double glass cylinder fitted with stainless steel plates as top and bottom (Fig. 1). The top plate supported the mixer, mixer motor, feed tube, and effluent tube, temperature measuring port and sampling port. The bottom plate had one sampling port. Stable reactor temperature was maintained at 55^{o}C by pumping hot water, from a water bath, in the space between the reactor glass walls. Reactors were fed semi-continuously at six hour interval by pumping the feed from feed bottles into R1 and R2. R3 was fed directly by the pressure developed from the produced biogas and the added feed in R2. Similarly, serial CSTRs of 30/70 and 13/87 were operated using reactors R4 and R5 respectively. R5 was fed directly from the effluent from R4. Reactors contents were stirred by mechanical mixers operated on a cycle of 40 seconds mix followed by 1 minute stop. The effluent was collected in the effluent bottle. Biogas from the effluent bottle flowed further to the gas meter for measuring biogas production. The gas was measured by using liquid displacement with a 100-mL reversible cycle and registrations, 18 VDC power supply (Angelidaki et al., 1992). The system setup is shown in Figure 1.

Table 1. Composition of cow manure (3 batches)

Parameter	Range
VS (%)	4.7-5.1
TS (%)	5.8-6.3
pH	7.3-7.5
Total VFA (g/l)	4.5-7.3
Acetate (g/l)	2.7-4.2
Propionate (g/l)	1.1-1.9
Butyrate (g/l)	0.2-0.3
Iso-butyrate (g/l)	0.2-0.5
Valerate (g/l)	0.04-0.32

[a]Values after dilution with water (1:1 ratio)

Pulse load tests

The test was conducted at steady-state and when serial reactors were operated with 70/30 or 50/50 (R2+R3) and 13/87% (R4+R5) volume distributions. Lipid in the form of olive oil was fed directly in to the reactors, in the case of serial coupling in the first reactor (98 g/l feed). The pulse load for the one-step CSTR was 19.6 g/l-reactor volume. The pulse load for serial reactors was similar based on total volume, but related to first reactor only. Pulse load for reactors operated

with 90/10 and 80/20 are shown in Table 3. While the load for reactor operated at 70/30, 50/50 and 13/87% volume distribution was 28, 39.2 and 65.3 g/l-reactor volume respectively. Biogas production, VFA concentrations and pH were followed by sampling everyday for 10 days.

Table 2 Process operation data during single- and serial CSTR anaerobic digestion manure with various volume distribution ratios.

Parameter	Single step CSTR	Serial CSTR digestion (90:10)			Serial CSTR digestion (80:20)		
	R1	R2	R3	R2+R3	R2	R3	R2+R3
Retention time, d	15	13.5	1.5	15	12	3	15
Working volume, L	3200	3200	356	3556	3200	800	4000
Feed rate, mL/d	213	237	237	237	267	267	267
Parameter	Single step CSTR	Serial CSTR digestion (70:30)			Serial CSTR digestion (50:50)		
	R1	R2	R3	R2+R3	R2	R3	R2+R3
Retention time, d	15	10.5	4.5	15	7.5	7.5	15
Working volume, L	5	3.5	1.5	5	2.5	2.5	5
Feed rate, mL/d	333	333	333	333	333	333	333
Parameter	Single step CSTR	Serial CSTR digestion (30:70)			Serial CSTR digestion (13:87)		
	R1	R4	R5	R4+R5	R4	R5	R4+R5
Retention time, d	15	4.5	10.5	15	2	13	15
Working volume, L	5	1.5	3.5	5	0.54	3.5	4.0
Feed rate, mL/d	333	333	333	333	270	270	270

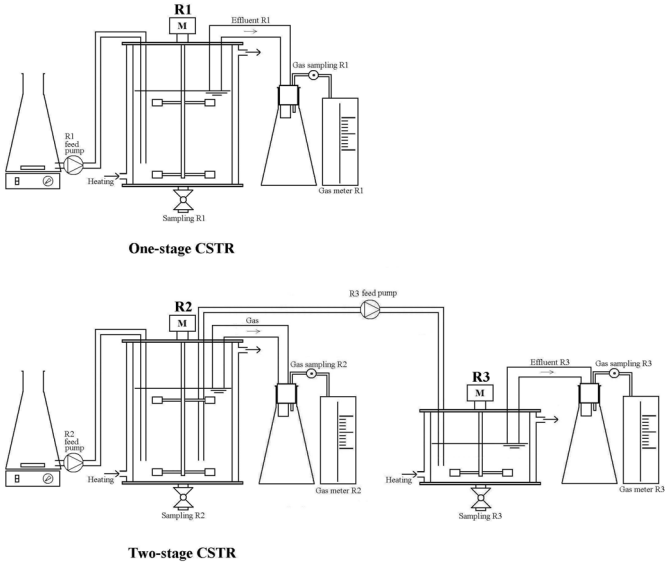

Figure 1 Reactor setup for the single- (above) and serial-CSTR experiments with 90/10, 80/20, 70/30 or 50/50% volume distribution.

Table 3 Data on different pulse loads tested during serial digestion of manure in 80/20 reactor configuration.

Pulse no.	Compounds	Organic load	Pulse load concentration(g/L reactor volume)
1	Crystalline cellulose	Carbohydrate, insoluble	31.3*
2	Crystalline cellulose	Carbohydrate, insoluble	31.3
3	Ispaghula (Vi-Siblin)	Carbohydrate, insoluble	46.8
4	Gelatin	Protein, insoluble	30
5	Gelatin	Protein, insoluble	9.4
6	Olive oil	Lipid	9.4

*Diluted with 300 mL water

Analytical methods

Samples of 15-20 ml were withdrawn from the reactors for various analyses. TS, VS, pH, ammonium- and total- Kjeldahl-nitrogen were determined using Standard Methods (Greenberg et al. 1998). CH_4 production from reactors and VFA were determined using a gas chromatograph (GC) HP 5890 Series II equipped with flame ionization detector (Angelidaki et al., 1991).

Microbial analyses

At steady state and a few days after each pulse organic load, the microbial community in each reactor was observed using fluorescence *in situ* hybridization (FISH) technique as described in Hugenholtz et al. (2001). The *Bacteria* probe EUB338mix and the *Archaea* probe ARC915 were used to identify the two main groups of microorganisms in the reactor which are acidogens/acetogens and methanogens, respectively. Both were used with 20% formamide.

Calculations

For lab-scale experiments, specific methane yield (ml/gVS_{fed}) was calculated as daily methane produced divided by the actual feed VS.

Results

Effect of serial CSTR digestion on process performance and methane production

The operation and process performance of the eight lab-scale CSTR reactors are summarized in Fig. 2-4 and Table 4-5. Results showed that serial CSTR processes with 90/10 and 80/20% volume distribution had 11% more biogas production

than the single CSTR process. A similar process performance was noticed also at 70/30, 50/50 or 30/70% volume distribution. The increase in the biogas production at 70/30, 50/50 or 30/70 was 13-17.8%. However, the process at 30/70 volume distribution was very unstable. At 13/87% volume distribution, no difference in biogas production in serial digestion was noticed.

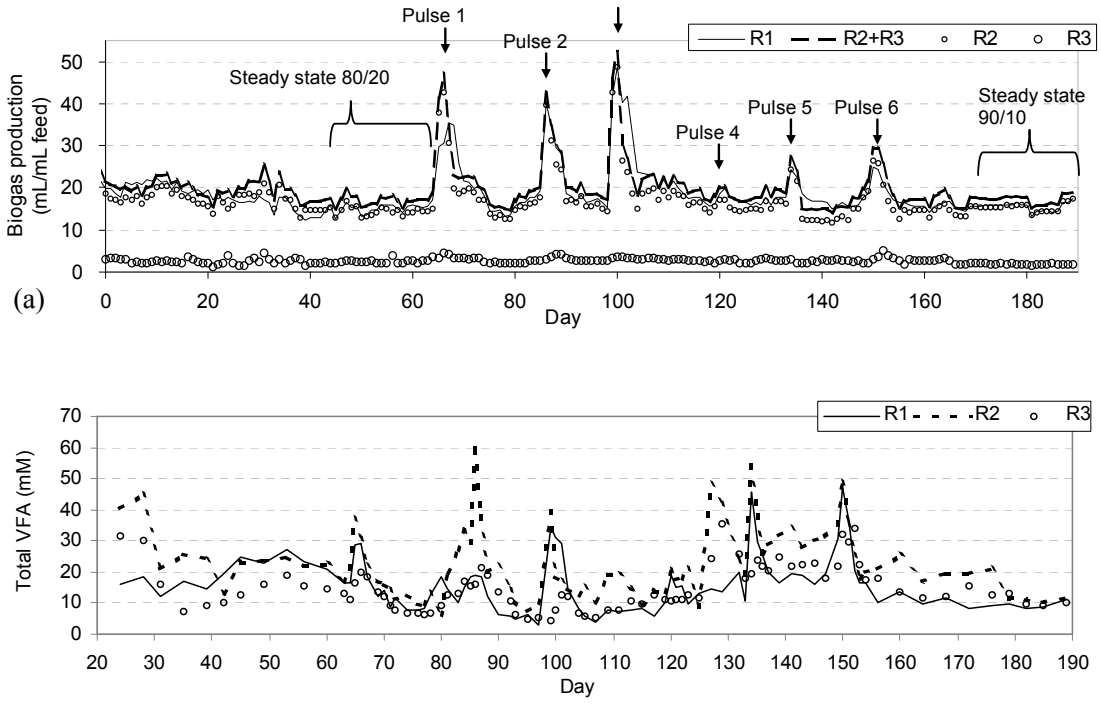

Figure 2 Process performance and biogas and VFA production during the anaerobic digestion of manure in control (R1) and serial reactors (R2+R3) with 90/10 or 80/20% volume distribution.

During steady state, pH in all reactors was in the range of 7 to 8 with slightly lower values for the 30/70 and 13/87% volume distributions. Ammonia values were more or less similar in all the reactors (1.3-1.5 g/l). VFA concentrations ranged between 0.7 and 2 g/l in one-step CSTR process (Fig. 2-4). The corresponding VFA values in serial digestion i.e. in post-digester were 24-43% lower than that noticed in one-step CSTR process. Among the different tested volume distributions of serial reactors, the order of magnitude for VFA levels was highest with 30/70% followed by with 13/87, 50/50, 70/30, 80/20, 90/10%.

Table 4. Experiment results at steady state and different pulse load

Parameter	Unit	Single CSTR	Serial CSTR			%Different	Note
		R1	R2	R3	R2+R3		
Steady state 90/10							average day
Biogas production	mL/mL-feed	15.6	15.4	1.9	17.3	11%	170-189
Relative biogas yield		100%	95%	16%	111%		
Steady state 80/20							average day
Biogas production	mL/mL-feed	15.1	14.3	2.5	16.8	11%	49-62
Relative biogas yield		100%	99%	12%	111%		
Pulse test 1 (100 g/l Crystalline cellulose + water)							
Biogas production	mL/mL-feed	24.1	22.6	3.3	25.9	7%	Average day 64-75
Relative biogas yield		100%	94%	14%	107%		
Pulse test 2 (100 g/l Crystalline cellulose)							
Biogas production	mL/mL-feed	26.9	24.6	3.5	28.0	4%	Average day 85-91
Relative biogas yield		100%	91%	13%	104%		
Pulse test 3 (150 g/l Biofibre)							
Biogas production	mL/mL-feed	24.8	20.7	3.0	23.8	-4%	Average day 98-118
Relative biogas yield		100%	83%	12%	96%		
Pulse test 4 (30 g/l Gelatin)							
Biogas production	mL/mL-feed	17.6	15.6	2.5	18.1	3%	Average day 120-126
Relative biogas yield		100%	89%	14%	103%		
Pulse test 5 (60 g/l Gelatin)							
Biogas production	mL/mL-feed	17.5	14.2	2.6	16.8	-4%	Average day 134-147
Relative biogas yield		100%	82%	15%	96%		
Pulse test 6 (30 g/l Olive oil)							
Biogas production	mL/mL-feed	19.8	18.9	3.2	22.1	12%	Average day 149-156
Relative biogas yield		100%	96%	16%	112%		

Microbiological analyses

Microbiological analyses showed no significant difference in the microbial ecology between one-step and serial digestion (data not shown). However, the relative abundance of the organisms varied between the two reactors of the serial digestion. Small short rod shaped bacterial cells along with a few cells belonging to *Methanosarcinacaea* and *Methanobacteriacea* were noticed in one-step CSTR (R1). A similar microbial ecology and abundance was also noticed in the main reactor of serial digestion (90/10, 80/20 or 70/30%; R2). The abundance of these microorganisms was however relatively low in the post-digester (R3). At 30/70% volume distribution, short and long rod shaped bacterial cells with a few cells of *Methanosarcinacaea* and *Methanobacteriacea* were noticed in the post-digester (R5).

Effect of different pulse load on methane production in a serial CSTR process

The data on average biogas production at steady-state and during organic pulse load are shown in Figures 2-4 and summarized in Table 4-5. In general, stable biogas production was noticed in all reactors and at all tested volume

distributions. On comparison to single CSTR, 3 to 12% extra methane was obtained in serial CSTR when operated with 80/20 volume distribution and subjected to pulse load of carbohydrates (pulse load 1 & 2), protein (pulse 4) or lipid (pulse 6). However, an increase in pulse loads for carbohydrate (pulse 5) or protein (pulse 5) did not result in extra gas production in serial digestion. Lipids gave highest extra methane production compared to protein and carbohydrates. Conversion efficiency also varied with substrate concentration and process (data not shown). For instance, the conversion efficiencies for carbohydrates (pulses 1 and 2) at lower concentration were more or less similar between the two processes. On the contrary, at a higher concentration of biofibre (pulse 3) and protein (pulse 5) conversion efficiency was significantly low in serial digestion than in single CSTR process. On the other hand, an increase in lipid pulse load (Table 5) no extra biogas production was noticed when serial reactors were operated with 70/30% volume distribution while 3.1% extra biogas was obtained with 50/50% volume distribution.

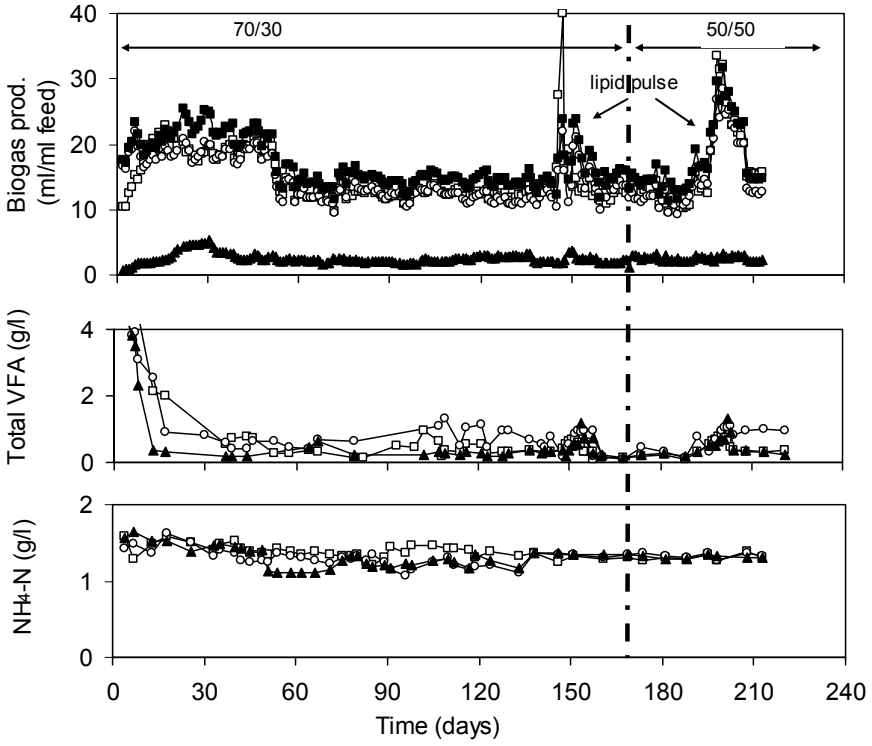

Fig. 3. Process performance and biogas production during anaerobic digestion of cow manure in one-step (□ R1) and serial digestion with 70/30 and 50/50% volume distribution (○ R2, ▲ R3 and ■ R2+R3) in lab-scale at 55°C.

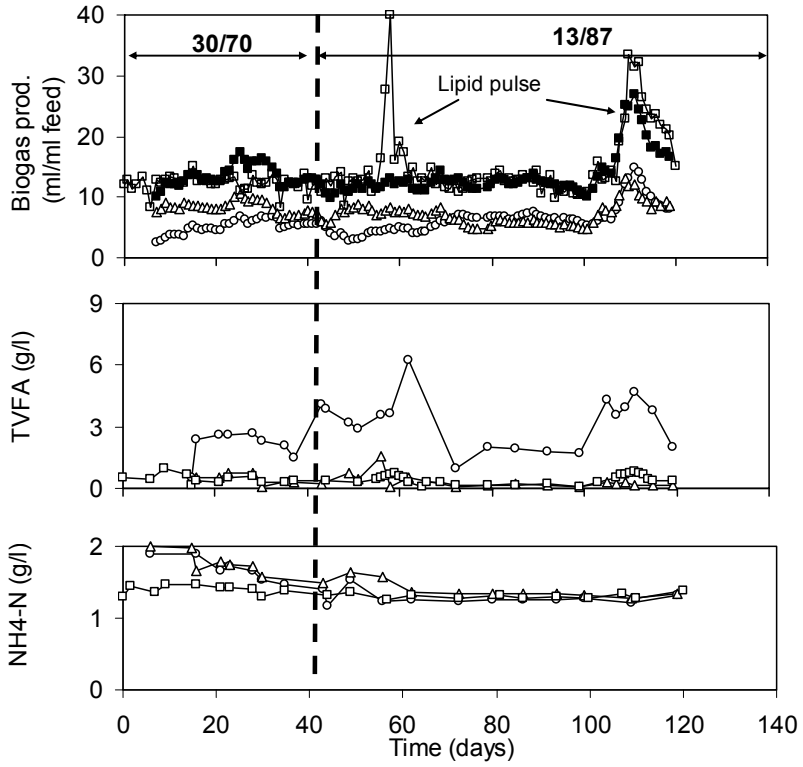

Fig. 4. Process performance and biogas production during lab-scale thermophilic digestion of cow manure in one-step CSTR (□ R1) and serial digestion processes with 30/70 and 13/87% volume distribution ratios (○ R4, Δ R5 and ■ R4+R5).

