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Optimisation of biogas production from manure through serial digestion: Lab-scale and pilot-scale studies

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ABSTRACT

In the present study, the possibility of optimizing biogas production from manure by serial digestion was investigated. In the lab-scale experiments, process performance and biogas production of serial digestion, two methanogenic continuously stirred tank reactors (CSTR) connected in series, was compared to a conventional one-step CSTR process. The one-step process was operated at 55 °C with 15 d HRT and 5 l working volume (control). For serial digestion, the total working volume of 5 l was distributed as 70/30%, 50/50%, 30/70% or 13/87% between the two methanogenic reactors, respectively. Results showed that serial digestion improved biogas production from manure compared to one-step process. Among the tested reactor configurations, best results were obtained when serial reactors were operated with 70/30% and 50/50% volume distribution. Serial digestion at 70/30% and 50/50% volume distribution produced 13–17.8% more biogas and methane and, contained low VFA and residual methane potential loss in the effluent compared to the one-step CSTR process. At 30/70% volume distribution, an increase in biogas production was also noticed but the process was very unstable with low methane production. At 13/87% volume distribution, no difference in biogas production was noticed and methane production was much lower than the one-step CSTR process. Pilot-scale experiments also showed that serial digestion with 77/23% volume distribution could improve biogas yields by 1.9–6.1% compared to one-step process. The study thus suggests that the biogas production from manure can be optimized through serial digestion with an optimal volume distribution of 70/30% or 50/50% as the operational fluctuations are typically high during full scale application. However, process temperature between the two methanogenic reactors should be as close as possible in order to derive the benefits of serial coupling.

1. Introduction

Anaerobic digestion of animal slurry for biogas production is commonly practiced in continuously stirred tank reactor (CSTR) and occasionally in plug-flow reactor (Wilkie et al., 2004). To improve the economics of biogas systems, the amount of energy produced per unit manure treated and the value of the digested material as fertilizer should be maximized while the investment and operation costs should be minimized (Kaparaju and Rintala, 2008). In a biogas process with a typical hydraulic retention time (HRT) of 15–30 d, only 50–70% of organic matter is converted to biogas producing an average methane yield of 0.20–0.25 m^3/kg volatile solids $(VS)_{added}$ (Hartmann et al., 2000). Reasons for the low methane recovery are presence of recalcitrant material in the residual organic matter and/or loss of degradable matter with the effluent, especially for particulate matter which requires a long time for solubilisation and hydrolysis. The latter phenomenon, commonly noticed in CSTRs, is due to "short-circuiting" of a portion of the feed, which has a much shorter retention time than the nominal average retention time in the reactor.

Conventional one-step CSTR is simple to operate but less efficient in terms of the effluent quality compared to other reactor configurations such as upflow anaerobic sludge blanket (UASB) reactor or two-phase reactor system (Speece et al., 1997; Azbar et al., 2001). High viscosity and particulate content in manure makes UASB reactors unsuitable for manure treatment. On the other hand, two-phase system, where a short acidogenic step is followed by a long methanogenic step, often with separation in between the two reactors to withhold particulate matter in the acidogenic step (Demirel and Yenigun, 2002), is considered to be sensitive to high organic load and separation processes are costly. Operation and control of a two-phase system is considered complicated as effluent characteristics of the acidogenic reactor (pH, volatile fatty acids (VFA) or nutrients) need to be adjusted prior to feeding the methanogenic reactor (Sachs et al., 2003; Babel et al., 2004). Although biodegradability of recalcitrant materials may be improved in a two-phase system, the syntrophic relationship

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between bacteria and methanogens is disrupted – a limitation of the system – and can cause product inhibition in the acidogenic reactor. For example, acetate can inhibit propionate degradation, or high hydrogen partial pressure can lead to accumulation of higher molecule VFA than acetate (Smith and McCarty, 1989).

An alternative approach to overcome the above mentioned problems with one-step CSTR and two-phase system is to operate two methanogenic reactors connected in series (serial digestion). In a recent study, Boe (2006) demonstrated that serial digestion, with percent volume distributions of 90/10 or 80/20 between the two methanogenic reactors, improved biogas production by 11% compared to a traditional one-step CSTR process. Furthermore, modeling results from the above study also confirmed that the longer the retention time in the post-digester (second reactor of serial process), the higher the methane recovery of the overall serial digestion (Boe and Batstone, 2005). However, the volume allocated to the main reactor (first reactor in serial digestion) must be sufficient to maintain a stable process with a reasonably low VFA level, as a healthy first step is a precondition for a successful serial digestion. In the present study, in continuation of the Boe (2006) study, four more serial volume distribution ratios were investigated. The tested volume distribution ratios between the two methanogenic reactors of serial digestion were 70/30%, 50/50%, 30/70% and 13/87% in the lab-scale study and 77/23% in the pilot-scale study. The latter two volume distributions, with a relatively small first reactor volume, were included to determine the limit of satisfying the criteria for a healthy initial methanogenic process and transition to a two-phase process. The process performance and stability of a serial digestion was compared to that of a one-step CSTR process operated under similar conditions. In order to compare the process stability between one-step and serial digestion, the effect of a lipid pulse load was also investigated. This experiment was performed in the lab-scale study only.

