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# Effects of mixing on methane production during thermophilic anaerobic digestion of manure: Lab-scale and pilot-scale studies

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## Abstract

The effect of mixing on anaerobic digestion of manure was evaluated in lab-scale and pilot-scale experiments at 55 °C. The effect of continuous (control), minimal (mixing for 10 min prior to extraction/feeding) and intermittent mixing (withholding mixing for 2 h prior to extraction/feeding) on methane production was investigated in three lab-scale continuously stirred tank reactors. On comparison to continuous mixing, intermittent and minimal mixing strategies improved methane productions by 1.3% and 12.5%, respectively. Pilot-scale studies also supported the lab-scale results with an average 7% increase in biogas yields during 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 min 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.
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## 1. Introduction

Anaerobic digestion of high solid slurries such as livestock manures is typically performed in continuously stirred tank reactors (CSTR). The performance of CSTRs is dependent on the hydraulic retention time (HRT) of the substrate and the degree of contact between the incoming substrate and a viable bacterial population (Karim et al., 2005). Both these parameters are a function of the hydraulic regime (mixing) in the reactors.

Mixing creates a homogeneous substrate preventing stratification and formation of a surface crust, and ensures solids remain in suspension. Further, mixing also enables heat transfer, particle size reduction as digestion progresses and release of produced gas from the digester contents (Meynell, 1976). With continuous mixing conditions, bacteria, substrates and liquid consequently have an equal retention time where solids retention time (SRT) will be equal to HRT. However, under non-continuous mixing conditions, as a consequence of stratification of solids and formation of dead zones (Stenstrom et al., 1983), SRT is not equal to HRT. This stratification could be employed as an operational strategy to retain solids in the reactor for a longer time than the average HRT (Kaparaju and Angelidaki, 2007). Such an operational strategy would not only retain a larger proportion of volatile solids (VS) for a more complete breakdown but also retain microbial biomass in the reactor and improve biogas production from manure (Ong et al., 2000; Zitomer et al., 2005).

Mixing is usually accomplished through various methods, including mechanical mixers, recirculation of digester

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contents, or by recirculation of the produced biogas using pumps (Karim et al., 2005). The importance of mixing in achieving efficient substrate conversion has been reported by several researchers (Smith et al., 1996; McMahon et al., 2001; Stroot et al., 2001; Kim et al., 2002; Karim et al., 2005; Vavilin and Angelidaki, 2005; Vedrenne et al., 2007). The main factors affecting digester mixing are the mixing strategy, intensity and duration and also the location of the mixer. However, the effect of mixing duration and intensity on the performance of anaerobic digesters are contradictory. Adequate mixing was shown to improve the distribution of substrates, enzymes and microorganims throughout the digester (Chapman, 1989; Lema et al., 1991) whereas inadequate mixing was shown to result in stratification and formation of floating layer of solids (Stenstrom et al., 1983; Chen et al., 1990). Continuous mixing was shown to improve biogas production compared to unmixed (Ho and Tan, 1985). The opposite results were also reported by several researchers (Ben-Hasson et al., 1985; Ghaly and Ben-Hassan, 1989; Chen et al., 1990). Nevertheless, intermediate mixing appears to be the most optimal for substrate conversion (Smith et al., 1996; Dague et al., 1970).

Mixing intensity was also shown to affect digester performances and biogas production (Stroot et al., 2001; Vavilin and Angelidaki, 2005). Minimal mixing was found to be sufficient to distribute the feed adequately and stimulate the formation of new initiation centers (Vavilin and Zaikin, 1971) that are required for autocatalytic reactions (Field and Burger, 1985). On the other hand, vigorous continuous mixing was shown to disrupt the structure of microbial flocks, which in turn disturbs the syntrophic relationships between organisms thereby adversely affecting the reactor performance (Stroot et al., 2001; McMahon et al., 2001; Kim et al., 2002).

Despite the importance of mixing in achieving efficient substrate conversion, there is no clear picture about the effects of mixing on anaerobic digestion of manure. Therefore, there is a need for further research on evaluating the optimum mixing strategy and/or duration. In the present study, the effect of three different mixing strategies viz., continuous mixing, minimal mixing (10 min before extraction/feeding) and intermittent mixing (continuous mixing but withholding mixing for 2 h before extraction/feeding) were tested in lab-scale experiments while the effects of continuous mixing (5 min on and 5 min off) and intermittent mixing were tested in a pilot-scale plant. Mean specific biogas yield was used as the primary evaluation parameter. In addition, the effect of mixing intensity (gentle, minimal and vigorous) under different substrate to inoculum ratios was also investigated in batch assays.