Discussion

The results from the present study demonstrates that serial CSTR process (R2+R3) compared to conventional single step CSTR (R1) process could improve the process stability and biogas production from manure based biogas reactors. Serial digestion of manure with a combined HRT of 15 d and with volume distributions of 90/10, 80/20, 70/30, 50/50 or 30/70% could give 11-17.8% additional biogas production compared to a traditional single step CSTR process. The increased biogas production in a serial digestion process was mainly contributed by the biogas produced from the second reactor, which could recover up to 16-18% of total biogas production in the serial system. The reasonable high biogas production although unstable when serial CSTR (R4 and R5) digestion was operated at 30/70% volume distribution suggest that both reactors behaved like methanogenic reactors rather than phasing out as

hydrolysis/acidogenesis stage in the first reactor and methanogenesis stage in the second reactor. This was evident from FISH analyses which showed that methanogenic bacteria were found in the first reactor of 30/70% volume distribution. Moreover, no hydrogen was produced during the entire experimental period. Process parameters such as pH, NH_4-N and TVFA in 30/70% serial digeston process were similar to those noticed in 90/10, 80/20, 70/30 or 50/50% serial CSTR configurations. The slightly high VFA levels (Fig. 2-3) in the main reactor of 30/70% serial configuration suggests that the volume allocated to the main reactor (4.5 d of HRT) was insufficient to maintain a stable process. On the other hand, the decreased biogas production with a corresponding increase in VFA levels in the main reactor upon redistributing the volume to 13/87% indicates a shift from methanogenic to hydrolysis/acidogenic process.

Table 5. Biogas production during lab-scale thermophilic digestion of cow manure in one-step and serial CSTR processes at different volume distribution ratios.

Volume distribution (%)	Steady-state	Biogas production (ml/ml feed)				Increase in biogas production (%)
		One-step CSTR	Serial CSTR			
		R1	**R2**	**R3**	**R2+R3**	
70/30	Day 45-140	12.8	12.6	2.3	14.9	16.4%
Lipid pulse	Day 144-154	18.4	15.9	2.5	18.4	0%
50/50	Day 173-194	12.3	12.0	2.5	14.5	17.8%
Lipid pulse	Day 195-209	22.9	20.9	2.7	23.6	3.1%
		R1	**R4**	**R5**	**R4+R5**	
30/70	Day 20-40	12.3*	5.7	8.2	13.9	13%
13/87	Day 85-100	12.2*	6.5	5.5	12.0	-1.7%
Lipid pulse	Day 106-119	23.4*	10.7	9.9	20.6	-13.6%

*Corresponding values from control (R1); Lipid pulse test was carried out with olive oil at organic load of 19.6 g/l/l reactor volume in R1 and 28, 39.2 and 65.3 g/l/l reactor volume in R2 when operated with 70/30, 50/50 and 13/87% volume distributions respectively

The stable biogas production noticed during the lipid pulse test suggests that both one-step and serial CSTR processes can overcome an organic overload ranging from 19.6 to 65.3 g/l reactor volume. However, the extra biogas production noticed only with 80/20 and 50/50% volume distribution suggests that the serial digestion could produce more biogas than the one-step CSTR as long as the main reactor was not inhibited (VFA build-up) and HRT of the post-

digester was sufficiently long (Table 2). The low extra biogas produced noticed at a higher pulse load of carbohydrate (biofibre 150 g/l) indicated that hydrolysis was the rate limiting step during the digestion of biofibre and long retention time was needed. Similarly, the low gas production at high protein pulse load could be due to free ammonia inhibition. The slight decrease in biogas production immediately after each pulse test suggest that the tested lipid load could have affected the process temporarily through accumulation of long chain fatty acids (not measured). The increased VFA levels however never reached to levels that could induce process inhibition. The highest VFA levels noticed during the lipid pulse test were <2 g/l with 70/30 or 50/50% and 5 g/l with 13/87% volume distribution (Fig. 1-2) indicating that the main reactor in the latter configuration was slightly inhibited. Nevertheless, the low VFA levels (0.3 g/l) noticed after 10-14 d of the test suggest that the added lipid load was completely removed from the system.

Conclusions

The results from the present study showed that the biogas production can be improved by a serial digestion, where the reactor volumes between the two reactors in series were distributed 90/20, 80/20, 70/30 or 50/50%. The increase in biogas production in serial CSTR process could be up to 11-17.8% than the traditional single step CSTR process as long as the first reactor of the serial digestion was not inhibited. Process at 30/70% volume distribution was very unstable while no significant difference in biogas production was noticed at 13/87% volume distribution. In addition, no phasing of the process to separate hydrolysis/acidogenesis and methanogenesis was noticed at 30/70 or 13/87% volume distributions. The results from microbial observations showed similar in abundance of microorganisms between the two reactors (R2 and R3) of serial process. The performance of reactor R3 was found to depend on the quality of effluent of R2 as microbial composition in R3 was mainly obtained from R2. The results from organic pulse loads showed that that both single step- and serial-CSTR processes can overcome an organic overload of up to 65.3 g/l reactor volume without any visible inhibition due to VFA build-up. Lipids would give higher extra methane production compared to protein or carbohydrates.

1.1. The effect of reactor configuration on the process performance and biogas production in a serial process (I)

Pilot-scale experiments

Pilot-scale experiments were conducted under more realistic conditions in order to support the results from lab-scale experiments, where reactors were fed with blended manure. In addition, mixing and point of effluent extraction in pilot-scale plant is more representative for a full-scale operation.

Feed preparation

Fresh cow manure was obtained in 800 lt batches from a centralized biogas plant (Hashoj biogas plant, Denmark). Batches were collected directly from the incoming delivery trucks (i.e. uncut) and always from the same cattle farm in order to ensure as uniform feed characteristics as practically possible. Feed was prepared by diluting the manure with water, whenever necessary, to attain a consistent total solids (TS) of 6.5-7.5%. Characteristics of feed are presented in Table 1.

Table 1. Average composition of feed cow manure

	Pilot expt [b].
VS (%)	6.5-7.3
TS (%)	4.8-4.9
pH	7.5-7.7
Ammonia (g/l)	1.8-2.4
Total VFA (g/l)	2.8-6.8
Acetate (g/l)	1.8-5.2
Propionate (g/l)	0.6-0.9
Butyrate (g/l)	0.06-0.21
Iso-butyrate (g/l)	0.24-0.40
Valerate (g/l)	0.10-0.13

[a]Values after dilution with water (1:1 ratio);

Reactor setup

The experiment was carried out in a pilot-scale plant built at the Institute of Environment & Resources, Technical University of Denmark. Two stainless steel reactors, referred to as R1 (800 lt) and R2 (200 lt) were used in the study. The

reactors were fitted with a stainless steel top plate, which supported the vertical low speed mixer, mixer gearmotor, gas sampler, safety and pressure valve and a safety level switch. Feed valve, effluent valves (3), temperature probe and sampling ports (3) were fitted to the reactor wall. Process temperature was maintained at 54±1°C by pumping hot water through a stainless steel coil fitted inside the reactor using an electric flow heater and circulation pump (Kaparaju and Angelidaki, 2007).

Feed was thoroughly mixed in the feed pallet prior to each feeding by a high speed motor (for 15 minutes). Reactor contents were mixed by a low speed gear motor fitted with two impellers aligned just below the liquid surface and above the bottom of the reactor. Reactor mixers were operated in a 5 minutes on/off mode. Two eccentric pumps with a flow rate of 10 lt/min were used to pump feed and effluent. Pumps were operated 3 times per day and for 50 or 65 seconds each time depending upon the feed rate. Prior to each feeding, an equal amount of effluent was removed from the middle part of the reactor. Effluent removal always preceded feeding to minimize short-circuit loss. Biogas from the reactor was measured continuously using a diaphragm gas meter. The pumps and mixers were controlled automatically by relay timers and a 2 channel 24 hours/7 day programmable time switch.

Reactor operation

Detailed description of start-up and operation of R1 (main reactor) has been described elsewhere (Kaparaju et al., 2007). Briefly, 450 lt of thermophilically digested manure (Centralized biogas plant, Denmark) and 30 lt of fresh cow manure were transferred to R1 on Day 0. Daily feeding in R1 commenced approximately 10 d after seeding. During Day 0 to 22, R1 was operated at a feed rate of 25 l/d and working volume of 500 lt corresponding to HRT of 20 d.

R2 (post-digester) was connected to R1 in series and started-up separately with a limited amount of initial inoculum, (i.e. 16.6% of the final working volume of 150 lt) and operated in a fed batch mode until the reactor was filled (6 days). The post-digester was fed directly with effluent from the main reactor by gravitation. During Days 0 to 22, digestion was operated at 25 l/d corresponding to a HRT in R1 of 20 d. On Day 23, feed rate was increased from 25 to 32.5 l/d to attain a combined HRT in R1+R2 of 20 d. The corresponding HRTs for the main reactor and post-digester were 15.4 and 4.6 days respectively. Data obtained from R1 during Days 0 to 22 and 64 to 97 were used as one-step CSTR process (reference period) while the data obtained from R1+R2 during Days 23 to 63 and 98 to 119 represented serial digestion.

Residual methane potential

Residual methane potential of digested materials was conducted for lab-scale experiments. At steady-state, effluent samples were collected from one-step (R1) and serial reactors (R2 and R3 operated at 70/30% volume distribution). Serum glass bottles of 118 ml total volume were used. To each bottle, 20 ml digested material was used. The headspace in the bottles was then flushed with a mixture of N_2/CO_2 gas mixture (80/20 ratio) and sealed immediately with butyl rubber stoppers and aluminium crimps. The prepared bottles were incubated at 55ºC. The experiment was carried out in triplicate. Methane production was measured in the headspace of the vials using Gas Chromatograph (GC) with Flame Ionization Detector (FID) as described elsewhere (Greenberg et al., 1992).

Analytical methods

TS, volatile solids (VS) and pH were determined according to Standard Methods (APHA, 1998). Total nitrogen and ammonium nitrogen (NH_4^+-N) were analyzed following Kjeldahl-N method (Greenberg et al., 1992). Methane content in biogas and VFA were measured by gas chromatograph (GC) HP 5890 Series II equipped with flame ionization detector..

Calculations

Specific biogas yield (ml/gVS_{fed}) was calculated as the daily biogas production, divided by a weighted average of VS fed over a period stretching 8 days backward. This 8 day weighted average was used to minimize the fluctuations when load was changed to obtain one-step/serial operation with similar retention time in the main reactor or total in both reactors. The weighted average was defined as effective VS basis for daily degradation to be represented by 57% of VS fed from the 3 most recent days, 29% of VS fed from the previous 3 days and 14% of VS fed from the last 3 days, a correlation which has previously proven to reflect daily biogas production relatively well in periods with fluctuating loading.

Results

The experiments were carried out over a period of 119 d covering four batches of feed. The results are presented in Fig. 1 and Table 2. As there were only one set of reactors available, one-step CSTR process and serial digestion process performance were compared off-set in time by alternately changing the feed rate. Data obtained from the main reactor when fed at 25 l/d and with total HRT of 20 d was used as reference reactor (R1). Data obtained from both reactors (R1+R2) when fed at 32.5 l/d and with a combined HRT of 20 d represents the data for

serial digestion. For more reliable comparison, both one-step and serial processes were operated within the same feed batch, which lasted for a maximum of 40 d. In Fig. 1, batch changes and periods with changes in reactor configuration/load are indicated. Temperature profiles showed that the mean temperature during the first 52 days of operation was slightly higher in the main reactor (54.5±0.1°C) than in the post-digester (52.3±1°C). Between Days 53 and 63, temperature in the main reactor and post-digester reached within 0.3°C difference but at a slightly lower level operating temperature (52.3°C). From Days 64 to 119, both reactors had a mean temperature of 54.2±0.2°C.

Table 2. Process performance and biogas yield during anaerobic digestion of manure in pilot-scale plant, subdivided into characteristic batch/volume distributions.

	Feed batch 1	Feed batch 2		Feed batch 3		Feed batch 4	
Period	One-step CSTR (R1) Day 0-15	One-step CSTR (R1) Day 16-22	Serial CSTR (R1+R2) Day 23-50	Serial CSTR (R1+R2) Day 51-63	One-step CSTR (R1) Day 64-88	One-step CSTR (R1) 89-97	Serial CSTR (R1+R2) 98-119
Biogas Prod. (l/d)	634.5	626.2	790.7	794.2	611.2	614.3	800.3
Biogas prod. (l/l feed)	25.4	25.0	24.3	24.4	24.5	24.5	24.6
Spec. biogas yield lt/kg-VS	468.3	449.8	464.5	461.4	452.7	460.7	488.7
Relative yield		=100%	3.3%	1.9%	=100%	=100%	6.1%
Methane content (%)	72.7	71.2	67	67	69.2	70.7	71.1
Effluent VS (%)	3.5	3.7	3.3	3.3	3.5	3.6	3.3
VFA (g/l)	0.28	0.27	0.25	0.25	0.32	0.31	0.30
Temp. (°C)	54.7	54.7	52.3	52.3	54.3	54.4	54.2

The mean biogas yield, the primary evaluation parameter, obtained during periods with serial digestion was 1.9-6.1% higher than those obtained during one-step CSTR process, with an average value of 3.8%. The trend in biogas yields upon changing from one-step to serial digestion was the same for each change when the results obtained within the same feed batch were compared. However, the best results were obtained during days 98 to 119 when the difference in temperature between the two serial reactors was small (0.3°C). The variations in biogas yield observed during the initial phase of the experiment (Days 0-88) were most likely the result of difference in temperature between the two serial reactors, temporarily affecting process performance of the post-digester and thus the overall biogas yield.

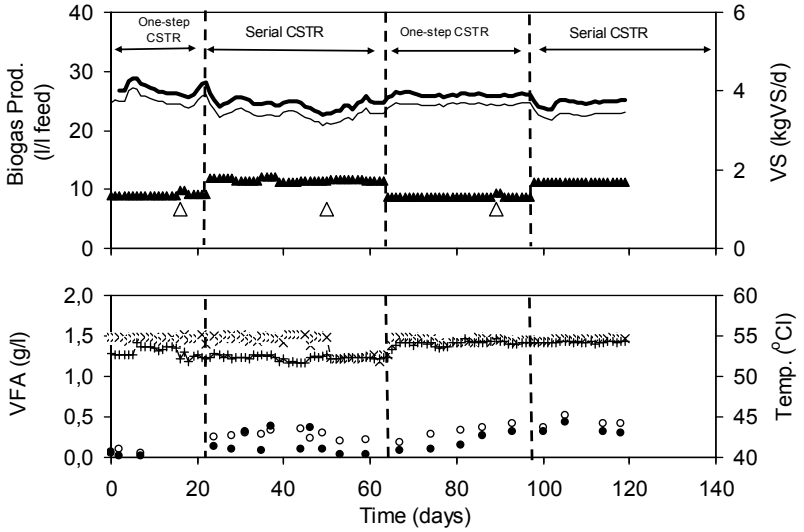

Fig. 1. Process performance and biogas production during pilot-scale thermophilic digestion of cow manure in one-step (R1) and serial digestion (R2+R3) with 77/23 volume distribution: biogas production in R1 (—) and R2+R3 (▬); Feed VS (▲); Feed batch changes (Δ); total VFA in R1 (○) and R2+R3 (●); Temperature in R1 (×) and R2+R3 (+).

Effluent VS, VFA and ammonia levels were in general lower during serial digestion than one-step CSTR process indicating that solids had much longer retention time than nominal average retention time with better conversion efficiency and thus minimized VS loss during serial digestion.

DISCUSSION

The present study demonstrated that serial digestion, i.e. two methanogenic reactors connected in series, could improve the conversion efficiency and thus optimize biogas production compared to a traditional one-step CSTR process. Results from the present study are in agreement with previous lab-scale studies where serial digestion with 90/10, 80/20, 70/30 or 50/50% volume distribution between the two serial reactors gave up to 11-17% extra biogas production compared to one-step process. Both these results suggest that the best conversion efficiency, with a given overall digester volume, can be achieved by a serial process with a relatively large main digestion step and a smaller post-digestion step. Further these results in practice suggests that under full-scale conditions, with daily variations in feed stock amount and quality, it is considered safer to

adopt a serial concept with a relatively large first step, in order to ensure process stability.

The relatively low biogas yield improvement in pilot-scale study compared to that obtained in lab-scale study was most likely the result of the unintentional small variation in temperature between the two reactors of the pilot-scale plant, temporarily affecting process performance and biogas yield of post-digester and/or due to the differences in reactor operation/construction between the two experiments. For instance, biogas production from post-digester was low (35-50% of normal level) when the temperature of the post-digester was 1°C lower than that of main reactor (Data not shown). This difference in temperature may have led to low biogas production from the post-digester as methanogenesis is usually more sensitive to a decreased process temperature than hydrolysis resulting in imbalance in VFA turnover, especially under thermophilic conditions (Angelidaki et al., 2005). On the other hand, post-digester in the lab-scale study accounted for up to 15-19% of total biogas production in the serial system when operated at the same temperature as that of the main reactor. These results in practise suggest that post-digester must be operated at temperature as close as possible to that of main reactor in order to maintain optimum activity in the post-digestion step. Secondly, the difference in the reactor operation/construction between the two experiments also suggests that the VS loss from the one-step lab-scale reactor (R1) may have been higher when effluent was "pressed out" from the surface layer than drawn out from the middle of the reactor (R2). Thus, the effect of serial operation in the lab-scale reactors therefore would be higher than what can be expected in the pilot-scale plant, where effluent was removed from the middle layer. Under full-scale conditions, technically more resembling the pilot scale experiment but where temperature stability and control can be better than in the present pilot-scale experiment, a result somewhere between present lab-scale and pilot-scale could be expected, i.e. most likely with improved biogas production in the range 7-10%. This range was based on cow manure, where a relatively large fraction of VS was presumed to be present in undissolved fibres/particles.

