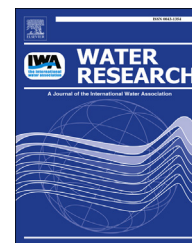




ELSEVIER

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.elsevier.com/locate/watres

High rate manure supernatant digestion

Wenche Hennie Bergland ^{a,*}, Carlos Dinamarca ^a,
 Mehrdad Toradzadegan ^{a,1}, Anna Synnøve Røstad Nordgård ^b,
 Ingrid Bakke ^b, Rune Bakke ^a

^a Telemark University College, Kjølnes Ring 56, 3918 Porsgrunn, Norway

^b Norwegian University of Science and Technology, Department of Biotechnology, Sem Sælands vei 6/8, 7491 Trondheim, Norway

ARTICLE INFO

Article history:

Received 3 December 2014

Received in revised form

18 February 2015

Accepted 22 February 2015

Available online 4 March 2015

Keywords:

Pig manure supernatant

Energy from slurries

AD stability

High organic load

Microbial communities

ABSTRACT

The study shows that high rate anaerobic digestion may be an efficient way to obtain sustainable energy recovery from slurries such as pig manure. High process capacity and robustness to 5% daily load increases are observed in the 370 mL sludge bed AD reactors investigated. The supernatant from partly settled, stored pig manure was fed at rates giving hydraulic retention times, HRT, gradually decreased from 42 to 1.7 h imposing a maximum organic load of 400 g COD L⁻¹ reactor d⁻¹. The reactors reached a biogas production rate of 97 g COD L⁻¹ reactor d⁻¹ at the highest load at which process stress signs were apparent. The yield was -0.47 g COD methane g⁻¹ COD_T feed at HRT above 17 h, gradually decreasing to 0.24 at the lowest HRT (0.166 NL CH₄ g⁻¹ COD_T feed decreasing to 0.086). Reactor pH was innately stable at 8.0 ± 0.1 at all HRTs with alkalinity between 9 and 11 g L⁻¹. The first stress symptom occurred as reduced methane yield when HRT dropped below 17 h. When HRT dropped below 4 h the propionate removal stopped. The yield from acetate removal was constant at 0.17 g COD acetate removed per g COD_T substrate. This robust methanogenesis implies that pig manure supernatant, and probably other similar slurries, can be digested for methane production in compact and effective sludge bed reactors. Denaturing gradient gel electrophoresis (DGGE) analysis indicated a relatively fast adaptation of the microbial communities to manure and implies that non-adapted granular sludge can be used to start such sludge bed bioreactors.

© 2015 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

1. Introduction

Governments promote anaerobic digestion (AD) of manure because it can reduce greenhouse gas (GHG) emissions and odors, produce renewable energy as methane and improve fertilizer properties (Masse et al., 2011). The largest potential source of methane by anaerobic digestion (AD) of wet organic

waste is manure, e.g. ~40% in Norway, but an insignificant fraction of this is realized (Berglann and Krokann, 2011). The main reason for this is the low energy density of manure, implying low production rates in continuous flow stirred tank reactors (CSTR) currently used for manure AD. Such solutions are not sustainable because the costs of construction and operation of such plants are larger than the value of the methane produced (Berglann and Krokann, 2011). Large scale

* Corresponding author.

E-mail address: wenche.bergland@hit.no (W.H. Bergland).

¹ Present address Yara Norge AS, Herøya Industripark, Postbox 1123, 3905 Porsgrunn, Norway.

<http://dx.doi.org/10.1016/j.watres.2015.02.051>

0043-1354/© 2015 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

farms may have their own CSTR AD solutions that are economically sustainable (Raven and Gregersen, 2007) but agriculture is dominated by smaller farms where such systems may not be rentable. Manure transport to central AD treatment plants is used to some extent, especially in Germany, but the sustainability of such solutions is questioned due to transport cost of manure with low biogas potential.

More efficient process solutions for AD treatment of manure are therefore required. High rate AD reactors may treat waste in smaller and presumably much cheaper digesters. A high rate AD manure treatment technology that is well integrated with existing farm infrastructure for slurry based manure handling systems, common for cattle and pig farms (Burton and Turner, 2003), is therefore investigated here. Manure from farms using slurry based handling systems has 61% of the total theoretical Norwegian manure energy potential of 2480 GWh/a (Raadal et al., 2008). The situation vary some around the world but it is assumed that the case investigated here is relevant for a large fraction of modern global agriculture, as well as aquaculture and other activities producing organic waste slurries.

Manure storage tanks with 8 months minimum HRT capacity, already installed in cold climate countries (e.g. Norway, to comply with government regulations to avoid use as fertilizer outside the short growth season), may serve as a first step in an AD treatment line and/or be used for effluent storage. It has been observed that manure particles disintegrate and hydrolyze during such storage, thereby improving its quality as AD feed (King et al., 2011; Bergland et al., 2014). In such tanks manure separates spontaneously into a floating layer (straw, wood chips, etc.), a bottom sediment layer and a middle layer with much less suspended solids than the floating and bottom layers (Fig. 1). Potentially suitable high rate AD feed can be taken out from the middle layer at no extra cost. A main issue of the present study is to determine if this middle layer, termed manure supernatant, can be used as feed for high rate AD. The assumption is that, if a sludge blanket high rate AD works well on such feed, this process can become economically feasible.

The original and most extensively used high rate reactor is the UASB (upflow anaerobic sludge blanket), developed by Lettinga et al. (1980). Such sludge blanket reactors are used to



Fig. 1 – Pig manure sample collected near the bottom of a pig manure storage tank.

treat the liquid fraction of organic waste containing small amounts of suspended solids (Tchobanoglous et al., 2003). The particle content of settled manure (Fig. 1) is higher (>6 g TSS L⁻¹) than recommended for UASB treatment (Tchobanoglous et al., 2003). Alternative high rate AD designs, such as fixed biofilm reactors, have been tested on such wastes but solids build up blocking the void spaces in the filter medium making such alternatives less promising (Bolte et al., 1986). Hybrid UASB (Lo et al., 1994) and a suspended particle-attached growth (SPAG) reactor (Cobb and Hill, 1989), are also available. The UASB is, however, the standard of high rate AD, so a small UASB like sludge bed reactor design was chosen for the present study to test the possibilities of high rate AD slurry treatment.