## 2. Methods

### 2.1. Lab-scale experiments

#### 2.1.1. Substrate preparation

Fresh cow manure collected from a full-scale biogas plant (Snertinge biogas plant, Denmark) was used as sub-

strate. 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 fresh cow manure after dilution are presented in Table 1.

#### 2.1.2. 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. 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. Effluent samples were drawn from the middle layer of the digester contents using the sampling port located on the top plate. 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. Reactors were fed semi-continuously at 12 h interval. Feed rate was 233 ml/d. 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 the effluent bottle. Biogas from the effluent bottle flowed to the gas meter to register biogas production as described elsewhere (Angelidaki et al., 1992).

#### 2.1.3. Reactor operation

The reactors were inoculated with 3.4 l of thermophilically digested inoculum obtained from a centralized biogas

Table 1
Characteristics of cow manure used in the experiments (after dilution)

| Parameter | Inoculum | Lab-scale expt. | Pilot-scale expt.[a] |
|---|---|---|---|
| TS (%) | 4.2 | 8.1 | 6.5–7.5 |
| VS (%) | 3.1 | 6.2 | 5–6.3 |
| pH | 7.3 | 7.2 | 7.5–7.7 |
| Total VFA (g/kg-waste) | 9.6 | 8.1 | 16.9–18.9 |
| Acetate (g/kg-waste) | 4.3 | 5.2 | 10.3–11.2 |
| Propionate (g/kg-waste) | 3.1 | 1.9 | 4.4–5.1 |
| Iso-butyrate (g/kg-waste) | 1.4 | 0.2 | 0.25–0.3 |
| Butyrate (g/kg-waste) | 0.1 | 0.3 | 0.95–1.1 |
| Iso-valerate (g/kg-waste) | 0.6 | 0.4 | 0.54–0.64 |
| Valerate (g/kg-waste) | 0.1 | 0.1 | 0.48–0.53 |
| Total-N (g-N/kg-waste) | 2.2 | 4.1 | 3.2–4.2 |
| $NH_4^+$-N (g-N/kg-waste) | 2.1 | 2.2 | 1.7–2 |
| Lipids (g/kg-waste) | n.a. | 6.3 | 6.3–7 |
| Protein (g/kg-waste) | n.a. | 12.5 | 8.1–12.5 |
| Carbohydrates | n.a. | 36.2 | 19.6–25.5 |

[a] Range of four batches; n.a.: not analysed; Protein = 6.25 × (TKN − $NH_4^+$-N); Carbohydrate = VS − lipids − proteins − VFA.

plant (Snertinge, Denmark) treating cow manure and pig manure with industrial wastes (Table 1). The experiment was carried out for a period of 70 days after start-up and stable gas production had been attained. During the initial 19 days of operation, reactors were mixed in a continuous mode by mechanical mixers. This was referred to as continuous mixing. Upon attaining steady-state under continuous mixing mode, various mixing strategies were introduced on day 20. The strategies adopted were minimal mixing (10 min mixing prior to extraction/feeding) in R2 and intermittent mixing (continuous mixing but withholding mixing for 2 h before extraction/feeding) in R3. From Day 44 onwards, R2 and R3 were operated again in continuous mixing mode as mentioned above. On the other hand, R1 was mixed in a continuous mode throughout the experimental run.

## 2.2. Pilot-scale experiments

Pilot-scale experiments were conducted under more realistic conditions in order to support results from lab-scale experiments, where reactors were fed with blended manure.

### 2.2.1. Substrate

Fresh cow manure was obtained in 800 l batches from a centralized biogas plant (Hashoj biogas plant, Denmark). Five different batches were collected directly from incoming delivery trucks (i.e. uncut) and always from the same cattle farm in order to ensure as uniform feed characteristics as practically possible. For each batch, feed was prepared by diluting the manure with water, whenever necessary, to attain a consistent TS of 6.5–7.5%. Characteristics of fresh cow manure after dilution are presented in Table 1.