Previous studies have also showed that improved biomass conversion efficiency and biogas yield can be obtained by selectively retaining the solids within the reactor by withholding mixing prior to effluent removal (Kaparaju et al., 2007) or post-treatment, in order to improve biodegradability and accessibility, of solids separated from digested material (Kaparaju and Angelidaki, 2007). However, serial digestion concept seems to have some economic gain over above two options when the extra process cost or complexity involved is compared to the costs involved in arranging reactors in a series, especially in an already existing plant with multiple reactors. Serial digestion

concept could therefore be considered as the first choice in optimising biogas conversion efficiency from manure compared to traditional one-step CSTR process, which may be further improved by other techniques if additional investment, operating cost and process complexity can be justified by extra yield.

CONCLUSIONS

The present results showed that the biogas production from manure can be optimized by operating two CSTR reactors connected in series. The increase in biogas production in serial digestion could be up to 1.9-6.1% compared to traditional one-step CSTR process. In addition, temperature was found to influence the methanogenesis and thus the post-digester should be operated at the same temperature as that of the main reactor. Thus, serial digestion can be considered a method to improve conversion efficiency. However, the extra installation costs and process complexity in executing serial digestion concept should be evaluated with the economic gain achieved due to extra biogas produced.

Experiment 1

1.2. Effect of temperature on the second step of a serial operation process

Background

The results of a previous project showed that the residual gas potential in the after storage reactors had good potential for additional gas production and the temperature of the after storage reactors had profound influence on the amount of biogas produced. In addition, the study also showed that after storage reactors should be operated either at 30°C under thermophilic conditions or at 25°C under mesophilic conditions in order to get an acceptable amount of biogas within a tolerable time limit. If the temperature is too low, gas production seems to cease. To further investigate and verify these results in continuous reactor experiments, the present project was carried out.

Objective

The main objective of this study was to investigate the influence of temperature on the efficiency of recovering biogas in after storage reactors. Further, the effects of different temperatures on various steps in the process were also investigated.

Methodology

The project was carried out in a laboratory as a serial CSTR process with a main reactor and three parallel after storage reactors operating at different temperatures (15°C, 37°C and 55°C). Furthermore microbial analyses by FISH were also used to understand the influence of temperature on the microbial ecology of the reactors and an activity test was conducted to show the immediate activity of the reactors during steady state.

Reactor experiment setup

The main experiment was carried out as a serial CSTR process. The first-step consisted of a main reactor (9 L CSTR) with working volume of 7.2 L and a HRT of 15 d and operated at 55°C (R0). R0 was connected to a second-step process, which was split in to three 1 L glass reactors with working volumes of 800 ml and a HRT of ca. 5.3 days each (Fig. 1). The three reactors were operated at temperatures of 15°C (R15), 37°C (R37) and 55°C (R55) respectively. The operating temperatures were ensured by circulating heated water (to R37 and R55) from water baths between the double walls of the reactors. All reactors were connected to an effluent flask where the produced biogas was also measured. Temperature in R15 was started out ca. at 25°C but before reaching steady state the temperature was decreased to 15°C to simulate a more realistic look at how a

real unheated after storage reactor of biogas plants in Denmark would operate. This was done by connecting a cooling bath, (with cooling liquid) to the reactor. All of the reactors were continuously stirred throughout the experiment. R0 was fed semi-continuously four times a day at six hour interval with a volume of 120 mL of manure per time. The three after storage reactors were fed once in a day using peristaltic pump at the rate of 150 mL/d with the effluent from R0.

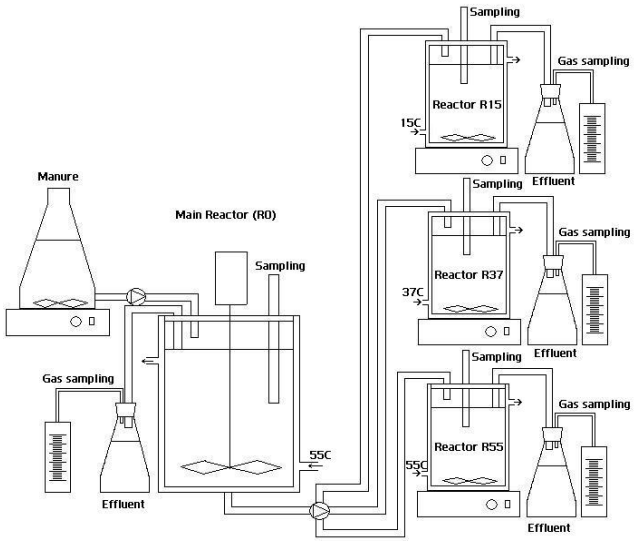

Figure 1. Conceptual drawing of the experimental setup.

Table 1. Operational parameters during experiment

Reactor	Temperature (°C)	total volume (mL)	liquid volume (mL)	flow (mL/day)	HRT (days)	feed (times/day)
R0	55	9000	7200	480	15	4
R15	15	1000	800	150	5.3	1
R37	37	1000	800	150	5.3	1
R55	55	1000	800	150	5.3	1

Feed preparation

Manure consisted of a mixture of cattle and swine manure and industrial waste procured from a thermophilic centralized biogas plant (Lemvig Biogas plant, Denmark). Upon arrival at the laboratory, manure was blended and mixed to ensure homogeneity of feed throughout the experiment and to prevent clogging of the pipes and pumps. The prepared feed was stored at -20°C until use.

Table 2 Mean values for the manure characteristic.

Parameter	**Mean value**
VS (%)	4.78
TS (%)	8.21
pH	6.87
Total VFA (g/l)	17.6
Acetate (g/l)	10.6
Propionate (g/l)	3.1
Butyrate (g/l)	2.1
Iso-butyrate (g/l)	0.6
Valerate (g/l)	0.6
Iso-valerate (g/l)	0.7
Total-N(g/l)	4.62
Ammonia-N(g/l)	3.26
Free Ammonia (g/l)	0.01
Carbohydrates (g/l)	26.6
Lipids (g/l)	5.0
Proteins (g/l)	8.4
Content of carbohydrates (% of VS)	64.2
Content of lipids (% of VS)	12.1
Content of proteins (% of VS)	20.4

Activity test

At steady state, an activity test was carried out to test the immediate activity of a specific inoculum on a specific substrate. On day 62, assays consisting of effluents from the reactors together with BA medium and different substrates (acetate, propionate, hydrogen/carbon dioxide, butyrate and glucose) were prepared in duplicates. A batch of BA-medium with no substrate was used as control (blank). Vials with 40 mL of BA medium were inoculated with 10 mL of sample from the four different reactors. The substrates were added to these vials and the vials were then kept in the respective temperatures of the inoculum (Angelidaki & Schmidt, 2002). Methane production and VFA levels were monitored closely for the first 10 d and thereafter at larger intervals for 50 days.

FISH

Fluorescence *in situ* hybridization (FISH) analysis was carried out to investigate the microbial ecology in the reactors and in that way to get an overview of the processes in the 4 reactors. Samples for FISH analyses were prepared as described elsewhere (Hugenholtz, et al., 2001). Different probes were used to get an idea of the distribution of microorganisms in the samples. These were:

- EUBMIX-cy3 probe (red) for all bacteria
- ARC915-FITC probe (green) for all archaea
- MX825-cy3 probe (green + red) for methanosaeta (acetate utilizing bacteria)
- MB1174-cy5 probe (blue) for methanobacterials (hydrogen utilizers)
- MG1200-cy5 probe (blue) for methanomicrobiales (hydrogen utilizers)
- MC1109-cy5 probe (blue) for methanococcales (hydrogen utilizers)

The FISH samples were observed in an epifluorescent microscope and pictures were taken from a Confocal Laser Scanning Microscope (CLSM) at DTU.

Pulse load experiment

On day 94, a pulse load experiment was conducted in order to test whether reactors in series (serial operation) were capable of overcoming an organic overload. This was done by injecting 219g of oil corresponding to 14.6 times the normal VS load, in the main reactor R0. Methane production, VFA concentrations and pH were followed as in the main experiment, however more frequently (twice a day).

Apparatus and Methods

Samples of 15-20 ml were withdrawn from the reactors. pH was measured immediately using a standard pH-meter. TS, VS and ammonium- and total-Kjeldahl-nitrogen were determined using standard methods (Greenberg et al. 1998). CH_4 production from reactors and VFA were determined using a gas chromatograph (GC) HP 5890 Series II equipped with flame ionization detector (Angelidaki et al., 1991).

Results

The effect of temperature on the methane production from second step of a serial operation process is presented in Table 3 and Figure 2. The experiment was carried out for 111 days and steady-state was achieved after 45 days of operation. Methane production significantly increased as the process temperature increased from 15 to 55°C during the post-digestion. Mean methane production in R0 (55°C) was 17.2 ml/ml feed (0.36 m^3/kgVS). Methane produced from after storage reactors was 2 ml/ml feed (0.079 m^3/kgVS) in R55 and 1.64 ml/ml feed (0.065

m^3/kgVS) in R37 whilst a mere 0.21 ml of methane per ml feed (0.008 m^3/kgVS) was obtained from R15. The additional methane obtained in R55 was 11.7% and was significantly higher than that obtained in R37 (8.4%) or R15 (1.2%). However, methane content in the gas produced in the post-reactors increased significantly with the decrease in post-digestion temperature.

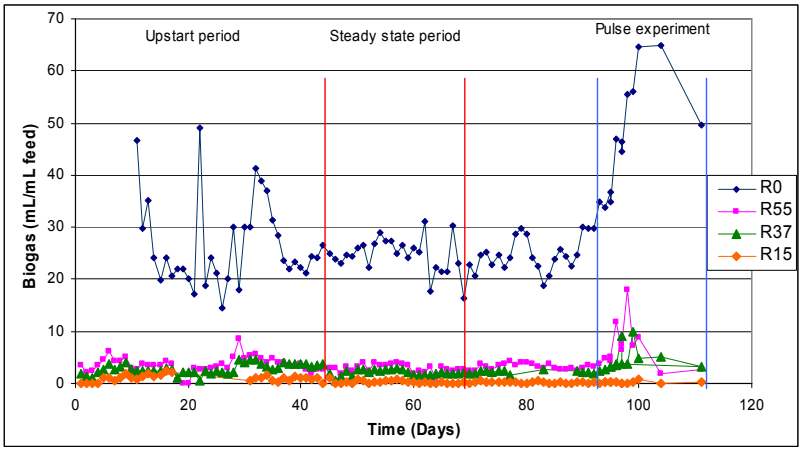

Figure 2. Biogas production during the experimental period.

The VFA concentrations in R0, R15 and R37 were between 1.2 and 1.5 g/l compared to 0.3 g/l noticed in R55 (Fig. 3-4). Acetate was the predominate VFA ranging between 0.7 to 1 g/l followed by propionate (0.4 g/l). In order to evaluate which process temperature combination was optimum, methane equivalents from VFA and the actual production were compared. The results showed that R37 had the highest total methane equivalent of 280 ml/d followed by R55 (276 ml/d) while the total methane equivalent in R15 was only 123 ml/ d.

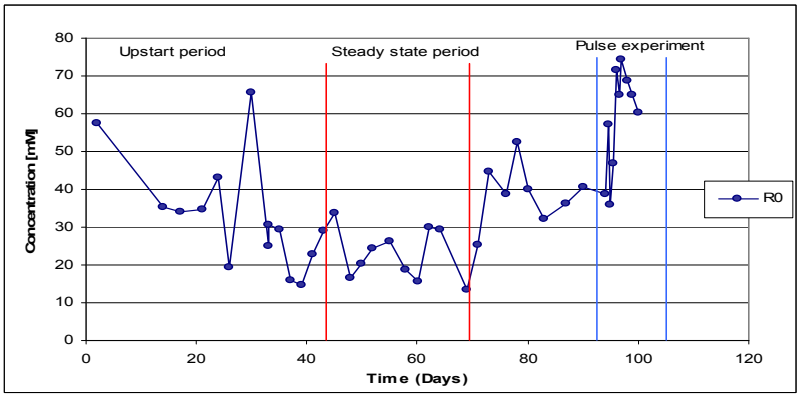

Figure 3. VFA concentration in R0 during the experimental run

Table 3. Measured parameters in the reactors at steady state (45 days of operation).

Parameters	**units**	**R0**	**R15**	**R37**	**R55**
VS	%	2.52	2.92	2.74	2.39
TS	%	5.50	6.28	5.88	5.23
pH		7.89	8.09	7.72	7.87
VFA total	mM	22.8	22.8	19.5	4.0
Acetate	mM	15.8	15.4	12.3	3.4
Propionate	mM	5.8	5.8	6.1	0.4
Butyrate	mM	0.2	0.2	0.1	0.02
Iso-butyrate	mM	0.5	0.5	0.5	0.04
Valerate	mM	0.1	0.1	0.1	0.02
Iso-valerate	mM	0.3	0.3	0.3	0.05
VFA total (0.1-2.5g/l for a steady process)	g/l	1.5	1.5	1.2	0.3
Acetate (< 0.8 g/l for a steady process)	g/l	1.0	0.9	0.7	0.2
Propionate	g/l	0.4	0.4	0.4	0.03
Butyrate	g/l	0.02	0.03	0.01	0.002
Iso-butyrate	g/l	0.05	0.06	0.04	0.003
Valerate	g/l	0.01	0.01	0.01	0.002
Iso-valerate	g/l	0.03	0.07	0.03	0.005
Total-N	g/l	4.7	4.8	4.8	4.7
Ammonium-N	g/l	3.3	3.3	3.8	3.6
Free Ammonia	g/l	0.85	0.13	0.22	1.10
Proteins	g/l	8.5	9.2	6.5	6.8
Biogas Production	ml/ml feed	24.6	0.3	2.1	2.9
Extra biogas production compared to R0	%	-	1.2	8.4	11.7
Methane percentage *	%	69.9	70.1	77.9	68.8

*The methane percentages are very high.

3.3.1. Methane equivalents

One way to examine how the different stages in the biogas process are working is by the use of methane equivalents. Methane equivalents are calculated from VFA and the produced methane in the reactors. Results showed that R55 had the highest hydrolysis/acidogenesis followed by R37 and R15. VFA content in R37 and R15 was much higher than in R55. The calculated methane equivalents (Table 3 & Fig. 5) indicated that amount of CH_4-eq produced between R55 and

R37 was more or less the same (Table 4). On the other hand, CH_4-eq produced in R15 was approx 126% lower than R55 and R37.

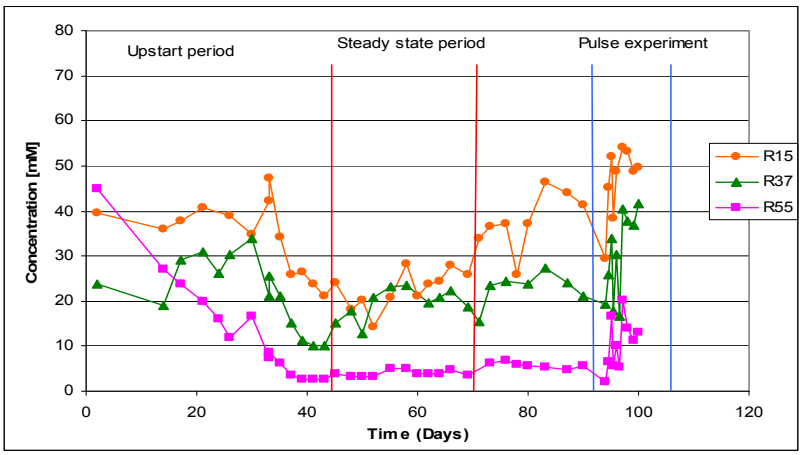

Table 4. Methane equivalents from the three after storage reactors.

	CH_4-eq from VFA (ml/day)	CH_4 from biogas (ml/day)	Total CH_4-eq (ml/day)
R15	97.8	26.1	123.9
R37	93.3	186.3	279.6
R55	17.6	259.2	276.8

Figure 4. VFA concentration in R15, R37 and R55 during the experimental run

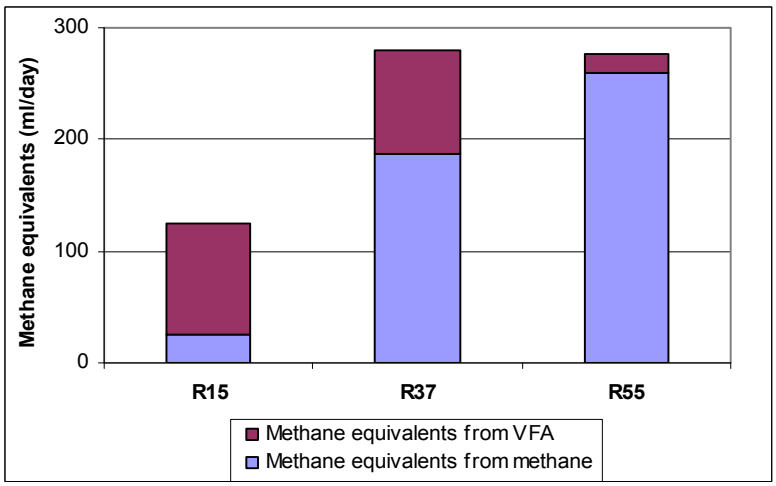

Figure 5 Methane equivalents from VFA and methane in R15, R37 and R55

Potential CH_4 yield

The methane yields calculated using amount of methane produced per added VS (4.78%) are presented in Table 5. A methane content of 60% was considered for all reactors while calculating the methane yields. The results showed that methane yields of 0.34-0.35 l CH_4/gVS were obtained when the reactors in series were operated at 37 or 55°C. On the other hand, up to 0.31 l CH_4/gVS was obtained when the process was operated at 15°C. The experiments also showed that 68-70% of the methane potential was realized at higher process temperatures of 35-55°C than at 15°C.

Table 5. Calculated and theoretical methane yields obtained during the experiment.

Serial reactor	Theoretical CH_4 yield (l CH_4/g-VS)	CH_4 yield (l CH_4/g-VS)	Utilized potential (%)
R0+R55	0.490	0.345±	70
R0+R37	0.490	0.335±	68
R0+R15	0.490	0.313±	64

Activity Test

Methanogenic activity tests revealed that the degree of degradation for the tested substrates increased with increase in temperature from 15 to 55°C. Higher rates of degradation for acetate, H2/CO2, butyrate and propionate were noticed in R55 and R0, which were both operated at 55°C than in R15 and R37. However, a higher acetate activity was noticed in R37 than in R55. No or very low degradation rate was noticed for all the tested substrates in R15.