The objective of this study was to examine the efficiency, flexibility and stability of manure supernatant AD treatment in sludge bed reactors. The process capacity and robustness was evaluated by measuring manure degradation and product formation for a wide range of loading rates, including loads that are much higher than what is expected to be required or optimal. A PCR/DGGE strategy was employed to characterize the microbial communities, and to evaluate the time needed for adaption of the granular inoculum to the conditions in the manure-fed AD reactors. The study is relevant for the development of efficient wet organic waste AD with low energy density and high particulates contents in general (e.g. manure, wastewater treatment plant sludge, aquaculture waste sludge) and it may be decisive for the development of sustainable solutions to recover energy for slurry type manures.

2. Materials and methods

2.1. Manure properties and handling

The process feed was pig manure slurry supernatant regularly collected from a production farm in Porsgrunn, Norway. The manure comes from barns that contains 105 sows, 315 “farrow to finish” and 545 weaners that are fed protein concentrate (14.6% crude protein) added some grass/straw. Wood shavings and straw are used as bedding material. The manure is transported into a storage pit where it is diluted about 30% by wash water from regular barn washing routines. This mixture is what we define as manure slurry, according to Burton and Turner (2003). The HRT of the storage pit varies from 70 to 90 days, which has no significant effects on manure composition (Bergland et al., 2014). The manure separated by gravity in the storage pit into three distinct layers. The top layer is wood shavings and straw. Heavy particles settled to form a bottom layer (Fig. 1). The middle layer, termed the manure slurry supernatant (Table 1), was siphoned and used as feed without any filtering. Fresh manure supernatant was thus collected frequently and stored at 4 °C until use.

2.2. Reactor design and start up

The reactor is a simplified UASB (Fig. 2a) made of a 370 mL glass vessel with 345 mL liquid volume, height 130 mm and diameter 60 mm. The substrate inlet is a central tube ending

Table 1 – Properties of the pig manure slurry supernatant used as substrate (Average and Std. Dev.).

Property	Average \pm SD
pH	7.3 \pm 0.3
COD _T (g L ⁻¹)	28.1 \pm 2.7
COD _S (g L ⁻¹)	16.0 \pm 2.8
COD _{VFA} (g L ⁻¹)	12.2 \pm 1.1
Acetate (g COD L ⁻¹)	5.7 \pm 0.9
Propionate (g COD L ⁻¹)	2.7 \pm 0.6
Butyrate + iso-butyrate (g COD L ⁻¹)	2.1 \pm 0.3
NH ₄ ⁺ – N (g L ⁻¹)	2.35 \pm 0.04
Alkalinity (g L ⁻¹)	8.7 \pm 0.8
TS (g L ⁻¹)	14.5 \pm 1.5
VS (g L ⁻¹)	7.3 \pm 1.5
TSS (g L ⁻¹)	6.2 \pm 2.7
VSS (g L ⁻¹)	5.1 \pm 1.8

10 mm above the reactor bottom, with a horizontal plate at the end to improve distribution of the substrate below the sludge bed. The lab-scale process line is presented in Fig. 2b. Suspended solids are separated inside the reactors to retain biomass while the gas and liquid is separated outside the reactors to ease operation in such small scale reactors. The substrate tank is kept at 4 °C and the four reactors at 35 °C.

Four identical reactors were operated for 68 days. The inoculum was based on granules (70 g L⁻¹ VSS) from a UASB reactor treating pulp and paper process wastewater at “Norske Skog Saubrug” in Halden, Norway. Half of the reactor volumes were filled with granules. Two of the reactors had been fed pig manure for 6 months as an adaption period prior to the experiment. The other two were inoculated using granules without any adaptation (these granules were stored at 11 °C for 6 months with no feed prior to the experiment). The reactors with granules not adapted to pig manure were started at a HRT of 42 h (medium rate) while the reactors with adapted biomass were started at 8.5 h HRT (high rate). Nearly constant HRT was maintained after start up until stable biogas production was established. Then an increase of the feed flow of 5% was imposed every day.

The reactors were fed intermittently, 25 mL each time which is < 1/10 of reactor liquid volume implying >10 feedings

for each HRT. It is therefore reasonable to assume continuous flow in the mass balance analysis of the process. Feed flow increases were obtained by increasing the feeding frequency.

2.3. Analysis

Biogas, inflow and outflow liquid samples were collected twice a week. Total chemical oxygen demand (COD_T), soluble COD (COD_S), total solids (TS), volatile solids (VS), total suspended solids (TSS), volatile suspended solids (VSS), pH, alkalinity, NH₄⁺–N, VFA's (acetate, propionate, butyrate, iso-butyrate, valerate, iso-valerate, iso-capronate and capronate) and gas composition were analyzed.

Gas production (L d⁻¹) and reactor temperature were monitored continuously online. The biogas flow was measured using a volumetric gas meter working according to the same principle as used by Dinamarca and Bakke (2009). The reactors were kept at 35 °C in a water bath.

COD was measured according to US standard 5220D (APHA, 1995). For COD_S determination the samples were first centrifuged at 10,000 rpm for 30 min and then filtered (0.45 µm). Alkalinity was measured by titration according to US standard 2320B (APHA, 1995).

NH₄⁺ – N concentration was analyzed on filtered samples (0.2 µm) by ion chromatography using an DX-500 ion chromatographic analyzer equipped with a conductivity detector, a SCS1 cation-exchange column (4 × 250 mm) in combination with a Dionex IonPac PCG1 (4 × 50 mm) guard column. 4 mM methane-sulfonic acid was used as the mobile phase. The oven temperature was kept constant at 35 °C.

VFA's were measured by gas chromatography (Hewlett Packard 6890) with a flame ionization detector and a capillary column (FFAP 30 m, inner diameter 0.250 mm, film 0.5 µm). The oven was programmed to go from 100 °C, hold for 1 min, to 200 °C at a rate of 15 °C min⁻¹, and then to 230 °C at a rate of 100 °C min⁻¹. The carrier gas used was helium at 23 mL min⁻¹. The injector and detector temperatures were set to 200 °C and 250 °C, respectively.