### 2.2.2. Reactor setup

The experiment was carried out in a pilot-scale plant built at the Institute of Environment and Resources, Technical University of Denmark. The reactor (800 l) was built from a used stainless steel tank. The reactor was fitted with a stainless steel top plate, which supported the mixer, mixer motor, gas sampler, safety and pressure valve and a level switch. Feed valve, effluent valves (3), temperature probe and sampling ports (3) were fitted to the reactor wall. Sampling ports and effluent valves were located at positions corresponding to the top, middle and bottom layer of digester contents. Effluent was removed from middle valve. The reactor had one outlet at the bottom for sediment removal. Reactor temperature was maintained at $54 \pm 1\,°C$ by pumping hot water through a stainless steel coil fitted inside the reactor. An electric flow heater (Spare-system 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 between the heater and the reactor was circulated using a 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.

Reactor was fed semi-continuously at an 8 h interval by pumping the feed from a feed tank (800 l). Feed was thoroughly mixed for 15 min prior to each feeding using a high speed mixer (DriveIT, 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). Two 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 had a flow rate of 10 l per 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 two channel 24 h/7 day programmable time switch (SC 28, Muller).

### 2.2.3. Reactor operation

The reactor was operated with a liquid working volume of 500 (total digester volume 800 l) with a HRT of 20 days and at $54 \pm 1\,°C$. At start-up, 450 l of thermophilically digested manure (see section 2.1.3) and 30 l of fresh cow manure were transferred to the reactor. Daily feeding was commenced approximately 10 days after start-up. Reactor was fed three times a day (8 h interval). Feed rate was 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 min on/off mode. While under intermittent mixing, the mixer was operated similar to that of continuous mixing but mixing was completely withheld for 2 h prior to each extraction/feeding. 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 to a nominal TS level of 6.5–7.5%. However, feed batch changes remained the main source of disturbances. For this reason, intermittent mixing periods were arranged relative to feed batch changes to obtain periods without feed batch change some time before/after changing mixing strategy.

### 2.2.4. Residual methane potential

The experiment was carried out in a 118 ml glass bottles. Digested material was sampled from the top, middle and bottom layers of the reactor on Day 102 (continuous mixing) and Day 123 (intermittent mixing) of operation. All samples were collected just before feeding, i.e., at the end of mixer blocking period for intermittent mixing. To each assay, 20 ml digested material were used. 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 55 °C. 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. Effect of mixing intensity on biogas process

The effect of mixing intensities and substrate to inoculum ratios on biogas production was tested in batch assays at 55 °C. Fresh cow manure obtained from a centralised biogas plant (Hashoj Biogas plant, Denmark) was used as substrate. Digested manure collected from the pilot-scale biogas plant was used as inoculum.

Batch experiments were conducted in 1 l serum bottles with a total working volume of 400 ml. The tested substrate to inoculum ratios were 10/90 and 40/60. Assays were mixed either vigorously and continuously on a shaker table (110 times per minute with a 3.5 cm stroke), gently and continuously on a shaker table (35 times per minute with a 1.2 cm stroke) or minimally – thoroughly shaken by hand for about 1 min every time a sample was taken. The experiment was carried out in triplicates and incubated at 55 °C. After 73 days of the run, mixing intensity of vigorously mixed assays was reduced to gentle while the mixing intensity of gently mixed assays was changed to vigorous mixing.

### 2.4. Chemical analyses

Biogas production and digester temperature were followed on daily basis. Process parameters such as pH, volatile fatty acids (VFA), TS, VS and ammonium nitrogen ($NH_4^+$-N) were followed by periodic sampling and analyses (twice a week). Sampling was performed before extraction/feeding. Effluent samples (20 ml in case of lab-scale reactors and 100 ml in case of pilot-scale reactor) were taken from the middle sampling port of the digester representing process parameters. Representative sample were obtained by wasting an initial sample of approximately 200–300 ml in case of pilot-scale and 10–20 ml in case of lab-reactors. Methane content in biogas and VFA were measured by GC with FID as described elsewhere (Sorensen et al., 1991). Samples were stored at −20 °C until analysis. TS, VS and pH were determined according to Standard Methods (APHA, 1998). Total-N and $NH_4^+$-N were measured following Kjeldahl-N method (Greenberg et al., 1992).