FISH analyses

FISH analyses revealed some difference in microbial ecology in the three reactors. CLSM picture showed the presence of fermentative bacteria, *Methanosaeta* and hydrogen utilizers in R0, R55 and R37 (Fig. 6). On the other hand, *Methanosaeta* was completely absent while only a few hydrogen utilizers and fermentative bacteria were noticed in R15 (Fig. 7).

Results from pulse experiment

On day 94 of the experiment, pulse load with oil was carried out in R0. A two fold increase in biogas production (Fig. 8) apparently due to higher organic load and subsequent production of VFAs was noticed. A similar process was also noticed in R55, R37 and R15 fed after the pulse load. However, the effect was more pronounced in R55 and R37 than in R15.

DISCUSSION

Comparison of the three after storage reactors at steady state

The results from the present study showed that temperature in the after storage reactors during anaerobic serial digestion of manure had a profound influence on process stability, biogas production and microbial dynamics. The extra biogas production of 8.4-11.7% with a utilized potential of 68-70% (R0+R55/37) reveals that in a serial CSTR process, after storage reactors should either be operated at temperature similar to main reactor's or at higher process temperatures of 37°C.

The more or less similar methane equivalents at 55 and 37°C suggest that the rates of hydrolysis and acetogenesis were equal while methanogenesis seems to be working at optimum in both R37 and R55. On the contrary, the low methane yield in R15, although total VFA levels in R15 were similar to those of R37, suggests that methanogenesis was inhibited. Further, the low (126%) methane equivalent in R15 indicates that not only the methanogenesis but also the hydrolysis and the acetogenesis were inhibited although the effect was more severe for methanogenesis. This was also evident from the low (1.2%) additional biogas production compared to R0 and the high variation in acetate concentration in R15, which exceeded 0.8 g/l at some time during steady state period. The probable reason for low methanogenic activity could be due to the inability of the methanogenic bacteria to be active at pyschrophilic temperatures. The pH distribution in the steady state period can support these observations. The optimum for most methanogenic bacteria is between 7 and 8 and the pH in R15 was just above 8.

The low VFA concentration and rather constant and high biogas production in R55 compared to other after storage reactors suggests that VFA concentration in R55 did not exceed the threshold limits of the process imbalance parameters (0.8g/l for acetate and between 0.1 and 2.5g/l for total VFA). However, the high free ammonia levels in R55 (1.1 g-N/l) suggests that the process might have been slightly inhibited. These free ammonia levels in R55 were within the limits that were reported to cause process inhibition (Hansen et al. 1998). Nevertheless, this does not seemed to be very significant based on the remaining results. On the other hand, the lower biogas production in R37 than R0 was probably due to inhibition of hydrolysis. This was indicated by the amount of VFAs in R37, which was slightly lower than that of the main reactor, R0.

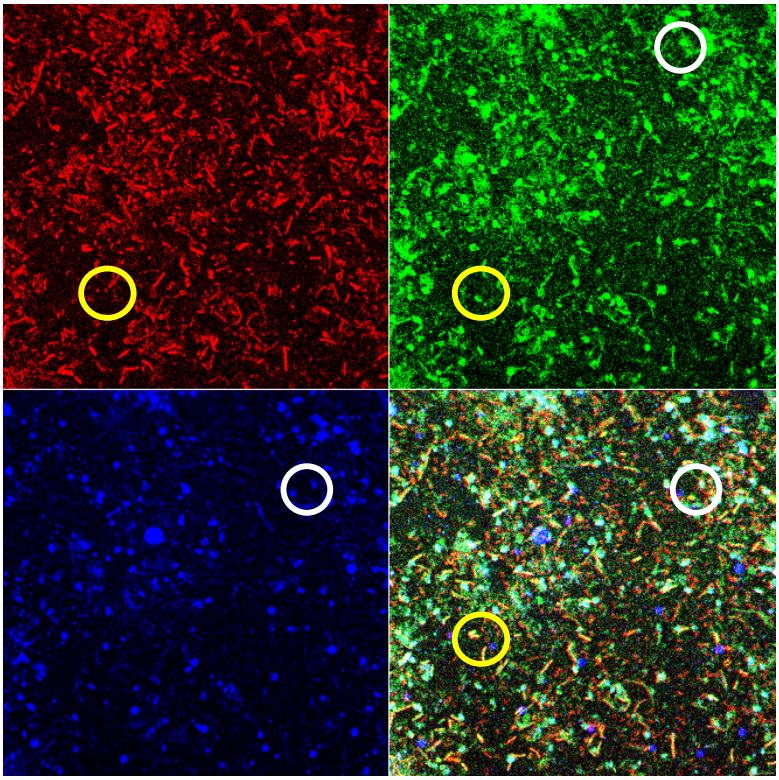

Figure 6 Picture from CLSM of sample from R0. The yellow rings show *Methanosaeta* and the white rings show hydrogen utilizers.

The slightly higher pH in R15 than in R37 and R55/R0 was in accordance to the lower VFA in the system. The study thus suggests that it would be unnecessary to build an additional second step in the biogas process if the after storage reactor has to be operated at 15°C. However, if the after storage reactor has to be heated, then arises the question whether it is technically feasible and economically viable to heat the reactor to around 55°C or if 37°C is sufficient. This is ultimately a question of economical estimations in the full scale biogas plants.

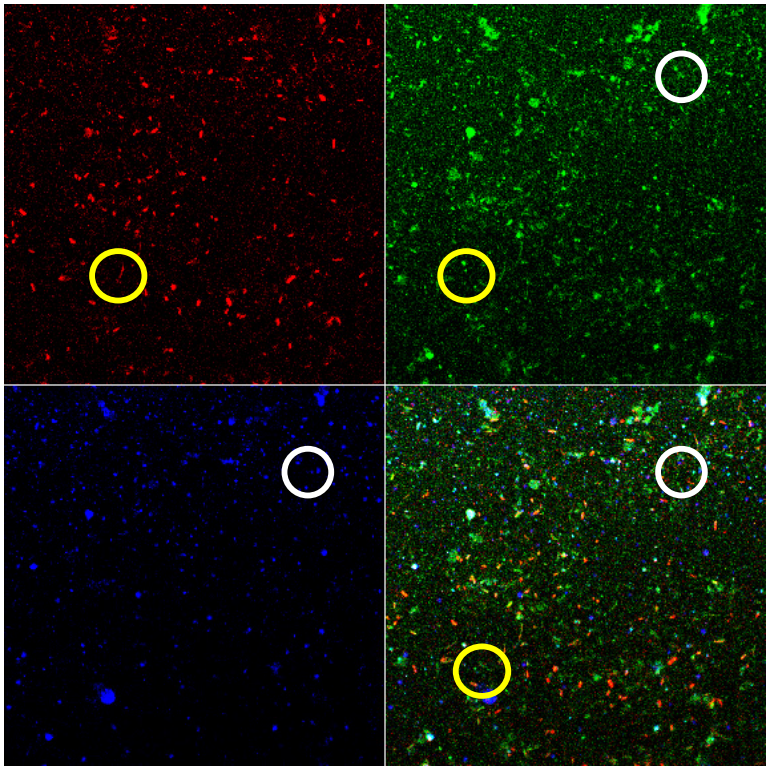

Figure 7 Picture from CLSM of sample from R15. The yellow rings show *Methanosaeta* and the white rings show hydrogen utilizers.

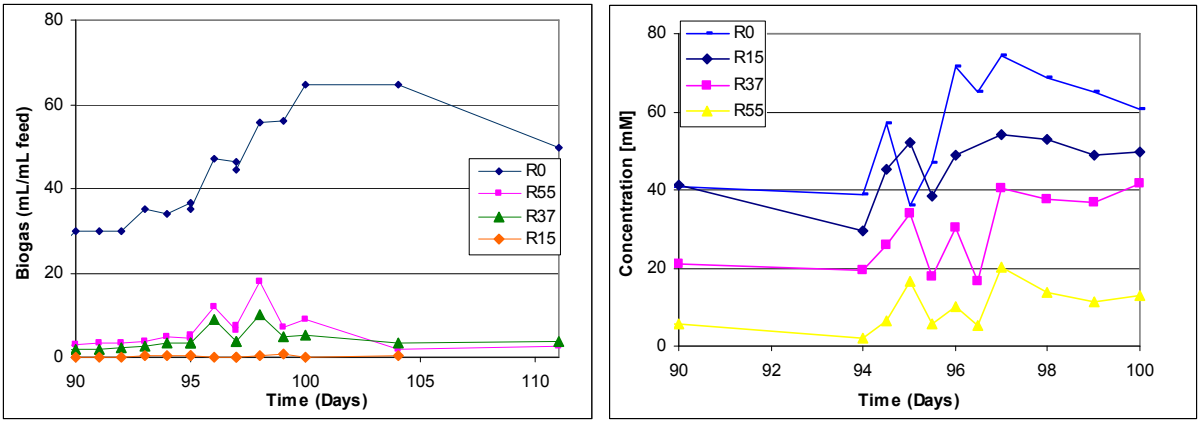

Figure 7 Biogas (left) and VFA (right) production during pulse experiment

Activity test

The results from the activity test showed a good correlation to the results obtained during the steady state. The high initial degradation rates for all tested substrates in R37, R55 and R0 suggested that both acetogenesis and

methanogenesis were working well. Moreover, the initial glucose activity in R37, which was as efficient as that in R55, indicates that hydrolysis and fermentation rates were equally good in these reactors. The high activity for acetate compared to other substrates such as H_2CO_2, butyrate and propionate in R37 than in R55 and R0 suggests that methanogenesis was slightly inhibited in R37. Conversely, the low activity for glucose at the beginning in R15 suggests that hydrolysis and fermentation rates were much lower in R15 than in R55 or R0. No methane production or very low degradation of butyrate, propionate, acetate and H_2CO_2 in R15 suggested that methanogenesis was severely inhibited.

FISH

FISH analyses revealed that R0, R37 and R55 had similar microbial consortia. The presence of a large number of fermenting bacteria, *Methanosaeta* and hydrogen utilizers in R0, R37 and R55 indicates that acidogenesis and methanogenesis were working properly in these reactors. On the contrary, the complete absence of *Methanosaeta* in R15, although few microorganisms that look like *Methanosaeta,* which were not synonymous with an active population of *Methanosaeta*, suggests that *Methanosaeta* was probably washed out from R15. Absence of *Methanosaeta* and presence of a few hydrogen utilizers in R15 than other reactors in fact explains the reason for almost no biogas production from R15. The presence of fermentative bacteria in R15 was however evident from the activity test, which showed that the hydrolysis and fermentation of organic compounds to VFA continued in R15 but at a lower rate than the other reactors.

Pulse experiment

Results from pulse load tests suggested that gas production could be enhanced and effect of the pulse load could last for about 5-7 days. Based on the gas production data, it could be presumed that R0, R37 and R55 are capable to handle a high VS overload. On the other hand, the process still seems to be unstable with respect to the amount of VFA produced. The low gas production in R15 suggests that R15 is very sensitive to pulse load. Unfortunately the experiment was not run for long enough to get results to clarify whether the reactors were able to overcome the extra VS load or not.

Conclusion

Results from the present study showed that process performance, microbial abundance and biogas production from the second step in a serial CSTR process was influenced by process temperature. Stable biogas production of 24.6 ml/ml feed with low VFA levels were noticed in R0, the main step in the process when operated at 55°C. Among the second step reactors, 2.1 and 2.9 ml/ml feed of

biogas was produced when the temperature was maintained at 37 and 55°C respectively. The additional biogas that could be contributed to the whole system was 11.7% and 8.4% by R55 and R37 respectively. On the contrary, no biogas was produced at 15°C indicating that the process would be inhibited very much at temperatures lower than main reactors' temperature. The process in R37 and R55 was relatively stable with slightly higher free ammonia concentration in R55 (1.1 g-N/l) and VFA levels in R37. Activity tests revealed that R0 had generally higher degradation and methane production rates than R55 followed by R37 and R15, with the latter having almost no methane production and low hydrolysis. These results were further confirmed by the FISH analysis which revealed that R0, R37 and R55 had similar microbial consortia. Hydrogen utilizers and *Methanosaeta* as well as fermentative bacteria were present in abundance at 37 or 55°C while the species were very much limited at 15°C.

Experiment 1.

1.3. Residual methane potential of digested manure from single step- and serial-CSTR processes.

Objective

To compare the process performance and conversion efficiency of single step CSTR (R1) with serial CSTR process (R2 and R3).

Methodology

Effluent samples were collected from R1 and R2 (day 160) and R3 (day 161) when the reactors were operated at 70/30 serial CSTR configuration.

Experimental set-up

The experiments were carried out in a 118 ml serum vials. To each assay, 50 ml digested material were transferred. No inoculum was used. The headspace in the bottles was then flushed with a mixture of N_2/CO_2 gas mixture (80/20 ratio) and sealed immediately with butyl rubber stoppers and aluminium crimps. The experiment was run in duplicate at 55°C. Methane production was measured in the headspace of the vials using GC (FID method).

Results and discussion

The residual methane potential experiment was run for 90 days (Fig. 1). Methane production started immediately in all assays and reached maximum after 73 d of incubation. The residual methane potential of one-step CSTR process was 113 ml/gVS_{added} (3.5 ml/ml sample) while that of serial digestion (70/30% volume distribution) was 99 ml/gVS_{added} (3.1 ml/ml sample). The low residual methane potential loss from serial CSTR process (0.4 ml/ml sample) than from single step CSTR process indicate that serial CSTR process had higher utilized methane potential than single step process.

Table 1. Residual methane potential and utilized methane potential of single step CSTR reactor (R1) and serial CSTR process (R2 and R3) with 70/30 volume distribution.

Parameter	Single step CSTR process	Serial CSTR process	
	R1	R2	R3
Residual methane production (ml/ml sample)	3.5	4.1	3.1
Expt methane production (ml/ml feed) [1]	19.8	19.3	2.6

[1]mean values during days 150 to 163 see section 1.1 of Expt 1;

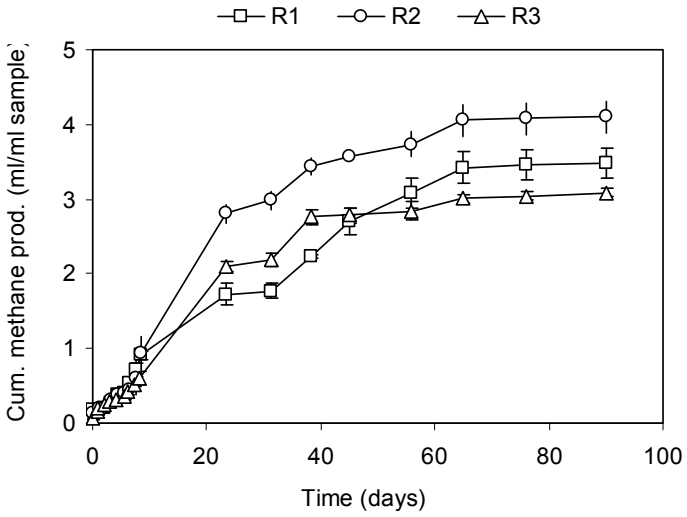

Fig. 1. Residual methane potential of digested material from single step CSTR process (R1) and serial CSTR process (R2 and R3) incubated at 55°C.

Experiment 2

2.1. Effect of temperature and microbial activity on passive separation of digested cow manure

Background

During non-stirring period in the reactor manure settles and stratification of solids takes place. Along with the solids, microbial distribution also occurs as microorganisms are often following the solid matter. This natural stratification could be employed as an operational strategy to improve the methane production from the manure by retaining the solids in the reactor for a longer time than average. Such an operational strategy would not only retain a larger proportion of VS effectively for more complete breakdown but also retains useful population of viable anaerobic bacteria in the digester (Zitomer et al., 2005). In the present study, the effect of temperature (10 & 55°C) and the importance of microbial activity, resulting in biogas formation, on passive separation of digested cow manure were investigated in order to optimise biogas production from manure.

Materials and methods

Digested cow manure from a centralized biogas plant (Snertinge, Denmark) operated at 52.5°C with a 22 d HRT was collected and stored at 4°C for further use. The characteristics of the digested cow manure are presented in Table 1.

Table 1. Characteristics of digested material used in passive separation experiments.

Parameter	Column A	Column B	Column C
TS (%)	3.9	3.9	4.1
VS (%)	2.7	2.9	2.7
pH	7.9	7.9	7.9
Total Kjeldahl-N (g/l)	2.1	2.5	2.0
NH_4^+-N (g/l)	1.5	1.4	1.4
Total VFA (mg/l)	1298	1423	600
Acetate	993	1031	153
Propionate	150	226	100
Iso-Butyrate	42	45	39
Butyrate	29	26	99
Iso-valerate	71	81	52
Valerate	12	14	157
Protein (g/l)	3.8	6.9	3.8
Carbohydrate (g/l)	69.2	24.6	44.3

Note: Column C material was autoclaved at 120ºC for 20 minutes.

Experimental design and set-up

The experiment was conducted in three plexiglass settling columns of 1 m deep and 9 cm inner diameter.

- Column A was filled with digested manure and incubated at 55°C. This column should indicate how digested manure would sediment during the biogas production. Biogas bubbles rising to the headspace of the column might actively influence the sedimentation process.
- Column B was filled with digested manure and was incubated at 10°C. By this column it should be possible to differentiate the influence of temperature on the sedimentation process.
- Column C was filled with autoclaved (120°C for 20 min; Systec V-65, Holm and Halby) digested manure and was incubated at 55°C. By this column experiment we expected to see whether microbial activity (and biogas production) would have influence on the sedimentation process.

Incubation temperature in Column A & C was maintained at 55°C by circulating warm water through the water jacket (silicon tube) fitted around the column and controlled thermostatically. In the unjacketed column B, material was allowed to cool and settle at 10°C in a temperature controlled room. Open end of the columns was sealed with a two hold rubber stopper. Outlets provided on the top of the rubber stopper were used for flushing the headspace of the column with nitrogen (70%) gas. Prior to the start of the experiment digested manure stored at 4°C was pre-incubated to 55°C for 1-2 d in order to reactivate the inoculum.