Gas composition (CO₂ and CH₄) was quantified by gas chromatography (Hewlett Packard 5890A) equipped with a thermal conductivity detector and two columns connected in

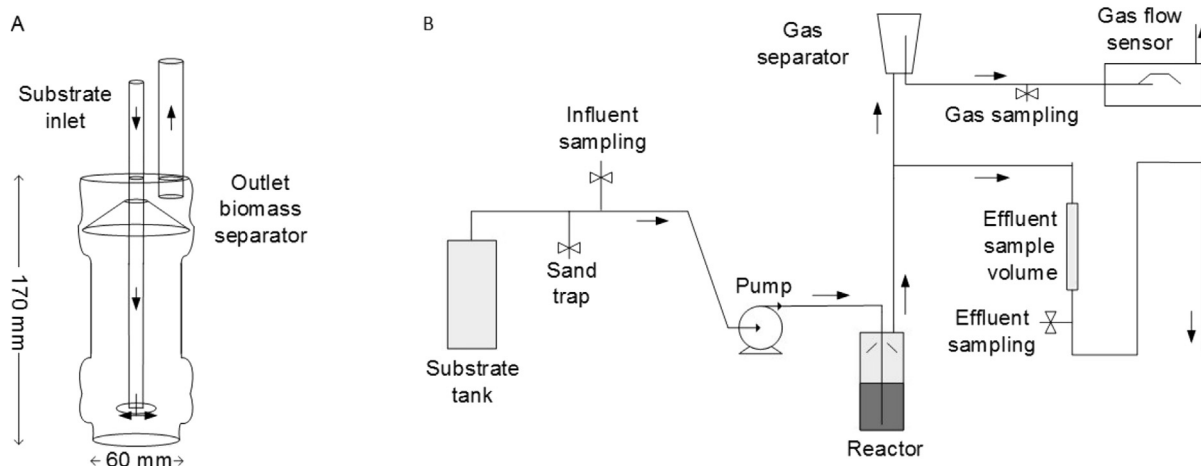


Fig. 2 – A) Sketch of lab-scale AD reactor with central inlet and separator. B) Diagram of lab-scale process line.

parallel: Column 1, CP-Molsieve 5A (10 m × 0.32 mm) and Column 2, CP-PoraBOND Q (50 m × 0.53 mm). The gas carrier was argon at 3.5 bar pressure. The oven temperature was kept constant at 40 °C.

2.4. DNA extraction, PCR, DGGE and statistical analysis

Samples for microbial analysis were taken from the sludge trap of the reactors at days 35, 61 and 68 of the experiment. Total DNA was extracted from the sludge samples by using the PowerFecal DNA Isolation Kit (MoBio) as described by the manufacturers. For bacteria, the v3 region of the 16S rRNA gene was amplified with the primers GC-338F (5'-cgcccgcgcgcgcccggggcgggggcgggggcagggggg actcctacggaggcagcag-3') and 518R (5'-attaccgctgctgctgg-3') (Muyzer et al., 1993). For methanogenic archaea, PCR primers targeting the 16S rRNA gene were designed. First, conserved regions of the 16S rRNA gene were identified by using alignments of methanogenic archaeal sequences downloaded from the Ribosomal database project (RDP). The Probematch tool of RDP was used for optimization of primer sequences and improving coverage. The resulting primers, GC-624F (5'-cgcccgcgcgcgcccggggcgggggcgggggcagggggg caccdrtggcgaagc-3') and 820R (5'-gccrattccttaagtcca-3'), was employed to amplify the v5 region of the 16S rRNA gene. PCR reactions were performed using the Taq PCR Core Unit Kit (Qiagen) and 0.3 μM of each primer, and run for 35 cycles of 95 °C for 30 s (s), 53 °C for 30 s, and 72 °C for 60/90 s for bacterial/archaeal PCR products, respectively. The PCR products were analyzed by denaturing gradient gel electrophoresis (DGGE) (Muyzer et al., 1993) with the INGENYphorU DGGE system (Ingeny) and 8% acrylamide gels with a denaturing gradient of 35–55 % for bacterial PCR products and 35–50 % gradient for methanogenic archaeal PCR products, as described in Bakke et al. (2013).

The Gel2K program (Svein Nordland, Department of Microbiology, University of Bergen, Norway) was used for converting band profiles in DGGE images to histograms, where the peaks correspond to DGGE bands. Peak area matrices, reflecting the band intensities, were exported to Excel spread sheets and used for statistical analysis. Individual peak areas were normalized by dividing on the sum of the peak areas for the relevant DGGE profile. Statistical analyses were performed using the program package PAST version 2.17 (Hammer et al., 2001). Bray–Curtis similarities (Bray and Curtis, 1957) were used to compare DGGE profiles, and was calculated based on square root transformed peak areas to reduce the impact of strong bands. Ordination based on Bray–Curtis similarities were performed using non-metric multidimensional scaling (NMDS; Taguchi and Oono, 2005). PERMANOVA was used for testing differences in average Bray–Curtis dissimilarities between groups of samples (Anderson, 2001).

3. Results and discussions

All four reactors produced biogas from day one and stabilized after 35 days of constant hydraulic load. The results are from the subsequent 33 days with 5% daily feed flow increase giving the reactors HRT from 42 to 8.5 h for “medium rate” and from

8.5 to 1.7 h for “high rate”. Biogas production increased with load during the whole experiment with low standard deviations between the parallel reactors.