### 2.5. Microbiological analyses

The effect of mixing scheme on stratification of microorganisms was investigated through Fluorescence in situ hybridization (FISH) analyses. FISH was performed using oligonucleotide probing (Hugenholtz et al., 2001). Samples were collected from the top, middle and bottom layers of pilot-scale reactor and from the middle layer of lab-scale reactors. Sampling was done at the end of each mixing strategy and within the same feed batch. The specific probes used have been described elsewhere (Kaparaju and Angelidaki, 2007). Briefly, the probes used were EUB-MIX-CY3 for Bacteria; ARC915 Alexa488 for Archaea; MX825 CY3 for Methanosaetaceae; MS1414 CY3 for Methanosarcinaceae; MG1200 CY5 for Methanomicrobiales; MB1174 CY5 for Methanobacteriaceae; MC1109 CY5 for Methanococcaceae. The slides were examined using a Zeiss microscope.

### 2.6. Calculations

For lab-scale experiments, specific methane yield was calculated as daily methane produced divided by the actual feed VS. Theoretical methane yield (STP l $CH_4$/g VS) was calculated based on the stoichiometric conversion of organic matter to methane and carbon dioxide and estimated as $0.496 \times$ proteins (%) + $1.014 \times$ lipids (%) + $0.415 \times$ carbohydrate (%) + $0.373 \times$ acetate (%) + $0.53 \times$ propionate (%) in the substrate.

For pilot-scale experiments, specific biogas yield was calculated as the daily biogas production, divided by a weighted average of VS fed 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 fed from the three most recent days, 29% of VS fed from the previous 3 days and 14% of VS fed from the last 3 days.

## 3. Results

### 3.1. Lab-scale experiments

#### 3.1.1. 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. 1 and Table 2. Process performance was evaluated by presenting the mean values of parameters such as biogas production and VFA over 1 HRT. During the pre-run continuous mixing period (Day 0–19), biogas production was relatively stable with an average production of 10.91, 10.59 and 10.88 ml/ml feed in R1, R2 and R3, respectively (Table 2). At the end of the pre-run continuous mixing period, mixing strategies in R2 and R3 were changed (Day 20) to minimal and intermittent mixing, respectively, while R1 was continued in continuous mixing mode. Biogas production (Day 20–43) in R1, R2 and R3 were 10.0, 11.28 and 10.15 ml/ml feed. On comparison with R1, the increase in biogas production in R2 (minimal mixing) and R3 (intermittent mixing) during the same period was 12.5% and 1.3%, respectively (Table 2).

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
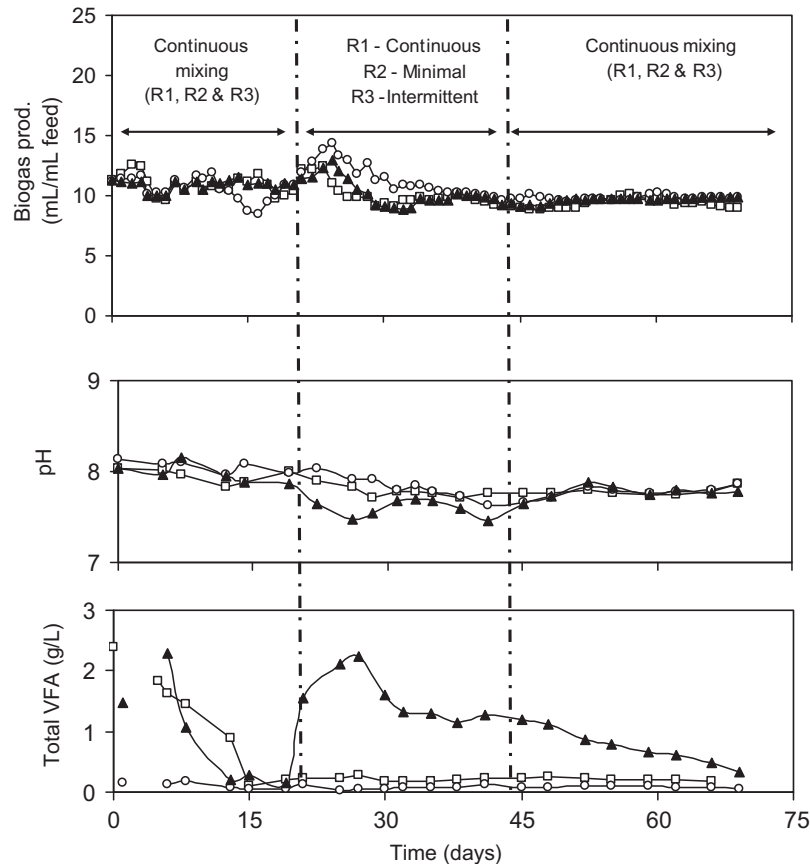
Fig. 1. The effect of mixing strategies on process performance and biogas production during anaerobic digestion of manure in CSTR at 55 °C: R1 (□, continuous), R2 (○, minimal) and R3 (▲, intermittent stirring).