Sampling procedure

In all experiments, *t=0* samples were taken prior to filling the columns. Samples (20 to 40 ml) were collected from 3 zones: at surface (top), at 60 cm (middle) and 97 cm (bottom) of column depth. Sampling intervals were 15, 30, 60 and 120 min (short time) and after 1, 5, 9 and 15 d (long time). Short time intervals would identify the optimum time interval for effluent pumping before stirring could resume. While the long time interval would represent the maximum settling that would likely to happen in a 15 d HRT reactor. Top samples were always taken before the lower ones using a disposal syringe. Samples from middle and lower layers were collected through the sampling port fitted to the column wall. Each sampling port, made of a steel tube (10 cm length and 10 mm diameter), consisted of two valves (diameter ¾ inch) fitted on either end. Before each sampling, material retained in the tube was scraped out and ca. 10 ml of initial volume flowing out was discarded. Material flowed by gravity into the sampling

tube was then collected into a sample bottle and stored at 4°C for further analyses.

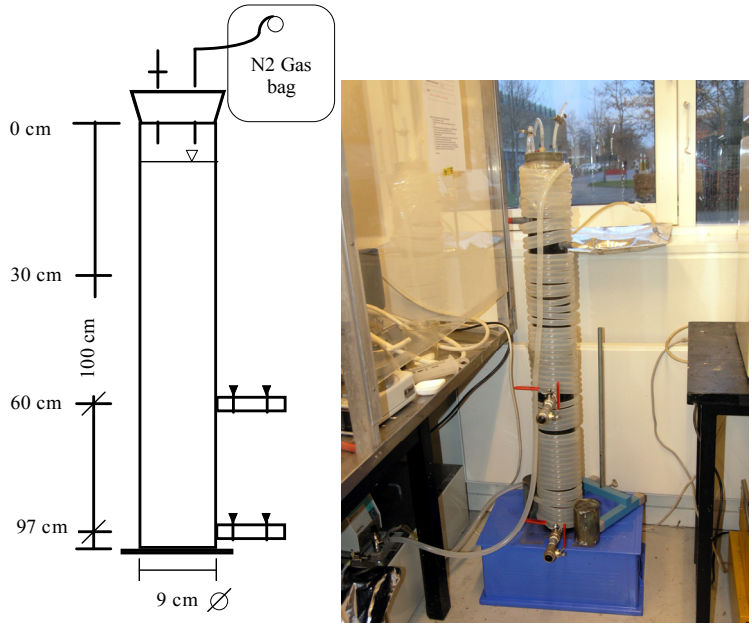

Fig. 1. Experimental set-up to study the effect of temperature and microbial activity on the passive separation of thermophilically digested cow manure.

Analyses

Samples were analyzed for TS, VS, Total Kjeldahl-N (TKN) and ammonium nitrogen (NH_4^+-N) according to Standard Methods (APHA, 1998). Total volatile fatty acids (VFA) concentration were determined by using a gas chromatograph (HP 5890) equipped with a flame ionization detector and HP FFAP column (dimensions 30 m · 0.53 mm · 1.0 μm).

Results

Effect of temperature on passive separation of digested cow manure.

The effect of temperature (10 and 55°C) on passive separation of the digested cow manure is shown in Figure 1 and 2. In both Columns, settling started immediately and continued throughout the experiment. The greatest degree (distance between the values in the individual sampling points with respect to mean sample value) and rates (how quickly separation takes place) of passive separation took place when the temperature during the settling was maintained at 55°C (Column A) than at 10°C (Column B). In Column A, most readily

settleable solids started to settle in about 15 min and reached maximum after 24 hrs although some additional settling occurred for hours. On the contrary, in Column B, peak settling was noticed after 60 min of incubation. Within the columns, the rates and degree of settling varied with depth and incubation time. In Column A, separation process was more pronounced in the upper layer than at the bottom layer while the opposite was true with Column B. Settling rates in the middle layer increased steadily for up to 2 hrs in Column B and up to 24 hrs in Column A and thereafter decreased in both Columns respectively.

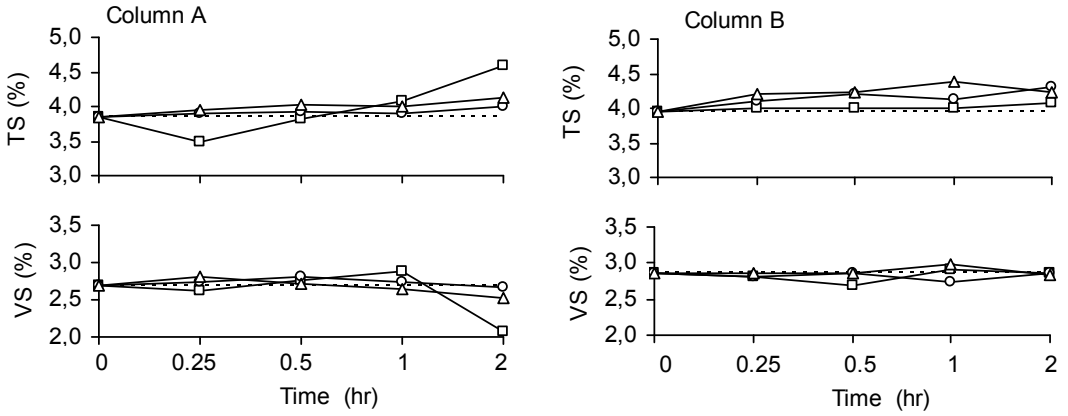

Fig.1 Changes in solids content at □ top, ○ middle and Δ bottom layers with respect to mixed sample (dotted line) of digested cow manure incubated (2 hrs) statically under anaerobic conditions at constant temperature of 55ºC (Column A) and 10ºC (Column B).

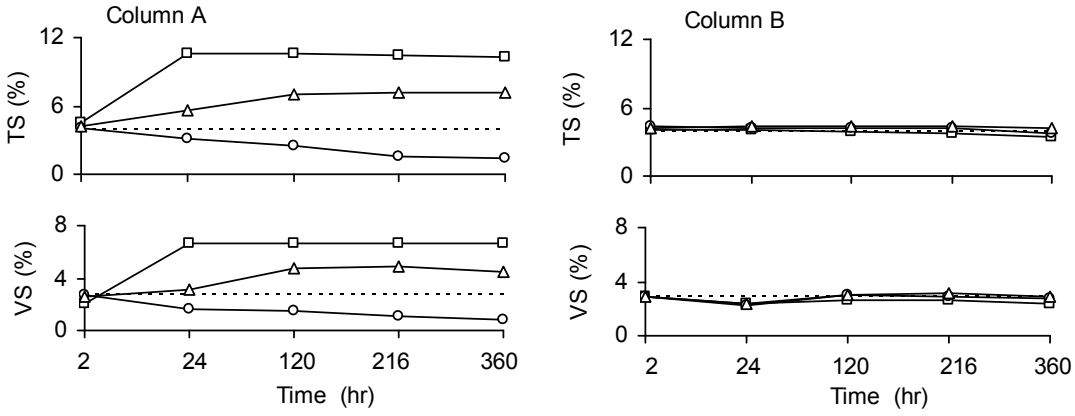

Fig.2 Changes in solids content at □ top, ○ middle and Δ bottom layers with respect to mixed sample (dotted line) of digested cow manure incubated (up to 360 hrs) statically under anaerobic conditions at constant temperature of 55ºC (Column A) and 10ºC (Column B).

The mean highest TS of 10.6% and VS of 6.6% were achieved after 24 hrs of incubation in the top layer of slurry held at 55°C (Column A). Correspondingly, the highest TS and VS values in Column B were 4.4% and 3% respectively and were noticed in the bottom layer after 1 hr. When compared with mixed sample values, the increase in TS and VS was 63.5% and 59.9% respectively at 55°C, whilst the increase at 10°C was 10.5% for TS and 4.7% for VS.
Acetate was the predominant VFA in both Column A and B, and constituted ca. 60 to 80% of the total VFA. TVFA levels in Column A decreased steadily from the initial value of 1298 to 240 mg/l within 1 hr and thereafter increased to reach a maximum value of 1387 mg/l after 5 d. A similar trend in TVFAs levels was also noticed in Column B. TVFA levels in Column B decreased more sharply from the initial levels of 1422 to 130 mg/l within 2 hrs and thereafter increased steadily to reach the highest levels of 725 mg/l at the end of the experiment.

NH_4^+-N stratification also varied with time and depth. NH_4^+-N concentration in top sampling point of Column A increased during the initial 30 min (from 1.5 to 2 g N/l) and thereafter dropped dramatically, reaching a value of 1 g-N/l. A similar trend was also noticed in the bottom sampling point but the NH_4^+-N levels were much lower (28%) than those noticed in the top layer. On the contrary, NH_4^+-N content at the middle layer remained more or less unchanged. NH_4^+-N concentration in Column B was more or less the same at the three sampling points and showed an increasing trend with incubation time. The highest concentration of 2 g NH_4^+-N /l was noticed after 1 d, especially in the top layer.

Effect of inactivation of microorganisms on passive separation of digested cow manure.

Results on the effect of inactivation of microorganisms on passive separation of digested material at 55ºC (Fig. 3) showed that autoclaving (120ºC for 20 min) had affected significantly the biological and chemical characteristics of the treated manure. Autoclaving apparently resulted in the decrease of VS from 2.9 to 0.75% (26%). Upon incubation, significant separation took place only after a lag period of 30 min. Highest TS of 11% was noticed in the bottom sample after 1 hr of incubation. When compared with mean sample values, TS increased by 1.2 folds in the bottom sample, whilst TS decreased by 3.4 folds in the top sample. Nevertheless, after 2 hrs of incubation, a gradual increase in VS over mean sample value was noticed (4.81%) in the bottom sample. On comparison with mixed sample VS values, the increase in VS concentration was 38.5% at the bottom layer. While a more dramatic decrease in VS was noticed in the middle and top layers.

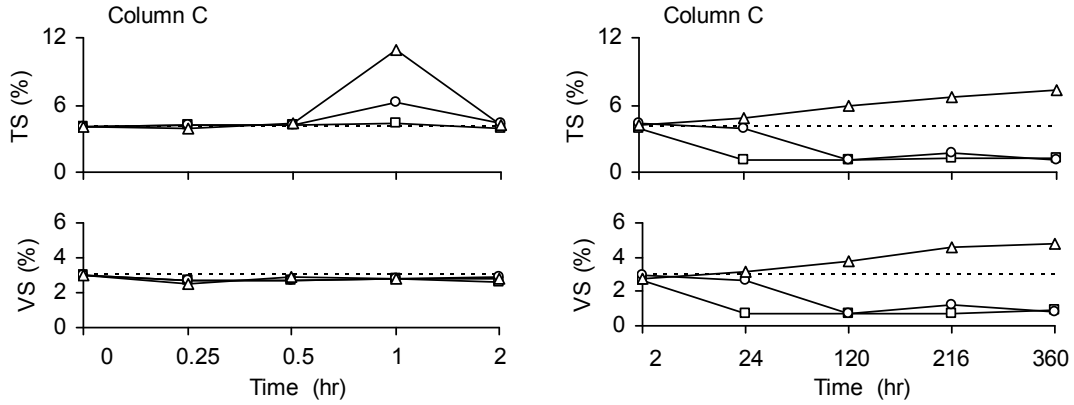

Fig.3 Changes in solids content at □ top, ○ middle and Δ bottom layers with respect to mixed sample (dotted line) of autoclaved digested cow manure incubated (up to 360 hrs) statically under anaerobic conditions at constant temperature of 55ºC.

Although NH_4^+-N concentrations increased slightly with increase in sampling depth (data not shown), the values in Column C were however significantly below the mixed sample value. The highest NH_4^+-N concentration of 0.973 g-N/l was noticed at 60 cm depth after 1 d.

Acetate was the predominant VFA in Column C. Interestingly, TVFA levels at the three depths showed more or less a similar trend. After the initial slump from 600 mg/l to 237 mg/l (30 min), TVFA levels remained more or less the same for up to 2 hrs before increasing sharply to reach 1582 mg/l at the end of the run.

Discussion

The results of the present study showed clearly that some passive separation occurred both at 10 and 55°C even though the digested material had already produced significant amounts of methane under normal full-scale digestion conditions (HRT approx. 20 d at 52.5°C). The higher rates and degree of separation along with continued settling process at 55°C than at 10°C suggests that higher temperature and lower viscosity, and probable higher biological activity at 55°C aided the separation process. This was further confirmed by the results from the Column C, where the advantage of an increase in viscosity due to high temperature was outweighed by the absence of flotation and gas production due to inactivation of microorganisms.

The small degree of settling noticed in Column B was caused probably by the low temperature (10°C) of the material and consequently the higher viscosity of the already pre-incubated digested material (55°C). The low degree of

separation in the upper layer of Column B was because biological activity and gas production, which were stopped quickly, so mixing and gas floatation effects were reduced. On the other hand, the high degree of separation at the bottom layer was due to sedimentation of smaller heavier solids by gravity.

Samples taken from Column A after 24 hrs settling time showed clear visual difference between top and bottom layers. The top layer appeared to contain light, fibrous particles and was almost fluffy in texture, with many entrapped gas bubbles. The bottom sample was relatively sludgy when compared with the mixed sample, and appeared to contain smaller, denser particles. These observations are much as would be expected, with gas bubbles (by floatation) bringing the larger cellulose-based particles to the surface and the smaller, denser particles settling to the bottom (by gravity). On the other hand, no visible changes were observed during the tests in Column B or C neither through the plexiglass column walls as changes in colour or fibre distribution, nor as differences in the consistency of samples taken from the top and bottom depths.

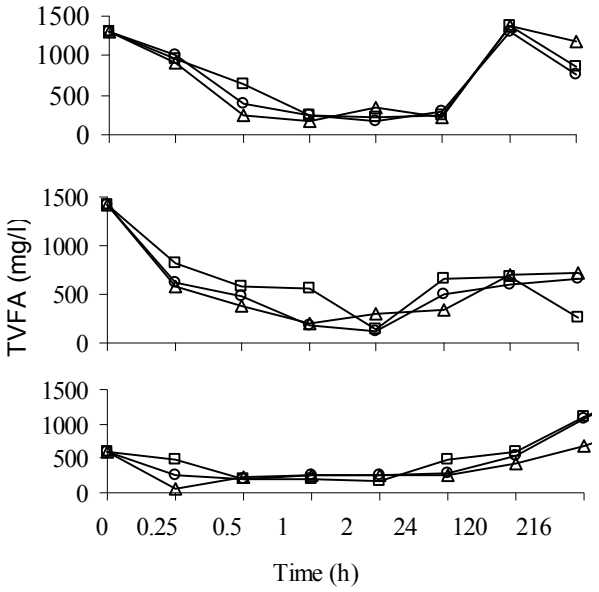

Fig. 3. Total VFA content at □ top, o middle and Δ bottom sampling points during passive separation of digested cow manure in column A and B and autoclaved digested cow manure in column C incubated statically under anaerobic conditions at 55, 10 and 55ºC respectively.

The present results in practice suggests that biogas production can be optimized if the unmixed period could be used effectively as a means of cell and solid material retention within the digester, without the need for any additional

settling and recycling equipment. A minimum of 2 hrs unmixed period is essential before pumping out the effluent from the middle layer. If treated and the least polluting slurry was discharged from the middle level of the digester, at the end of the "unmixed" period, then the upper and lower most productive zones would be retained for further treatment. No extra equipment would be required, and the different retention times desired for the solid and liquid would be achieved. Some sludge build-up may occur in the bottom of the digester, but this could probably be discharged of or controlled by occasional discharging from the bottom rather than the middle, or periodically discharging from the digester whilst the contents were mixed. Thus, passive separation of digester contents within the digester can be used effectively as an operating strategy in a full-scale, small digester to achieve increased biomethanation.

Conclusion

The results of the present study showed that temperature had profound influence on passive separation of digested manure obtained from a full-scale biogas plant. Higher rates and degree of separation occurred in slurry incubated at 55°C than at 10°C. Separation rates also varied with the depth of the column. Maximum TS of 10.6% and VS of 6.6% were achieved at 55°C after 24 hrs of incubation in the top layer. The correspondingly values at 10°C were 4.4% and 3% respectively but noticed in the bottom layer after 1 hr. The increase in TS and VS over mean values was 63.5% and 59.9% respectively at 55°C and 10.5% for TS and 4.7% for VS at 10°C. Inactivation of microorganisms resulted in loss of VS and poor separation. Thus, for effective passive separation within the reactor a minimum of 2 hrs of unmixed period should be the optimum before effluent pumping. Effluent should be pump out from the middle layer (high in ammonia nitrogen and low solids content) while retaining the top and bottom layers.

Experiment 2

2.2. Effects of mixing strategy on methane production from manure in CSTR

2.2.1 Lab-scale studies

In the present study, the effect of mixing strategies on process performance and methane production in CSTR fed with cow manure was investigated. Three different mixing strategies viz., continuous mixing, minimal mixing (10 minutes before feeding) and continuous mixing but withholding mixing for 2 hours before feeding were tested.

Background

Mixing of digester contents creates a homogeneous substrate preventing stratification and formation of a surface crust, and ensures solids remain in suspension. Bacteria, substrates and liquid consequently have an equal retention time where solids retention time (SRT) will be equal to hydraulic retention time (HRT). Further, mixing also enables heat transfer, particle size reduction as digestion progresses and in release of produced gas from the digester contents. Despite the importance of mixing in achieving efficient substrate conversion, optimum mixing pattern and duration of mixing is a subject of much debate.

Materials and methods

Substrate preparation

Fresh cow manure collected from a full-scale biogas plant (Snertinge biogas plant, Denmark) was used as substrate. To prevent blocking of feed tubes, substrate was blended using a kitchen blender (Braun, Germany). The homogenized manure was transferred into 2 l containers and frozen at -20ºC until further use. Frozen manure portions were thawed at room temperature and the prepared feed was stored at 4ºC for 2-3 days. Feed was prepared once or twice in a week by diluting fresh manure with distilled water in a 1:1 ratio.

Experimental setup

Three CSTR reactors referred to as R1, R2 and R3, with a working volume of 3.6 l were operated at a 15 d HRT. Thermophilically digested inoculum from full-scale biogas Plant (Snertinge, Denmark) treating livestock manure and industrial organic wastes was used as inoculum. Each reactor system consisted of a feed bottle, a CSTR reactor, a feed pump, an effluent bottle and a gas meter (Fig. 1). Reactors were built from double glass cylinder (5 l) fitted with stainless steel plates as top and bottom. The top plate supported the mixer, mixer motor, feed

tube, and effluent tube, temperature measuring port and sampling port. The bottom plate had one sampling port. Stable reactor temperature was maintained at 55°C by pumping hot water, from an electrically heated thermostatic water bath, in the space between the reactor glass walls. All three reactors were fed semi-continuously at 12 hour interval by pumping the feed from feed bottles into the reactors (top end). An equal amount of effluent was removed automatically due to the pressure developed from the produced biogas and the added feed. The effluent along with biogas was then collected in the effluent bottle (Fig. 1). Biogas from the effluent bottle flowed to the gas meter to register biogas production. The produced biogas was measured by using liquid displacement with a 100-mL reversible cycle and registrations, 18 VDC power supply (Angelidaki et al., 1992).