2. Methods

2.1. Lab-scale experiments

2.1.1. Feed 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 −20 °C until further use. Frozen manure portions were thawed at room temperature and the prepared feed was stored at 4 °C for 2–3 days. Feed was prepared once or twice a week by diluting fresh manure with distilled water (1:1 ratio). Characteristics of feed are presented in Table 1.

Table 1
Average composition of feed cow manure

	Lab expt.[a]	Pilot expt.[b]
TS (%)	5.8–6.3	6.5–7.3
VS (%)	4.7–5.1	4.8–4.9
pH	7.3–7.5	7.5–7.7
Ammonia (g/l)	1.7–2.2	1.8–2.4
Total VFA (g/l)	4.5–7.3	2.8–6.8
Acetate (g/l)	2.7–4.2	1.8–5.2
Propionate (g/l)	1.1–1.9	0.6–0.9
Butyrate (g/l)	0.2–0.3	0.06–0.21
Iso-butyrate (g/l)	0.2–0.5	0.24–0.40
Valerate (g/l)	0.03–0.32	0.10–0.13

[a] Values after dilution with water (1:1 ratio).
[b] Values after dilution with water to attain 6.5–7.5% solids.

2.1.2. Reactor set-up and operations

Experimental set-up is shown in Fig. 1. One-step CSTR process was carried out in a 7 l reactor (R1, control) with 5 l working volume and 15 d HRT. Serial digestion (R2 and R3) was constructed using two CSTR reactors connected in series with a total HRT of 15 d and combined working volume of 5 l distributed at 70/30% or 50/50% between the two reactors (Fig. 1). Reactors were built from double glass cylinder 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. Stable reactor temperature was maintained at 54 ± 1 °C by pumping hot water, from an electrically heated thermostatic water bath, in the space between the reactor glass walls. R1 and R2 were fed semi-continuously at 6 h interval. R3 was fed directly with the effluent from R2. Feed rate was 333 ml/d (Fig. 1). An equal amount of effluent was removed automatically due to the pressure developed from the produced biogas and the added feed. Effluent along with produced biogas was collected in an effluent and gas separation bottle. Biogas from the effluent bottle flowed to the gas meter to register biogas production as described elsewhere (Angelidaki et al., 1992). Reactors were stirred by mechanical mixers operated on a cycle of 40/60 seconds on/off. In parallel, another serial CSTR set-up consisting of two reactors, referred to as R4 and R5, were also operated as described above. Total working volume in R4 and R5 was 4 l and was distributed as 30/70% or 13/87%, respectively. R5 was fed directly with the effluent from R4. Feed rate was 270 ml/d.

2.1.3. Pulse load tests

The effect of a pulse organic load on process stability and behavior was evaluated for both one-step CSTR and serial digestion. 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 into the reactors, in the first reactor in the case of serial coupling. 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. Pulse load for serial reactors operated with 70/30%, 50/50% and 13/87% volume distributions was 28, 39.2 and 65.3 g/l-reactor volume, respectively, calculated with respect to the first reactor volume only. Biogas production, VFA concentrations and pH were followed by sampling every day for 10 days.

2.2. 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.

2.2.1. Feed preparation

Fresh cow manure was obtained in 800 l 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 for the pilot-scale plant by diluting the manure with water, to attain a consistent total solids (TS) of 6.5–7.5%. Characteristics of feed are presented in Table 1.

2.2.2. Reactor set-up

The experiment was carried out in a pilot-scale plant built at the Institute of Environment and Resources, Technical University of Denmark. Two stainless steel reactors, referred to as R1 (800 l) and R2 (200 l) were used in the study and shown in Fig. 2. The reac-