in all three reactors and pH remained stable around 8 throughout this period (Fig. 1).

Mixing strategies also affected the methane yields (Table 2). Highest methane yield of 0.245 m³/kgVS was obtained in R2 compared to the yields obtained in R1 and R3.

The utilized methane potential calculated based on the theoretical methane yield of the fresh manure (0.4 m³/kgVS) 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 (56.4%) than R1 or R3 (50%).

On Day 44, mixing strategies were reverted to continuous mode in R2 and R3. The average biogas production during Days 55–69 (post-test continuous mixing period) was approximately 9.5–9.8 ml/ml feed in all three reactors (Table 2). VFA and ammonia levels remained more or less the same in all three reactors.

### 3.1.2. Microbiological analyses

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

### 3.2. Pilot-scale experiments

#### 3.2.1. Effect of mixing strategies on process performance and biogas production

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 Figs. 2 and 3 and Table 3. In Fig. 2, batch changes and periods with intermittent mixing period are indicated. Fig. 2 illustrates the level of stability obtained. Daily loading (data not shown) was 25 l/day and temperature was maintained at 54 ± 1 °C 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 with no significant variation in relation to mixing strategy.

Table 3 summarizes the results subdivided into intervals with same feed batch and mixing strategy. Mean specific biogas yield, the primary evaluation parameter, is shown in Fig. 3. 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% when results obtained within the same feed batch was compared. In addition, the effluent VS level was generally lower during the periods of intermittent mixing than

Table 2
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 production (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 production (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 production 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 |

continuous mixing, which indicate that this mixing strategy resulted in stratification of digester content and thus minimized VS loss. The trend in biogas yields upon changing mixing strategy, 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 shift from continuous to intermittent mixing was relatively short and was affected by a disturbance (shift in specific biogas yield) related to feed batch change (Fig. 3). Furthermore, there was a VFA build-up in the initial phase of the intermittent mixing (Fig. 2), 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 45) 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 on Day 70, must have been with a high biogas potential. However, the process appeared to have

stabilized before the second intermittent mixing period was initiated.

### 3.2.2. Microbiological analyses

FISH analyses showed abundance of small short rods-shaped bacterial cells during continuous mixing mode with Methanomicrobiales being the dominant methanogens (data not shown). However, no stratification of microorganims between top, middle and bottom layers of the reactor was noticed. Conversely, stratification of microorganisms was noticed in the samples taken from top, middle and bottom layers of the reactor during intermitent mixing period. Small irregular shaped archaeal cells belonging to Methanomicrobiales were abundant in the middle layer. On the other hand, thin and short rod-shaped Archaea belonging to Methanobacteriaceae, strict hydrogen utilising methanogens, were observed in the top layer, while clusters of Methanosarcinacae and cells belonging to Methanomicrobiales were found in the bottom layer.

### 3.2.3. Residual methane potential

The experiment was carried out for more than 76 days. Overall, methane production started immediately in all assays. Residual methane potential of samples collected during continuous mixing was more or less the same between top, middle and bottom layers (4.6–5 ml/ml sample). On comparison with continuous mixing period, higher residual methane potential was noticed in samples collected during intermittent mixing, especially with respect to top and bottom samples. Methane potential of samples during intermittent mixing was 5.86, 4.0 and 6.33 ml/ml sample in the top, middle and bottom samples, respectively.