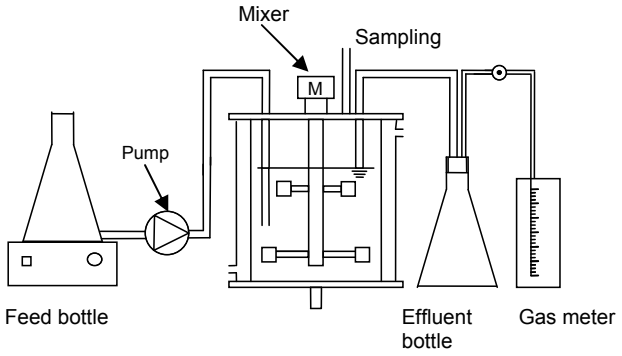

Figure 1 Conceptual drawing of the experimental setup.

Reactor operation

The experiment was carried out for a period of 109 days. To permit a clearer evaluation, the experiment was divided in to four different periods. These include, continuous mixing pre-test period (days 1 to 19), mixing strategies scheme test period (days 20-43) and continuous post-test period (days 44-69).

During the initial 19 days of operation, reactors were mixed in a continuous mode by mechanical mixers operated on a cycle of 40 seconds on and 1 minute off. This was referred as continuous mixing. Upon attaining steady-state under continuous mixing mode, various mixing strategy were introduced on day 20. R2 was operated at minimal mixing strategy (10 minutes mixing before feeding) while R3 was operated at constant mixing but withholding mixing for 2 hours before feeding. From Day 44 onwards, all three reactors were again operated in continuous mixing mode as mentioned above.

Results

Effect of mixing strategies on process performance and biogas production

The process performance and biogas production during different phases of the experiment is presented in Fig. 2 and Table 1. During the steady-state, biogas production was relatively stable with an average production of 10.81, 10.56 and 11.45 ml/ml feed in R1, R2 and R3 respectively (Table 1). At the end of pre-run continuous mixing (day 19), mixing strategies in R2 and R3 were changed to minimal and intermittent mixing respectively while R1 was operated in continuous mixing mode. Biogas production (day 20-43) in R1, R2 and R3 were 9.98, 11.28 and 10.12 ml/ml. On comparison with R1, the increase in biogas production in R2 (minimal mixing) and R3 (intermittent mixing) during the same period was 12.4% and 1.8% respectively (Fig. 3).

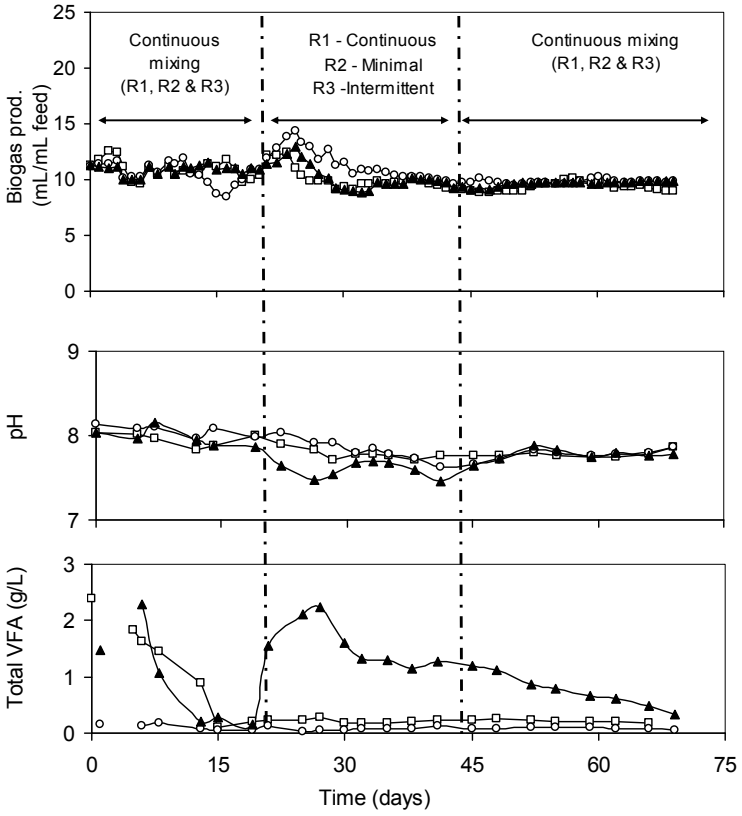

Fig. 2. The effect of mixing strategies on process performance and biogas production during anaerobic digestion of manure in CSTR at 55°C: R1 (continuous), R2 (minimal) and R3 (intermittent stirring).

Table 1. Process performance, biogas production and methane content in biogas during anaerobic digestion of manure in CSTR at 55°C under continuous (R1), minimal (R2) and intermittent mixing (R3).

	Continuous mixing Pre-run	Various mixing strategy	Continuous mixing Post-run
	Day 0-19	Day 20-43	Day 55-69
Biogas Prod. (l/d)			
R1	2.62±0.457	2.42±0.186	2.29±0.188
R2	2.56±0.507	2.71±0.346	2.37±0.346
R3	2.62±0.391	2.45±0.284	2.35±0.218
Biogas prod. (ml/ml feed)			
R1	10.91±1.51	10.03±0.59	9.51±10.61
R2	10.59±2.19	11.28±0.92	9.85±0.51
R3	10.88±1.48	10.15±0.88	9.77±0.39
Increase in gas prod. over R1 (%)			
R2	-	12.5	3.6
R3	-	1.2	2.7
Methane content (%)			
R1	62.8	64.1	61.7
R2	62.7	64.1	61.7
R3	61.6	63.0	61.3
Methane yield (m^3/kgVS)			
R1	0.198±0.035	0.217±0.032	0.180±0.055
R2	0.193±0.048	0.246±0.031	0.187±0.059
R3	0.195±0.035	0.218±0.030	0.185±0.058
Utilized methane potential (%)			
R1	45.5	50.1	41.5
R2	44.3	56.4	43.0
R3	44.7	49.8	42.4
VFA (g/l)			
R1	1.379	0.209	0.210
R2	0.104	0.076	0.087
R3	0.911	1.564	0.786
pH			
R1	7.9	7.8	7.8
R2	8.1	7.8	7.7
R3	7.9	7.7	7.8

VFA levels were slightly higher in R3 (intermittent mixing) than in R1 (continuous mixing) and R2 (minimal mixing). Ammonium nitrogen levels were more or less the same in all three reactors and pH remained stable around 8 throughout this period (Fig. 2).

Mixing strategies also affected the methane yields (Fig. 4). Highest methane yield of 0.246 m^3/kgVS was obtained in R2 compared to 0.218 m^3/kgVS in R1 and 0.217 m^3/kgVS in R3.

The utilized methane potential calculated based on the theoretical methane yield of the fresh manure (data not shown) and the experimental values obtained during the

treatment period (days 20-43) is presented in Table 2. Results showed that R2 had a better utilized potential (61.5%) than R1 (54.5%) or R3 (54.3%).

Table 2. Utilized methane potential during the anaerobic digestion of manure at 55°C in CSTR with continuous (R1), minimal (R2) and intermittent (R3) stirring conditions.

Reactor	Theoretical CH4 yield (m^3/kgVS)	CH_4 yield (m^3/kgVS)	Utilized potential (%)
R1	0.40	0.218	54.5
R2	0.40	0.246	61.5
R3	0.40	0.217	54.3

On day 44, mixing strategy was reverted to continuous mode in R2 and R3. The average biogas production during days 44-69 (continuous post-test period) ranged between 7.90 and 8.64 ml/ml feed (Table 1). The increase in biogas production over R1 was ca. 9% in R3 and 5% in R2. VFA and ammonia levels remained more or less the same in all three reactors.

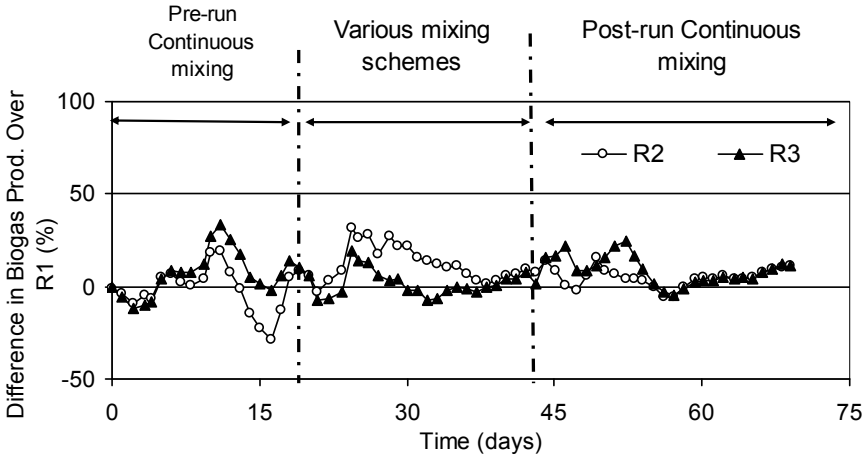

Fig. 3. The difference in biogas production in R2 (minimal) and R3 (intermittent stirring) over R1 (continuous) under different mixing strategies.

Microbiological analyses

FISH analyses results showed the presence of both *Archaea* and *Bacteria* in all three reactors (data not shown). Higher abundance of small rods shaped bacterial cells were noticed in R1 and R3 while long rods were more abundant in R2. Among the methanogens, *Methanosarcinacea* was noticed in all three reactors.

Discussion

Results from the present lab-scale study showed that mixing strategy had some influence on process performance and methane production in CSTR reactors treating cow manure. Among the three tested treatments, minimal mixing was found to improve biogas production compared to intermittent or continuous mixing. The increased methane production under minimally mixed conditions (R2) was probably due to better syntrophic association. These results are in accord with Stroot et al., (2001) who suggested that the improved performance under minimally mixed conditions was related to the difference in feed spatial distribution. It is presumed that in a CSTR with semi-continuous mode of feeding, continuous mixing might promote rapid hydrolysis and fermentation and at the same time affect the syntrophic association between H_2 producing and consuming organisms. This may result in poor performance as the turn over of fermentation products is lower than the rate of formation leading to acid accumulation immediately after feeding. On the other hand, minimally mixed conditions would result in slower hydrolysis and fermentation without affecting the spatial association between syntrophs. This would allow syntrophs and methanogens to consume the fermentation products without any VFA build-up.

Experiment 2

2.2. Effect of mixing strategy on biogas production from manure in CSTR

2.2.2. Pilot–scale studies

Objective

To investigate operational mixing procedures to retain degradable material as well as biomass in a CSTR reactor treating cow manure with an aim to improve biogas production. In this study, the effect of mixing strategy on process performance and biogas production in a pilot-scale plant fed with cow manure was investigated. The mixing strategies investigated were a) continuous mixing (5 min on and 5 min off) and b) intermittent mixing i.e. continuous mixing but withholding mixing for 2 hours before effluent removal from mid level of the digester. The experiment in pilot-scale level was conducted under more realistic conditions in order to support previous lab-scale experiments with blended manure.

Background

Previous studies under EFP 04 project showed that passive separation of digested manure within a reactor was possible, especially at a reactor working temperature of 55°C (refer to EFP annual report 2005). In addition, previous lab-scale experiments (section 2.1.1 of this report) showed that mixing strategy could positively affect methane yields.

Material and methods

Substrate and inoculum

Cow manure was obtained in 800 l batches from a centralized biogas plant (Hashoj biogas plant, Denmark). Batches were obtained directly from incoming delivery trucks (i.e. uncut) and always from the same cattle farm in order to secure as uniform feed as practically possible. Feed was prepared by diluting the manure with water to attain TS of 6.5 to 7.5%. Characteristics of fresh cow manure after dilution are presented in Table 1.

Thermophilically digested material from a centralized biogas plant (Snertinge biogas plant, Denmark) treating manure and industrial wastes was used as inoculum. Inoculum for various lab-scale experiments was collected during stable periods of the pilot-scale reactor.

Reactor setup

The experiment was carried out in a pilot-scale plant built under EFP-04 project. The pilot-scale plant was assembled at the Institute of Environment & Resource, Technical University of Denmark. The Reactor (800 l) was built from a used stainless steel tank. The top of the reactor was fitted with a stainless steel top plate. The top plate supported the mixer, mixer motor, gas sampler, safety and pressure valve and a level switch. Feed valve, effluent outlet valves (3), temperature probe and sampling ports (3) were fitted to the reactor wall. The reactor had one outlet at the bottom for sediment removal. Stable reactor temperature was maintained at 53±1°C by pumping hot water through a stainless steel coil fitted inside the reactor. An electric flow heater (Sparesystem HO 3B, JEVI A/S, Denmark) mounted with an electric cartridge (3 kW; 8 W/cm^2) and a control box (thermostat 30-85°C/thermofuse 110°C) was used as heat source. Hot water was circulated between the heater and the reactor using a circulation pump (Grundfos Alpha+ pump, Grundfos, Denmark). A standard expansion vessel (Type Cubex 2/1, Flamco Flexcon) with a manometer and a safety valve pressurized to an initial pressure of 0.5 bar was also connected to the sealed heating installation with a maximum working pressure of 3 bars.

Table 1. Characteristics of fresh cow manure after dilution (4 batches)

Parameter	Range
TS (%)	6.5-7.5
VS (%)	5-6.3
pH	7.5-7.7
Total VFA (g/kg-waste)	16-18
Acetate (g/kg-waste)	10-11
Propionate (g/kg-waste)	4-5
Iso-butyrate (g/kg-waste)	0.25-0.3
Butyrate (g/kg-waste)	0.95-1.1
Iso-valerate (g/kg-waste)	0.54-0.64
Valerate (g/kg-waste)	0.48-0.53
Total-N (g-N/kg-waste)	3-4
NH_4^+-N (g-N/kg-waste)	1.7-2
Lipids (g/kg-waste)	6.3-7
Glucose (g/kg-waste)	0.45-0.51
Xylose (g/kg-waste)	0.83-0.94
Arabinose (g/kg-waste)	0.09-0.11

Reactor was fed semi-continuously at eight hour interval by pumping the feed from a feed container (800 l). Feed was thoroughly mixed for 15 minutes prior to each feeding using a high speed mixer (Drive^{IT}, 1.5 kW, 950 rpm)). Reactor contents were mixed by a low speed top mounted mixer shaft with two impellers driven by a geared DC motor (0.25 kW, 5000 rpm) operated on a cycle of 5 minutes mix followed by 5 minute stop. The effluent was removed from the middle portion of the reactor. Restch eccentric pumps (RB 30 D) fitted with a standard gear (Nord Gear; SK 01-80L/4; 0.75 kW; Gear ratio: 14.75; rpm 95) were used for pumping feed and effluent. The pumps were operated for 50 seconds every 8 hours with a flow rate of 10 litre minute. Biogas from the reactor was measured continuously using a residential diaphragm gas meter (Gallus 2000, Flonidan DC, Denmark). The pumps and mixers were controlled automatically using relay timers (Timer TC 14, Muller) and a 2 channel 24 hours/7 day programmable time switch (SC 28, Muller).

Reactor operation

The reactor was operated with a liquid working volume of 500 l (total digester volume 800 l) with a HRT of 20 days and at 54±1 °C. At start-up, 450 l of inoculum and 30 l of fresh cow manure were transferred to the reactor. Daily feeding was commenced after approximately 10 days when methane content in the biogas reached 50%. Fresh cow manure was fed to the reactor 3 times a day (8 hour interval) and gradually increased to 25 l/day corresponding to 20 days HRT. Prior to each feeding, an equal amount of effluent was removed from the middle part of the reactor. During continuous mixing, mixer was operated in a 5 minutes on/off mode. While under intermittent mixing, the mixer was operated similar to that of continuous mixing but mixing was completely withheld for 2 hours prior to each effluent removal. Thus, three mixer blocking periods were introduced per day. The length of intermittent mixing period, introduced upon attaining steady-state, varied between 1 (same feed batch) and 2 (two different feed batches) HRTs.

Attempts were made to maintain more or less similar feed characteristics throughout the run by diluting the fresh manure to a nominal TS level (6-7%). However, feed batch changes remained the main source of disturbances. For this reason intermittent mixing periods were sequenced with feed batch changes to obtain periods without feed batch change before/after changing the mixing strategy.

Chemical analyses

The performance of the CSTR was followed by daily measurement of biogas production and digester temperature as well as process parameters such as pH,

VFA, TS, VS and NH_4^+-N by periodic sampling and analysis. Samples were taken from the effluent tube and from the middle layer of the digester representing process parameters. Methane content in biogas and VFA were measured by Gas Chromatograph (GC) using Flame Ionization Detector (FID). Samples were stored at -20ºC until analysis. TS, VS and pH were determined using APHA Standard procedures (APHA, 1998). Total-N and NH_4^+-N were measured following Kjeldahl-N method. Lipids were analysed by Soxhlet method. Sugars were determined using weak acid hydrolysis by High Performance Liquid Chromatography (HLPC).

Calculations

Specific biogas yield was calculated from the detailed data as the daily biogas production, divided by a weighted average of VS feed over a period stretching 8 days backward. The weighted average was defined as effective VS basis for degradation to be represented by 57% of VS feed from the 3 most recent days, 29% of VS from the previous 3 days and 14% of VS from the last 3 days.

Results

After the initial start-up, experiments were carried out over a period of 150 days covering five batches of feed. The results are presented in Fig. 1 and Table 2. In Fig. 1, batch changes and periods with intermittent mixing period are indicated. Fig. 1 illustrates the level of stability obtained. Daily loading (data not shown) was 25 l/day and temperature was maintained at 54±1ºC throughout the period. VFA level was higher during the first part of the experiment (2-4 g/l), probably due to remaining start-up adaptation, but fell to a low and constant level (1 g/l) in the latter part of the run.