3.1. Stability

In all the reactors the biogas production was still increasing, due to the increasing load, when the experiment was stopped. No foaming, typically experienced in manure AD (Hill and Bolte, 2000), or significant pH changes were observed. The average effluent pH in all 4 reactors was 8.0 ± 0.1 with influent average pH of 7.3 ± 0.3 and no active pH control. The alkalinity was also stable with similar effluent alkalinities of 10.6 ± 0.8 g L⁻¹ (high rate case) and 11.0 ± 0.9 (medium rate case). No visual signs of process failure or instability were observed even at the highest organic load rate (OLR) of 400 g COD L⁻¹ reactor d⁻¹ tested, implying that pig manure slurry supernatant sludge blanket AD can be a very robust process (chemical signs of process instability are discussed below). The reactors also showed remarkable stability and adaptation to the daily loading rate changes. Stable performance has also been reported for attached growth reactors fed liquid pig manure (Bolte et al., 1986) at loads in the lower range tested here. The observed robustness and process stability is especially important for farm and other small scale AD applications without dedicated process operators.

3.2. Capacity

The methane production rate and yield, VFA and COD results are evaluated to establish process efficiency and capacity of the process. The daily average methane production rate during the daily 5% load increases are given in Fig. 3a with a rate of $58 \cdot \text{HRT}^{-0.79}$ NL CH₄ L⁻¹ reactor d⁻¹ (HRT in hours; R² is 0.99). The highest measured rate was 34 NL CH₄ L⁻¹ reactor d⁻¹ (= 97 g COD L⁻¹ reactor d⁻¹) at HRT 1.7 h. This is about fifty times higher production rate than reported for conventional stirred tank AD processes operated on manure alone (Chynoweth et al., 1999; Summers and Bousfield, 1980). The methane yield on liter basis (Fig. 3b) was $0.75 \cdot \ln(\text{HRT}) + 2.2$ NL CH₄ L⁻¹ feed (HRT in hours; R² is 0.88) with a maximum of 4.7 NL methane per liter feed at HRT 42–17 h, decreasing to 2.4 NL methane per liter feed for the lowest HRT. This is 0.47 g COD methane g⁻¹ COD_T feed at HRT 42–17 h and a decrease to 0.24 at HRT 1.7 h (0.166 NL CH₄ g⁻¹ COD_T feed decreasing to 0.086). The biogas methane content was 76–81 % for all HRT. The COD removal, measured as COD_T, COD_S and COD_{VFA}, varied between 24 and 68 %, 38–65 % and 46–90 %, respectively, with increasing effluent concentrations with load (Fig. 4). An observed 49% COD_T reduction at HRT 17 h corresponds well with results from similar cases reported by Kalyuzhnyi et al. (1999) and Kang et al. (2003). No published results are found to compare the highest loads (400 g COD L⁻¹ reactor d⁻¹) investigated here but OLR up to 72.5 g COD L⁻¹d⁻¹ using cow manure supernatant have been run at steady state obtaining higher yield (Rico et al., 2011). The effluent COD concentrations achieved here are probably not as low as achievable in a steady feed operation. This can be seen in Fig. 4 where the medium rate reactors at the end of the experiment removed significantly less COD_T, COD_S and COD_{VFA}, at

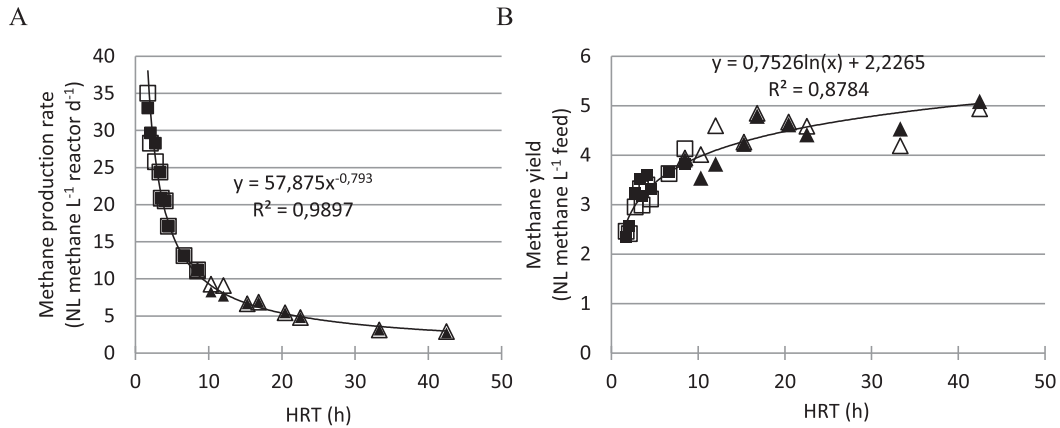


Fig. 3 – Average methane production rate (A) and yield (B) in both medium (Δ ▲) and high (□ ■) rate reactors. One parallel reactor filled symbols and the other one empty.

HRT = 8.5 h than the high rate reactors that started at steady state at this HRT. The daily 5% load increases used here to test the robustness of the reactors are not conducive to maximize transformation efficiency.

3.3. VFA

Process efficiency can be further elucidated from the measured VFA concentrations during the experiment. COD_{VFA} was removed by 86 %–90 % in all the reactors at the start of the load increase and reduced to 46% at the highest load. The effluent acetate concentration (Fig. 4) increased

with load but remained quite low during the experiment, implying robust methanogenesis. The reduced methanogenesis with load may be caused by ammonia inhibition, according to the inhibition factors proposed for ADM1 (Batstone et al., 2002) which in this case (measured effluent ammonia = 2.32 ± 0.03 g NH₄-N L⁻¹ and pH 8.0) can cause 90% reduction in the acetate removal rate. Such strong effect was, however, not observed, implying that some adaptation to high ammonia (e.g. as explained by Schnürer and Nordberg, 2008; Hattori, 2008) may have occurred. This suggests that inhibited methanogenesis is not the main cause of reduced methane yield with load.