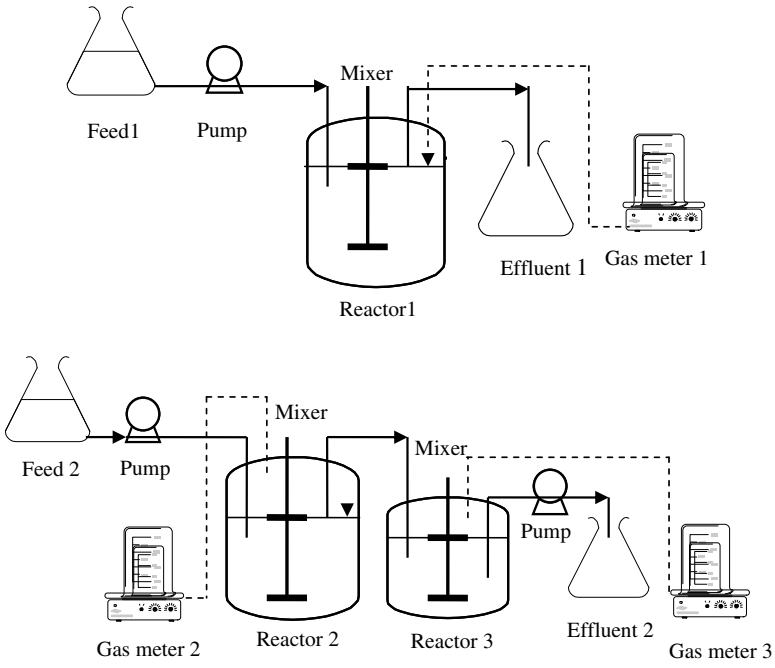

Fig. 1. Lab-scale experimental set-up for one-step (R1) and serial digestion with 70/30 or 50/50% volume distribution (R2 and R3).

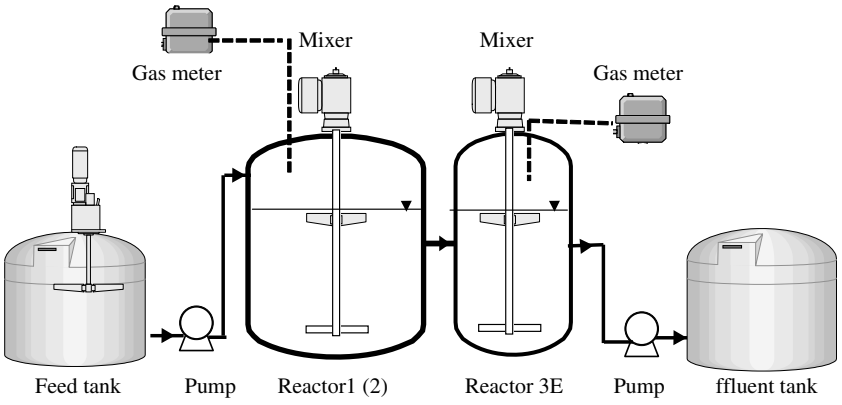

Fig. 2. Pilot-scale experimental set-up for one-step (R1) and serial digestion (R2 and R3) with a volume distribution of 77/23%.

tors 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 in R1 was maintained at 54 ± 1 °C (except for a short period of disturbance) by pumping hot water through a stainless steel coil fitted inside the reactor using an electric flow heater and circulation pump (Kaparaju et al., 2008). However, it should be noted that it was more difficult to control temperature in the post-digester as accurately as that of main reactor.

Feed was thoroughly mixed in the feed pallet prior to each feeding by a high speed motor (for 15 min). Reactor contents were mixed by low speed gear motors fitted with two impellers aligned just below the liquid surface and above the bottom of the reactor. Reactor mixers were operated in a 5 min on/off mode. Two eccentric pumps with a flow rate of 10 l/min were used to pump feed and effluent. Pumps were operated three times per day and for 50 or 65 s 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 h/7 day programmable time switch.

2.2.3. Reactor operation

Detailed description of start-up and operation of R1 (main reactor) has been described elsewhere (Kaparaju et al., 2008). Briefly, 450 l of thermophilically digested manure (Centralized biogas plant, Denmark) and 30 l of fresh cow manure were transferred to R1 on Day 0. Daily feeding in R1 commenced approximately 10 d after seeding and loading was gradually increased to attain the desired level (Fig. 2). 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 l) 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. After the initial start-up/stabilisation, R1 was operated for 22 days at a feed rate of 25 l/d and working volume of 500 l corresponding to HRT of 20 d. Later, 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 25 l/d feed rate were used as one-step CSTR process (reference period) while the data obtained from R1 + R2 during 32.5 l/d feed rate represented serial digestion with comparable overall HRTs.

2.2.4. 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 55 °C. 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).

2.3. 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.

2.4. Microbiological analyses

The variation in the microbial community during one-step and serial digestion (70/30 and 30/70% volume distribution) was investigated through flourescence *in situ* hybridization (FISH) analyses. FISH was performed using oligonucleotide probing (Hugenholtz et al., 2001). The specifics probes used has been described elsewhere (Kaparaju and Angelidaki, 2008). Briefly, the probes used were EUBMIX-CY3 for *Bacteria*; ARC915 Alexa488 for *Archaea*; MX825 CY3 for *Methanosaetaceae*; MS1414 CY3 for *Methanosarcinaceae*; MG1200 CY5 for *Methanomicrobiales*; MB1174 CY5 for *Methanobacteriaceae* and MC1109 CY5 for *Methanococcaceae*. The slides were examined using a Zeiss microscope. Similar analyses were also performed with samples from pilot-scale plant.