Table 2 summarizes the average results subdivided into intervals with same feed batch and mixing strategy. The primary evaluation parameter is average specific biogas yield, which is also shown in Fig. 2. Results (Table 2) indicate that the specific biogas yield obtained during periods with intermittent mixing was 2.5-14.6% higher than those obtained with continuous mixing, with an average value of 7%. In addition, the effluent VS level was generally lower during the periods of intermittent mixing, which indicate that this mixing strategy resulted in stratification of digester content and thus minimized VS loss. Although there were significant variations in biogas yield when changing mixing strategy, the tendency was the same for every change, and thus considered as a statistically reliable observation. Variations observed were most likely the result of other disturbances, temporarily affecting process performance. The period prior to the first change from continuous to intermittent mixing was relatively short and was affected by a disturbance (shift in specific biogas yield) related to feed batch

change. Furthermore, there was a VFA build-up in the initial phase of the intermittent mixing, which may not be related to the mixing strategy, reducing biogas yield during the intermittent mixing period and thus resulting in a relatively small change in yield. Likewise, the relatively large step observed when switching back to continuous mixing (day 32) may be affected by a temperature disturbance shortly before shifting the mixing strategy (which was also the case second time at day 135), and the general shift in VFA level in this period. It was obvious that the 3rd feed batch started at day 70 must have been with VS with higher biogas potential, however the process appear to have stabilized before the second intermittent mixing period was initiated.

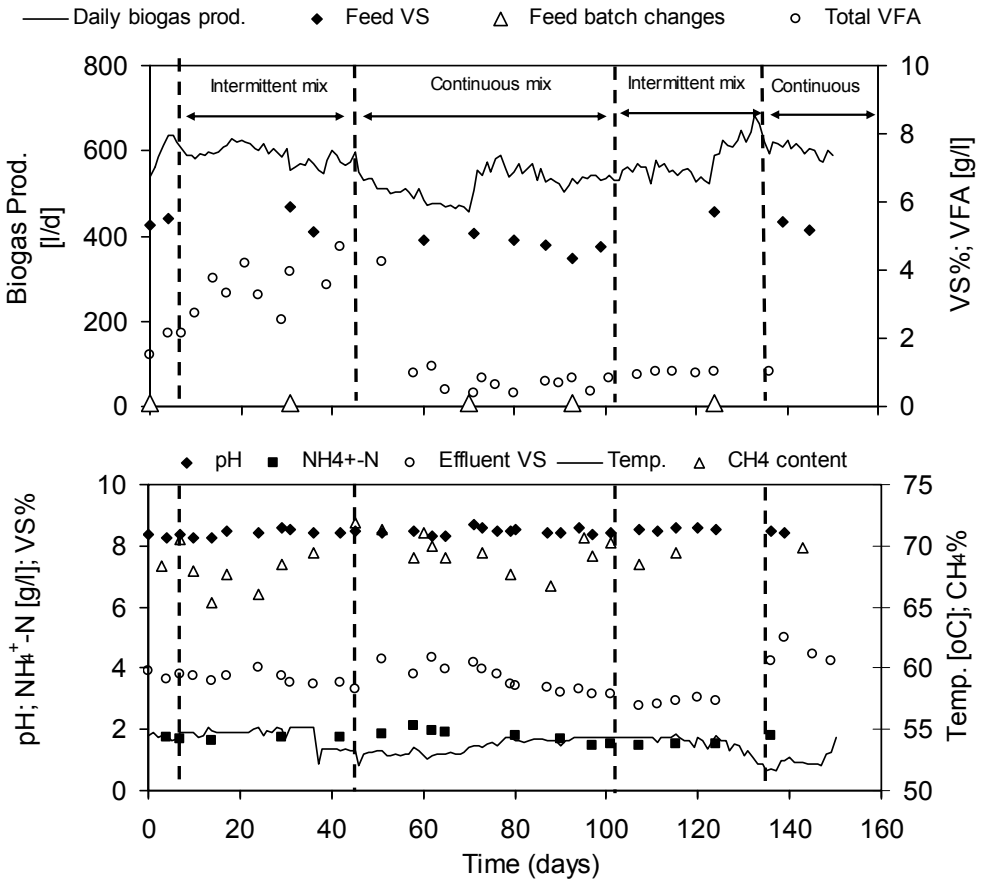

Figure 1. Process performance during anaerobic digestion of manure with continuous and intermittent mixing (mixer blocking) strategies at 55°C.

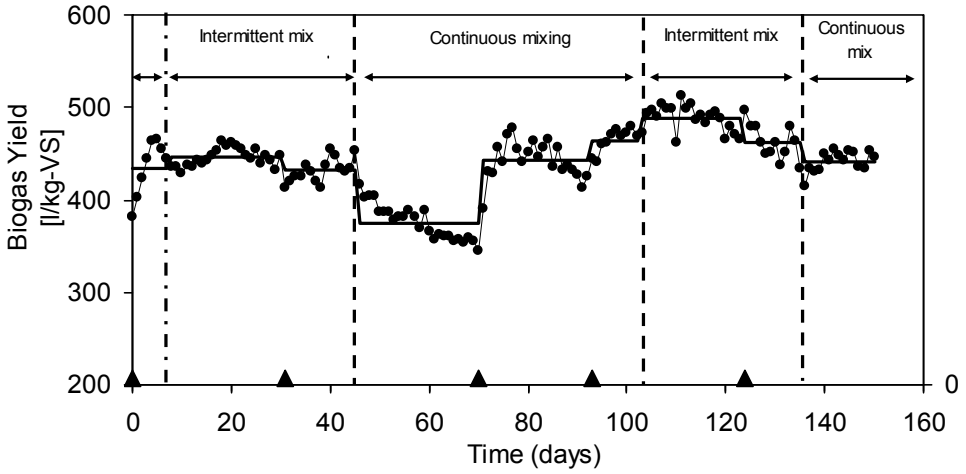

Figure 3. Specific biogas yield during anaerobic digestion of manure with continuous and intermittent mixing (mixer blocking) strategies at 55°C.

Discussion

The results of the present study indicate that mixing strategy can positively affect biogas production in CSTR reactor treating manure. The improved biogas production under intermittent mixing compared to continuous mixing can be attributed to better solids and biomass retention in the reactor. Biogas yield improvement appear to be of the order 7% from the present experiments, which is of significant importance for existing and new full scale biogas plants, as the improvement method involves virtually no cost, except a revised control philosophy for digester mixing. These results are in accord to Dague et al. (1971) who have also reported that shifting from continuous mixing to intermittent mixing (2 min of mixing/h) resulted in significantly higher gas production during the anaerobic treatment of a liquid waste stream. The increase in biogas production in the above study was attributed to improved solids retention due to better bioflocculation in the intermittently mixed reactor. Smith et al., (1996) have also showed that intermediate degree of mixing was optimal for substrate conversion.

Table 3. Process performance during anaerobic digestion of manure in pilot-scale digester, subdivided into characteristic batch/mixing strategy periods.

	Feed batch 1		Feed batch 2		Feed batch 4		Feed batch 5	
	Continuous mixing	Intermittent mixing	Intermittent mixing	Continuous mixing	Continuous mixing	Intermittent mixing	Intermittent mixing	Continuous mixing
Period	Day 0-7	Day 8-31	Day 32-45	Day 46-69	Day 70-102	Day 103-123	Day 124-135	Day 136-150
Biogas Prod. (l/d)	599	603	572	497	540	550	628	602
Biogas prod. (l/l feed)	24.0	24.1	22.9	19.9	21.6	22.0	25.1	24.1
Spec. biogas yield l-CH_4/kg-VS	435	446	432	377	451	465	477	442
Relative yield	=100%	102.5%	114.6%	=100%	=100%	103.1%	107.3%	=100%
Methane content (%)	69.4	67.1	70.7	70.1	69.0	69.0	69.4	69.9
Effluent VS (%)	3.79	3.77	3.48	4.10	3.52	2.89	2.95	4.48
VFA (g/l)	1.8	3.2	4.1	1.8	0.7	1.0	1.0	1.0
Temp. (°C)	54.5	54.8	54.0	53.0	54.1	54.2	53.2	52.5

The choice of a 2 hour mixer blocking period ("non-stirring") was based on previous settling experiments (EFP -04 report, 2006) showing that most stratification of solids occurred within this period, but also considering practical possibilities on most full-scale biogas plants operating with discharge intervals in the range of 4-10 hours. During this "non-stirring" period, the lighter fiber fraction floats to the surface (by floatation) while the heavier solids settle to the bottom (by gravity) leaving the middle portion with low solids content. Removal of effluent from the middle layer with lowest solids content at the end of the "non-stirring" period had resulted in retaining the solids from upper and lower layers with higher VS content for further degradation and minimizing effluent loss. Previous research also showed that optimal biomethanation of cattle manure was obtained when the effluent discharge point was at the middle of the liquid level in a biogas plant (Schofield and Rees 1988; Ong et al., 2000). Hence, separation of digester contents within the reactor through withholding mixing can be used effectively as an operating strategy to optimize biogas production in full-scale plants. However, care should be taken when operating at high TS/VS mixtures as high straw/fiber content can lead to formation of solid floating layer which may overload mixer when starting after a "non-stirring" period.

Moreover, accumulation of solids in the bottom can reduce the effective working volume. To avoid these two operational problems, effluent should be discharged either from the middle layer with reactor periodically operated in continuous mixing mode or by occasional discharging from the bottom rather than the middle layer

Experiment 2.

2.2.3. Residual methane potential of samples from pilot-scale plant under contnuous and intermittent mixing

Residual methane potential test was performed with the samples taken from the three different depths of the reactor in order to evaluate the reactor utilization efficiency during continuous and intermittent mixing of the pilot-scale plant.

Methods and materials

The experiments were carried out in a 118 ml serum vials. Samples were collected during day 104 (continuous mixing) and day 135 (intermittent mixing) of the reactor operation (Section 2.1.2 of experiment 2). To each assay 20 ml digested material from top, middle and bottom layer were transferred. The headspace in the bottles was then flushed with a mixture of N2/CO2 gas mixture (80/20 ratio) and sealed immediately with butyl rubber stoppers and aluminium crimps. The prepared assays were incubated at 55ºC. Methane production was measured in the headspace of the vials using GC (FID method).

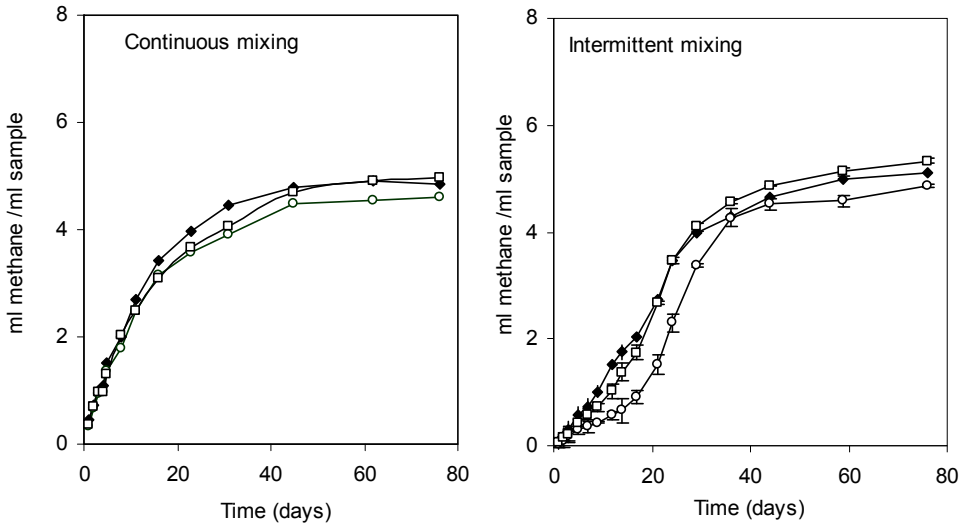

Figure 1. Residual methane potential of samples taken from (♦) top, (○) middle and (□) bottom depths of pilot-scale reactor operated during continuous and intermittent mixing.

Results and conclusions

The experiment was carried out for more than 76 days and the results are shown in Table 1 and Figure 1. Overall, methane production started immediately in all assays. Residual methane potential of samples during continuous mixing ranged between 4.6 and 5 ml/ml sample, with a slightly low methane potential from middle layer. The methane potential of samples obtained during intermittent mixing ranged between 4.86 and 5.33 ml/ml sample. On comparison with continuous mixing period, higher residual methane potential noticed from the samples collected during intermittent mixing. These results indicate stratification of solids within the reactor during intermittent mixing (2 hr withholding mixing prior to feeding). Thus, removal of effluent from the middle layer with lowest solids content at the end of the "non-stirring" period would result in retaining the solids from upper and lower layers with higher VS content for further degradation.

Table 1. Residual methane potential of samples taken from top, middle, bottom depths and effluent tube of pilot-scale plant operated during continuous and intermittent mixing.

	Continuous mixing			Intermittent mixing		
	Top	Middle	Bottom	Top	Middle	Bottom
Methane potential (ml/ml sample)	4.86	4.6	4.96	5.11	4.86	5.33
[1]Comparison to reactor production (%)	20.9	19.8	21.4	21.3	20.2	22.2

Note: [1]Reactor methane production during sampling period was 23.2 (continuous) and 24 ml/ml feed (intermittent mixing).

Experiment 2.

2.3. Effect of initial subtrate to inoculum ratio and mixing intensity on batch start-up of manure digesters

Lab-scale experiment was performed to study the effect of initial substrate to inoculum ratio and mixing intensity on batch start-up or re-inoculation of reactors. The studied substrate to inoculum ratio was 10:90 and 40:60. Methane and VFA production was followed during the experiment.

Experimental work

In all, 28 reactors (1 l serum bottles with a total working volume of 400 ml) in parallel, i.e. 14 reactors in duplicate, were used for batch digestion (Table 1). The digestion was carried out at 55°C under various mixing conditions as shown in the Table 2. the digesters were either operated vigorously and continuously mixed on a shaker table (110 times per minute with a 3.5 cm stroke) gently and continuously mixed on a shaker (35 times per minute with a 1.2 cm stroke) or "no mixing" – thoroughly shaken by hand for about 1 min every time a sample was taken.

Table. 1. Digester operating conditions.

Mixing level	Digester	substrate to inoculum ratio	Analyses
No mixing	1-2	10:90	CH_4
	3-4	10:90	VFA
Gentle mixing	5-6	10:90	CH_4
	7-8	10:90	VFA
Vigorous mixing	9-10	10:90	CH_4
	11-12	10:90	VFA
No mixing	13-14	40:60	CH_4
	15-16	40:60	VFA
Gentle mixing	17-18	40:60	CH_4
	19-20	40:60	VFA
Vigorous mixing	21-22	40:60	CH_4
	23-24	40:60	VFA
Control	25-26	0:100	CH_4
	27-28	0:100	VFA

Digested manure obtained from the pilot-scale biogas plant of Institute of Environment and Resources, Technical University of Denmark was used as inoculum. Fresh cow manure was obtained from Hashoj Biogas plant, Denmark. Methane production was measured in the headspace of the bottles by GC using a

FI detector. The major VFA components were measured by taking samples from a separate set of bottles and by using GC with FID detection. TS, VS and pH were determined using APHA Standard procedures (APHA, 1998). Total-N and NH_4^+-N were measured following Kjeldahl-N method.

Results

The effect of initial substrate to inoculum ratio and mixing intensity on start-up of batch reactors is illustrated in Figure 1 & 2.

Low initial substrate to inoculum ratio (10:90)

According to Figure 1, Methane production in assays subjected to "no mixing" and "gentle mixing" started after a lag phase of 3-4 days and reached maximum within 40 days of incubation. No significant difference in methane and VFA production was noticed when assays were mixed either gently or occasionally (no mixing). Final methane yields produced in these two treatments were 0.20 m^3/ kg VS. The VFA production also followed more or less a similar trend in these two treatments (Fig. 1). On the contrary, methane production in "vigorously mixed" assays was delayed and remained low for up to 20 days resulting in lowest final methane yield of 0.12 m^3/ kg VS. The low methane production in vigorously mixed assays was due to the build-up of VFAs, especially acetate (data not shown). The possible reason for the delayed methane production could be due to inhibition of methanogenesis when high VFA was distributed homogenously.

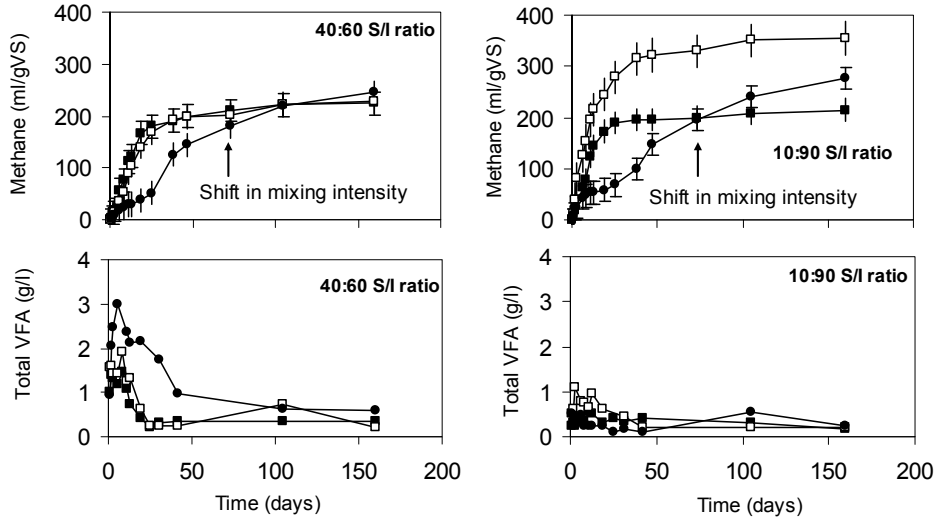

Figure 1. Methane production at different substrate to inoculum (S/I) ratios of 10:90 and 40:60 and mixing strategies: ■ No mixing, □ gentle mixing and ● vigourous mixing.

High initial substrate to inoculum ratio (40:60)

The effect of mixing intensity on methane and VFA production under a high initial substrate to inoculum ratio was significantly different. Methane production started immediately in all assays but remained low for up to 25 days in vigourously mixed assays. Maximum methane yields of 0.31 m^3/ kg VS were obtained in assays subjected to gentle mixing. On the other hand, methane yields in "no mixing" and "vigorously mixed" assays were 0.15 and 0.12 m^3/ kg VS respectively.