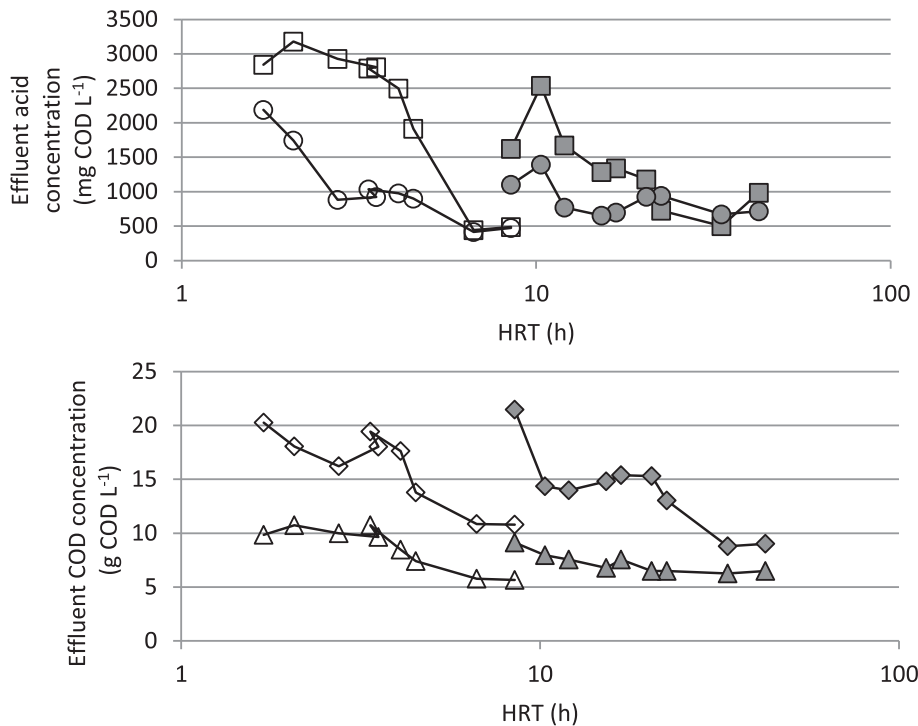


Fig. 4 – Effluent acetate (○), propionate (◻), CODT (◊) and CODS (Δ). Medium rate symbols are filled and high rate symbols are unfilled.

The fraction of removed acetate from the influent COD_T remained constant during the experiment (Fig. 5). Propionate removal on the other hand was reduced with the load increase, but this did not cause other instability symptoms than lowered methane yield ($\text{g COD CH}_4 \text{ g}^{-1} \text{COD}_T \text{ feed}$) even though virtually no propionate was removed at the highest loads (Figs. 4 and 5). The reduced propionate removal can be explained by low growth rate and inhibition due to high levels of acetate and/or hydrogen. High concentrations of these propionate removal products are thermodynamic unfavorable for propionate reduction (Batstone et al., 2002) and can occur during load increase. During constant feed operation propionate accumulation may be avoided. The increasing feed flow rate used to induce the load increase could also have caused a washout of some dispersed biomass especially at the higher flows, worsening the situation for the slow growing propionate removal organisms.

Propionate has been recommended as state indicator, together with acetate and biogas production, to monitor manure digesters due to the slow growth of propionate degraders (Boe et al., 2010). The observations discussed above confirm that propionate degradation can be an AD rate limiting step and propionate therefore is a useful state indicator.

The reduced conversion efficiency with load, attempted explained by inhibition above, may alternatively have a physical cause. Mass transfer effects on the observed kinetics of substrate uptake have been studied in detail by several authors, as summarized and evaluated for AD by Pavlostathis and Giraldo-Gomez (1991). Given that granular sludge bed processes decouple sludge retention time from HRT they can be mass transfer limited rather than reaction limited. Diffusion of molecules from the liquid phase into the granules and entrapment of small particles may be influenced by hydraulic load: Low HRT allows little time for such mass transfer. Coitois kinetics proposed to describe substrate uptake AD kinetics predicts effluent substrate concentrations similar to those observed here, typical for mass transfer limited processes (Pavlostathis and Giraldo-Gomez, 1991), but the results are not decisive. Distinguishing mass transfer and reaction limitation in such processes is a challenge for future research.

3.4. Microbial communities

The microbial communities in the reactors were compared at three different time points. Non-metric multidimensional scaling of Bray–Curtis similarities indicated that the bacterial and archaeal communities of the reactors differed with respect to the type of granule inoculum used (Fig. 6).

A PERMANOVA test confirmed that there were significant differences in microbial communities between the reactors inoculated with pre-adapted granules and the reactors inoculated with non-adapted granules both for bacteria ($p = 0.003$) and archaea ($p = 0.002$) hence the six months pre-adaptation period of the high rate reactors had a significant impact on the reactor microbial community. The average Bray–Curtis similarities show that the microbial communities in the high rate reactors and the medium rate reactors became more similar with time. The average Bray–Curtis values increased from 0.63 ± 0.03 to 0.77 ± 0.06 from day 35 to day 68 for bacteria and from 0.64 ± 0.04 to 0.75 ± 0.05 for archaea. This implies that a long-lasting adaptation of the granular inoculum from pulp and paper mill UASB wastewater treatment is not needed to make it capable of treating manure. This can perhaps be explained by the diverse microbial community generally found in manure (Hagen et al., 2014; Liu et al., 2009; Barret et al., 2012) such that the AD process is continuously inundated by manure adapted organisms in the feed.

3.5. Process implications

The results show that settled pig manure supernatant is a suitable substrate for sludge bed AD in spite of having particulates content above the recommended range for UASB feeds (Tchobanoglous et al., 2003). The manure fraction tested here has similar composition to other slurries, such as wastewater sludge, fish pond aquaculture sludge and other types of manure, encompassing nearly half of all wastes deemed suitable for AD (Berglann and Krokann, 2011). This does not necessarily imply that all such slurries can be treated by high rate AD. Lettinga and Hulshoff Pol (1991) warned that suspended matter can have adverse effects.