The number of *Bacteria* and *Archaea* were assessed using a subjective scale ranging from 0 (none) to 5 (abundance). A similar subjective scaling for the assessment of the proportions of *Bacteria* and *Archaea* and subgroups of *Bacteria* was reported by Bjornsson et al. (2002) and Kong et al. (2002). Results were presented based on approximately 20 microscopic fields examined with the 63 × 1.4 objective, representing 2000–10,000 individual cells.

2.5. Calculations

For lab-scale experiments, specific methane yield (ml/gVS_{fed}) was calculated as daily methane produced divided by the actual feed VS. Theoretical methane yield (STP m^3 CH_4/kg VS) was calculated based on the stoichiometric conversion of organic matter to methane and carbon dioxide (Angelidaki and Sanders, 2004). The calculated theoretical methane yield of manure was 0.40 m^3/$kgVS_{added}$.

Total methane equivalents (total CH_4 eq) were calculated as sum of methane equivalents obtained from individual VFAs (VFA CH_4 eq) and biogas (Biogas CH_4 eq) produced at steady-state. VFA CH_4 eq was calculated as sum of CH_4 eq for acetate, propionate, butyrate and valerate in the reactor times the amount of feed/effluent (l) at STP. For the serial process, calculation was based on VFA in the second reactor only, as this represents the effluent loss. Biogas CH_4 eq was calculated as the amount of biogas (ml) produced per ml feed multiplied by the amount of feed (ml) and the methane content (%) in the biogas.

For pilot-scale experiments, specific biogas yield (l/$kgVS_{fed}$) 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.

3. Results

3.1. Lab-scale experiments

3.1.1. Biogas production in one-step CSTR and serial digestion processes

The operation and performance parameters of the five lab-scale reactors are illustrated in Figs. 3 and 4. Table 2 summarizes the steady-state data for one-step and serial digestion processes. The mean biogas production for one-step CSTR process (R1) was 12.2–12.8 l/l feed (337–370 l/$kgVS_{fed}$). At the same time, biogas production in serial digestion (R2 + R3) operated with 70/30% volume distribution was 14.9 l/l feed (436 l/$kgVS_{fed}$). The increase in biogas production for serial digestion over one-step CSTR process was 16.4%. A similar process performance and biogas production was also noticed when serial reactors were operated with 50/50% or 30/70% volume distribution (Fig. 4). The increase in biogas production with volume distribution ratios of 50/50% and 30/70% was 17.8 and 13%, respectively. However, the process at 30/70% volume distribution was very unstable with respect to biogas production. For 13/87% volume distribution, no significant difference in biogas production in serial digestion was noticed.

VFA concentrations ranged between 0.7 and 2 g/l in one-step CSTR process (Fig. 3). The corresponding VFA values in serial digestion i.e. in post-digester were 24–43% lower than that noticed in one-step CSTR process (Fig. 4). Among the different tested volume distributions of serial reactors, the order of magnitude for VFA levels was highest with 30/70% followed by 13/87%, 50/50% and 70/30% reactor configurations. In R4, significantly high levels of propionate at 30/70% and both acetate and propionate at 13/87% volume distributions were noticed (data not shown). pH in all reactors was in the range of 7–8 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).

3.1.2. 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. Large number of fermenting bacteria (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 composition and abundance was also noticed in the main reactor of serial digestion (70/30% volume distribution, R2). The abundance of these microorganisms was however relatively low in