### 3.3. Effect of mixing intensity on biogas process

### 3.3.1. High substrate to inoculum ratio (40/60)

The experiment was carried out for 160 days and the average methane yields and VFA production are illustrated in Fig. 4. Results showed that mixing intensity had profound influence on the methane production rates and yields. Methane production started immediately in gentle and minimal mixed assays and reached a maximum of 0.21 $m^3$/kgVS after 73 days of incubation. On the other hand, methane production in vigorously mixed assays was delayed and remained low for up to 25 days before reaching 0.18 m3/kgVS of methane at the end of 73 day run (Fig. 4). However, when mixing intensity was reduced (Day 73), methane yields in vigorously mixed assays was improved by 36% compared to 13% in gently mixed assays. Mixing intensity also affected the acetate and propionate concentrations and their turnover during the digestion (data not shown). High levels of acetate and propionate with persistence of propionate was mainly noticed in vigorously mixed assays compared to gentle or minimal mixed assays. However, by switching from vigorous to gentle mixing conditions, propionate was quickly consumed.
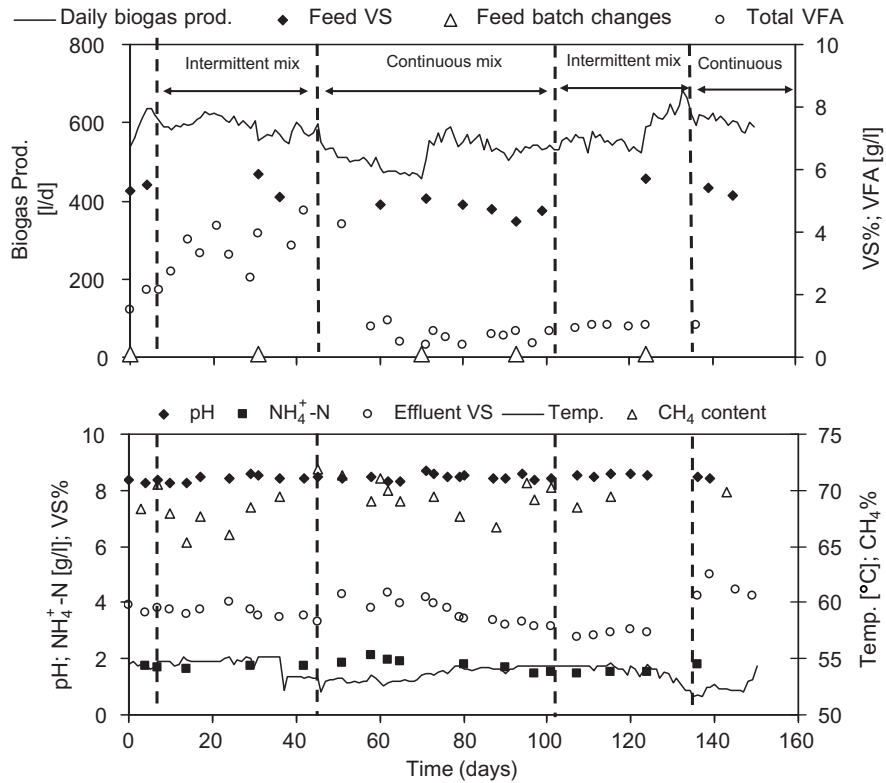
Fig. 2. Process performance during anaerobic digestion of manure with continuous and intermittent mixing (mixer blocking) strategies at 55 °C.

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 Production (l/d) | 599 | 603 | 572 | 497 | 540 | 550 | 628 | 602 |
| Biogas production (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 |
| Temperature (°C) | 54.5 | 54.8 | 54.0 | 53.0 | 54.1 | 54.2 | 53.2 | 52.5 |

### 3.3.2. Low substrate to inoculum ratio (10/90)

The effect of mixing intensity on methane and VFA production under a low substrate to inoculum ratio was significantly different. Methane production started immediately in all assays but remained low for up to 25 days in vigorously mixed assays (Fig. 4). At the end of 73 day run, methane yields were 0.32, 0.20 and 0.19 m$^3$/kgVS in gently, minimally and vigorously mixed assays, respectively. However, methane yields improved by 34% when mixing intensity was reduced in vigorously mixed assays.