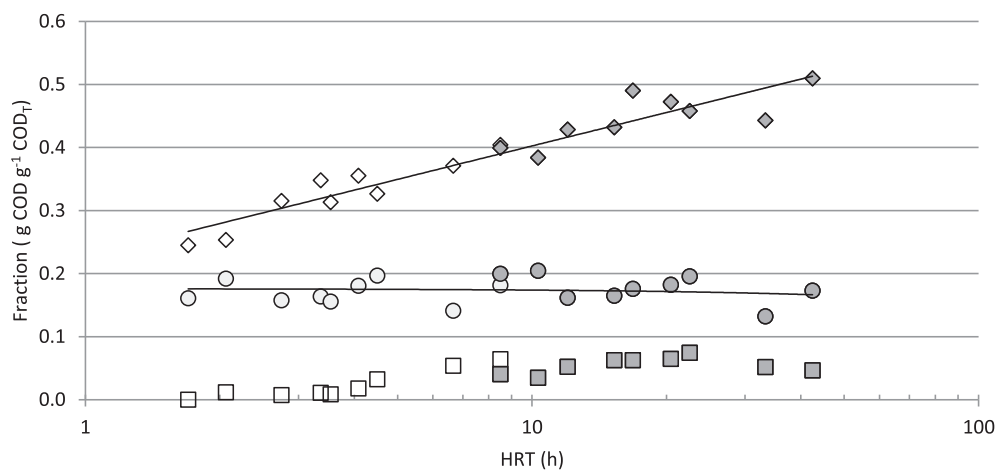


Fig. 5 – Produced biogas (\diamond), removed acetate (\circ) and removed propionate (\square) as fractions of influent COD_T . Medium rate symbols are filled and high rate symbols are unfilled.

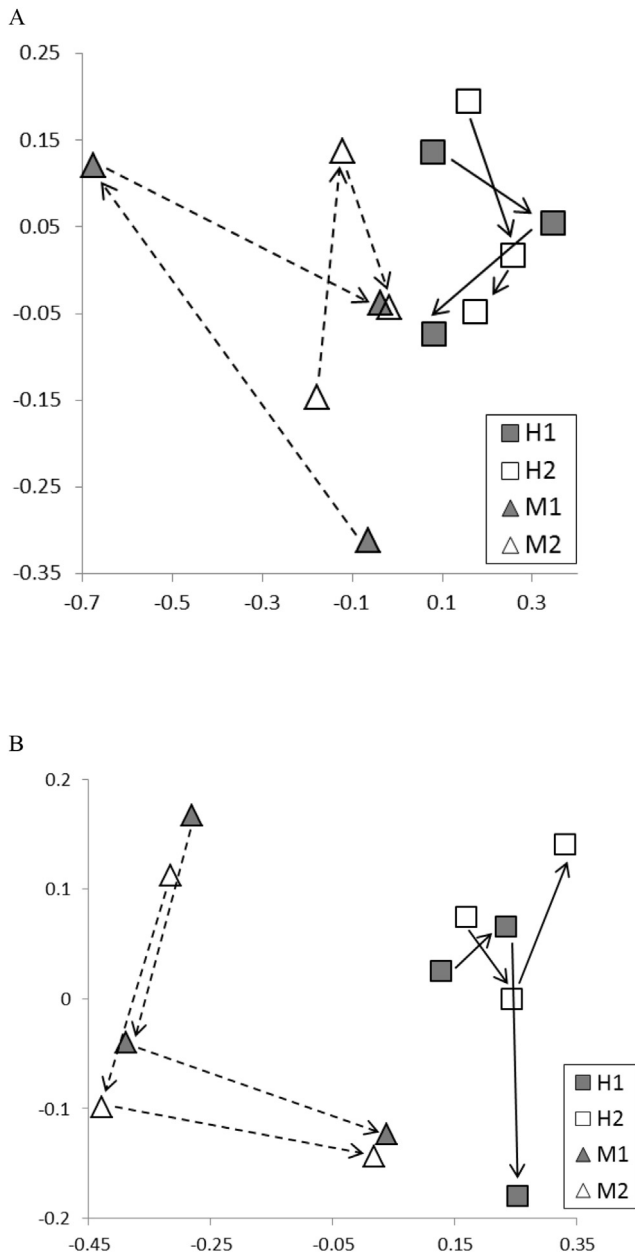


Fig. 6 – NMDS ordination based on Bray–Curtis similarities for comparisons of bacterial (A) and archaeal (B) communities in the high (H) and medium (M) rate reactors at day 35, 61, and 68 of the experiment. The arrows indicate the time course of the samples.

The biogas yield was $0.47 \text{ g COD methane g}^{-1} \text{ COD}_T \text{ manure}$ from HRT 42 to 17 h, decreasing to 0.24 at HRT 1.7 h (Fig. 5). This implies that HRT > 17 h is adequate to obtain high energy recovery yield and production rates up to $20 \text{ g COD methane L}^{-1} \text{ reactor d}^{-1}$.

There is a large trade-off between production rate and yield at the highest loads imposed. This can partly be explained by propionate degradation lagging behind in the AD chain reactions. It is likely that this limitation would lessen if steady state was allowed to establish, but some yield loss at high

production must be expected. It is still likely that high production during periods of high demand can have greater value than the loss in total production caused by temporary low yield, at least down to HRT = 4 h (Figs. 3 and 5).

Very high and changing loads imposed here did not cause process failure. This suggests that such processes can be operated safely without much monitoring in the whole range tested, up to $400 \text{ g COD L}^{-1} \text{ reactor d}^{-1}$. The result also demonstrates that it is possible to turn biogas production up and down depending on energy demands, but this must be done with caution. The reduced propionate removal caused by a 5% load increase (Fig. 5) can be seen as a stress symptom, suggesting that faster changes can be risky but achievable.

The microbial communities in the reactors inoculated with pre-adapted granules and non-adapted granules were significantly different with respect to both bacteria and archaea, but became more similar with time. The relatively fast adaptation to manure implies that non-adapted granular sludge may be used to start sludge bed bioreactors for treatment of pig manure supernatant.

Cheap and mechanically simple processes are also required to make manure AD economically sound. The extreme high rate AD obtained here demonstrates that it is possible to treat manure in small and thereby presumably cheap digesters. Mechanical simplicity was achieved by not using recycle flow to fluidize the active biomass (as opposed to standard UASB design). The inflow, controlled with a timer (on/off), hit the reactor bottom in pulses as an alternative way to fluidize the sludge (Fig. 2). The strongest mixing occurred during feeding while it was visually observed that gas production maintained mixing between feedings. It was also observed that the feed flow stirred and mixed well with the lower sludge bed layers during each pulse feed while the upper sludge bed fluidized but was not much stirred. This suggests that the process behaves more like a plug flow than a stirred tank reactor and is thus, in this respect, similar to a conventional UASB. A full scale AD sludge bed reactor without recycle will be tested next. Pulse feeding has been demonstrated to favor the development of efficient granular sludge for wastewater treatment (Franco et al., 2003).