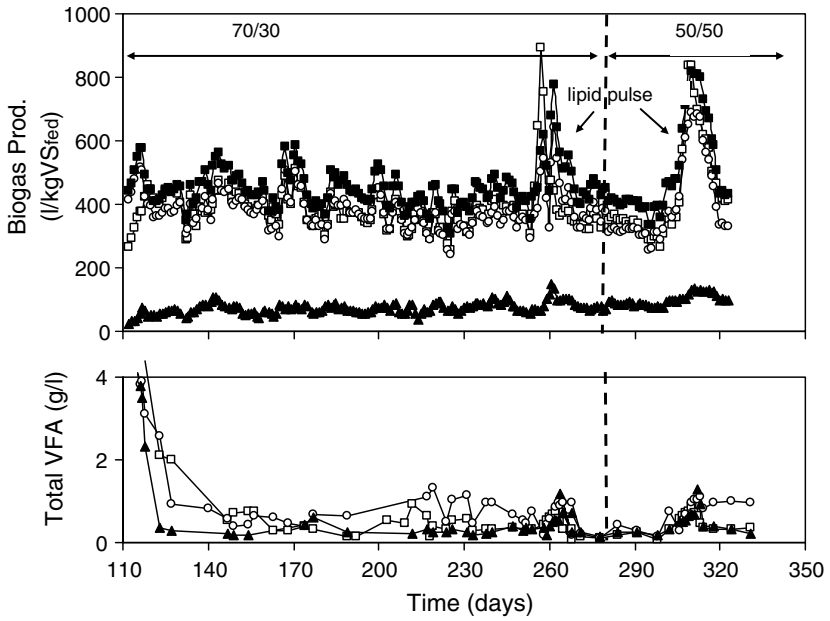

Fig. 3. Process performance and biogas production during anaerobic digestion of cow manure in one-step ((□) R1) and serial digestion with 70/30% and 50/50% volume distribution ((○) R2, (▲) R3 and (■) R2 + R3) in lab-scale at 55 °C.

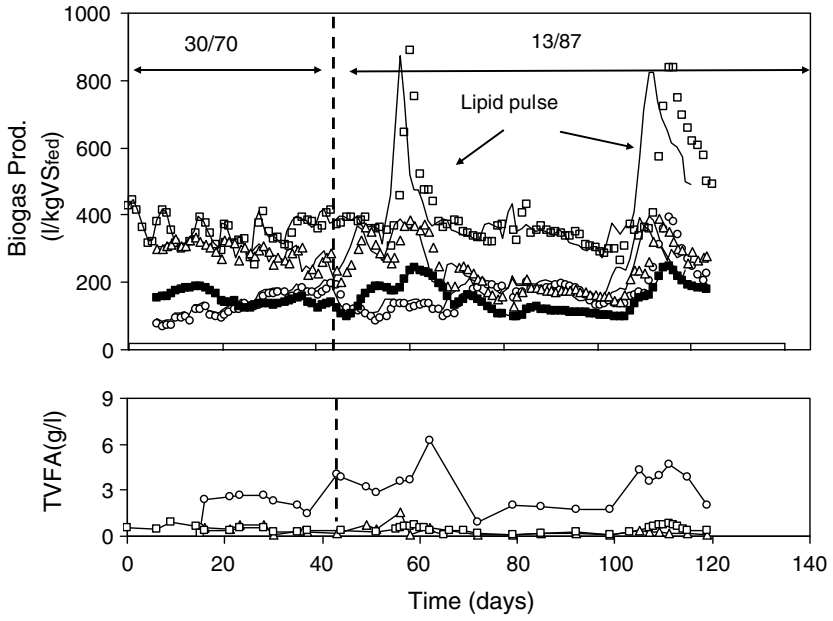

Fig. 4. Process performance and biogas production during lab-scale thermophilic digestion of cow manure in one-step CSTR ((□) R1) and serial digestion processes with 30/70 and 13/87% volume distribution ratios ((○) R4, (Δ) R5 and (■) R4 + R5).

the post-digester (R3). The presence of fermentative bacteria in R3 was however evident from the activity test (data not shown), which showed that the hydrolysis and fermentation of organic compounds to VFA continued in R3 but at a lower rate than the other reactors. 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).

3.1.3. Residual and utilized methane potential of one-step and serial digestion processes

The experiment was carried out in batch assays and methane production was followed for 90 d. During the run, methane production started immediately in all assays and reached maximum after 73 d of incubation. Mean residual methane potential of one-step CSTR process was 113 l/kgVS$_{added}$ (3.5 l/l sample) while that of serial digestion (70/30% volume distribution) was 99 l/kgVS$_{added}$ (3.1 l/l sample).

3.1.4. Methane equivalents of one-step and serial digestion processes

The hydrolysis efficiency of one-step CSTR and serial digestion was evaluated through methane equivalents calculated from the VFA and the produced biogas in the reactors. Results are presented in Table 3. Results showed that serial digestion had better hydrolysis/acidogeneses than one-step CSTR process. One-step CSTR

Table 2
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 (l/l feed)				Increase in biogas production (%)
		One-step CSTR	Serial CSTR			
		R1	R2	R3	R2 + R3	
70/30	Day 45–140	12.8 ± 2.5	12.6 ± 2.5	2.3 ± 0.5	14.9 ± 2.8	16.4%
Lipid pulse	Day 144–154	18.4 ± 8.2	15.9 ± 3.9	2.5 ± 0.7	18.4 ± 4.0	0%
50/50	Day 173–194	12.3 ± 1.6	12.0 ± 1.7	2.5 ± 0.4	14.5 ± 2	17.8%
Lipid pulse	Day 195–209	22.9 ± 6.2	20.9 ± 4.8	2.7 ± 0.3	23.6 ± 5	3.1%
		R1	R4	R5	R4+R5	
30/70	Day 20–40	12.3 ± 1.2[a]	5.7±1.0	8.2 ± 1.6	13.9 ± 2.2	13%
13/87	Day 85–100	12.2 ± 1.2[a]	6.5 ± 0.7	5.5 ± 0.6	12.0 ± 1.3	−1.7%
Lipid pulse	Day 106–119	23.4 ± 6.1[a]	10.7 ± 2.9	9.9 ± 2.6	20.6 ± 4.9	−13.6%

[a] 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.