## 4. Discussion

Results from the present study showed that mixing strategies and intensities affect process performance and methane production during anaerobic digestion of cow manure. Lab-scale studies in CSTR showed that minimal mixing improved biogas production compared to intermittent or continuous mixing. These results are in accord with Stroot et al. (2001). The increased methane production under minimally mixed conditions was attributed to a better syntrophic association between H2 producing and consuming organisms. It is presumed that in a CSTR with semi-continuous mode of feeding, minimal mixing might result in slower hydrolysis and fermentation without affecting the syntrophic association (Stroot et al., 2001). This would thus allow the syntrophs and methanogens to consume the fermentation products without any VFA build-up (Vavilin and Zaikin, 1971; Vavilin et al., 2007).
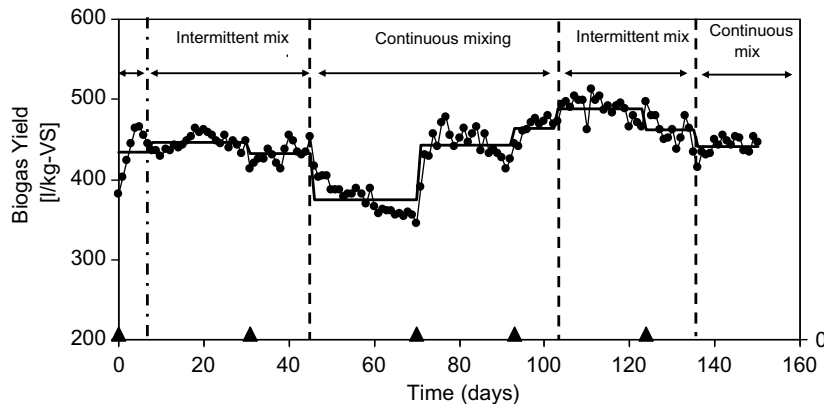
Fig. 3. Specific biogas yield (●) during anaerobic digestion of manure with continuous and intermittent mixing (mixer blocking) strategies at 55 °C. ▬, Period average; ▲, feed batch change.
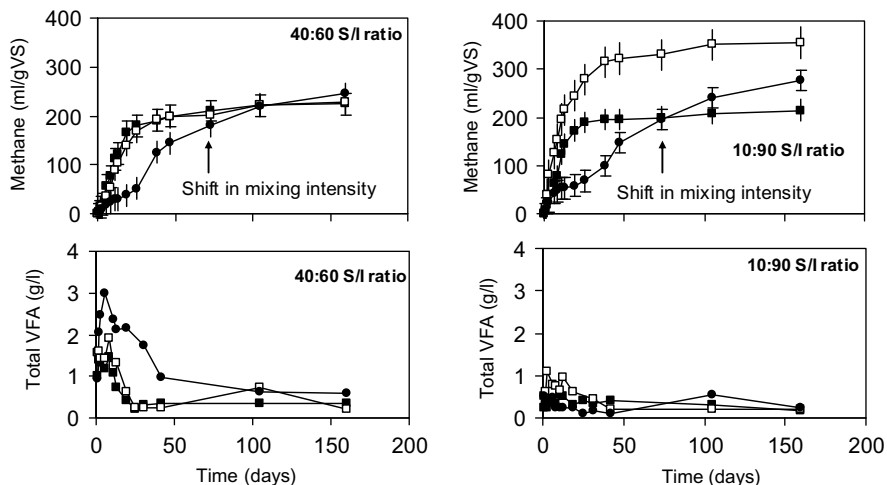


Fig. 4. Methane (above) and volatile fatty acids (below) production at different loading rates (substrate to inoculum (S/I) ratios of 10/90 and 40/60) and mixing intensities: ■ No mixing, □ gentle mixing and ● vigorous mixing.

The improved biogas production under intermittent mixing compared to continuous mixing in the pilot-scale plant can be attributed to better solids and biomass retention in the reactor. A similar observation was made by Dague et al. (1970). The different response to intermediate degree of mixing – found to be optimal for substrate conversion in pilot-scale plant but not in lab-scale study – could be due to the use of blended manure in lab-scale study and also due to differences in reactor construction and scale-up. Nevertheless, the improved biogas yield (7%) during intermittent mixing in the pilot-scale study is significant for existing and new full scale biogas plants, as the improved method involves virtually no cost, except a revised control philosophy for digester mixing.