A rather compact sludge bed was observed at the lowest loads while a more expanded bed was observed as the loading increased. The biomass was fluidized to almost fill the whole reactor volume at the highest load, with the potential for biomass washout. This did not occur to any great extent but VFA data suggest a slight loss of biomass with increasing flow, especially at the highest flows, as discussed above.

Expanded beds not fully fluidized could trap organic particulates (Tchobanoglous et al., 2003). This was the case here judging from the removal rate of COD_T (Fig. 7) which is slightly larger than the methane production rate. Particles evidently contributed to the methane production since the COD_S removal rate was less than the methane production rate. This effect appears, however, to be valid for fully fluidized sludge beds also, since the relationships between COD_T , methane and COD_S transformation were the same in the whole range tested.

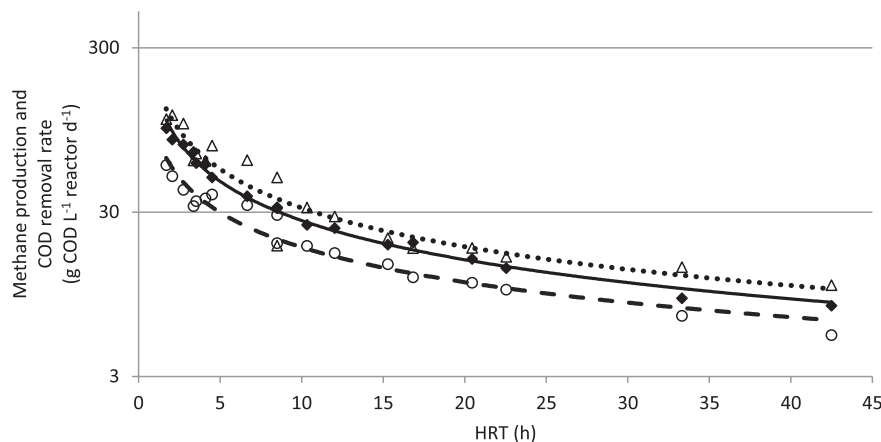


Fig. 7 – Methane production rate (- ◆) compared to the removal rate of CODT (... Δ) and CODS (- - ○). All data points are average from the two parallel reactors.

Practical challenges regarding AD feed handling in full scale at the farm will be met through cooperation with farmers, equipment suppliers and agriculture research teams. The two main issues are: 1. How to operate the AD through cycles of manure availability, spreading etc, 2. The high dry matter fraction from bottom and floating layers must regularly be removed to avoid technical problems. When to remove these fractions (and how to do it) depends on a variety of local conditions, especially its final use as fertilizer. Infrequent removal is advantageous for the overall biogas yield as it allows more degradation of particulates compared to shorter storage (Bergland et al., 2014). An AD reactor volume of about 10 m³ has been identified to be appropriate for the treatment of up to 5000 m³/y, which covers almost all Norwegian pig farms. Farmers express interest in such solutions to improve their abilities to manage the manure as fertilizer while recovering energy.

4. Conclusion

Sludge bed AD reactors can treat settled pig manure supernatant efficiently.

Biogas production rate of 97 g COD L⁻¹ reactor d⁻¹ was obtained at the highest load tested (HRT = 1.7 h and OLR = 400 g COD L⁻¹ reactor d⁻¹) with no physical signs of process failure.

The process handled 5% daily load increases well with reduced methane yield as the only stress symptom down to HRT = 4 h.

Propionate accumulation was observed at the highest OLRs.

A relatively fast adaptation to manure of the microbial communities implies that non-adapted granular sludge can be used as inoculum for sludge bed pig manure treatment.

High process capacity and robustness in mechanically simple manure supernatant treatment suggests a general potential for sustainable sludge bed slurry treatment.

Acknowledgment

The project was supported by the Norwegian Agricultural Authority, Innovation Norway, The Research Council of Norway (Grant no. 208818) and Telemark University College. The authors wish to thank Associate Professor Finn Haugen and Eivind Fjeldalen for designing and implementing automatic process monitoring and control.

REFERENCES

- Anderson, M.J., 2001. A new method for non-parametric multivariate analysis of variance. *Austral Ecol.* 26, 32–46.
- American Public Health Association (APHA), 1995. *Standard Methods for the Examination of Water and Wastewater*, 19th ed. American Public Health Association, American Water Works Association and Water Pollution Control Federation, Washington D.C.
- Bakke, I., Skjermo, J., Tu Anh, V., Vadstein, O., 2013. Live feed is not a major determinant of the microbiota associated with cod larvae (*Gadus morhua*). *Environ. Microbiol. Rep.* 5, 537–548.
- Barret, M., Gagnon, N., Morissette, B., Topp, E., Kalmokoff, M., Brooks, S.P., Matias, F., Massé, D.I., Masse, L., Talbot, G., 2012. *Methanoculleus* spp. as a biomarker of methanogenic activity in swine manure storage tanks. *Fems Microbiol. Ecol.* 80 (2), 427–440.
- Batstone, D., Keller, J., Angelidaki, I., Kalyuzhnyi, S., Pavlostathis, S., Rozzi, A., et al., 2002. The IWA anaerobic digestion Model No. 1 (ADM1). *Wat. Sci. Technol.* 45 (10), 65–73.
- Bergland, W.H., Dinamarca, C., Bakke, R., 2014. Effects of psychrophilic storage on manures as substrate for anaerobic digestion. *Biomed. Res. Int.* 8. ID 712197. <http://dx.doi.org/10.1155/2014/712197>.
- Berglann, H., Krokann, K., 2011. Biogassproduksjon på basis av husdyrgjødsel – rammebetingelser, økonomi og virkemidler. Norsk institutt for landbruksøkonomisk forskning (NILF), p. 71.
- Boe, K., Batstone, D.J., Steyer, J.P., Angelidaki, I., 2010. State indicators for monitoring the anaerobic digestion process. *Wat. Res.* 44 (20), 5973–5980.