Table 3
Total methane equivalents produced during anaerobic digestion of manure in lab-scale one-step CSTR (R1) and serial digestion (R2 and R3) at 55 °C

Volume distribution (%)	Methane equivalent from VFA (ml/d)	Methane equivalent from biogas (ml/d)	Total methane equivalent (ml/d)	Total methane equivalent yield (l/kgVS)	Utilized methane potential[b] (%)
70/30 (Days 45–140)					
R1	54	2519	2572	243	60.7
R2 + R3	44	2921	2965	280	70
50/50 (Days 173–194)					
R1	25	2562	2587	250	62.5
R2 + R3	32	2937	2969	293	73.3
30/70 (Days 20–40)					
R1	66 [a]	2353 [a]	2419 [a]	235[a]	58.8
R4 + R5	69	1760	1829	202	50.5
13/87 (Days 85–100)					
R1	25 [a]	2295 [a]	2319 [a]	244 [a]	60
R4 + R5	22	1942	1964	203	50.7

[a] The corresponding values from R1.
[b] The calculated theoretical methane yield of manure was 400 l/gVS.

process had total CH_4 eq of 2.3–2.5 l/d with less than 3% accounted by VFA. On comparison to one-step CSTR process, the values for serial digestion were 14.8–16% higher with 70/30 or 50/50% and 32.2% and 18.1% lower with 30/70 and 13/87% volume distributions, respectively.

The utilized methane potential, calculated based on the theoretical methane yield of the fresh manure (0.40 $m^3/kgVS_{added}$) and the experimental methane yields, is presented in Table 3. Results showed that serial digestion had high percentage of methane potential utilized compared to one-step CSTR process (61%) when operated with 70/30% or 50/50% volume distributions.

3.1.5. Effect of lipid pulse load on biogas production in one-step and serial digestion processes

The data on average biogas production during lipid pulse load are shown in Figs. 3 and 4 and summarized in Table 2. In general, stable biogas production was noticed in all reactors and at all tested volume distributions. However, the response to a lipid pulse load in serial digestion was different. No extra biogas production was noticed with 70/30% volume distribution while 3.1% extra biogas was obtained when serial reactors were operated with 50/50% volume distribution. During the test, VFA levels reached a maximum value of 0.8 g/l in one-step CSTR (after 5 d) and 1.1–1.2 g/l in the serial reactors (after 7 d). These elevated VFA levels remained for up to 7 d before restoring to the values prior to the pulse test (<0.3 g/l). On the contrary, 13.6% lower biogas was produced when serial reactors were operated with 13/87% volume distribution despite noticing highest biogas production of 9.9 ml/ml feed in the post-digester.

3.2. Pilot-scale experiments

3.2.1. Biogas production in one-step CSTR and serial digestion (77/23% volume distribution) processes

The experiments were carried out over a period of 119 d covering four batches of feed. The results are presented in Fig. 5 and Table 4. 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 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. 5, 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 (52.3 °C). From Days 64 to 119, both reactors had a mean temperature of 54.2 ± 0.2 °C.