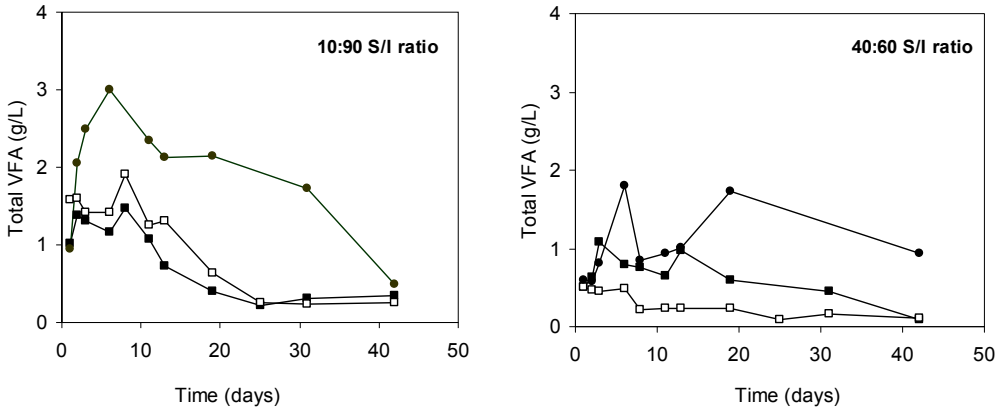

Figure 2. VFA production at different substrate to inoculum ratios (S/I) of 10:90 and 40:60 and mixing strategies: ■ No mixing, □ gentle mixing and ● vigourous mixing.

Discussion

The results showed that the initial biomass concentration and mixing intensity had some affect on production of VFA and the subsequent methane from the produced VFA. The long delay in methane production noticed under vigorous mixing indicate that methanogenesis was inhibited as the produced VFA was distributed homogenously in the reactor. Moreover, under low initial biomass concentration, vigorous mixing could have inhibited the biomass growth in the methanogenic centres due to diffused VFA from acidogenic to methanogenic zones. These results in practice suggest that vigorous mixing should be avoided during the batch start-up of anaerobic digestion of manure, especially under high loading conditions. Additionally, under high initial inoculum concentration, low mixing intensity may facilitate to realize maximum methanogenesis. For initial start-up, partial filling with pure inoculum followed by a fed batch period according to the "activated biomass concept" (Angelidaki et al., 2006) is recommended. However, when having to re-inoculate the existing reactor after a process failure, where it may not be practical to empty the reactor contents above

illustrate that reduced mixing intensity after reinoculation may be helpful to start-up the process faster.

Experiment 3

3.1. Effect of post-treatments on methane production from solids separated from thermophilically digested cow manure

Background

The biogas potential of manure could be significantly increased by treatment of the recalcitrant organic matter (biofibers) contained in the manure. A large part (40–50%) of the total solids in manure and other wastes consists of biofibers. Biofibers can only partly be degraded during the biogas process. Increase of the biodegradability of biofibers will be an important factor for the economic feasibility of manure based full scale biogas plants. (Angelidaki and Ahring, 2000).

To improve the biodegradability of lignocellulosic materials, several treatment methods have been investigated, having as a primary goal to decrease the association of lignin with the degradable part of biofibers and thereby increase the substrate accessibility to the bacteria. Other less important goals are to break down the crystalinity of cellulose to increase the pore size of cellulose and to increase the specific surface area. Previously, treatments such as physical, chemical and biological methods were used. In the present study, the effect of grinding, wet oxidation and chemical treatments (NaOH, $Ca(OH)_2$ or CaO) on improving the biodegradability and biogas from fibers separated from digested manure was investigated.

Objective

The effect of different post-treatments on biogas production from fibers separated from thermophilically digested cow manure

Materials and methods

Solid-liquid separation

Solid fraction from thermophilically digested manure, obtained from a full-scale biogas plant (Snertinge biogas plant, Denmark) was used as substrate. The solids were separated in a stepwise fractionation using 3 different sieves of mesh size 2, 1 and 0.5 mm. Solids retained on each sieve (fibers) and the liquid (filtrate) passed through 0.5 mm sieve were weighed separately to estimate the relative size fractions. The solids retained on each sieve were then washed with tap water.

Post-treatment

The post-treatment methods applied are shown in Table 1. The experiment was carried out in 118 ml glass serum bottles. Thermophilically digested material from full-scale biogas plant (Snertinge Biogas plant, Denmark) was used as inoculum. To each assay, 30 ml of inoculum, 7 g of treated fibers and 10 ml of distilled water were added to have final liquid volume of 47 ml. The headspace of the assays was then flushed with N_2 gas and a further 0.2 ml of $Na_2S{\cdot}8H_2O$ was added to assure optimum anaerobic conditions. The bottles were closed with butyl rubber stoppers and aluminium crimps. Assays without fibers were used as controls. The assays where then incubated statically at 55C. The methane produced in the headspace of each bottle was measured every two to three days for a period of 30 days. All experiments were run in duplicate.

Table 1. Set-up of the batch experiment

Post-treatment	Treatment conditions	SubstrateVS to InoculumVS
Control (inoculum)	--	--
Untreated fibers	--	1.5
NaOH	40 g/kg VS incubated statically at 20ºC for 48 hours	1.5
CaO	40 g/kg VS incubated statically at 20ºC for 48 hours	1.5
Grinding	Manual grinding in motor and pistle	1.5
Wet oxidation	100 g of fibers mixed with 1 lt of water and subjected to 195ºC and 12 bars of O_2 for 10 min.	1.5

Calculations

Specific methane yields ($m^3/kgVS_{added}$) were calculated as the cumulative methane (ml) produced per g VS_{added} before employing the treatments.

Results

Characteristics of fibers

Chemical characteristics of the fibers sieved out from thremophilically digested manure before and after post-treatments are presented in Table 2. Untreated fibers had a VS of 17.6%. Chemical treatment with NaOH or CaO resulted in a decrease in VS from 17.6 to 15.3% and to 15.7% respectively. A significant decrease in VS content was noticed after

wet oxidation (from initial VS of 17.6 to 7.2%). Grinding the fibers with pistle and motor did not affect fiber VS.

TABLE 2 Characteristics of the fibers separated from digested manure before and after employing various post-treatments.

	VS (%)	
Post-treatment	before	After
Untreated fibers	17.6	17.6
NaOH	17.6	15.3
CaO	17.6	15.7
Grinding	17.6	17.4
Wet oxidation	17.6	7.2

With all treated materials, significant methane production started immediately at 55°C (Fig. 1). Untreated fibers had a final methane yield of 0.20 $m^3/kgVS_{added}$. The mean specific methane yields ($m^3/kgVS_{added}$) obtained for treated fibers during 183 d of incubation were 0.02, 0.23, 0.17 and 0.19 for wet oxidation, grinding, NaOH and CaO treatments respectively (Table 3). Among the treatments, only grinding gave higher methane yields while chemical treatments gave more or less the same methane yields as that of untreated fibers.

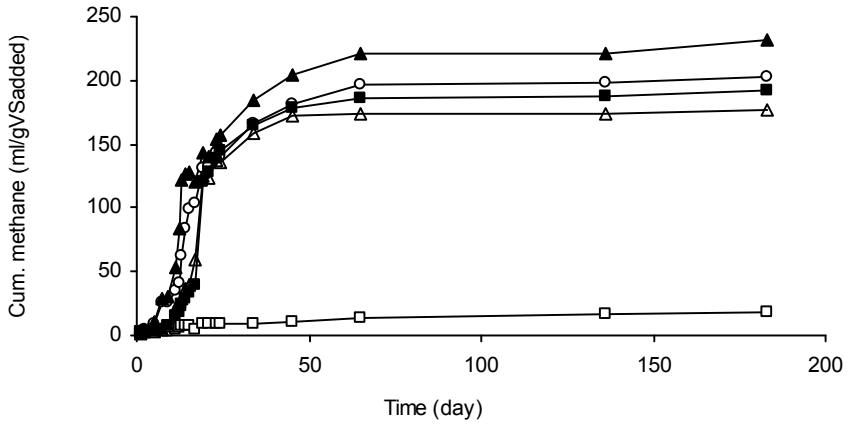

Fig. 1. Effect of post-treatments on the fibers separated from digested manure: ○ untreated fibers, □ wet oxidation, ▲ grinding, Δ CaO and ■ NaOH.

Discussion

The present study indicate that there is a significant residual methane potential still available in the solids fractions of a thermophilically digested manure. Insufficient retention time of fibers during the anaerobic digestion of manure in the full-scale CSTR for initial hydrolysis could be the probable reason for this

loss of methane potential. The fibers used in the present study were separated from digested manure obtained from a full-scale biogas plant operated at 55°C with 20 d HRT. In practice, significant conversion efficiency could be improved by securing extended solids retention times through active separation of the solids in the process or by recirculation of fibers separates from digested manure. From the present study only mechanical grinding appears to be able to increase the benefit of simple solids retention time (SRT) increase both by making more of the recalcitrant fibers available for degradation but most likely also by increasing rate of recovery which is important in view of the practical limits in obtaining very long SRT (mixing and thickening problems in the process reactors).

Table 3. Effect of treatments on methane production of fibers separated from digested cow manure.

Treatment	Methane yield[1] ($m^3/kgVS_{added}$)	Methane yield (m^3/t)	Change in methane yield (%)
Untreated fibers	0.20	35.6	--
Wet oxidation	0.02	8.3	-90.9
Grinding	0.23	40.9	+14.9
NaOH	0.17	38.9	-12.8
CaO	0.19	34.9	-4.5

[1]Yields based on the original VS present in the substrate

The low or no improvement in methane yields of the treated fibers over untreated fibers could be due to the differences in hydrolysis of treated fibers induced by various treatments and/or limitations in conversion of solubilized COD during several steps of anaerobic degradation. From the present study and literature it appears that the effects of treatments are different for fibers separated from already digested manure compared to fibers from untreated manure. Previous studies have shown an increase in methane yields after employing some of the tested treatments either on fresh manure (up to 25%) or on the fibers separated from the manure (16-20%) as pre-treatments (Angelidaki and Ahring, 2000; Hartmann et al., 2000; Bonmati et al., 2001). On the other hand, Kaparaju and Rintala (2005) reported that only NaOH chemical treatment with or without thermal treatment (80°C for 3 h) enhanced the methane yields while some treatments even decreased the yields of solids separated from mesophilically digested cow manure.

The improved methane yields noticed for grinded fibers are in accord with an earlier work where the physical treatment was used to increase the biogas potential of fibers. The physical treatment had a significant effect on the degradability of the fibers and the treatment reduces the crystalinity of lignocellulose (Angelidaki and Ahring, 2000). Moreover, the lignocellulosic structure of fibers in manure is easier to open by

mechanical treatment as the fiber structure has been softened by the high water content of the manure and by partial pre-digestion of the fibers (Angelidaki et al. 2000).

The low methane yields obtained after a wet oxidation was probably due to loss of VS after the treatment apparently due to exposure to high temperatures of 190°C for 10 min. Wet oxidation is usually performed to convert enzymatically the cellulose and hemicellulose fractions of fibers to hexoses and pentoses, respectively. Although much of the carbohydrate fractions should remain stable in order to enable the separation of the cellulose and hemicellulose fractions (Bjerre et al. 1996), wet oxidation also results in the loss of CO_2.

The slightly low or similar methane yields obtained with chemical treatment indicate that the duration or concentration of the chemicals was insufficient or probably due to sodium ion inhibition (Kim et al. 2000). Bases such as NaOH and CaO were reported to cause some degree of de-lignifications of the cellulosic structures and affect solids' degradation through swelling/dissolving of the cellulose and thereby its hydrolysis (Fan et al., 1982; Hobson and Wheatley, 1993). Previous researchers have reported a 13 and 23% increase in methane potential at a dosage of 20 and 40 g kg^{-1} VS of NaOH respectively for fresh cattle manure (Angelidaki and Ahring, 2000) or fibers separated from mesophilic digested manure (Kaparaju and Rintala, 2005).

Experiment 4

4.1 Effect of temperature on recovering the residual methane potential of digested manure from lintrup biogas process

Background

In the previous EFP Annual report 2005 (Kaparaju et al., 2005), it was reported that less than 5% additional biogas was being harvested from the post-digester. The low biogas production from post-digester was due to the fact that digestion in the post-digester was continued without additional heating, apparently at 42°C (incoming effluent). In the present study an attempt was made to evaluate the loss of residual methane potential if post-digester is not operated at or near main process temperature.

Objective

To determine the loss of methane potential from the main reactor and to assess the recovery efficiency of the post-digester if operated at main process temperature i.e. from 42 to 53ºC.

Methodology

Grab samples were obtained from various process steps of Lintrup biogas plant (Denmark). Samples were taken from the three main reactors (R1, R2 and R3), post-digester (R4) and from the post-storage tank (R5). The three main reactors were operated in parallel at 53°C. Effluents from the main reactors were conveyed to a post-digester, established as biogas collection systems, and further down-stream to a post-storage tank. No additional heating was provided to post-digester or post-storage tank. The temperature in the post-digester was measured to be 42ºC and that of post-storage tank was slightly above the ambient temperature (15-20ºC).

Experimental set-up

The experiments were carried out in a 118 ml serum vials. To each assay 50 ml digested material were transferred. No inoculum was used. The headspace in the bottles was then flushed with a mixture of N_2/CO_2 gas mixture (80/20 ratio) and sealed immediately with butyl rubber stoppers and aluminium crimps. Two sets of assays were incubated at the main process temperature applied at the plant (53ºC) while another set was incubated at post-digester temperature (42ºC). After 15 days of incubation, one the two sets incubated at 53ºC was moved to 42ºC. This enables to evaluate the effect of lowered temperature on degradation activity and methane recovery efficiency. The experiment was run in duplicate.

Methane production was measured in the headspace of the vials using GC (FID method).

Results and discussion

The experiment was run for 250 days. Methane production at various temperatures is shown in Figure 1 and Table 1. Methane production started immediately at both 42 and 53ºC. No significant difference in methane yields was noticed at 42ºC or at temperature closer to that of original process of 53ºC. However, a decrease in temperature from 53 to 42ºC after 15 days of incubation resulted in a corresponding decrease in methane potential ca. 35 to 55%. The lower end of this range represents the post-digester where the effluent temperature was originally at 42ºC. The low methane yields upon decreasing the temperature was due to the fact that methanogenesis is more sensitive to a decreased process temperature than hydrolysis, especially for thermophilic main process temperatures. This might have led to an imbalance between VFA production and consumption. This imbalance, especially on thermophilic plants can lead to less efficient biogas recovery especially if post-digesters are operated at low or ambient temperatures.

Table 1. Effect of temperature on residual methane potential of digested material from Lintrup full-scale biogas plant.

Treatment	Methane yield (m^3/kgVS)			
	42°C	53°C	53 to 42°C[1]	Loss in methane potential (%)
Reactor 1 (R1)	0.16	0.18	0.09	50
Reactor 2 (R2)	0.18	0.17	0.07	41
Reactor 3 (R3)	0.19	0.18	0.10	55
After storage tank (R4)	0.16	0.17	0.06	35
Post-storage tank (R5)	0.17	0.18	0.08	44

[1]Temperature was changed after 15 days of incubation at 53°C.

The fact that the highest specific methane yields were achieved at 42°C, somewhat below the actual process temperature could be due to temperature dependent ammonia concentration. The high residual methane potential at both 42 and 53ºC indicate that insufficient retention time in the main process. Post-digester operated at process temperature can only retain the biomass for a few hours and recovery from this step could therefore be very much limited. On the

other hand, the retention times in post-storage tanks is usually 5-10 days and operated at or slightly above ambient temperature either due to heat recovery from the incoming effluent or due to natural cooling in un-insulated tank. The methane recovery from post-storage tank is however relatively low and temperature dependent. Previous studies have shown that methane potential of effluent from a post-storage tank can constitute up to 20-30% of the main digester effluent (Kaparaju and Rintala, 2003).

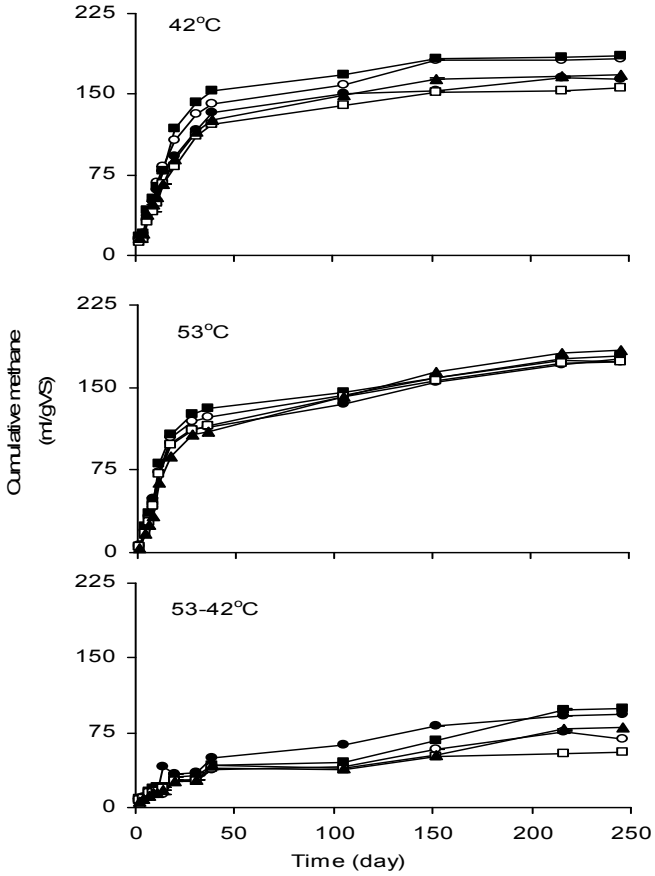

Fig. 1. Residual methane potential of effluent from three main reactors (● R1, ○ R2 & ■ R3), □ post-digester and Δ post-storage tank incubated at 42 and 55ºC.

The present results demonstrate the importance of operating the post-digester at the same temperature as that of the main reactor (53C) or at least to operate at a temperature within +/- 5°C of original process. Otherwise long adaptation periods are required for the development and retention of high concentrations of active and well balanced biomass inside the reactor.

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