- Bolte, J., Hill, D., Wood, T., 1986. Anaerobic-digestion of screened swine waste liquids in suspended particle-attached growth reactors. *Am. Soc. Agr. Eng.* 29 (2), 543–549.
- Bray, J.R., Curtis, J.T., 1957. An ordination of the upland forest communities of Southern Wisconsin. *Ecol. Monogr.* 27, 326–349.
- Burton, C.H., Turner, C., 2003. *Manure Management: Treatment Strategies for Sustainable Agriculture*, second ed. Silsoe Research Institute. Editions Quae, p. 451.
- Chynoweth, D., Wilkie, A., Owens, J., 1999. Anaerobic treatment of piggery slurry – review. *Asian Australas. J. Anim. Sci.* 12 (4), 607–628.
- Cobb, S., Hill, D., 1989. A comparative-analysis of 2 synthetic media for suspended particle-attached growth anaerobic fermentation. *Am. Soc. Agric. Eng.* 32 (1), 223–231.
- Dinamarca, C., Bakke, R., 2009. Apparent hydrogen consumption in acid reactors: observations and implications. *Wat. Sci. Technol.* 59 (7), 1441–1447.
- Franco, A., Roca, E., Lema, J., 2003. Improvement of the properties of granular sludge in UASB reactors by flow pulsation. *Wat. Sci. Technol.* 48 (6), 51–56.
- Hagen, L.H., Vivekanand, V., Linjordet, R.A., Eijsink, V.G., Horn, S.J., 2014. Microbial community structure and dynamics during co-digestion of whey permeate and cow manure in continuous stirred tank reactor systems. *Bioresour. Technol.* 171, 350–359.
- Hammer, Ø., Harper, D.A.T., Ryan, P.D., 2001. PAST: paleontological statistics software package for education and data analysis. *Palaeontol. Electron* 4, 1–9.
- Hattori, S., 2008. Syntrophic acetate-oxidizing microbes in methanogenic environments. *Microbes Environ.* 23 (2), 118–127.
- Hill, D., Bolte, J., 2000. Methane production from low solid concentration liquid swine waste using conventional anaerobic fermentation. *Bioresour. Technol.* 74 (3), 241–247.
- Kalyuzhnyi, S., Sklyar, V., Fedorovich, V., Kovalev, A., Nozhevnikova, A., Klapwijk, A., 1999. The development of biological methods for utilisation and treatment of diluted manure streams. *Wat. Sci. Technol.* 40 (1), 223–229.
- Kang, H., Moon, S., Shin, K., Park, S., 2003. Pretreatment of swine wastewater using anaerobic filter. *Appl. Biochem. Biotech.* 109 (1–3), 117–126.
- King, S.M., Barrington, S., Guiot, S.R., 2011. In-storage psychrophilic anaerobic digestion of swine manure: acclimation of the microbial community. *Biomass Bioenerg.* 35 (8), 3719–3726.
- Lettinga, G., Hulshoff Pol, L.W., 1991. UASB-process design for various types of wastewaters. *Wat. Sci. Technol.* 24 (8), 87–107.
- Lettinga, G., Vanvelsen, A., Hobma, S., Dezeew, W., Klapwijk, A., 1980. Use of the upflow sludge blanket (USB) reactor concept for biological wastewater-treatment, especially for anaerobic treatment. *Biotechnol. Bioeng.* 22 (4), 699–734.
- Liu, F.H., Wang, S.B., Zhang, J.S., Zhang, J., Yan, X., Zhou, H.K., Zhao, G.P., Zhou, Z.H., 2009. The structure of the bacterial and archaeal community in a biogas digester as revealed by denaturing gradient gel electrophoresis and 16S rDNA sequencing analysis. *J. Appl. Microbiol.* 106 (3), 952–966.
- Lo, K., Liao, P., Gao, Y., 1994. Anaerobic treatment of swine wastewater using hybrid UASB reactors. *Bioresour. Technol.* 47 (2), 153–157.
- Masse, D.I., Talbot, G., Gilbert, Y., 2011. On farm biogas production: a method to reduce GHG emissions and develop more sustainable livestock operations. *Anim. Feed Sci. Tech.* 166–67 (SI), 436–445.
- Muyzer, G., Dewaal, E.C., Uitterlinden, A.G., 1993. Profiling of complex microbial-populations by denaturing gradient gel-electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16s ribosomal RNA. *Appl. Environ. Microbiol.* 59, 695–700.
- Pavlostathis, S.G., Giraldo-Gomez, E., 1991. Kinetics of anaerobic treatment: a critical review. *Crit. Rev. Environ. Cont.* 21 (5–6), 411–490.
- Raadal, H.L., Schakenda, V., Morken, J., 2008. *Potensialstudie for biogass i Norge*. Østfoldforskning AS and UMB, p. 55.
- Raven, R.P., Gregersen, K.H., 2007. Biogas plants in Denmark: successes and setbacks. *Renew. Sustain. Energy Rev.* 11 (1), 116–132.
- Rico, C., Garcia, H., Rico, J.L., 2011. Physical-anaerobic-chemical process for treatment of dairy cattle manure. *Bioresour. Technol.* 102 (3), 2143–2150.
- Schnürer, A., Nordberg, A., 2008. Ammonia, a selective agent for methane production by syntrophic acetate oxidation at mesophilic temperature. *Wat. Sci. Technol.* 57 (5), 735–740.
- Summers, R., Bousfield, S., 1980. Detailed study of piggery-waste anaerobic-digestion. *Agric. Wastes.* 2 (1), 61–78.
- Taguchi, Y., Oono, Y., 2005. Relational patterns of gene expression via non-metric multidimensional scaling analysis. *Bioinformatics* 21, 730–740.
- Tchobanoglous, G., Burton, F.L., Stensel, H., 2003. *Wastewater Engineering: Treatment and Reuse*, Advanced Wastewater Treatment, fourth ed. McGraw-Hill Series in Civil and Environmental Engineering. Metcalf and Eddy Inc, New York, pp. 983–1027.