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. How-

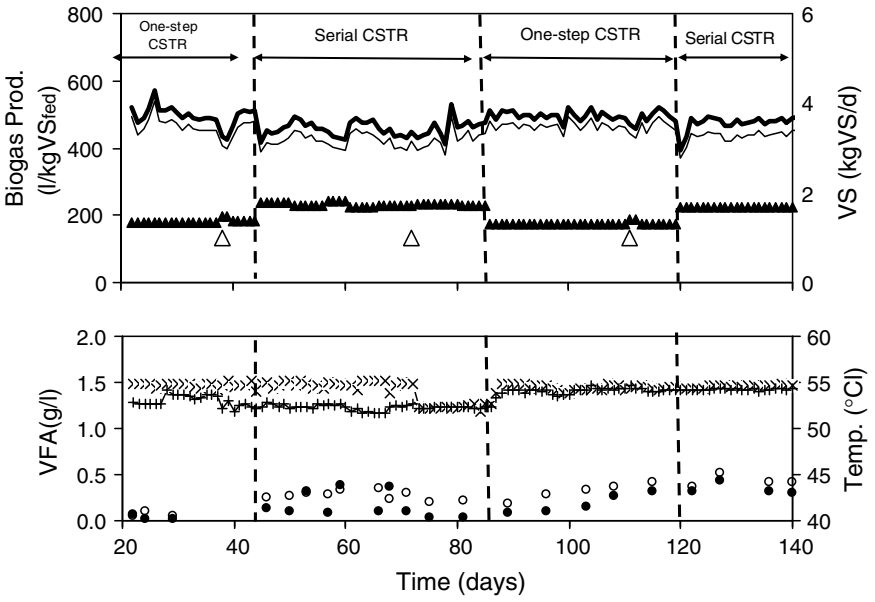

Fig. 5. Process performance and biogas production during pilot-scale thermophilic digestion of cow manure in one-step (R1) and serial digestion (R2 + R3) with 77/23 volume distribution: biogas production in R1 (—) and R2 + R3 (▬); feed VS (▲); feed batch changes (Δ); total VFA in R1 (○) and R2 + R3 (●); temperature in R1 (×) and R2 + R3 (+).

Table 4
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	
	One-step CSTR (R1)	One-step CSTR (R1)	Serial CSTR (R1 + R2)	Serial CSTR (R1 + R2)	One-step CSTR (R1)	One-step CSTR (R1)	Serial CSTR (R1 + R2)
Period	Day 22–37	Day 38–44	Day 45–72	Day 73–85	Day 86–110	111–119	120–141
Min. retention time (d)	15	7	28	13	25	9	21
Biogas prod. (l/d)	634.5 ± 32.9	626.2 ± 29.2	790.7 ± 30.5	794.2 ± 49.4	611.2 ± 16.7	614.3 ± 19.6	800.3 ± 36.3
Biogas prod. (l/l feed)	25.4 ± 1.3	25.0 ± 1.2	24.3 ± 0.9	24.4±1.5	24.5 ± 0.7	24.5 ± 0.8	24.6 ± 1.1
Spec. biogas yield (l/kgVS_{fed})	468.3 ± 24.4	449.8 ± 35.3	464.5 ± 20.8	461.4 ± 29.4	452.7 ± 12.7	460.7 ± 20.8	488.7 ± 22
Relative yield		=100%	+3.3%	+1.9%	=100%	=100%	+6.1%
Methane content (%)[a]	72.7	71.2	67	67	69.2	70.7	71.1
Effluent VS (%)[a]	3.5	3.7	3.3	3.3	3.5	3.6	3.3
VFA (g/l)[a]	0.28	0.27	0.25	0.25	0.32	0.31	0.30
Temp. (°C)[a]	54.7 ± 0.2	54.7 ± 0.4	52.3 ± 0.4	52.3 ± 0.1	54.3 ± 0.5	54.4 ± 0.2	54.2 ± 0.1

[a] Values for serial digestion are from R2 only.

ever, the best results were obtained during days 98–119 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.

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. The apparent random shifts in methane content when changing from one-step to serial operation is not considered reliable, a.o. since samples had to be carried from pilot plant premises to laboratory before analysis with possible air contamination underway. The low effluent VS and VFA values during serial operation is more systematic and consistent with the indication from measured biogas production.

3.2.2. Microbiological analyses

FISH analyses showed that the microbial ecology in the pilot-scale plant reactors was similar to that noticed in the lab-scale reactors (data not shown).

4. 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. Lab-scale experiments showed that serial digestion with an overall retention time of 15 d and volume distribution ranging from 70% (11.5 d) to 50% (7.5 d) in the main reactor and 30% (4.5 d) to 50% (7.5 d) in the post-digester and operated at same temperature could give 13–17.8% additional biogas production compared to a traditional one-step process. The improved biogas production was consistent with the low effluent VFA level and residual methane potential of serial operation compared to one-step operation. Although serial reactors with 30/70% and 13/87% volume distributions performed relatively well, results from VFA (high acetate and propionate concentrations in R4), microbial composition and in particularly methane yield indicated that process performances at these configuration was poorer than combination with a larger first step. These results illustrate that the volume allocated to the main reactor in a serial digestion must be sufficiently large to maintain a stable process with a reasonably low VFA level, as a healthy main reactor is a precondition for a successful serial digestion. Results

from the present study are in agreement with previous lab-scale studies where serial digestion with 80/20% or 90/10% volume distribution between the two serial reactors gave up to 11% extra biogas production compared to one-step process (Boe, 2006). 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, the 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 practice 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. It should also be noted that the HRTs in the lab-scale was 15 d compared to 20 d in pilot-scale experiments. A longer retention time usually result in a more complete degradation of organic material which could diminish the advantages obtained in serial digestion at lower HRT.