The choice of a 2 h mixer blocking period ("non-stirring") was based on previous settling experiments (Kaparaju and Angelidaki, 2007) 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 h. During this "non-stirring" period, the lighter fibre fraction floats to the surface (by floatation) while the heavier solids settle to the bottom (by gravity) leaving the middle portion with low suspended 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 VS loss. This result was further evident from the residual methane potential study where the samples collected during intermittent mixing had higher methane potential, especially from the top and bottom samples, than those sampled during continuous mixing. 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.

The higher abundance of Bacteria, the main phylogenetic group involved in hydrolytic–acidogenic stages of anaerobic degradation (Madigan et al., 2000), in the top and bottom layers than in the middle layer of the pilot-scale plant indicate that active hydrolytic–acidogenic degradation of solids takes place in these zones and some binding of these microorganisms to the solid material is essential (Cirne, 2006).

The high 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 evolution of Methanomicrobiales in the pilot-scale reactor indicates that intermittent mixing facilitated syntrophic association and that Methanomicrobiales were preferred syntrophic partners for syntrophic propionate-oxidizing bacteria (SPOB) (McMahon et al., 2001). It is presumed that during the periods of rapid priopionate comsumption, SPOB were dependent on aceticlastic methanogens (Methanosarcinaecae and Methanosaetaceae) and hydrogenotrophic methanogens (such as Methanobacteriaceae) to consume their metabolites (McMahon et al., 2001). The presence of Methanomicrobiales during continuous mixing suggests that Methanobacteriaceae were out competed by Methanomicrobiales and could have hindered SPOB activity.

The low and delayed methane production noticed under vigorous mixing, especially under high substrate to inoculum ratio in batch experiments, indicate that methanogenesis was inhibited as the produced VFA was distributed homogenously in the bottle. 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 (Vavilin and Angelidaki, 2005) and destroy the synthrophic interactions (Stroot et al., 2001). In a similar study, high VFA concentration was showed to inhibit strongly the methanogenesis at any mixing conditions when high substrate to methanogenic biomass ratio (41 g/g) was used during anaerobic digestion of undiluted municipal household solid waste (Vavilin and Angelidaki, 2005).

The persistence of propionate during vigorous mixing indicates that mixing may also play a detrimental role in the turnover of propionate, possible because of the disruption of synthrophic association. Similar observation was also made by Stroot et al. (2001). However, by switching from vigorous to gentle mixing conditions, propionate was quickly consumed, while continued vigorous mixing resulted in propionate persistence (Stroot et al., 2001). Both these results in practice suggest that vigorous mixing should be avoided during the batch start-up of manure digesters, especially under high substrate to inoculum ratio conditions. It also suggest that a further improvement in process performance might be obtained by limiting mixing intensity for a period after feeding, in cases where CSTRs are operated semi-continuously with interval between feed batches. Additionally, under low substrate to inoculum ratio, 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 existing reactors after a process failure, where it may not be practical to empty the reactor contents, the above illustrate that a reduced mixing intensity after reinoculation may be helpful to start-up the process faster. Thus, mixing schemes not only improved solids and biomass retention but also affected spatial distribution of substrate and biomass (Kaparaju and Angelidaki, 2007).

## 5. Conclusion

Results from both lab-scale and pilot-scale experiments showed that mixing strategies had some influence on process performance and methane production in CSTR reactors treating cow manure. Among the three mixing strategies tested in the lab-scale experiments, minimal mixing was found to improve methane production by 12.5% compared to continuous mixing. Withholding mixing for 2 h prior to extraction/feeding would result in stratification of solids with higher solids content in the top and bottom layers compared to middle layer. Similar results were also obtained in pilot-scale plant. The increase in biogas yield was from 2.5% to 14.6% during intermittent mixing compared continuous mixing. However, further experimental work is needed to elucidate this result as the mixing strategies overlapped each other and the retention time for each mixing strategy was probably too short. The effect of organic load and mixing intensities (minimal, gentle or vigorous) during biogas process showed that gentle or minimal mixing under low substrate to inoculum ratio or gentle mixing under high substrate to inoculum ratio would result in higher methane production. On the contrary, vigorous mixing would result in delayed and low methane production, especially under high initial substrate to inoculum ratio.

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