The main reason for improved biogas production in serial digestion was due to a more optimal retention time distribution for particulate matter than the nominal average retention time minimizing the loss of relatively fresh feed due to "short-circuiting", commonly noticed in CSTRs (Angelidaki et al., 2005). However, it should be noted that serial digestion process with same total volume as that of one step process does not result in any extra (average) retention time. During serial digestion, the portion of particulate matter with short retention time, which represents the major part of loss of biogas potential, is changed significantly. Previously, this particulate matter reduction was estimated to be 5–10% per day (Angelidaki et al., 2005). By shifting the retention time for a portion of this particulate matter in the lower end of the "age profile" of a one step process can result in a significant change in utilized biogas potential. The higher total CH_4 eq along with lower VFA, residual methane potential and VS values noticed for serial process (70/30% volume distribution) than for one-step process suggests that the former process had longer retention time for hydrolysis of particulate matter. In addition, the microbiological analyses also showed that the syntrophic relationship between acetogens and methanogens in reactors operated in series was not lost (data not shown), which inevitably aided to reduce intermediates inhibition and maintain process stability.

The reasonable high biogas production when serial process was operated with a volume distribution of 30/70% suggests that both reactors behaved like two methanogenic reactors rather than phasing out as hydrolysis/acidogenesis stage in the main reactor and methanogenesis stage in the post-digester. These results were further confirmed by the presence of methanogens in the main reactor (data not shown). No hydrogen production was noticed. However, the slightly higher VFA levels (Figs. 3 and 2) and under utilized methane potential (Table 3) in the main reactor 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. This was evident from the high total CH_4 eq values (Table 3). But the high CH_4 eq along with the low utilized potential in the post-digester (R5) suggests that methanogenesis was not optimum although phasing of the process to hydrolysis/acidogenesis and methanogenesis was noticed (Table 3). This eventually led to unstable process as hydrolysis step is the yield limiting step for methanogenesis, while conversion of VFA is the rate limiting step for achieving stable process.

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 (3.3%) noticed only with 50/50% volume distribution suggests that the serial digestion could produce slightly 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 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 (Figs. 3 and 2) indicating that the main reactor in the latter configuration was temporarily 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.

The low abundance of *Bacteria* and *Archaea* in the post-digester compared to the main reactor (70/30% serial volume distribution) was attributed to low biomass accumulation in the post-digester, which was operated at a short retention time of 4.5 d. Nevertheless, the microbial ecology in both serial reactors was similar as the post-digester received the effluent directly from the main reactor. The abundance of *Methanosarcinaceae* in both lab-scale and pilot-scale reactors was in agreement to previous researchers who reported that *Methanosarcinaceae* were the most abundant aceticlastic methanogens in reactors with a history of high VFA and ammonia levels (Karakashev et al., 2005). The presence of *Methanobacteriaceae* in all reactors indicates that syntrophic association was not lost and that *Methanobacteriaceae* were the preferred syntrophic partners for syntrophic propionate-oxidizing bacteria (SPOB) (McMahon et al., 2001). It is presumed that during the periods of rapid propionate consumption, SPOB were dependent on aceticlastic methanogens (*Methanosarcinaecae* and *Methanosaetaceae*) and hydrogenotrophic methanogens (such as *Methanobacteri-*

aceae) to consume their metabolites (Stams et al., 1992; McMahon et al., 2001).

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., 2008) or post-treatment, in order to improve biodegradability and accessibility, of solids separated from digested material (Kaparaju and Angelidaki, 2008). However, serial digestion concept seems to have some economic gain over the 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. By employing serial reactor configuration, with same total working volume as that of one step process, an improvement in biogas production could be achieved through a reduction of particulate matter as the age distribution of particulate matter in the effluent in a serial digestion is modified. Alternatively, a decrease in overall load in an already existing plant with two reactors operated in parallel could also be adopted. Serial digestion concept could therefore be considered as the first choice in optimizing 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.

5. Conclusions

The present results showed that the biogas production from manure can be optimized by operating two CSTR reactors connected in series. Best results were obtained when the total working volume was distributed with 70–50% (7.5–10.5 d) in the main reactor and 30–50% (4.5–7.5 d) in the second (post-digester) reactor. The increase in biogas production in serial digestion could be up to 16.4–17.8% compared to traditional one-step CSTR process. The 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. Although a relatively large post-digestion step was found beneficial, the volume allocated to the main reactor must be sufficient to maintain a stable process with a reasonably low VFA level, as a healthy first step was found essential for a successful post-digestion. 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. Pulse load tests showed that both one-step- and serial digestion can overcome an organic overload up to 65.3 g/l reactor volume without any serious inhibition due to VFA build-up. Results from pilot-scale study confirmed the lab-scale study with 1.9–6.1% increase in biogas yield obtained with serial digestion compared to one-step CSTR process. 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.

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